Health effect of household fuel pollution on

young children in semi-urban and urban areas of Bangladesh

by

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Submitted in partial fulfillment of the requirements
for the award of
Doctoral Thesis of Loughborough University

23rd October 2009

Abstract: Household fuel pollution from the use of low quality biomass fuels is considered as a risk factor for respiratory tract infections (RTI) in women and children. Inhalation of fuel-derived pulmonary toxins (e.g. particulate matter ($PM_{2.5}\mu m$), and carbon monoxide (CO) can harm the lungs of young children, due to their under-developed immune defences. In Bangladesh acute respiratory infections (ARI) are the leading cause of child mortality (< 5years of age). This thesis aimed to examine the relationship between RTI and household fuel pollution exposure using measured pollution data and medical diagnoses. During an 18-month longitudinal health intervention in northern Bangladesh households (n=408) were interviewed (3 times) on cooking/fuel-use practices and child health. Anthropometric data (height/weight) and finger-prick blood samples for analysis of immune status (c-reactive protein, alpha-1-acidglycoprotein (AGP) and albumin) were collected (n=321 < 5years of age). All unwell children (62.4%) were medically examined. Household pollution levels (particulate matter ($PM_{2.5}\mu m$) and carbon monoxide (CO) were monitored for a 24-hour period (n=61). Moderate/ severe RTI was common (24.8%) (youngest child only n=213). Poor child growth (stunted: 43.8%, underweight=66.7%, wasted: 38.4%) and immunity were detected. 98% of the households used inefficient chimneyless mud stoves and low quality biomass fuels (wood, golden, dung). The measured indoor pollution levels exceeded the WHO safety thresholds ($PM_{2.5}\mu m$ range: 85 to $3020\mu g/m^3$ CO range: 0-16 ppm) ($PM_{2.5}\mu m>25\mu g/m^3$, CO>9ppm). Longitudinal multivariate GLM showed that cooking practices were associated with child immune status: haemoglobin levels (F=1.555, p=NS) were significantly associated with Bihari ethnicity and a fixed stove use (F=3.718 and F=3.716, p<0.05 respectively). Elevated log_{10}-AGP levels were found (F=4.371, p<0.05) in Saidpur in households using a fixed stove (F=4.123, F=3.780, p<0.05). The patterns in child growth z-scores were due to age only (stunting: F=7.413, p<0.01, underweight F=5.787, p<0.05). Interestingly, poorer change score for weight-for-age (F=34.893, p<0.01) was associated with low age and more frequent cooking (F=6.441 and, F=6.553, p<0.05 respectively). Logistic regression (healthy vs. RTI) identified the presence of child by the stove during cooking as the sole risk factor for RTI (absent OR= 0.257, 95% CI: 0.097 – 0.676, p<0.01). Indoor cooking and the use of a fixed stove were associated with low SES. Education may help to reduce behaviours associated with high household fuel pollution exposure via the introduction of simple healthy cooking practices.

Keywords:

Household fuel pollution

Respiratory tract infections

Child health

Immunity

Particulate matter

Carbon monoxide

Intervention

Acknowledgements:

I would like to thank Concern Worldwide (Bangladesh), Winrock International, Village Education Research Group and Exonics (Bangladesh) for their great work to make this fieldwork so successful! Thank you for all your efforts. Thanks to my interviewer team from the Associates of Community and Population Research for your hard work in the field. It was fun to work with you!

Thank you Emily for being such a supportive, patient and fun supervisor. I enjoyed working with you on this project. Hopefully we can continue this work together!

Nashtara: thank you for responding to my advert when I needed someone to translate some text written in Bangla! Our great friendship began from than! Thanks for your continuing support and encouragement throughout the work, and the great insight into the life in Bangladeshi you have given me.

Special thanks go to Dr Rie Goto who got me so interested in child health issues in Bangladesh. I thoroughly enjoyed the South Asia-training you arranged for me! Especially eating nice Bangla food was really enjoyable, and of course our trips to the Piggy!

I would like to thank my family for all the support throughout my PhD!

I am forever grateful to my husband Rod who supported me from the beginning of the project, until the very end! Proof-reading would have never been done so well without my own Clippet! It was so nice to share the field experiences with you too! Thank you for that! Thank you for being so interested in it all and believing in it, when I lost the hope (happened occasionally ☺ !). This would have never happened without!

Thank you Freya (Muikku) for keeping everything in perspective, especially during the data analysis and the final write-up when I was in danger of being buried under the work load. Your chuckles and squeaks always make me smile!

Thank you Oskar (in the belly) for making it all very real that the work needed to be completed! Thanks for speeding me along so nicely! ☺

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Chapter 1: Introduction.

1.1. Introduction

Household fuel pollution is defined as smoke indoors produced as a result of daily burning of biomass fuels such as wood, dung and crop residues indoors for domestic cooking and heating. Use of inefficient stove designs can lead to incomplete combustion of fuels causing fuel wastage, longer cooking times and release of high levels of pollutants such as particulate matter, carbon monoxide and nitrogen oxides in the indoor air. An epidemiological study has suggested that long-term exposure to particulate matter levels slightly above the background levels (3-$5\mu g/m^3$) may be a cause of chronic ill-health in exposed individuals **(WHO, 2005).**

The industrialised world is currently struggling to reduce the levels of outdoor air pollution from transportation and industries, which is a one of the most worrying environmental threats today. However approximately half of households globally use highly pollutant biomass fuels for domestic energy which is the main cause of indoor air pollution. The focus on the health effect from chronic household fuel pollution is essential as people spend the majority of their time indoors sleeping or doing domestic work including cooking **(Weisel, 2002),** particularly in the developed world. Household fuel pollution is a highly significant problem in rural parts of the developing world where up to 90% of the households rely on biomass fuel for domestic energy for cooking and heating **(Reddy, Williams and Johansson, 1997).** Also, due to a lesser degree of industrialisation in developing countries the health threat from household fuel pollution is likely to be far more significant than from outdoor air pollution. Due to high poverty and scarcity of resources, switching to cleaner burning fuels is often impossible in societies in these areas. Especially in poorer segments of societies low quality biomass fuels have often continued to be the main source of domestic energy and household fuel pollution continues to be a significant health problem among women and children in particular. There is even evidence that the use of biomass fuel is increasing among the poor **(WRI, 1999)** due to increased urbanisation and urban migration as migrants often face poverty and a

lack of amenities in the urban areas, which leads to an adoption of low-cost biomass fuels **(von Schirnding** *et al,* **2002).**

Household fuel pollution exposure has been associated with adverse health outcomes such as more frequent and more severe respiratory infections and poor immunity in children, and chronic obstructive pulmonary disease (COPD) and lung cancer in women **(Smith** *et al,* **1994: Ellegård, 1996, 1997; Xu** *et al,* **1996; Perez-Padilla** *et al,* **1999; Ezzati and Kammen, 2001A,B; Naeher** *et al.,* **2001; Rollin** *et al,* **2001; Balakhrisna** *et al.,* **2002; Kleinerman** *et al,* **2002, Smith and Mehta, 2004; Mishra, Retherford and Smith, 2005).** Women and young children are most likely to be affected by the adverse impacts of household fuel pollution as in traditional societies women have the main role as a household cook and child minder. Currently however, only a limited number of studies have quantified air pollution levels within households, as well as assessing the prevalence and incidence of respiratory infection within the community **(e.g. Melsom** *et al,* **2001: Ekici** *et al,* **2005).**

1.2. Bangladesh as a study setting

Bangladesh is located in South Asia between India and Myanmar (see Figure 1.1, p.7). Bangladesh became an eastern part of Pakistan following the end of the British rule over India in 1947. Bangladesh was formed in 1971 when the former East Pakistan gained independence from Pakistan. At the time of India's separation the Muslim Bihari (speakers of Urdu, the language of Pakistan) who had previously inhabited the Bihar state of Hindu-India were forced to emigrate and they settled in the then East Pakistan (today Bangladesh). During the separation process to form a new independent country 'Bangladesh', Pakistan refused to receive the Bihari in fear of instability from ethnic uprising of minority ethnic groups within its lands. Bangladesh refused to give citizenship to the Bihari (as they supported Pakistan); however they were allowed to stay within the borders of Bangladesh. Now many of the Bihari live in poor camp-like settlements around Dhaka and in the Rangpur district in the north-west of Bangladesh. Integration of the Bihari into Bangladeshi society is slow but steady.

Bangladesh is one of the poorest countries in the world with 80 percent of the population living in rural areas. Bangladesh has one of the highest population densities in the world which increases at the rate of 2.7% every year (**BDHS, 2007**). The under-five mortality rate is high at 65 per 1000 live births and maternal mortality rate is one of the highest in the world (3 per 1000 live births) (**BDHS, 2007**). Solid biomass fuels are widely used around Bangladesh for cooking whereas kerosene or electricity is used for lighting. Seasonal and economic factors greatly influence the availability and the use of low quality biomass fuels in Bangladesh. People in Bangladesh have very poor access to adequate health care services: over 60 % of the population lack access to adequate health care (**MOH and FW, 2003**). Currently Bangladesh ranks as the 146th country (out of 182 countries) on the Human Development Index 2009. Acute respiratory infections (ARI) are the leading cause of morbidity and mortality in children under the age of five years in Bangladesh (37 percent among children aged under one) (**BDHS, 2004**). The incidence rate of ARI among children (aged under five years) is high (5.5 episodes per year) (**Zaman *et al*, 1997**).

Research into the health impacts of indoor air pollution in Bangladesh is very limited despite a high prevalence of respiratory illnesses such as respiratory infections and asthma in the country (Bangladesh Demographic and Health Survey or **BDHS, 2004**). A community-based project to control and reduce child mortality from ARI in Bangladesh was set up by the Bangladesh Rural Advancement Committee (or BRAC). Health promotion to increase maternal awareness of ARI through health workers was very successful in controlling and reducing ARI mortality in children at grass root level (**Hadi, 2002**). However one in five children less than five years old still suffers from an acute respiratory infection in Bangladesh (**BDHS, 2007**). A quarter of infant deaths in Bangladesh during a survey in 1993-4 were due to acute respiratory infection (**Baqui *et al*, 1998**). Despite an immense improvement in child survival rates in Bangladesh, respiratory infections still remain as one of the leading contributors of mortality in children under five in Bangladesh (**BDHS, 2004**).

Several studies on the levels of household fuel pollution in communities have shown very high daily exposures to particulate matter and carbon monoxide levels. One study in Bangladesh reported that households commonly experienced very high particulate matter levels from 68 to 4864 $\mu g/m^3$ compared with the WHO recommendations for safe particulate matter exposure ($PM_{2.5}\mu m<25\mu g/m^3$) and the variation was probably due to quality of the fuel used **(Dasgupta *et al*, 2006).** Households with no ventilation methods experienced the highest particulate matter pollution levels. The use of biomass fuels is very prevalent throughout Bangladesh **(Dasgupta *et al*, 2006).**

This PhD research investigated household fuel use and cooking practices in semi-urban and rural communities in northern Bangladesh. This research aimed to assess the prevalence of respiratory infections and the general health status in children under five and measure the extent of domestic indoor air pollution from biomass fuels. Child health status was assessed via medical diagnosis and an analysis of selected immune markers which have been successful at assessing the population health status in many epidemiological studies globally **(Manary *et al*, 1997; Lunn *et al*, 1998; Panter-Brick, 2001; Baqui *et al*, 2003; De Silva *et al*, 2003; McDade, 2003; Najera *et al*, 2004; McDade, Hawkling, Cacioppo, 2006).** The use of selected biomarkers of immunity was expected to help to ascertain the degree of poor health in the communities, which are often over-looked when defining health status through verbal biopsies and medical diagnoses.

1.3. Chapter descriptions:

Brief details are provided on the content of each of the chapters to follow.

Chapter 2: Literature review: This chapter provides a review on previous studies on health impacts of household fuel pollution around the world.

Chapter 3: Household-health survey methods and air-quality monitoring: This chapter describes the survey methods used to collect data on household characteristics and child health.

Chapter 4: Immune analysis: This chapter provides detailed information about the blood sample collection methods applied in the field and the laboratory techniques

used to analyse finger-prick blood samples from children for selected biomarkers of immunity. This chapter also describes the techniques used and developed to study the usefulness of filter-paper-technique for collecting blood samples in a field situation as well as how environmental factors may affect the integrity of the blood samples.

Chapter 5: Sample background descriptives: Chapter 5 provides a description of the sample features of the study community in terms of age, housing, occupational and income details, as well as the growth details of children and health descriptives of mothers and children under the age of five years.

Chapter 6: Child health and immunity: The main focus of this chapter is to examine whether or not indoor air pollution exposure influences the prevalence of respiratory tract infections and other respiratory-related illnesses among young children in Bangladesh.

Chapter 7: Household fuel pollution: This chapter describes observed and experienced fuel pollution levels in the study households (where air-quality was monitored). This chapter also attempts to find household variables which may influence the pollution levels that the household members are exposed to on a daily basis. Also the technical and behavioural/education intervention which was introduced into the study community is briefly described.

Chapter 8: Thesis discussion and conclusion: This chapter discusses the research findings of this thesis in the context of current research into household fuel pollution and its adverse impact on health.

1.4. List of abbreviations used in this thesis:

AGP= Alpha-1-acidglycoprotein

ARI= Acute respiratory infection

CO= Carbon monoxide

COPD= Chronic obstructive pulmonary disease

CRP= C-reactive protein

IAP= Indoor air pollution

Hb= Haemoglobin

PM= Particulate matter

Ppm= Parts per minute

RTI= Respiratory tract infection

URTI= Upper respiratory tract infection

Tables and Figures:

Figure 1.1: Map of Bangladesh
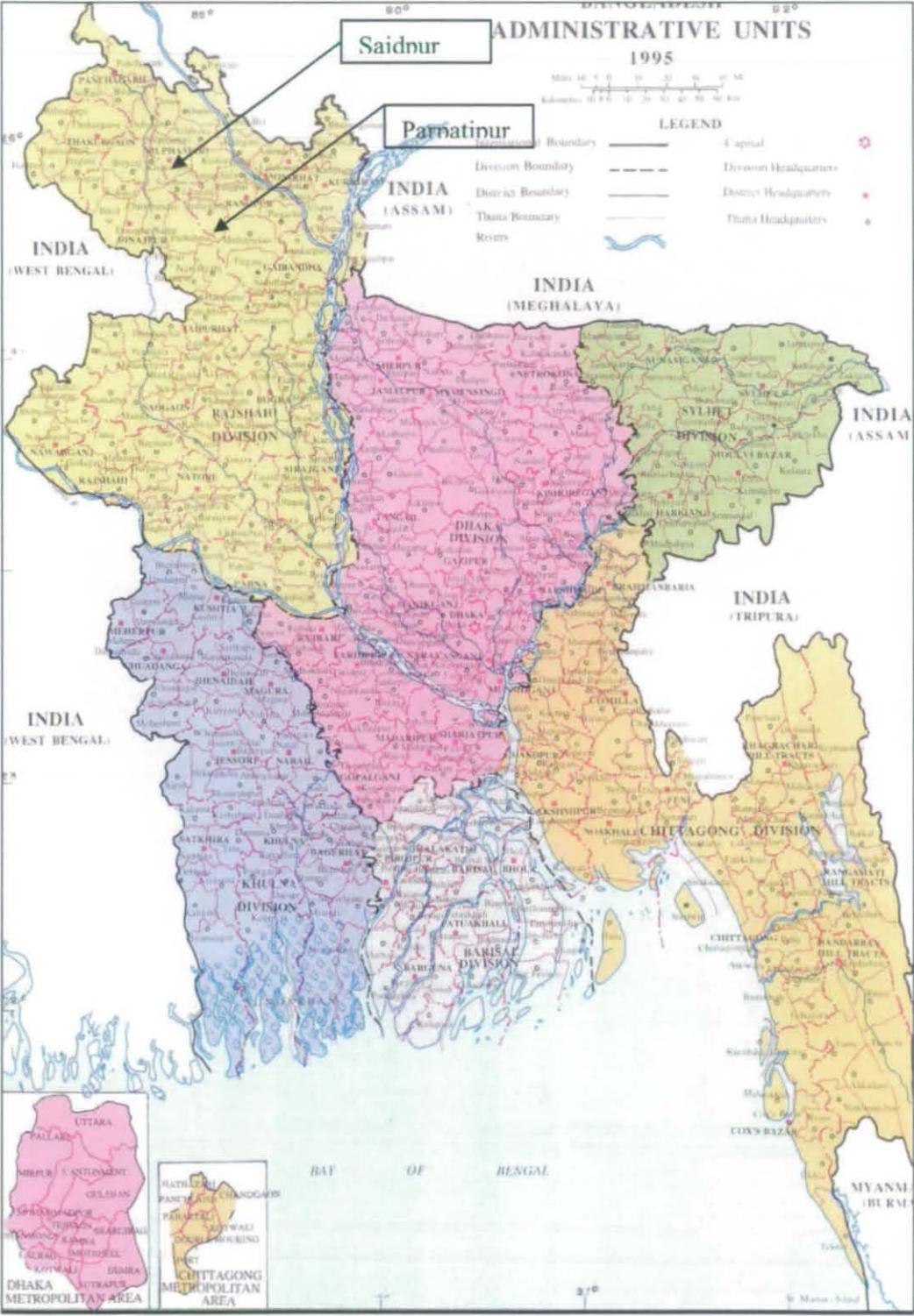
Chapter 2:   Review of current research on household fuel pollution and its impacts on health.

2.1. Introduction to methods applied in the literature search

This chapter reviews the current and previous research carried out in the field of indoor air pollution The articles reviewed in this chapter were found using 'Google Scholar', Science Direct, Pubmed websites and search engines such as Metalib provided by the Pilkington library of Loughborough University using general search terms such as "indoor air pollution" and "biomass fuels". These research terms were very useful in identifying what was meant by indoor air pollution, mechanisms by which indoor air pollution may act as a health hazard, as well as guidelines for safe exposure to inhalable environmental toxins. However these keywords were not specific enough to meet the topic of this study and search criteria needed to be narrowed to target any articles related to the aim of this PhD.

The use of keywords "indoor air pollution and developing countries" and "indoor air pollution and South Asia" helped to specify articles directly related to third world countries such as Bangladesh which was the focus of this PhD. These research terms helped to bring up publications on the use of low quality biomass fuels, stove designs and any interventions aiming to reduce the smokiness of household air by improving stoves. Most publications aimed to deal with the actual daily exposure to indoor air pollution with little interest on health outcome.

"Indoor air pollution or household fuel pollution and health" This search term brought up the most significant works carried out in the field of indoor air pollution; for example those carried out in Guatemala and China as well as current health research on occupational health hazards of chronic exposure to indoor air pollution. The most current work from developed countries came from the United States of America and included a wide range of occupational health research and chronic obstructive pulmonary disease. Many meta-analyses on the quality of past research in the field of

indoor air pollution were also found using this research term. These articles were very helpful in identifying the past and the current focus of indoor air pollution research and any shift in the research interest in the field. Use of meta-analyses also helped to identify key research groups in the field of indoor air pollution.

The search term "indoor air pollution and child health" and "indoor air pollution and child health in Bangladesh" and "household fuel pollution and respiratory infections in children" finally identified some key articles within the interest of this PhD thesis and any research on early life exposures to indoor air toxins. This also brought up publications on intra-uterine exposures to household fuel pollution and maternal pollution exposure during pregnancy; interesting points to remember when considering the extent of the health impacts of indoor air pollution. The search terms given showed the notable lack of current work on health impacts of household fuel exposure on very young children, with indoor air pollution as the main focus. Often indoor air pollution and frequent respiratory infections were investigated as part of an authors' work on childhood malnutrition and socio-demographic determinants of poor nutrition and related health outcomes.

Articles identified were used to clarify the past and current focus of indoor air pollution research globally and in developing countries such as Bangladesh. The review identifies the strengths and weaknesses of each study chosen for the review. The studies chosen for the review have been selected on the basis of their good study design, and where possible for their large enough sample size to strengthen the statistical analyses performed.

2.2. Indoor air pollution

2.2.1. What is indoor air pollution?

Indoor air pollution or household fuel pollution is smoke indoors that results from the burning of solid biomass fuels such as wood, dung and crop-residues as fuel for domestic cooking and heating. Smoke from the burning of solid unprocessed biomass fuels contains many toxins and chemical compounds which are likely to be harmful to a person's health when inhaled. The lungs are particularly vulnerable to the toxins from indoor air pollution exposure, but deteriorating general health may also be observed as a result of chronic air pollutant exposure **(Kulkarni and Grigg, 2008).** Current toxicological research on indoor air pollution has focused on studying chemical behaviour and the health effect of inhalable particulate matter (PM), oxides of nitrogen, sulphur dioxide, ozone, and volatile organic compounds and formaldehydes. The mechanisms of how these common biomass-derived toxins influence pulmonary health and immune status are described in detail below.

2.2.1.1. Nitrogen oxides

Combustion of solid biomass fuels is a great source of nitrogen oxides (NO) which readily react with ozone to form nitrogen dioxide, nitric acid and nitrate particles. Nitrogen dioxide (NO_2) is a non-water soluble compound which, when inhaled, can get deposited on the mucosal surface of the lower pulmonary tract. Exposure to nitrogen dioxide can irritate the eyes and the mucosa of the lungs. A very high exposure to nitrogen oxide has been associated with diffuse lung injury and pulmonary oedema, whereas low chronic exposure has been associated with acute and chronic bronchitis and an increased bronchial reactivity in some asthmatics **(Lotz *et al*, 2008)**. It has been suggested that exposure to nitrogen dioxide may increase the risk of bacterial and viral lung infections and reduce normal lung function **(Bruce, Perez-Padilla, Albalak, 2000).**

2.2.1.2. Volatile organic compounds

Volatile organic compounds are compounds which are found in paints and household solvents as well as road and biomass fuel pollution, and they are often gaseous and genotoxic **(Harrison, 1999)**. Volatile organic compounds are known irritants of the airways and the mucosal surface of the lungs and have been linked with many severe respiratory conditions such as lung cancer, asthma and respiratory distress. **Formaldehyde** is a carcinogenic chemical which can cross-link DNA and proteins of the nasal and the pulmonary mucosa when inhaled **(Swenberg, Barrow and Starr, 1983)**. Formaldehyde can increase allergic sensitisation and cause asthma as well as increase susceptibility to infections **(Delfino, 2002)**. Carcinogens derived from solid biomass fuels have been associated with an increased risk of lung cancer, and the cancers of the larynx and naso-pharynx in exposed individuals **(Pintos *et al*, 1998)**.

2.2.1.3. Sulphur dioxide (SO_2)

Sulphur dioxide (SO_2) is a colourless heavy gas with a strong odour, which is emitted from the burning of solid biomass fuels. Burning of coal is a rich source of sulphur dioxide. This highly water soluble gas forms an acid when in contact with water. The inhalation of sulphur dioxide results in a deposition of acidic compounds on the surface of the upper respiratory tract, and mucosal irritation can cause broncho-constriction **(Bruce, Perez-Padilla, Albalak, 2002)**. The bodily response to an exposure to sulphur dioxide is coughing as a result of broncho-constriction, even at relatively low exposure doses. Asthmatics and those individuals with sensitive respiratory systems and lowered pulmonary functions are likely to experience airway irritation at a lower exposure to sulphur dioxide which would not affect healthy individuals. Functional changes in the pulmonary system have been suggested as a result of an exposure to sulphur dioxide **(Altshuller, 1987)** as well as an increase in the respiratory symptoms and poorer pulmonary function **(Peters *et al*, 1996)**.

2.2.1.4. Carbon monoxide (CO)

Carbon monoxide is a colourless, odourless and water soluble gas which is a common by-product of fuel combustion (including biomass fuels). The toxicity of carbon monoxide is caused by its ability to combine with blood haemoglobin, which causes

the formation of carboxyhaemoglobin (COHb). The presence of carboxyhaemoglobin can interfere severely with normal oxygen transportation to tissues with the highest oxygen demand, such as cardiovascular and pulmonary systems. Oxygen starvation may disrupt the energy transportation of the cell. An exposure to a 1:500 concentration of carbon monoxide to oxygen can cause unconsciousness and a 1:100 concentration causes immediate death. Cellular damage of nerve cells from carbon monoxide exposure resulting in neurological and behavioural changes has been reported **(Jasper *et al*, 2005).** Health symptoms of carbon monoxide exposure can vary from mild ones such as fatigue, dizziness, headaches and nausea, which could be mistaken for a common cold or flu, to severe health damage such as cognitive impairment. An exposure to biomass fuel pollution can increase the oxidative stress and tissue damage due to a high concentration of nitrogen oxides as carbon monoxide will bind more readily with *haem*, blocking the removal of nitrogen oxides by the red blood cells **(Omaye, 2002).**

The greatest health damage from carbon monoxide exposure can be experienced by the elderly with poorer lung function or young children whose pulmonary system is still undeveloped. A lower birth weight has also been observed in babies born to women exposed to carbon monoxide from highly polluting fuels (solid biomass fuels) **(Boy, Bruce and Delgado, 2002; Mishra *et al*, 2004; Mishra, Retherford and Smith, 2005).** Studies have also shown that exposure to carbon monoxide may lead to an onset of angina in individuals who have ischemic heart disease and exercise regularly. Exercise tolerance has also shown to be reduced in individuals with chronic obstructive pulmonary disease (COPD) when exposed to carbon monoxide **(American Thoracic Society, 1990).**

2.2.1.5. Particulate Matter (PM)

Particulate Matter (PM) pollution is a term used to describe any air-borne particles of different sizes and compositions (organic and inorganic) which are released from burning fuels. The main source of particulate matter is an incomplete combustion of fossil fuels. Wind-blown particulates are often larger (diameter larger than 10μm) and are filtered out by the upper airways without causing any tissue damage **(Kulkarni**

and Grigg, 2008), and they are not therefore termed as particulate matter. Inhalation of smaller particulate matter (under 10μm in diameter) can lead to a deposition of particles in the lungs resulting in the irritation of the bronchial mucosa. The smallest particles, in particular those less than 2.5μm in diameter ($PM_{2.5}$), are able to penetrate very deeply into the pulmonary bronchioles and even alveoli when inhaled, leading to a deposition of the particles in the lungs. This can result in irritation of the bronchial mucosa and tissue tear causing great pulmonary damage **(USEPA, 1997).** The presence of particulate matter on the surface of the lungs may induce macrophageal immune responses which may also reduce the mucosal defences of the lungs. Particulate matter exposure therefore may contribute to the development of respiratory infections and worsen the symptoms in asthmatics **(Nordenhäll *et al*, 2001).** Research has identified short-term and long-term adverse health effects from particulate matter exposure, however the cellular mechanism by which the adverse health outcomes have been caused is not yet clear. Chronic inflammation of the lung tissue which may cause tissue leakage and a release of toxins into the blood stream have been suggested **(Ware and Matthay, 2000).**

2.2.2. Determining factors of exposure

The health effect from household fuel pollution is likely to be dependent on the nature of exposure. An exposure to indoor air pollution has been defined as a function of time of exposure per individual **(Lioy, 1990).** In order to simplify the quantification of unhealthy air pollution exposure the World Health Organisation (WHO) in 1985 defined safety thresholds for levels and length of exposure for various air pollutants. The safety guidelines were updated in 1997 and again in part in 2005 (particulate matter). Table 2.1 provides details of the safety thresholds for carbon monoxide and particulate matter (PM_{10} and $PM_{2.5}$) by the WHO and the US-Environmental Pollution Agency (US-EPA) as these pollutants are the main interest in this thesis (Table 2.1, p.48). WHO guidelines provide a lower end of the range at which adverse health effects due to short-term pollution exposure have been identified. They have been created using multi-city data which makes them highly applicable for use in urban or rural settings globally. The US-EPA pollution criteria standards were created to protect the general public including the most sensitive individuals from adverse health

effects of carbon monoxide and particulate matter. This data set is US-specific and probably most applicable to developed country settings.

2.3. The health effects of household fuel pollution:

Current research suggests a link between exposure to household fuel pollution from the burning of solid biomass fuels and health outcomes such as more frequent and more severe respiratory infections and poor immunity in children, chronic obstructive pulmonary disease (COPD) and lung cancer in women **(Smith *et al*, 1994; Ellegård, 1996, 1997; Xu *et al*, 1996; Perez-Padilla *et al*, 1999; Zhong *et al*, 1999; Ezzati and Kammen, 2001B; Naeher *et al*., 2001; Rollin *et al*, 2001; Balakhrisna *et al*., 2002; Kleinerman *et al*, 2002; Smith and Mehta, 2004; Mishra, Retherford and Smith, 2005).** Links between health outcomes such as asthma, cataracts, adverse pregnancy outcomes and tuberculosis have also been suggested **(Kelly, 2003: Bobak, 2000; Chen *et al*, 2002; Lee, 2003; Mannes *et al*, 2005).** Eye irritation and headaches have been reported in relation to household fuel pollution exposure **(Ellegård *et al*, 1997; Jalaludin, 2007)**, however currently very little evidence exists for this **(Kulkarmi and Grigg, 2008).**

Health problems from indoor air pollution exposure are significantly heightened in developing countries where over 75% of the rural populations rely on low quality solid biomass fuels for energy **(Reddy, Williams and Johansson, 1997; Smith and Mehta, 2003).** Cooking and domestic activities expose women and young children to fuel fumes from daily cooking and heating for many hours each day. It has been suggested that up to 45 percent of COPD in women in developing countries is due to household fuel pollution exposure **(WHO, 2002)**. Acute respiratory infections are the most significant cause of death among children under the age of five years globally, causing 20% of deaths annually **(Tomaskovic, Boschi-Pinto, Campbell, 2004; Rudan *et al*, 2004; Wardlaw, Salama, Johansson, 2006).** Studies where the suggested health outcomes are linked to household fuel pollution exposure will be discussed in detail below:

2.3.1. Acute respiratory infections

Acute respiratory infections (or ARIs) are defined as infections of the airways including upper respiratory infections, tracheitis, and bronchial infections as well as influenza (**International statistical classification of diseases and related health problems, tenth revision, WHO, 1993**). The World Health Organisation classification of acute respiratory infections has categorised ARI cases into mild, moderate and severe on the basis of the symptoms listed below (**WHO, 1984; Pio, 2003**):

- **Mild:** Cough, with sore throat, runny nose and possibly ear discharge for over two weeks in length, no fast breathing or chest in-drawing (as defined by Campbell *et al*, 1989). Home treatment with rest and proper nutrition enough and no antibiotics required.
- **Moderate:** Cough and fast breathing but no chest in-drawing. Antibiotics required for the treatment of moderate bacterial respiratory tract infection.
- **Severe:** Cough, fast breathing and chest in-drawing, or stridor at rest. Hospital treatment is required due to severe bacterial or viral infection of the respiratory tract (clinical pneumonia).

(Note: Fast breathing was identified as over 60 breaths per minute in under two month old babies, over 50 breaths per minute in babies between two to 11 months of age and over 40 breaths per minute in children from one to four years of age (**Berman, Simoes, Lanata, 1991; Pio, 2003).**

Acute respiratory infections are the leading cause of mortality among young children in developing countries (**UNICEF, 2009**). The majority of child deaths related to acute respiratory infections occur in developing countries where the use of solid biomass fuels for domestic cooking and heating is prevalent. Pneumonia prevalence is particularly high in Sub-Saharan Africa, India, China and South East Asia (including Bangladesh), over two thirds of the global pneumonia deaths occurring in these areas (**Singh, 2005).**

Pollutants from biomass fuels have been reported as irritants of the mucosa of the lungs and the airways **(Kulkarni and Grigg, 2008).** Pollutant exposure and the presence of particulate matter and the gaseous pollutants in the cilial surface of the airways is likely to trigger the immune response, reducing the continuous immune defences in individuals who are chronically exposed to household fuel pollution **(Brauer, 1998; Bruce, Perez-Padilla, Albalak, 2002; WHO. 2004)** making them more vulnerable to any infectious diseases. Young children have under-developed pulmonary functions which makes infants potentially more vulnerable to tissue damage from chronic pollution exposure **(Chan *et al*, 1989; Palta *et al*, 2007).** Low birth weight children are at particular risk of pulmonary damage from household fuel pollution exposure because of their very under-developed immune defences and immature lungs and alveolar development as well as their very narrow airways **(Chan *et al*, 1989; Palta *et al*, 2007).** Also the volume of air exchange of a child lungs is considerably higher than in adults due to their higher metabolic rate: for this reason inhalation of air pollutants is likely to cause greater inflammatory action and tissue damage in children than in adults **(Kulkarni and Grigg, 2008)** and greater tissue damage of the lungs in very young babies **(DiFranza, Aligne, Weitzman, 2004; Jedrychowski *et al*, 2007A,B).** Further problems may arise as any deficit in the lung function from early childhood is unlikely to be restored as the lungs mature even in the absence of air pollution **(Gauderman, 2004).**

2.3.2. Studies examining acute respiratory infections, tuberculosis and pneumonia in terms of household fuel pollution exposure:

Many studies have attempted to find a link between respiratory tract infections and household fuel pollution infection. A Colombian case-control (n=104 within each group) study examined patients hospitalised for chronic bronchitis (CB), identified as forced expiratory volume of the lungs (or FEV) less than 70%. Exposure to household wood smoke increased the risk of CB by 3.43 times (95% CI: 1.7 – 9.1, p<0.001). Household fuel pollution exposure (yes vs. no) was verbally reported **(Dennis *et al*, 1996),** which reduced the power of this analysis. Also significantly lower tobacco smoking (p<0.01) was identified within the control group, which confounded the

study. A hospital based clinical case-control study in Mexico city involving women patients aged 40 or over referred to a chest hospital for chronic bronchitis showed a higher prevalence of chronic bronchitis with airway obstruction in individuals continuously exposed to wood smoke (case=yes) (OR=9.7, 95% CI: 3.7- 27) than those who were not (control group=no) (**Pérez-Padilla** *et al*, **1996**) (even after adjusting for income, age, education and smoking habit). However, again wood pollution exposure was verbally reported. A case-control study in Nepal studied the health of women aged 35-75 years of age using dirty biomass fuels (wood or dung) and clean fuels (biogas and kerosene). This study examined women attending an eye hospital for the first time. Exposure to household fumes from biomass fuels showed an increased risk of cataract among women using flueless solid-fuel stoves without ventilation (n=206) compared with women using clean fuels (n=203) (OR 1.90; 95% CI 1.00–3.61). Lack of ventilation increased the risk of cataract independently (OR 1.96; 95% CI 1.25–3.07) (**Pokhrel** *et al*, **2005**). Again, this study relied on women attending hospital for a chronic eye condition and used crude pollution exposure data based on household fuel choice rather than measured pollution levels. The study design again reduces the power of the study.

A study using data on children's hospital admissions examined the effect of ambient air pollution on the prevalence of respiratory symptoms (aged newborn to five years of age) in five cities in Australia and two in New Zealand between 1998 – 2001 (sample size in the cities varied from just over a million to almost four million inhabitants of which about 20% were children under the age of fifteen years). The study found that the number of hospital admissions for respiratory infections among children under the age of one year increased significantly with higher ambient particulate matter (24-hour levels) (OR=2.4, 95% CI: 1.0 – 3.8) (**Barnett** *et al*, **2005**). The respiratory symptoms were more prevalent among children from five years and above with higher nitrogen dioxide levels (24-hour levels) (OR=5.8, 95% CI: 17 – 10.1) (**Barnett** *et al*, **2005**). This study used pollution data from weather stations as an average over two days. This method therefore ignored any variation in the pollution exposure of children in their personal living surroundings. No accurate data on the reasons for hospital admissions of the children or the number of visits per year per child were reported or taken into account in the analysis, which weakens this study.

A longitudinal health study from Kenya examined the prevalence of acute respiratory infections (ARI) among children using measured daily (24-hour) pollution levels and child health data from weekly examinations by a nurse over a period of at least two years **(Ezzati and Kammen, 2001A)**. 93 children from 55 households participated in this study. Under five-year old children from households with particulate matter emissions higher than 1000-2000μg/m^3 showed a significantly higher risk of developing ARI than those with PM exposure less than 200μg/m^3 (control group) (OR= 4.3, 95%CI: 2.63 – 7.04) **(Ezzati and Kammen, 2001A)**. The use of health data from nurses' examination and the measured pollution measures gives this study strength over other studies reported here.

A large study in Guatemala (RESPIRE study) which consisted of a randomised stove intervention trial (which included setting up an improved stove called 'plancha') has a strict methodology for the health assessment including lung x-rays, spirometry as well as air quality monitoring. This randomised case-control study investigated the effect of the use of 'plancha' over an open fire on exhaled carbon monoxide and the lung function. 503 Mayan village women from Guatemala with an average age of 27.7 years±7.2SD were recruited for this epidemiological case-control study. The sample included women (n=350) who had been exposed to household fuel smoke from birth. Personal carbon monoxide exposure (48hours) was also measured. Chronic coughs (over 3 months in duration), wheeze and tightness in the chest were highly prevalent among the women indicating a high risk of COPD. A 1ppm increase in exhaled carbon monoxide was associated with a significant increase in the risk of cough and chronic cough (OR= 1.07, 95% CI: 1.01 to 1.15 and OR= 1.10, 95% CI: 1.01. to 1.18 respectively) **(Diaz *et al* 2007B)**. This study indicated a link between an increased risk of chronic bronchitis among women with higher carbon monoxide exposure (by a single measure of exhaled carbon monoxide). The strengths of this study came from the use of actual lung function assessment and real pollution exposure measures, which is still fairly rare in the field.

An Indian nation-wide epidemiological case-control study examined the prevalence of acute respiratory infections among children under 35 months of age from households where biomass fuels were used (biomass only: 64.2% and mixed fuels: 23.5%) and

those children from households using clean fuels (12.3%) (data from National Family Health Survey 1998-9, n= 29,768 children). This study showed a significantly higher risk of acute respiratory infections among children from households using biomass fuels (OR=1.68, 95% CI: 1.26 to 2.23) (controlling for tobacco smoking, child age and sex, birth order, mother's age at child birth, religion, caste/tribe, housing type, separate kitchen, crowding, education, urban/rural residence and geographical region) **(Mishra, Smith, Retherford, 2005).** This study used health and pollution data from the National Family Health Survey obtained through verbal interviews. The presence of ARI was identified as a Yes answer to the presence of cough and fast breathing in a child. The fuels were classified as biomass or clean fuels. The verbally obtained health and pollution data lacks in accuracy as the data is very subjective and influenced by the understanding of the person being interviewed. It is very difficult to compare the verbally obtained data with other studies.

2.3.2.1. Tuberculosis

Tuberculosis is a serious lung infection caused by *Mycobacterium tubercle* which particularly affects people with a weaker immune system such as the young and the old. Bacterial infestation in the upper respiratory tract causes lesions which stimulate a strong granulomatous immune response and results in pulmonary tissue-caseation, which when healed forms calcified scar-tissues in the lung **(Lakhani, Dilly, Finlayson, 1993).** *M. tubercle* can remain latent in the body and a reactivation of latent bacteria may result in secondary tuberculosis. This makes tuberculosis a difficult health issue to solve, particularly in poorer socio-economic groups where the lack of appropriate health care facilities hinders the efficient care and prevention of tuberculosis as well as other severe respiratory infections **(Baris and Ezzati, 2004).**

A nation-wide epidemiological study of over 260,000 adults (over the age of 20 years) in India participating in the National Family Health Survey during 1992-3 showed a higher incidence of tuberculosis among women who reported the use of unprocessed biomass fuels than women who used clean fuels (adjusted OR= 2.58, 95% CI: 1.98 to 3.37), even after correcting for socio-economic factors **(Mishra, Retherford and Smith, 1999).** However, the data in this study was obtained through self-reporting of

symptoms and the pollution exposure was estimated on the basis of the household fuel choice therefore this study lacks in reliability. A Mexican cross-sectional case-control study examined urban women who had been referred to a local chest hospital due to an active tuberculosis smear for their personal wood smoke exposure. The pollution exposure (case=yes, n=288, control=no, n=545) which was reported verbally provided evidence for the association between tuberculosis and wood smoke exposure (current exposure OR=5.2, 95% CI: 3.1 – 8.9, past exposure OR 1.8, 95% CI: 1.1-3.0) (**Perez-Padilla** *et al*, **2001**). Smoking was rare among the study women. The place of birth, education level and household crowding were significantly associated with the risk of tuberculosis. Again, this study lacks in accuracy of wood smoke exposure due to the use of verbally reported pollution measures and recruitment of hospital follow-up patients. The cross-sectional study design reduces the depth of the results in this study.

An Indian hospital-based case-control study involving child patients aged under five years with moderate or severe acute respiratory infection (case=201, control=311) reported that the use of biomass fuel instead of biogas, malnutrition and lack of breast-feeding were strongly associated with the presence of severe pneumonia (OR=2.24, 95% CI: 1.51-3.34, OR=2.08, 95% CI: 1.21- 3.59, OR=2.24, 95% CI: 1.55 -3.23 respectively) (**Broor** *et al*, **2001**). The authors suggested that interventions which lowered the infant exposure to biomass smoke reduced the prevalence, frequency and severity of respiratory infections. The role of appropriate nutrition and exclusive breast-feeding within the first four months of life in lowering further the number of respiratory infections in communities was also suggested (**Broor** *et al*, **2001**). This indicates that the health damage from exposure can be moderated by the provision of improved nutrition and a better socio-economic background (**Gupta, Kumar and Singh, 1999**).

Many studies examining the effect of household fuel pollution exposure on acute respiratory infections, tuberculosis and bronchitis unfortunately rely on verbally reported health and pollution data and therefore these studies do not provide very reliable information about the health effect of household fuel pollution exposure.

Often studies which use real health data use patients admitted to a hospital due to moderate or severe respiratory symptoms. These studies can provide useful information about the extent of lung damage or pulmonary irritation due to household fuel pollution exposure, however they only include the most severe cases observed in a population. Hence data from these studies cannot be applied at the community level. Commonly, pollution exposure is verbally reported and the employment of indirect pollution measures reduces the accuracy of the health outcome.

2.3.3. Asthma

Asthma is a condition where chronic insults of the pulmonary mucosa has led to inflammatory responses, bronchial responsiveness and chronic changes in the lung function e.g. irreversible airflow obstruction in the worst case scenario (**Erlevyn-Lajeunesse *et al.*, 2008**). Only a few studies have published the association between asthma and air pollution exposure. The reason is most likely that it is very difficult to efficiently differentiate between the asthma and respiratory conditions in people subjected to air pollution. However, Weiland and Forastiere (WHO, 2005) concluded that there is enough evidence to state an increase in the prevalence of cough and asthma, as well as worsening of the asthma and lung function among people chronically exposed to air pollution (**WHO, 2005**).

A few child health studies in Kenya, Malaysia and China have found an association between childhood asthma and indoor air pollution from the use of dirty biomass fuels (**Ezzati and Kammen, 2001A,**). It has been suggested that an early-life exposure to air pollution leads to a sensitization to allergens among asthmatics (**Björksten, 1999**). Breastfeeding has been suggested to have a protective effect against asthma, allergies and acute respiratory infections (**Ratageri *et al*, 2000**). Epidemiological studies show evidence for worsening of the asthma symptoms in people exposed to air pollution and higher hospital admissions for asthma in areas of high traffic pollution (**Peden, 2001: Teague and Bayer, 2001; Bardana, 2001**). A Southern Californian traffic pollution study revealed a higher risk of prevalent asthma and wheeze (OR=1.50, 95% CI: 1.16-1.95 and OR=1.40, 95% CI: 1.09-1.78, $p < 0.01$ respectively) among kindergarten children (aged 5-7 years) whose homes were less than 75 meters from a

main motorway (**McConnel** *et al*, **2006**). This study used life history and medical diagnosis of asthma of children from 13 communities in Southern California and the health data was obtained through verbal interviews. A comparative study in Bangladesh measured carbon monoxide levels in households using biomass fuels (wood or crop residues) and cleaner fuels (biogas or kerosene). Health symptoms were verbally obtained. A significantly higher risk of coughs (OR=2.2, 95%CI:1.0 to 4.6, biomass fuel n=45, cleaner fuel n=26), shortness of breath (OR=4.4, 95%CI: 1.6 to 11.8, biomass fuel n=24, cleaner fuel n=6) or wheezing (OR=5.0, 95%CI 1.9to 13.4, biomass fuel n=26, cleaner fuel n=6) were reported among children under the age of five years in households using biomass fuels than children from households using cleaner fuels (**Khaleguzzaman** *et al*, **2007**). However due to uneven and small group sizes (n=6) the value of this study is limited.

2.3.4. Chronic Obstructive Pulmonary Disease (COPD):

Chronic obstructive pulmonary disease (or COPD) is a non-reversible lung condition where the normal airflow to the lungs is hindered due to structural changes in the peripheral and central airways. Diagnosis of COPD is typically done using a lung function test using spirometry, as well as a clinical examination. Clinical examination reveals a chronic inflammation of the airways and the presence of T-cell and macrophage infiltration in the airways and neutrophils in the airway lumen. It has also been suggested that COPD may involve a systemic inflammation which can explain diagnosed muscle-wasting and fatigue in patients (**Wouters, 2005**). Chronic obstructive pulmonary disorder is defined as; forced expiratory volume: forced vital capacity (or FEV1: FVC ratio) less than 70% (**Global Initiative for Chronic Obstructive Lung Disease, 2001**). Male gender, advanced age, cigarette smoking, occupational exposure, and low socioeconomic status are well-known independent risk factors for COPD. Chronic obstructive pulmonary disease currently ranks as the 6th leading cause of mortality of men and women in the world (**WHO, 2002**). The World Health Organisation has now defined indoor air pollution as a significant risk factor for the development of chronic obstructive pulmonary disorder (**Global Initiative for Chronic Obstructive Lung Disease, 2001**). Women's health and the aetiology of COPD in developing countries are currently severely understudied.

Chronic obstructive pulmonary disease was identified as the main cause of death of hospital-based women over 30 years, who had been exposed to indoor air pollution in Colombia **(Dennis *et al*, 1996)**. However the adverse health impact from household fuel pollution is likely to cause the development of chronic obstructive pulmonary disease much earlier in life. Indoor air pollution exposure has been linked with the diagnosis of COPD in women in their early twenties **(Norboo *et al*, 1991)**. This longitudinal study interviewed all women in their twenties and above living in three Himalayan villages contacted using electoral registry. The subjects gave their responses to a medical interview and underwent spirometry. Exhaled carbon monoxide and ambient kitchen carbon monoxide were measured for each participant and their household. The effective study design gives this work its great strength.

A case-control study in 10 rural villages in Turkey measured the respiratory function (spirometry and health interview) in women over the age of 40 years exposed to biomass smoke. The study showed a significant risk of developing COPD as a result of biomass fuel pollution exposure (OR=1.4, 95% CI: 1.2 to 1.7, p<0.001) (adjusted for age, smoking habit, education) **(Ekici *et al*, 2005)**. The risk of developing COPD was almost 1.4 times higher among the women exposed to household fuel pollution than among women in the control group (using clean fuels such as LPG and kerosene) (OR=1.4, 95% CI:1.2 to 1.7). However, indoor air pollution levels which would strengthen the health associations were not measured in this study.

An earlier hospital based epidemiological case-control (n=104 in each group) study in hospitals in Bogota, Colombia showed that wood smoke, tobacco-smoking and passive-smoking were all significantly associated with chronic obstructive pulmonary disease (or COPD) in women aged 35 to 75 years (OR=3.43, 95%CI 1.7-9.1, p<0.001: OR=2.2, 95% CI 1.2 to 5.5, p<0.001 and OR=2.05, 95% CI 1.1 to 3.9, p<0.03 respectively), however the strongest association was found between wood smoke and COPD **(Dennis, 1996)**. This study used verbally reported pollution exposure data which reduces the link between health outcome and pollution exposure.

A cross-sectional epidemiological study in a village in Mexico examined the prevalence of COPD in women (n=841) aged 38+years and measured one-hour accumulative particulate matter (2.5 and 10μm) levels in kitchens using gas (n=67) or biomass fuels (n=778) for cooking. Lung function was studied using spirometry and a health questionnaire. The particulate matter levels were significantly lower in households where gas was used as a fuel. The risk of a chronic cough (over three months in duration) was significantly higher in households where the peak particulate matter concentrations were higher (under 2.6 mg/m^3 versus above 2.6 mg/m^3): OR=1.7, 95% CI: 1.0 2.7, p<0.05 **(Regalado *et al*, 2006).** No significant decrease in the lung function (FEV: FVC ratio) was detected on the basis of the pollution levels, however individual decreases in the FEV (4.7%) and FVC (3.9%) were detected among women from households with higher pollution levels (above 2.6mg/m^3). This study provided evidence to support a role of solid biomass fuel fume exposure in the development of COPD in the developing world. However, it did not provide a very clear indication of reduction in the lung function as a result of household fuel fume exposure. However, a significant decline in respiratory health was detected as a result of biomass fuel exposure, and these symptoms were very similar to COPD. Also an uneven group size (control versus case) may blur the results due to a possible lack of diversity observed within the smaller group. The smaller group may not be a good enough representative of a characteristic tested when compared with the larger group and this may add a bias to the analyses performed.

Currently research into the role of exposure to biomass fuels and the development of COPD still lacks quantitative measures of pollution. The lung function tests have often been carried out to adequate standards, however assessment of real-time pollution exposure is often missing **(Ezzati and Kammen, 2002B).** Also the research suffers from a vulnerability to confounding and misdiagnosis of the symptoms with other respiratory illnesses such as chronic bronchitis. The use of longitudinal data to examine the progression of the COPD linked with pollution data is still practically non-existent which is a great short-fall of research into COPD.

2.3.5. Cardiovascular diseases:

Only within the past ten years cardiovascular diseases have been listed as a potential health outcome of solid biomass fuel exposure. Particulate matter exposure may contribute to the development of cardiac disorders such as a stroke and deep vein thrombosis through bombardment of the endothelia which may trigger platelet activation involving fibrinogen and other blood clotting factors. This can lead to endothelial dysfunction which in turn may cause a release of an embolus into the blood stream and cause a stroke **(Polichetti *et al*, 2009)**. An Italian random case-control study involving adult patients diagnosed with deep vein thrombosis or pulmonary embolism showed a significant increase in the risk of men developing deep vein thrombosis with each $10\mu g/m^3$ increase in the level of area-specific ambient particulate matter measured the year before the diagnosis of a patient (OR=1.70, 95% CI: 1.30 to 2.23, p<0.01) (case=871, control=1210 men and women) **(Baccarelli *et al*, 2008)**. The risk for women was slightly lower, however significant (OR=1.40, 95% CI: 1.02-1.92, p<0.01). This study has an efficient methodology for the assessment of deep vein thrombosis. However this study loses its power through the use of annual levels of ambient particulate matter, without taking into consideration seasonal effects and other confounders of the risk of developing deep vein thrombosis. Miller and the team in 2007 showed that each $10 \mu g/m^3$ increase in the ambient particulate matter levels significantly increased the risk of a cardiovascular event (OR=1.24, 95% CI: 1.09-1.41, p<0.05) among women (n=65,893 aged between 50 and 79 years) without any history of cardiac disease prior to the survey (taking place between 1994-8) **(Miller *et al*, 2007)**. The ambient pollution data from the year 2000 was linked with the location of the residency of the participating women to draw association, which makes this study weak.

In general the evidence to support the role of particulate matter pollution exposure in the development of cardiovascular disease is through association studies (e.g. **Zanobetti and Schwartz, 2009)**. Most studies described the role of particulate matter pollution using nation-wide multicity data on the pollution levels and aimed to find any associations between the risk of cardiovascular conditions and the pollution levels. Hence this type of study design lacks in accuracy, specificity and reliability.

However association studies are very useful for looking at larger trends which then should be analysed using more targeted study designs such as an epidemiological design. More direct pollution measures are required in order to strengthen the suggested health outcome and air pollution. No studies have examined specifically cardiovascular diseases among women exposed to household fuel pollution in developing countries.

2.3.6. Poor Immunity:

The specific impact of chronic indoor air pollution on immunity has not been discussed as a separate issue in many studies. However a clear association has been found between chronic exposure to tobacco smoke and an impaired cellular immunity **(Houtmeyers *et al*, 1999).** Evidence of systemic inflammation as a result of chronic obstructive pulmonary disease **(Wouters, 2005)** may help to explain a reduction in the immune defences in individuals exposed to biomass pollution. It has been suggested that particulate matter (PM) inhalation as a result of biomass fuel pollution exposure is likely to activate PM phagocytosis by macrophages **(Mukae *et al*, 2001)** and an induction of pro-inflammatory cytokines **(Fuji *et al*, 2001).** This can then trigger local or systemic inflammation **(Pope *et al*, 1991).** A chronic immune-stimulation is likely to be a very energy-demanding process, and reduce the general immune defences in an individual **(Lunn *et al*, 2000: Yang *et al*, 2001).** A small comparative laboratory study between women and children from Ethiopia and Leicester, UK shows that the macrophage particulate matter load is associated with daily particulate matter exposures. Both women (n=10) and children (n=10) from Ethiopia had a significantly higher macrophage carbon load than the participants from Leicester (women: median 9.19 vs. 0.71 μm^2 /alveolar macrophages (or AM), p<0.01, n=10, and children: 3.32 vs. 0.44μm^2 /AM, p<0.005, n=10) **(Kulkarni *et al*, 2005).** This study has very promising results despite its small sample size which may affect the power of analysis. Another laboratory study by the same researcher on school children aged 8 to 15 years (n=114) from Leicester, UK showed that a 1.0μm^2 increase in the carbon load of the macrophages has a significant adverse effect on the lung function (forced expiratory volume or FEV was 17.0±11.2% lower and forced expiratory or FEF was 34.7±23.4% lower) (p<0.05) **(Kulkarni *et al* 2006).**

It may be that the health damage of indoor air pollution is likely to lead to a suppression or a reduction of the immune responses, which makes the person more vulnerable to respiratory infections (Smith and Mehta, 2003) without the presence of any obvious visible signs of an illness, the estimate of real damage can be difficult to obtain. An investigation into the general health and immune status of populations chronically exposed to indoor air pollution would help to estimate the degree of health damage from indoor air pollution. Few occupational health studies have used this method (Woodin *et al*, 1998; Backe *et al*, 2004). The analysis of serum immune markers (IgE, C-reactive protein, IgM, T-cells) in salt miners (aged 26 to 62 years) exposed to salt dust, diesel exhaust and nitrogen oxides showed raised IgE and T-cell levels which significantly correlated with the exposure levels (R= 0.139 p<0.05 and R=0.148 p<0.05. respectively) but no significant correlation was found between exposure and IgM, and c-reactive protein (p=NS) (Backe *et al*, 2004). A similar study among boilermakers exposed to fuel-oil ash for two weeks showed high levels of upper airways inflammation and interleukin-6 levels in response to a higher exposure to $PM_{10\mu m}$ (Woodin *et al*, 1998).

2.3.7. Eye Conditions and Otitis Media:

Biomass smoke can cause eye irritation (**Ellegård, 1997**) which can lead to an increased oxidative stress on the lens of the eye (**Rao *et al*, 1995**). A recent epidemiological case-control study on an adults aged 2- to 59 years (n=476) from small villages in India identified an increased risk of cataract by 2.2 (95% OR 1.03 – 4.34) in households primarily using wood as fuel (**Saha *et al*, 2005**). Older women in particular were affected by eye irritation and related eye conditions in this study when compared with women under 20 years of age (p<0.05) (**Saha *et al*, 2005**).

2.3.8. Pregnancy, still-births and low birth weight:

Adverse pregnancy outcomes such as low birth weight (**Maisonet *et al*, 2001**), still-birth and peri-natal deaths have been suggested as being partially due to maternal exposure to air pollution (**Boy, Bruce, Delgado, 2002; Mishra *et al*, 2004; Mishra, Retherford and Smith, 2005**). The role of particulate matter in the causation of adverse pregnancy outcomes is currently uncertain. It has been suggested that the

harmful effect of pollution on the developing foetus is caused by an inflammatory response as a result of particulate matter pollution exposure of a mother (**Dexter** *et al*, **2000**). This may increase blood viscosity and affect the placental nutrient and oxygen supply (**Lee** *et al*, **2003**). During pregnancy any toxins circulating in the maternal blood stream can readily cross the placenta. For this reason maternal exposure to household fuel pollution with high levels of gases such as carbon monoxide is likely to lead to 'intra-uterine' pollutant exposure. Also pro-inflammatory chemicals induced by particulate matter exposure may affect the cellular defences of the foetus.

2.3.8.1. Still-births and neonatal deaths:

Annually over three million children are born dead (**WHO report, 2005**). Nation-wide data from the National Family Health Survey from India between 1988-99 provides evidence that women aged 40 to 49 years (n=19,189) who cook using wood, dung and crop residues (53%) are 1.44 times more likely (OR: 1.44; 95% CI:1.04 to 1.97) to experience still-birth than those from households where cleaner fuels (27%) are used (**Mishra, Retherford and Smith, 2005**) (controlling for tobacco smoking, socio-economic status, women's nutritional status, household conditions (household type and crowding),urban/rural residence and geographical location). In the same study tobacco smoke had a positive but non-significant effect on the prevalence of still-births. The higher risk of still births could be due to the presence of carbon monoxide in the maternal bloodstream which may cause foetal hypoxia (**Gabrielli** *et al*, **1975**).

2.3.8.2. Low birth weight

Pollution exposure and carbon monoxide exposure in particular has been suggested as a cause of low weight at birth (**Boy, Bruce and Delgado, 2002; Mishra** *et al*, **2004; Mishra, Retherford and Smith, 2005**). A Zimbabwean study using data from the National Demographic Health Survey between 1994 to 1999 compared birth weights (n=3559 child births) from households using biomass fuels for cooking and electrified households. The study provides evidence that exposure to high levels of indoor air pollution from cooking fuels can cause a reduction in birth weight by 175 grams (95% CI: -50 to -300 grams) (p<0.01)(**Mishra** *et al*, **2004**) (controlling for child's sex and

birth order). When controlling for confounders (maternal age and body mass index (BMI), order of birth, maternal nutritional supplementation) a great reduction in the birth weight was observed (114grams, p<0.01) (**Mishra *et al*, 2004**). However the data was part of the Zimbabwean Demographic Health Survey and almost half of the birth weight details were collected by recall which reduced the reliability of the data. A Guatemalan epidemiological study with women and child participants from urban (n=1145) and rural (n=572) households found that the birth weight was significantly lower (p<0.05) for babies born in households using wood than those using cleaner fuels (biomass fuels: mean birth weight: 2,819g±29SD, n=861, clean fuels: mean birth weight: 2,948g±50SD, p<0.01, n=365), even when adjusted for socioeconomic status (**Boy, Bruce and Delgado, 2002**). Low birth weight is likely to indicate a more immature pulmonary development, which in turn may make a child living in a highly polluted house more prone to chronic lung damage and impaired lung function (**Green, Mead, Turner, 1974; Chan *et al*, 1989**). Broncho-pulmonary dysplasia is a condition which affects babies with low-birth weights. This suggests that the damaging effect of indoor air pollution will continue over the generations through a higher susceptibility to infections among children whose mothers were exposed to indoor air pollution, which subsequently resulted in them having lower birth weights.

The available evidence indicates that maternal health is a very strong determinant of the child's survival of an infantile infectious disease. This highlights the need to reduce household indoor air pollution exposure in order to reduce poor immunity and the prevalence of still births and neonatal death in developing countries. The studies in this field are often based on national cross-sectional data with indirect measures of maternal pollution exposure, which reduce their power. However the problem with studying adverse pregnancy outcomes in this way is that they rely on recall of pollution exposure. A long birth cohort study including continuous household air-quality measures could be set up to overcome the problem.

2.4. Household fuel pollution worldwide

The main source of household fuel pollution is the use of solid biomass fuels and incomplete combustion of low quality fuels. Currently over half of the world's populations still rely on the use of solid biomass fuels (wood, dung, leaves and crop residues) for domestic energy **(Rehfues, Mehta, Prüss-Üstün, 2006)**. The use of solid biomass in the developing world often exceeds 75 percent of the total energy usage in areas such as Africa, China and Asian countries such as India and Bangladesh **(Biswas and Lucas, 1997; Singh, 2005)**. Biomass use is often coupled with poverty and scarcity of resources such as electricity **(Smith and Mehta, 2003)**. The extent of household fuel pollution exposure may vary a great deal between households and it may be heavily dependent on the type of fuel used, type of stove used and its design, the length of cooking period, ventilation methods as well as the house material and the layout of the rooms **(Brauer and Saxena 2002)**. Often inefficient stove designs are used in households where household fuel pollution is an issue. In many societies traditional stove designs (often three stove design with a combustion chamber under the stove or a similar shape made out of mud or clay) are used which have relatively low thermal efficiency **(Berrueta, Edwards, Masera, 2008)**: a thermal efficiency of 10 to 30% has been suggested by some studies. Low thermal efficiency can lead to poor combustion and a production of high levels of particulate matter. Coupled with longer cooking time traditional stoves are likely to have a high impact on personal exposure to household fuel pollution. It has been suggested that the effect of different fuel types on the pollution levels is overridden by the ventilation methods **(Dasgupta *et al*, 2006; Begun *et al*, 2009)**. High pollution exposure in the household is likely to have a greater damaging effect on women and children at home than men who often work away from home **(Boy *et al*, 2000; Bruce *et al*, 2004)**. The fuel emissions vary a great deal throughout the day, peaking at cooking times. Pollution clearance is likely to be highly dependent on the ventilation methods and its extent, air-tightness of the house, wall and roof materials, as well as location of cooking **(World Bank 2002; Brauer and Saxena, 2002)**. Household air turn over should be higher than 0.35 times per hour **(Shaw, 2007)**.

It has been suggested that the use of the traditional stove design increases the risk of acute respiratory infections up to ten times **(Smith, 2000)** and the use of an improved stove or cleaner fuels could reduce the pneumonia in children up to 50% **(Larson and Rosen, 2002)**. Some research suggests the severity and the frequency of respiratory infections among people who are chronically exposed to household fuel pollution on a daily basis is not dependent on the intensity of the pollution exposure **(Ezzati and Kammen, 2001B)**. It may be that the health damage of indoor air pollution exposure is caused by a continuous stimulation of the immune defences by the pollutants which then increase the susceptibility to respiratory and other infections. Adverse health impact of particulate matter exposure has been reported at levels just slightly above the background exposure, around the level of 3–5 $\mu g/m^3$ in both the United States and Western Europe **(WHO, 2005)**.Epidemiological studies suggest that long-term exposure to particulate matter levels slightly above the background levels may be a cause of chronic ill-health in exposed individuals **(WHO, 2005)**.

2.4.1. Household fuel pollution exposures from field studies

Many studies have been carried out to estimate household fuel pollution levels in areas where solid biomass fuels are commonly used for domestic heating and cooking **(Albalak, Frisancho, Keeler, 1999; Ezzati and Kammen 2000; Dasgupta *et al*, 2006)**. A Bolivian study which looked at the indoor pollution levels in two villages with different cooking habits (indoors versus outdoors, n=12 in each) showed higher measured particulate matter (PM_{10}) pollution levels in the village which cooked indoors than in the village which cooked outdoors (geometric mean SE in kitchen indoors: $1830\mu g/m^3$, outdoors: $430\mu g/m^3$) **(Albalak, Frisancho, Keeler, 1999)**. Daily exposure of men in indoor cooking village ranged from $9840\mu g\text{-}h/m^3$ to $13\,680\mu g\text{-}h/m^3$, whereas the same range for women was $11\,280\mu g\text{-}h/m^3$ to $15\,120\mu g\text{-}h/m^3$. In outdoor cooking villages the daily PM exposures of men and women were quite similar (men: $5520\mu g\text{-}h/m^3$ to $6240\ \mu g\text{-}h/m^3$ and women: $5760\mu g\text{-}h/m^3$ to $6000\mu g\text{-}h/m^3$). However only 12 households in each village were monitored to represent household pollution levels in the study villages. The pollution data was then applied to all study household on the basis of household material, size and kitchen type. The

use of indirect pollution measures significantly weaken associations found in this study. A country-wide study in Bangladesh showed household fuel pollution levels ranging from 68 to 4864 $\mu g/m^3$ between households using low quality fuels and cleaner fuels for cooking (**Dasgupta et al, 2006**). A recent study in rural areas of southern India reported average values of particulate matter of 500-2000$\mu g/m^3$ in households which used biomass fuels. The highest particulate matter concentrations were obtained from households which used cow-dung, and then wood, charcoal, kerosene, and low pressure gas (LPG) respectively (**Balakrishnan et al, 2002**). In a South African study real-time kitchen carbon monoxide and particulate matter levels were measured in the kitchens in households using wood as fuels and those using electricity for cooking (**Röllin et al, 2001**). Carbon monoxide levels were associated with the fuel type used in households ($p<0.001$). Log-transformed carbon monoxide levels in un-electrified households were significantly higher than in electrified households (mean: 1.25 vs. 0.69 respectively, $p<0.001$) (**Röllin et al, 2001**).

A two-year longitudinal health study in rural Kenya monitored particulate matter (PM_{10}) and carbon monoxide levels in 55 households (using chimneyless stoves) for 200 days to estimate pollution exposure. The study observed great daily variations in the pollution levels and suggested that daily mean pollution levels could not provide accurate data on the pollution exposure of household members (**Ezzati and Kammen 2001B**). Households on average experienced daily mean particulate matter levels over 1000 to 2000$\mu g/m^3$ and the concentrations dropped very quickly when moving away from the fire (**Ezzati and Kammen 2001A,B**). A household fuel pollution study in Tanzania reported very high particulate matter (PM_{10}) levels in different types of kitchens (indoor kitchen, indoor kitchen in a separate building, or outdoor kitchen) using measurements during cooking times (2 hours): $791.1 \pm 638.9 \mu g/m^3$, $576.2 \pm 413.9 \mu g/m^3$ and $428.6 \pm 334.7 \mu g/m^3$ respectively. The levels were higher in indoor kitchens within the house than the outdoor kitchen ($p<0.05$), but not indoor kitchen versus the levels measured in a kitchen located inside a separate building ($p=NS$) (**Kilabuto, Matsuki, Nakai, 2008**). The pollution levels exceeded the recommended safety thresholds set by the World Health Organisation in 2005 for all types of kitchen at least for the period of 30 minutes for which the monitoring was done. A recent

study in Bangladesh reported particulate matter (PM_{10}) concentrations between 60-
1160μg/m³ measured in households in Dhaka and in Narayanganj. The study showed
a great effect of ventilation in reducing pollution levels: the highest concentrations
were measured in households with little or no ventilation **(Dasgupta *et al*, 2006)**. This
suggests that simple rearrangement of the kitchen is likely to reduce household fuel
pollution exposure effectively and in a way that is attainable for the poorest of the
families **(Begum *et al*, 2009)**.

Previous studies mostly show that the pollutant levels in households where poor
quality biomass fuels are used often greatly exceed the recommended guideline levels.
This suggests an adverse health outcome as a result of chronic household fuel
pollution exposure in such households is likely **(Perez-Padilla *et al*, 1999;**
Balakrishnan *et al*, 2002; Naeher *et al*, 1999; Ezzati and Kammen, 2001A,B,
2002A,B). The studies described above showed that household layout, ventilation
methods and activity patterns of household members were likely determinants of
pollution exposure of household members. No studies described here measured
personal exposure to household fuel pollution which is likely to give more accurate
information about the extent of the health impact of household fuel pollution
exposure.

2.4.1.1. Personal carbon monoxide exposure and household carbon monoxide levels

Use of the personal exposure measure of carbon monoxide exposure is a sensitive
method for indoor air pollution monitoring and could be used to estimate the personal
health risk from indoor air pollution. An epidemiological study in Guatemala
measured the personal carbon monoxide exposure in households using either an open-
fire or a plancha for cooking. A strong positive correlation was observed between the
mother's and child's carbon monoxide exposure in households using an open fire
(R=0.85: p<0.001) or a plancha (R=0.68 p<0.0002) **(Naeher *et al*, 2001)**. Maternal
carbon monoxide exposure has been suggested as a good proxy for the estimation of
the carbon monoxide exposure in children **(Naeher *et al*, 2001)**. However, the study
noted that the stove type had an effect on the relationship between carbon monoxide

exposure of a mother and a child. The strong correlation was observed in households using a plancha or open fire, but the relationship was more blurred in households using gas.

2.5. Interventions to reduce household fuel pollution exposure

The first interest in tackling the problem of household fuel pollution through intervention studies began in the 1980s. Interventions initially focused on designing more efficient stoves for households to use at community level (WHO, 1992; Smith, 1993). It was not until later that the adverse health effects of household fuel pollution exposure were raised as a key reason for interventional work.

2.5.1. Technical interventions

Technical interventions to tackle household fuel pollution exposure have so far involved various designs: designing a more efficient stove with higher thermal efficiency and better fuel combustibility, the use of chimneys or flues to expel the smoke, cooking in a separate building to reduce the family exposure, or improving the ventilation (Smith, Rogers, Cowlin, 2005), together with education on health hazards of indoor air pollution to support the success of a technical intervention (Barnes and Mathee, 2002; Schirnding et al, 2002). Significant reductions in the household particulate matter and carbon monoxide concentrations have been reported as a result of the use of an improved, more efficient stove design (Ezzati, Mbinda, Kammen, 2000; Albalak et al, 2001).

The Indian government subsidised a nationwide cleaner stove (or chulha) programme which included the implementation of 30 million stoves in rural households throughout the country. However this project failed to tackle indoor air pollution. The failure was due to inefficient burning and smokiness of the stove design. Further problems were encountered with the lack of infrastructure to maintain and market the stoves in communities (Rajvanshi, 2003).

The introduction of a more efficient ceramic charcoal stove in Kenya, which was designed locally using information about the local demands for the stove, was a high success due to efficient marketing of the stove and the education of local manufacturers on stove manufacture **(WHO, 1992)**. The ability to use a relatively cheap, locally available fuel and the local manufacture of the stove helped to maintain a lower price and ensured the continuity of the intervention **(Karekezi, 1994)**. The stove design has now been marketed throughout Africa. The Kenyan rural study with dissemination of ceramic coal burners has also been linked with a reduction of up to 65% in the prevalence of respiratory infections among young children (under the age of five) **(Ezzati and Kammen, 2002B)**.

A Chinese nationwide stove implementation programme in 1980 provided stoves with flues to 70% of rural households in order to conserve diminishing biomass resources **(Bruce, 1999)**. By 1995 over 172 million stoves had been successfully installed around the country **(Lin, 1998)**. The stove design and its durability made the stove desirable to the locals. Marketability of the stoves was the key to the successful implementation **(RWEDP, 1993a)**. Heavy government subsidies of the stove installation programme sealed the success of this stove programme in China **(Smith, 1993)**. The latest review of the Chinese stove programme has however shown a very small improvement in the air quality. This is due to the inefficiency of the design and the parallel use of other polluting stoves for domestic heating by families **(Jin *et al*, 2005; Edwards *et al*, 2007)**.

Recent interventions to tackle the health danger from household fuel pollution have included large-scale national schemes as well as small isolated projects. Currently the research into the use of improved stoves to reduce household fuel pollution exposures in order to improve health is still limited **(Wallmö and Jacobson, 1998)**. Also a review of the literature indicates a need to study the community before setting up a technical intervention to solve the health problems from fuel pollution exposure at the community level. Many attempts have been made to introduce interventions into communities where indoor air pollution has been identified as a health risk. Some

interventions have been unsuccessful due to a lack of sustainability and unsuitability for the society **(Muneer and Mohammed, 2003).**

In a Guatemalan study an improved woodstove with flue ('plancha') was set up in case households to measure the efficiency of the stove design in reducing pollution levels and to study the lung function of women and children **(Smith, Bruce, Arana, 2006).** The 'plancha' was less polluting than an open fire (PM_{10} $\mu m/m^3$ for plancha = $186\mu m/m^3$, open fires=$716\mu m/m^3$) **(Naeher *et al* 1996).** However this stove intervention suffered from a lack of success at its initial stages. The first model of the 'plancha' introduced to the houses was shown to be highly inefficient, on the basis of the standard water-boiling test **(WITA, 1985)** and it was highly fuel-consuming. It was not desirable to the household women which reduced its acceptance in the community. An improvement of the stove design has produced more promising outcomes in terms of acceptance by the household women as well as the reduction in the fuel pollution levels. The Guatemalan RESPIRE study also examined the respiratory health in women and children using lung x-rays and spirometry to study any change in the lung function as a result of switching to a plancha stove. Switching to a cleaner-burning 'plancha' stove was shown to reduce health symptoms such as back pain **(Diaz *et al*, 2007a),** eye irritation and respiratory symptoms including wheeze **(Schei *et al*, 2004)** significantly **(Bruce *et al*, 2004; McCracken *et al*, 2007).** This is one of the first interventions to fully investigate the association between health outcomes and direct pollution measures.

A Pakistani interventional study examined the effect of the use of an improved stove design on the health of women. The health was verbally reported and no pollution measures were taken. The authors reported a reduced prevalence of dry cough, sneezing and tears among women using the improved stove design **(Khushk *et al*, 2005).** However no significant differences were shown. This study suffered severely from design error as no actual effect of the improved stove on reducing pollution levels was measured and the health outcomes were verbally reported.

A Mexican randomised case-control stove intervention study examined the effect of pollution exposures between an improved PATSARI stove (case n=282) and a traditional stove (control n=270) and health outcomes such as cough, wheezing and lung function in women aged 20 or over (n=552) for a period of ten months in rural highlands. Health outcomes were reported by nurses at monthly visits to the households. The use of the PATSARI stove was linked with a lesser risk of respiratory problems among women (relative risk: 0.77, 95% CI: 0.62 to 0.95 for cough and wheeze RR: 0.29, 95% 0.11 to 0.77) (adjusted for confounders) (**Romieu** *et al*, **2009**).

Other benefits which have been associated with the introduction of an intervention into the communities with a high fuel pollution problem include time-saving and extra income generational activities. Katuwal and Bohara in their review on success and failings of a biogas installation in Nepal indicated a reduction of 1.5 hours of time spent on household chores including cooking when women used a biogas stove instead of a traditional stove design. The authors suggested that women would spend saved time on socialising, but also on social work and income generation (**Katuwal and Bohara, 2009**). From the research into interventions to solve the household fuel pollution issue it is evident that a technical intervention alone (in the form of an improved stove or a chimney) cannot solve the problem. Behavioural and education support is required for the efficient dissemination of the stoves to encourage their usage and to improve the understanding of the benefit of the intervention to the community (**Jin** *et al*, **2006**).

2.5.2. Behavioural intervention

Few behavioural interventions to reduce household fuel pollution exposure have been developed and evaluated. Most studies have provided a review of potential behavioural changes which could help to reduce household fuel pollution exposure. It has been suggested that successful interventions should be designed with poor families in mind. They should be simple, effective and culturally acceptable. The simplest way could be most effective in avoiding extensive exposure through a change in daily behaviours (**Torres-Duque** *et al*, **2008**). Some studies have suggested

keeping children away from the pollution source or from the house during cooking and cooking on a raised platform to improve ventilation as potentially simple but effective changes to reduce household fuel pollution exposure **(Ezzati, Saleh, Kammen, 2000; Dasgupta** *et al*, **2006)**. Also better use of ventilation and windows has been suggested as ways to reduce household fuel pollution exposure with minimum cost or interference with family lives **(Dasgupta** *et al*, **2006)**. Reviews have suggested the importance of cultural and behavioural considerations of household stove needs in ensuring a successful behavioural intervention. In some cultures smoke from different fuels may enhance the flavour of the food **(Budds, Biran and Rouse, 2001)** and it may be used as a method to keep insects and mould away **(Smith, 1987; Smith, 1996)**. Currently very little research into indoor air pollution has focused on a demand at the household level for indoor air pollution interventions. Torres-Duque and co-workers suggested an inclusion of members of all tiers of a society (health workers, doctors, schools and community leaders as well as government employees at the local levels) as a potentially successful route to a community-wide educational intervention **(Torres-Duque** *et al*, **2008)**. Adverse health impacts of household fuel pollution are likely to be most significant for the poorest of the society, who cannot afford buying cleaner fuels. They are most likely to be the most ignorant in terms of health knowledge regarding the increased risk of acute respiratory disease and related conditions as a result of household fuel pollution exposure **(Mehta and Shahpar, 2004; Jin** *et al*, **2006)**. Simple behavioural changes and educational support work are most likely to be the only really successful way for an introduction of an intervention into a community **(Dasgupta** *et al*, **2006)**.

2.6. State of current research

Increasing epidemiological evidence indicates an association between adverse health outcomes such as more prevalent acute respiratory infections, chronic bronchitis among young children and chronic obstructive pulmonary disease (COPD) and lung cancer in women who have been chronically exposed to domestic fuel pollution from the use of low quality biomass fuels and inefficient cooking stoves **(e.g. Smith, 1993; Pandey** *et al*, **1991; Ekici, 2005)**.

A review of recent literature on studies on household fuel pollution research and related health issues highlighted the research aims and the methods of health and exposure assessment used. However only a limited number of research publications are epidemiological study designs which have quantified air pollution levels within households, as well as assessed the prevalence and incidence of respiratory infection within the community (e.g. **Melsom *et al,* 2001; Ekici *et al,* 2005**). The review of studies have shown that current knowledge of potential health dangers of household fuel pollution rely on observational studies using reported pollution levels and health status. A large proportion of the published studies which examine the health effect of household fuel pollution have recruited patients from hospitals (**Dennis *et al,* 1996; Pérez-Padilla *et al,* 1996; Broor *et al,* 2001; Perez-Padilla *et al,* 2001; Barnett *et al,* 2005; Pokhrel *et al,* 2005; Miller *et al,* 2007; Baccarelli *et al,* 2008**). The hospital-based approach in diagnosing health outcomes of indoor air pollution has its draw-backs: by detecting the visible symptoms of diseases only the more severe cases are tracked. Data from hospital-based studies do not correctly reflect the health problem from household fuel pollution exposure at the community level. Milder and sub-clinical infections which may represent a majority of individuals in a community affected by domestic fuel pollution can go undiagnosed. Many community-based studies in turn rely on self-reporting of respiratory health (**Mishra, Retherford, Smith, 1999:2005; Mishra *et al,* 2004; Mishra, Smith, Retherford, 2005; McConnel *et al,* 2006; Khaleguzzaman *et al,* 2007; Rinne *et al,* 2007**), which may be inaccurate and do not allow an estimate of the severity of the health impact of household fuel pollution exposure. Only the more recent studies have measured the real-time exposure levels of PM and CO, due to the high financial and time-cost of air pollution monitoring methods (**Ezzati and Kammen, 2001A; Naeher *et al,* 2001; Kulkarni *et al,* 2005; Dasgupta *et al,* 2006; Diaz *et al* 2007B; Baccarelli *et al,* 2008; Kilabuto, Matsuki, Nakai, 2008**). Most studies so far have relied on verbally reported pollution measures (**Dennis *et al,* 1996; Pérez-Padilla *et al,* 1996; Mishra, Retherford and Smith, 1999, 2005; Broor *et al,* 2001; Perez-Padilla *et al,* 2001; Mishra *et al,* 2004; Ekici *et al,* 2005; Mishra, Retherford, Smith, 2005; Mishra, Smith, Retherford, 2005; Pokhrel *et al,* 2005**).

2.6.1. Rationale for the study

The World Health Organisation has introduced an analytical framework to aid in the assessment of determinants of child health in developing countries. This framework brings together socio-demographic, biological and nutritional determinants of child health in order to improve social and medical interventions to reduce child morbidity and mortality (**Moss and Chen, 1984**). The framework classifies the external determinants of child health such as socio-economic factors (productivity of the parents, traditions, household income and wealth, community's ecological and political settings) which all work through biological factors: maternal factors (age, parity, birth interval), environmental contaminants (air, water, skin, soil, insect vectors), nutritional deficiency (calories, proteins, nutrients) and injury (accident, intended) (**Moss and Chen, 1984**). The framework is very useful in clarifying complex hierarchical relationships between external determinants which will help researchers to target the essential determinants amongst multifactoral causes of outcomes of child morbidity and nutrition. This PhD work addressed the effect of household fuel pollution on child health in Bangladesh using a longitudinal health intervention design. The health impacts of household fuel pollution will be measured via health and nutrition status such as anthropometry and selected biomarkers of immune status (details provided in the sections below). The socio-demographic and maternal background characteristics of the study households were also examined. The Mosley-Chen framework was applied in planning the univariate and multivariate analyses in order to structure the analyses in a meaningful way of child health and growth in this PhD work.

2.6.1.1. The relationship between child nutritional status, exposure to household pollution and acute respiratory infections

Anthropometric measures such as height and weight are commonly used to assess child growth. In order to quantify child growth and put it in the context of standard growth for a particular age or sex in a population, z-scores are calculated against a set growth reference mean as a calibration standard. The growth z-scores (or standard deviations) are deviations of growth measure from the mean for a particular age or sex. Height and weight are considered as good indicators of the health and growth

status in young children. This information is often used in epidemiological studies as a measure of the population health in general. Infants who are small for the gestational age and fall into the less than 10th percentile on the reference growth chart have a higher risk of neonatal mortality **(Balcazar and Haas, 1990).**

Height-for-age z-score (or HAZ or stunting) is a child growth measure which describes the height appropriate for age and sex. Stunting of growth describes a linear growth rate which is slower than that expected for the particular age. A child whose height-for age is below -2 to -3SD is considered stunted, -3SD or below indicates severe stunting (or fall into the 2.5% of healthy children in the population whose height-for-age is more than two standard deviations lower than the population mean for children of a particular age or sex for the National Center for Health Statistics 2000 (or NCHS) reference population standard). The justification for the choice of growth reference used (CDC 2000) is provided in Chapter 3 (section 3.4.1, p.64). Stunting reflects a long-term malnutrition often due to a chronically inadequate nutrient supply. Malnutrition may also be due to a recurrent or chronic infection with an increased energy demand on an individual which has not been met. The Weight-for-age (WAZ) measures body mass in relation to age. Weigh-for-age below -2SD describes children whose weight at their particular age is lower than two standard deviations below the population mean weight-for-age (or within the 2.5% of the healthy population who fall into this group). They are considered as severely underweight. Being underweight may be a result of inadequate food intake or recent episodes of illness. Prevalence of low weight-for-age may be highly seasonal ($p<0.001$) **(Brown, Black, Becker, 1982).** Weight-for-height (or WHZ) describes the relationship between weight and height and children below –2SD for weight-for-height are considered as too light for their height or 'wasted'. Wasting is a sign of acute nutritional deficiency.

Anthropometric information described above provides useful information about the population health as well as allowing a comparison of the child growth with other epidemiological and nutritional studies. For instance a study carried out on acute respiratory infection prevalence and protein malnutrition in Papua-New Guinea

reported the risk of death to be 8 times higher among children who had less than 70% of the normal weight-for-age score **(Lehmann, Howard, Heywood, 1988).** Also the risk of acute respiratory infections in underweight children (weight-for-age less than - 2SD) was significantly higher than in children aged 18 to 23 months (n=559) with better weight-for-age status (more than -2SD) (OR=0.64, 95% CI: 0.45 to 0.92) **(Zaman *et al*, 1997).** A community-wide randomised controlled trial (n=11,728) of a vitamin A supplementation study in south India which studied children under the age of six months revealed a significantly increased risk of underweight (WAZ) and stunting (HAZ) among children exposed to biomass fuel smoke (verbally reported use of biomass fuel vs. clean fuel) at home (HAZ OR=1.13, 95%CI: 1.06 – 1.60, WAZ OR=1.45, 95% CI:1.20 – 1.75) **(Tielsch et al, 2009).** The Bangladesh Demographic and Health Survey 2004 reported 43% of children less than five years of age as stunted and 16% severely stunted, the highest prevalence of stunting (height-for-age less than -2SD) was among children aged 36 to 47 months (54%) **(BDHS, 2007).** No studies which clearly link wasting with respiratory tract infections could be identified.

2.6.2. Biomarkers of immunity

2.6.2.1. Haemoglobin

Haemoglobin is the iron-containing (*haeme*) oxygen-binding protein of the red blood cells and is responsible for oxygen transportation to the cells of the body. The oxygen-carrying capacity of haemoglobin can be disrupted by the presence of carbon monoxide which haemoglobin has a higher affinity for than oxygen molecules. Carbon monoxide exposure may therefore lead to oxygen starvation of cells due to the occupation of oxygen-carrying sites of the haemoglobin by carbon monoxide. Oxygen starvation may also be a result of a deficiency of haemoglobin proteins in the blood cells, which is termed as *anaemia*. One of the causes of anaemia is an inadequate *haeme* production due to low iron intake. Lower haemoglobin or anaemia may be a result of an infection which may cause a redirection of nutritional resources elsewhere **(Wieringa *et al*, 2002).**

Iron deficiency has been associated with lower neural and motor development and poorer cognitive function in children. A Bangladeshi study on children aged between 6 to 12 months showed an improvement in the motor function and exploratory behaviour in those children who received iron supplement instead of a placebo (p<0.05, Dunnett's test) **(Black *et al*, 2004)**. Long-term malnutrition due to a disrupted uptake of vitamins and nutrients as well as dietary nutrient deficiency can lead to iron deficiencies **(Nasaringa Rao, 1991)**. However, immune responses can alter the micronutrient levels (e.g. iron) due to up-regulation of immune pathways leading to low iron stores **(Roy and Enns, 2000)**. Deficient nutrient levels can therefore increase the risk of an infection **(Wieringa *et al*, 2002)**. An impaired immune-competency and increased morbidity due to iron deficiency have also been reported **(Thibault *et al*, 1993)**. A randomised control trial in Sri Lanka reported a significantly lower prevalence of respiratory tract infections among children between the ages of five to ten years who received iron supplements than the placebo group (p<0.05) **(De Silva *et al*, 2003)**. This suggests the beneficial role of iron in the maintenance of strong immune defences especially in the case of childhood respiratory infections **(De Silva *et al*, 2003)**. The World Health Organisation set the threshold for anaemia as 110g/L for haemoglobin **(WHO, 1992b)**.

Haemoglobin as an indicator of iron status can be a very useful marker of health and is commonly used to assess health status and immunity in epidemiological studies (e.g. **le Cessie *et al*, 2002: Siegel, 2006**). Poor nutritional status such as a low iron status may lead to an infection but also immune responses may disrupt the nutrient levels of the body **(Wieringa *et al*, 2002)**. For this reason, in areas of highly prevalent infectious diseases such as in developing countries, an alternative marker of health is required to support the evidence provided by haemoglobin. In a laboratory study by Wieranga *et al* the c-reactive protein and alpha-1-acidglycoprotein responses significantly altered the plasma ferritin levels in Indonesian under one year old children (32.1μg/L, 95% CI: 28.0 – 48.0, effect size: 17.2 (range: 13.4 – 20.1)). However haemoglobin status was not affected by the infection (109g/L ± 11, effect size =0.5, range: -3.5 to 2.4) **(Wieranga *et al*, 2002)**. Therefore the use of other biomarkers of infection and immunity is essential to clarify the causal pathway.

2.6.2.2. Albumin – nutritional status

Albumin is the most abundant serum protein, responsible for the maintenance of oncotic pressure of the blood. Albumin levels can decrease in periods of malnutrition or as a result of an infection (**Aburawi** *et al*, **2006**). For this reason albumin is commonly used as a clinical measure of health. Low albumin levels may indicate malnutrition or parasitic, viral and bacterial infection. Protein malnutrition, which is common in developing countries, can result from an inadequate intake of a protein-rich diet. A poor protein status can lead to an inefficient immune response, making an individual prone to further infections. Acute respiratory infections, and diarrhoeal diseases and protein deficient diet result in approximately 12 million deaths in children under five worldwide (**Najera** *et al*, **2004**). A Bangladeshi study showed a negative relationship between serum albumin levels and diarrhoea incidence among young children. Children under the age of five with diarrhoea had significantly poorer albumin levels than those without (p<0.01) (-2.28, 95% CI: -3.99, -1.66) (**Northrop-Clewes** *et al*, **2001**). Albumin levels have also been associated with socio-economic status in Nepal. Children from the lower socio-economic class had significantly lower albumin levels than those from better off families (p<0.0001) (**Panter-Brick** *et al*, **2001**). Very low levels of protein synthesis and breakdown were observed in children hospitalised for an acute respiratory infection and malnutrition in Malawi (**Manary** *et al*, **1997**).

2.6.2.3. C-reactive protein (CRP)

C-reactive protein is an acute phase protein synthesized by the liver as a response to an infection. CRP concentration increases very rapidly as a response to infection, trauma, and other acute inflammatory events (Roberts *et al*, 2001). The CRP levels peak at about 10 hours after the onset of an infection and the levels return to normal within 1 week. Low elevation in the CRP levels has been linked to chronic illnesses. The Sri Lankan iron supplementation trial among children from five to ten years of age showed a significant relationship between an improved immune status and lower prevalence of respiratory infections as a result of iron supplementation and a

significant drop in the c-reactive protein values as a result of better health status **(de Silva *et al* 2003).**

A cut-off point for inflammation of the CRP level below the 5 mg/L is commonly used but levels may increase up to 500 mg/L as a result to an infection **(Pepys and Hirschfield, 2003)**. Sub-clinical inflammation can be identified as consistent slightly raised CRP levels without any apparent sign of an infection **(Mishra, 2004)** whereas CRP levels of 50 to 100 mg/L indicate an acute infection **(de Ferranti and Rifai, 2002)**. Elevated CRP levels have been associated with chronic conditions such as cardiovascular disease, high blood pressure and diabetes. No significant age-related, seasonal or diurnal variations in the CRP concentration have been reported, although weight loss may results in a drop in CRP values **(Pepys and Hirschfield, 2003)**. The use of CRP levels to predict the type of infections (bacterial, viral, chronic) is currently debatable **(van der Meer *et al*, 2005).**

A study including children under the age of one year in Indonesia showed a positive correlation between the CRP levels, the alpha-1-acid- glycoprotein and the severity of acute respiratory infections (Pearson r =0.60, p<0.001 and Pearson r=0.22, p<0.05 respectively) **(Sankaranarayanan *et al*, 2006)**. The high sensitivity of CRP to bacterial infections makes it a useful method of assessment of the immune status in areas of high prevalence of bacterial infections such as Bangladesh. Even though C-reactive protein is not a specific marker for respiratory infection or inflammation, it is a very useful marker for screening different types of lung infections. This makes CRP a useful marker of chronic inflammation such as sub-clinical respiratory infections, caused by exposure to indoor air pollution.

2.6.2.4. Alpha-1-acidglycoprotein (AGP)

Alpha-1-acidglycoprotein is an acute phase protein produced by activated macrophages as a response to an inflammation. AGP response is relatively slow compared to the rapid CRP response. AGP is commonly used to test for an immune response in patients and it is an accurate indicator of current bacterial and viral infections as well as chronic inflammation. Normal AGP levels range between 25 –

135 mg/L and no age-specific variation has been reported. AGP responds to an infection or inflammation 24 hours after the onset and elevated levels remain for several weeks after the infection has cleared. This makes AGP a very useful maker for the detection of infections in the recent past which may not be evident from medical diagnosis or a CRP test due to the short-lived CRP response, where it has subsided. This is useful for the establishment of the health status in a population, especially when the sampling frequency is relatively low. The Indonesian study described earlier (in relation to CRP) showed the usefulness of AGP as a marker of infection **(Sankaranarayanan *et al*, 2006).** As particulate matter exposure has been linked with macrophageal immune response AGP could be a very informative marker for studying the health impact and immune damage following a chronic household fuel pollution exposure.

2.7. Aims and objectives of this PhD

2.7.1. General Aims

This project aimed to assess the health status of young children (under the age of five years) and pollution exposure in two semi-urban and rural communities in Bangladesh (in two towns: Saidpur and Parpatipur in the Rajshahi district of the north), where low-quality biomass fuels were used for cooking, together with traditional stove designs without chimneys. The field work was carried out in collaboration with the non-governmental organisation Concern Worldwide (Bangladesh), which has carried out significant development work and maternal and child health work in Bangladesh for decades (please see the next paragraph and Chapter 3, section 3.3.9. p.61 for further details of the work partners). The study communities were part of Concern's maternal and child health work .This project aimed to measure the levels of household fuel pollutants experienced by households. Children were measured and their immune status assessed via haemoglobin, c-reactive protein, alpha-1-acidglycoprotein and albumin.

The PhD work focused on the health and the prevalence of respiratory infections among under five year old children using maternally reported ill-health, medical

diagnosis of respiratory tract infections and immune analysis. This project also aimed to report on the uptake of an educational/behavioural and technical intervention introduced into the study communities. A randomised stove intervention included the fitting of new improved stove design in a sub-sample of households where air quality was also monitored for particulate matter (less than 2.5μm in diameter) and carbon monoxide. The implementation and running of the educational/behavioural intervention, together with fitting the improved stoves and the household interviews and health diagnoses were done in partnership with Concern Worldwide (Bangladesh). Concern has a strong positive relationship with communities and an extensive network at grassroots level which was very beneficial in building a trusting relationship with partaking households. Additional NGO partners in this study were Winrock International and Village Education Resource Centre (or VERC) who were responsible for the design and the dissemination of the technical and educational/behavioural interventions.

2.7.2. Research objectives

1. To assess the relative contribution of cooking practices, fuel-use and ventilation methods as well as housing structure to household pollution levels measured by particulate matter and carbon monoxide.
2. To examine whether household fuel pollution is a risk factor for respiratory infections in young children after controlling for household socio-demographic background and the cooking and fuel-use practices.
3. To examine risk factors for poor immune status and under-nutrition in young children, since these will also increase susceptibility to respiratory tract infections.

In addition this work aimed to report on the uptake of a behavioural intervention and introduction of an improved stove by the study households.

Tables and Figures:

Table 2.1: Safety guidelines for thresholds for particulate matter ($PM_{2.5\mu g/.m^3}$ and $PM_{10\mu g/.m^3}$) and carbon monoxide exposure by WHO (1999 and 2005) and US-EPA (1997 and 2006)

Pollutant	Period	WHO, 1999	WHO, 2005	US-EPA, 1997	US-EPA, 2006
Particulate matter (diameter less than 2.5μm)	Annual	$10\mu g/m^3$	$10ug/m^3$	$50\mu g/m^3$	$15\mu g/m^3$
	24-hr	$65\mu g/m^3$	$25\mu g/m^3$	$150\mu g/m^3$	$35\mu g/m^3$
Particulate matter (diameter less than 10μm)	Annual	$25\mu g/m^3$	$20ug/m^3$	$50\mu g/m^3$	Revoked
	24-hr	$20\mu g/m^3$	$50\mu g/m^3$	$150\mu g/m^3$	$150\mu g/m^3$
Carbon monoxide (ppm)	8 hrs	10ppm	*unchanged*	9ppm	9ppm
	1 hr	30ppm	*unchanged*	35ppm	35ppm
	15 min	1000ppm	*unchanged*	N/A	N/A

Chapter 3: Methods – household-health survey, household air-quality monitoring and intervention.

3.1. Introduction

Current research has attempted to study the health impact of domestic indoor air pollution, among women and young children in particular **(Hughes and Dunleavy, 2000; Bruce *et al*, 1998; Kleinerman *et al*, 2002, Mishra, 2003; Smith and Mehta 2003; Mishra, Retherford and Smith, 2005; Mannes *et al*, 2005)**. However, the causal pathway in which household fuel pollution may increase the health risk of respiratory infections is not well understood **(Baris and Ezzati, 2004)**. Smoke irritation of the bronchial mucus leading to a disruption of the mucosal immune   . defence of the lungs makes individuals more vulnerable to infections **(Smith and Mehta, 2003; WHO. 2004)**. Linking the potential health damage from inhalation of household fuel pollutants to adverse health outcomes can be challenging, due to complex socio-economic and behavioural factors and their interactions which may influence the results. So far no research has quantified the impact of household fuel pollution on child health under the age of five years in a community setting.

The previous chapter (Chapter 2) provided a few examples of studies which attempted to effectively solve the health danger from household fuel pollution exposure through the use of an intervention such as an improved stove. The chapter highlighted the importance of fully understanding the socio-economic settings, age, occupation and educational background of the community where the quantification and reduction of household fuel pollution are attempted. A thorough understanding of cooking and fuel use practices and their related behaviours within particular cultural settings is the most likely key to a successful intervention **(Budds, Biran and Rouse, 2001)**.

This chapter aims to describe the methods applied in this PhD fieldwork to generate information about the socio-economic, household occupational and educational background as well as the household structure, cooking- and fuel-use practices in two

semi-urban and rural towns in Bangladesh. Also the methods applied to measure the actual levels of household fuel pollution and the interventions introduced to this Bangladeshi community will be described in this chapter.

3.2. Study design

An 18-month longitudinal survey was carried out in communities living in Saidpur and Parpatipur, two semi-urban and rural towns of North-Western Bangladesh. 625 households of which 65.3% (n= 408) had children under the age of five years present were randomly recruited to take part in the three-stage household interviews including health survey and medical diagnosis. The study households were from five wards (three in Saidpur and two in Parpatipur). The selected wards were part of Concern's current maternal and child health work in the area. All households present in the selected wards were asked to participate in the study in order to obtain a manageable cluster of houses to which an intervention could be introduced smoothly. No sample size or power calculations were applied. The sample recruited from each five wards was the same (n=125) households as this was estimated as a possible household recruited from each of the wards on the basis of the size of the wards. A study sample size should be large enough so that the data is capable of producing statistically significant results. If the sample size is too low any important variation within the sample size may be too low for any statistical analyses. A too large sample size however may produce too many significant results and make the work in that way irrelevant. Power calculations are commonly used to identify a useful sample size required for effective statistical analysis. Despite the lack of power calculations applied in this study the selected sample size was expected to be large enough to provide enough variation for informative statistical testing. The sample size of 625 households including n=408 households with children aged under five years was expected to be large enough for data analyses. The survey households also benefited from behavioural and technical interventions in the form of educational workshops and new improved stove designs. Households were visited three times over the period of 18 months (baseline: October 2005, midterm: June 2006, final: February/March 2007) with nine month intervals in between the surveys. The field study was aimed to include an examination of the effect of seasonality on household fuel pollution.

However due to nature of the international collaboration the work in the field was delayed and the surveys did not fall into any specific seasons (the aim was to carry out the baseline and the final surveys during the dry season and the midterm survey as a monsoon study). The baseline survey consisted of full household interviews with examinations of child health and growth. Air-quality measurements were carried out in a sub-sample of houses (details will be provided later in this chapter, section 3.3.5: Household air-quality monitoring p.58). The mid-term survey examined household cooking and fuel-use practices as well as child health and growth. Household socio-economic status and occupational details were assumed to be as reported during the baseline survey and therefore not repeatedly recorded this time around. No air-quality monitoring was performed due to the very high cost of the monitoring and its related data processing. The final survey included again the full household interviews, together with the full child health and growth examinations. No air-quality measurements were taken during the final survey.

3.2.1. Household selection criteria

Households were asked to voluntarily participate in this study. The inclusion criteria were:

- Use of a traditional stove (Figures 3.1 and 3.2, p. 70)
- Use of biomass fuels (wood, dung, rice husk residues, golden, twigs and any dried plant-derived matter such as coconut husks and leaves).
- Having a child or children under the age of five years in the household.

Households were selected to represent all five socio-economic quintiles of a Bangladeshi town and the housing range represented the diversity observed in a typical semi-urban and rural setting around Bangladesh. Households were chosen only in the selected wards (the term 'ward' was used to an administrative unit of housing areas) in order to obtain a sample within a manageable community cluster. This was done to ensure a smooth introduction of an intervention in the study area. A sub-sample (n=67) of households were subjected to household fuel pollution measurements. The air-quality monitoring was carried out in approximately 13 households in each ward in order to have comparable results of the air quality across

the whole study community. The following section will provide a detailed description
of the methods applied during the fieldwork.

3.2.2. Household interviews

Household cooks or mothers were asked a list of questions on their household
educational, occupational, ethnic background, cooking and fuel-use practices as well
as child health status (only including children under the age of five years). The
interviews were carried out by a team of 12 experienced Bangladeshi females who
each completed four interviews per day (Figure 3.3, p.71). The team completed 48
interviews per day and it took approximately two weeks to complete each field survey
in the entire study community.

3.3. Training and pre-testing of data collection methods

The interviewer team underwent a one week training course held in Dhaka where the
interview techniques and child health and growth data collection methods were taught
and practiced. The interviewer team went through each question in the survey
questionnaire and discussed the meaning and the potential reaction by the household
members.

The techniques applied in the collection finger prick blood samples, blood spots on
filter paper and child growth data were demonstrated by the PhD candidate (**Näsänen-
Gilmore**) over a period of three days during the training week. The first day consisted
of a demonstration of blood collection methods and the equipment and discussion of
potential difficulties in the field in terms of blood sampling and anthropometric
measurements. The entire interviewer team took part in this first demonstration. The
collection of blood spot samples and the anthropometric measures were designated to
the supervisors of the interviewer team as they had the most experience in working in
the field and quality control. The second day of training of the collection of blood and
child growth data involved only the supervisors, who were once more demonstrated
the techniques by the PhD candidate (Näsänen-Gilmore) after which the supervisors
were able to practice the techniques a minimum of five times under the supervision of

Näsänen-Gilmore. Any questions that may have arisen were answered and solved immediately. For anthropometric measures height and weight recordings were practiced by the supervisor team during the second day of training in blood sampling. On the third day some young children (mostly family members of the interviewer team) were brought in for training purposes and growth measures were practiced (under the supervision of Näsänen-Gilmore).

On the last day the supervisors underwent a test on the blood sampling techniques including sterilization using alcohol wipes, collecting blood correctly on filter paper (ensuring that the blood spot soaked through the filter paper evenly), collection of blood using a HemoCue cuvette, the use of HemoCue machine for reading haemoglobin, appropriate waste disposal (used gloves and equipment) as well as height and weight measurements to an accuracy of 2mm for height and 100 grams for weight. The test consisted of carrying out the data collection procedure on 3 individuals and the recording of readings on the interview sheet. The candidates with the best overall techniques were designated for the job of blood sampling and the collection of child anthropometry. The backup team was formed of 3 other supervisors who had undergone the whole training.

After the training course the interview techniques as well as blood sampling and child anthropometric measurements were tested in Bihari slum settlements in Mirpur, near Dhaka (in areas which represented the actual community setting as closely as possible) prior to the baseline survey, in order to identify any weaknesses in the survey questionnaire and child health and growth data collection methods. Air-quality monitoring was also tested in the actual field site in Saidpur and Parpatipur. The details of the testing methods are provided below.

3.3.1. Household-Health Questionnaire

The household-health questionnaire was prepared in English and translated into Bangla. The format of the questionnaire was then tested in the test area of Dhaka, Bangladesh where the socio-economic status, indoor air pollution levels, stove and

fuel usage, cooking techniques, average maternal age and the birth-rate and number of pregnancies per year were similar to that of the study area in Saidpur and Parpatipur. The main problems identified through the pre-tests were that some questions were misleading and unclear (1-2%). Some regional variation in the stove and cooking terminology was identified. Interviews during cooking times were likely to lead to many disruptions as mothers were required to tend their stoves and cooking. The misleading and unclear questions were clarified and the translation into Bangla was adjusted to the local dialect of Saidpur and Parpatipur. After pre-testing the modified questionnaire was back-translated into English for a final check to ensure that nothing essential had been lost in translation. The interviewer team were trained on any changes in the questionnaire before the execution of the fieldwork.

3.3.2. Air-quality monitoring

Household air-quality monitoring tests were carried out in the field-site by Winrock International during a two-week field trip to the study sites. The monitoring equipment was tested in 10 households which represented the types of households (house material, house structure, congestion levels) included in this study in order to identify any problems associated with the field-setting and test protocol. It was observed that the household fuel pollution monitoring protocol needed to be modified to suit specific features of each household type. Data collection techniques were modified according to the comments from the pre-testing before the fieldwork began. The pre-testing identified that MiniVol (PM) and T28 monitors (CO) could not always be placed approximately five feet from the stove due to the small size of the kitchen or the house structure. The mother/primary cook sometimes found the monitor obstructive in the kitchen and it may have affected the normal movements of the primary cook during her daily activities in the household. Adjustments were made to suit the needs of the household during the air-quality monitoring: It was aimed to have the carbon monoxide and particulate matter monitors approximately 5 feet away from the stove, however due to space constrains the monitor was placed as close to 5 feet away from the stove and in a place that it was least affecting the normal movements of the family members. In order to estimate more accurately the pollutant load

household members potentially inhale daily as a result of household fuel pollution mothers wore a passive carbon monoxide stain tube (Dräger, UK) which measured accumulative carbon monoxide exposure. Personal carbon monoxide exposure was measured during the day only in 52 air-quality monitoring households at the time of the baseline survey in October 2005.

3.3.3. Interview control checks in the field

The work of the interviewers was controlled by a team of four supervisors who carried out random spot-checks on interviewers, checking the questionnaires after the completion of an interview and frequently sitting through an interview. 30% of randomly selected questionnaires were also checked by the supervisors at the end of each day to ensure the quality of the data and to correct any errors found in the forms. Any errors were corrected by re-visiting the households and checking the answers with household members on the following day.

3.3.4. Child health survey

All children under the age of five years at the time of the baseline survey in October 2005 participated in the health survey with their mother's consent. The age of the child was confirmed from the child's vaccination card or birth certificate (where available). Most mothers in this study sample were able to provide written details of the age of their child (percentage not recorded). All children selected for the survey were encouraged to participate in the health monitoring throughout the duration of the fieldwork (18 months in total) if possible. Child health and growth analyses (Chapter 6) were performed using data only from the youngest child in each household in order to avoid a potential household effect. Chapter 5 - Child health descriptives introduces data on child health and growth using all children under the age of five in this Bangladeshi sample.

3.3.4.1. Child health reported by mothers

Mothers were asked to provide a two week health history of all their children (aged under five years) and any incidences of sore throat, runny nose, ear ache, fever cough, wheezing, lower chest in-drawing as well as to provide some details on daily breast-feeding and complementary feeding (Appendix 1, p.277).

3.3.4.2. Medical check

Children who had been unwell up to two weeks prior to the survey according to their mother (excluding a runny nose and a sore throat) were examined by a medical doctor who worked in the field alongside the survey team. Local doctors were chosen to help overcome any problems with local language and cultural behaviour. Doctors carried out medical diagnoses when children were presented to them (Figure 3.4, p.71). Doctors performed a standard medical check on the children and asked specific questions about their respiratory health. Breathing rate, body temperature and details of chest auscultation were recorded during every medical examination as an additional measure to identify the presence/absence of a respiratory illness, according to the recommendation by the World Health Organisation **(WHO, 1991).** Doctors prescribed medication when required and severe cases of illnesses were referred to the local hospital for further care.

3.3.4.3. Immune analysis

The immune status of the children was analysed from finger-prick blood samples collected using the blood spot method. The blood was analysed for selected biomarkers of immunity in a laboratory at Loughborough University (UK). This is discussed in more detail in Chapter 4 (from p.77). The mother's consent was obtained for the blood collection from their child. Child haemoglobin levels were measured from the finger-prick blood sample using a HemoCue machine (HemoCue, UK) and the results were available immediately for the information of the parents.

3.3.4.4. Growth status

Anthropometric measurements (height and weight) were collected from all children under the age of five. Height boards were used to measure all children under the age of five. Recumbent length was measured on children under the age of 24 months by laying a child down on the height board in a horizontal position. The child was positioned so that the head touched the board, the legs were straightened and the shoulders and hip touched the board. The head was held in this position by one person and another person brought the foot board to the heel of the child's feet whilst keeping the knees straight. The length reading (in centimetres to the nearest millimetre) was taken by reading the scale on the side of the height board. Children over the age of 24 months were measured in an upright position with their heels and buttocks touching the board, head up and their necks straightened and shoulders pushing backwards. The headboard was gently lowered onto the head of the child and the measurement read (in centimetres). Child weight was measured using Salter digital weighing scales (Salter scales, County scales Ltd. UK), with an accuracy of 100grams. Children were first held by their mother and the mother plus child weight was recorded. Then the mother stepped on the scale alone and her weight was recorded. This method was most preferred by the mothers in the field and therefore used throughout the work. The child weight was calculated by subtracting the mother's weight from the mother and child weight (in kilos and grams to the nearest 100grams). The children's clothing was not taken into account in the measures as the children mostly wore very light clothing. No allowance was made for the children's clothing and this may have led to some erroneous recordings of child weight data. The style of measurement of child weight may have also led to slightly erroneous readings of the weight due to movements of a child in the mother's arms during the measurement. The measurements were taken in the household immediately after the interview in order to minimise any disruptions to the households caused by the lengthy presence of an interviewer. Two field assistants assisted in carrying the equipment needed for the blood collection as well as the anthropometric measurements, such as height boards and scales.

3.3.5. Household air quality monitoring

Indoor air pollution was monitored in 67 households which represented the housing diversity in terms of for example household building material in the study sample. 12 to 13 randomly selected households in each ward were selected for the air-quality monitoring. Household air quality was monitored for particulate matter (12-hour accumulative) and carbon monoxide (real-time, minute by minute measurements and personal accumulative exposure). The techniques are described in the sections below

3.3.5.1. Particulate matter

Household air was measured for particulate matter (less than 2.5μm in diameter). Levels of the smallest particulates were considered as the most harmful to the lung tissue due to their highly penetrative nature (Chapter 2, section 2.2.1.5., p.12). Particulate matter levels were measured using MiniVol equipment (Airmetrics, Oregon, US) (Figure 3.5, p.72) in which a pump sucked air at the rate of five litres per minute through a 47mm thick filter which trapped particulate matter from the air. Quantification of the particulate matter levels was done by comparing the pre- and post-measurement weights of the filters in μg/m³ (done by Exonics, Bangladesh) (Figure 3.6, p.72). The particulate matter measurements were obtained as 12-hour accumulative weights per volume of air sucked through the MiniVol machine over the period of 12 hours (μg/m³). Particulate matter levels were measured over 12 hours in the kitchen and the living room, during the day (cooking hours) and night (non-cooking hours). If the kitchen and living room were only separated partially (by a low wall or a thin curtain) the monitoring was assessed for one room only. The MiniVol pump was located in the vicinity of the stove (five feet from the stove at the height of a one metre off the floor, where possible).

3.3.5.2. Carbon monoxide

Carbon monoxide real-time 24-hour levels were measured using the T82 machine (Photon Machines, Washington, US). The T28 machine measures the carbon monoxide concentration (in particles per million or ppm) in the household air *in situ* at 1-minute intervals. The machine was placed on top of the MiniVol machine in the

vicinity of the stove. Carbon monoxide levels were assessed for one period of 24-hours in order to obtain information about daytime and night-time carbon monoxide exposure. Personal carbon monoxide exposure of the mother/primary cook was measured using personal carbon monoxide stain tubes (Draeger Safety, UK) over a period of 12 hours during the cooking hours (day-time). A colour change along a scale on the side of the tube was observed as a sign of carbon monoxide exposure (Figure 3.7, p.73). An hourly average of maternal carbon monoxide exposure could then be calculated using the following formula: carbon monoxide tube reading / length of time of measurement. The tubes were worn by the mother of the households selected for the air-quality monitoring in this study. Maternal carbon monoxide exposure was considered to be a reasonable indicator of carbon monoxide exposure of children. According to a recent Guatemalan study a relatively strong correlation between ambient kitchen 24-hour carbon monoxide levels and child personal carbon monoxide exposure exists (r=0.54, p<0.001) **(Bruce *et al*, 2004).**

3.3.6. Re-identification of households during the follow-up surveys

Identification of the households for each of the follow-up surveys was done by using the name and age details of the family members and cluster and ward numbers. Households were assigned a cluster number during the baseline survey to ensure that the families could be located again during the later surveys. The name and age details were always referred to during the data checks to confirm results. The name details were not used in any analysis. Data was treated anonymously in the statistical analyses.

3.3.7. Ethical approval for the project methods

Ethical approval was obtained from the Bangladesh Medical Research Center and Loughborough University Ethical Advisory Committee (Ref No: R05/P22) for blood sample collection using the finger-prick technique and the use of a carbon monoxide stain tube for the measurement of personal carbon monoxide exposure in primary cooks (**Appendix 2**). Anthropometric measures (height and weight) as well as household fuel pollution monitoring methods were non-invasive and required no

ethical consideration. Maternal written consent was obtained for the interviews, household fuel pollution monitoring, child anthropometry and blood sampling. Mothers were given full details of the information collected in the interviews and the details of child health and growth examinations, medical checks and finger-prick blood sampling. Households had full rights to withdraw from the study at any stage; the fieldwork relied entirely on a voluntary participation. Most mothers agreed to take part in the household and health interviews but a few did not give consent for their child to participate in the anthropometric measurements, medical diagnoses or blood sampling (or a combination of the three types of child health measures).

3.3.8. Household intervention

All study households benefitted from the technical and behavioural interventions which aimed to reduce household fuel pollution exposure. The technical intervention included the introduction of an improved stove into the study households. A small number of female members of the communities (often community health volunteers) underwent a one week training course in Dhaka on making an improved stove using clay and traditional techniques. The design of the improved stove was based on the traditional stove design with an improved efficiency and fuel combustibility (with a chimney made out of a cheap metal tube, where possible). The transfer of the technical intervention then relied on passive transfer (using a method of spreading the message through daily behaviours of community members as well as word by mouth) through an increasing interest of neighbouring households on having a better burning, less smoke-producing and more efficient stove which could be made by the household members themselves. A selected group of community volunteers attended a training course on how to make the improved stove themselves and they were encouraged to educate other women in the communities on their stove making skills. Educational and behavioural interventions ran alongside the technical intervention. Behavioural change messages were designed through an interaction with leaders at the local government level (municipality chairmen, ward commissioners, teachers, religious leaders and community health volunteers) and at the household level (mothers and primary cooks of households). Messages of behavioural change were developed to

actively promote cooking and fuel use practices which would reduce household fuel pollution exposure. Messages included better ventilation practices and keeping children away from the stove during cooking as possible simple ways to reduce household fuel pollution exposure and the prevalence of respiratory tract infections in young children. Health messages were introduced into the community through focus groups for household mothers, drama, bill boards and posters. The behavioural and educational intervention was an on-going process which began around the time of the midterm survey (June 2006) and continued at least until the final survey (February 2007). The intervention was in effect for the latter nine months of the 18 months of fieldwork. The behavioural / educational as well as technical interventions were aimed at the whole study sample in order to engage the entire community in this work.

3.3.9. Study collaborators and their roles

The different components of fieldwork (health status monitoring, household interviews, air-quality monitoring and interventional work) were carried out by different collaborators with the best knowledge and training in their own aspect of the work. Brief details of each collaborator and their role within the study are provided below:

Concern Worldwide (Bangladesh): Fieldwork planning, and operating in the field through some of Concern's own workers and community health volunteers. They provided an introduction of the survey team to the study community and maximised the acceptance of the work and activities in the field. Concern was also responsible for dissemination of the behavioural and educational intervention through drama and bill posters in the study community. Concern was also responsible for data entry, which was sub-contracted to Associates for Community and Population Research, Dhaka, Bangladesh.

Author of this thesis (Näsänen-Gilmore): Execution of the fieldwork, supervision of the interviews, child health checks, anthropometric measurements and blood collection. Training of the survey team to carry out these activities in the field. Immune analysis of the blood samples in the UK and statistical analysis of the data from household-health surveys, medical diagnoses, immune analysis as well as the air

quality monitoring.

Exonics, Bangladesh: Air-quality measurements. Monitoring of particulate matter (less than 2.5μm) and carbon monoxide levels in air-quality households.

Winrock International: Household questionnaire design and the design of the improved stove, improved stove pre-testing and dissemination of the improved stoves into the study households. Education and encouragement of stove makers in the local market to manufacture the improved stove in order to maximise the uptake and the sustainability of the intervention. Design of the parallel behavioural intervention to run alongside the technical intervention.

Village Education Research Group (VERG): Engagement of study households, local community leaders, community health volunteers and teachers in educating the study households of the health dangers of household fuel pollution exposure and simple behavioural changes to avoid the daily pollution exposure.

Associates of Community and Population Research: Commercial population research centre which was hired to carry out the household interviews for the fieldwork. They were also responsible for the data entry.

3.4. Data cleaning

Questionnaire sheets were photographed after each survey in order to have a hard back-up copy of the questions and answers for each household secured. Due to the length of the questionnaire only the child health section was photographed for further checks (as this was believed to contain the most errors and it was the most relevant to this thesis). The photographic questionnaire answers were held at Loughborough University, UK. Ten percent of the questions were chosen as a limit of error detection. If more than two errors were found in the child health section of the questionnaire a further five percent of the questionnaires were randomly selected for further checks. "Random selection method" of the SPSS programme (v.14) was used to generate a random selection of households which constituted ten percent of the whole dataset. The total sample size included in the ten percent check was 88 cases, which included some households more than once due to the presence of more than one child in the

household. This ten percent dataset was checked against the questionnaires from each of the three surveys.

After random selection checks the whole child growth data was checked longitudinally for any negative longitudinal discrepancies in the height and weight details for each child across the whole 18 month field survey. Differences between the height measurements for the three surveys were calculated in the following way to see if any changes in the growth data had occurred in the wrong direction.

1. Height Survey B – Height Survey A
2. Height Survey C – Height Survey A
3. Height Survey C – Height Survey B

Any negative values indicated a reverse growth data development (hence an error) and required checking from the actual data sheets. Once the data sheets were checked for the negative readings and the database corrected accordingly, suitable boundaries for the differences which could have realistically taken place in the height and weight readings were assigned. For height any change in the height details over a period of nine months over ten centimetres was excluded as an outlier. Growth z-scores were calculated using the height and weight information using Epi Info programme (version 3.3.2). Very high and low z-scores were lined up with age, height and weight raw data, in order to identify the source of error in the growth z-scores. Questionnaire sheets were checked for the details of birth date and growth measures to ensure that the correct details had been entered into the database and these were not the source of erroneous or strange z-scores. Table 3.1 (p.74) illustrates the number of children who provided data for the each of the surveys, the number of successful and unsuccessful corrections made in the database during the data checks and the final number of children with appropriate height measures in this study sample.

Erroneous readings for weight and height were recorded as 888 in the database in order to distinguish between missing data (which was coded as 999). In this way height detail could be used in the absence of weight data and vice versa for some of the analysis. This allowed for the maximum data inclusion in the data analysis.

The source of error in the child weight detail could be due to the method used for data collection. Field workers did not feel confident in measuring the child weight alone so a subtraction method was used. Mother and child weights were recorded for all mother-child pairs, after which the mother was weighed alone. The child weight was calculated by subtracting mother's (alone) weight from the mother+child weight.

3.4.1. Calculation of growth z-scores and choice of growth reference

In 2006, the World Health Organisation published new growth standards using longitudinal data from children under the age of 24 months who lived and grew in optimal conditions from six different countries (Brazil, Ghana, India. Norway, Omar and the U.S). The aim of this sampling method was to provide an internationally more representative sample on which the standards are based, which then would allow more globally applicable standards for population analyses. The WHO growth standard included children from all ethnic groups and places of birth and it aimed to describe how children should grow in optimum conditions (**Garza and de Onis, 2004**). In order to ensure the same optimal growth potential all children had been solely breastfed for the first four months of life, introduction of solid foods by six months, followed by partial breast-feeding until at least the age of one year. The aimed use of this growth standard was to catch any children whose growth was deviating from the optimum growth through the use of very stringent selection criteria such as family's SES status based on income and parental education, absence of significant morbidity, parental anthropometry, lack of maternal smoking habit before or after delivery, single term birth, as well as breastfeeding practiced as described above (**de Onis *et al*, 2004b**). Low birth-weight (less than 1500grams) (LBW) full-term babies were included to allow for an assumption that these babies grow normally in optimum conditions. Child anthropometry was recorded at short intervals (weekly measurements until the age of two months followed by monthly measures thereafter (**de Onis *et al*, 2004b**): therefore it is useful for the detection of rapid changes in the growth status of children.

The CDC 2000 reference charts include children (breast and formula-fed) from diverse ethnic backgrounds within the U.S. only aged newborn to twenty years of age. The data was collected during a period from 1963 to 1994 in five instalments (National Survey 3-3, National birth date from US vital statistics and Birth certificate data from Visconsin and Missouri State vital statistics, Fels Longitudinal Study data (**Kuczmarski** *et al*, **2000; 2002**). Low birth weight children were excluded due to their unusual growth pattern. Children who contributed to the CDC 2000 growth reference were from a range of nutritional and socio-economic backgrounds. This reference aimed to describe the growth of children in the whole range of settings including non-optimal settings.

The advantage of the WHO 2006 growth chart is the inclusion of only-breastfed children in the standard as this is the best nutrition for a child which is a one of the failings of the CDC 2000 (**de Onis and Onyango, 2003**). Bangladeshi mothers tend to continue breastfeeding up to the age of two years hence the use of the CDC2000 growth chart instead of the WHO2006 standard in this Bangladeshi study may lead to some misclassification of child growth due to the infant nutrition. However, it has been recommended that the WHO 2006 growth standard would be used for the children under 24 months of age, followed by the use of CDC2000 growth reference from the age of two years in order to maximise the accuracy and the benefit of each of the growth references (**Grummer-Strawn, Reinold, Krebbs, 2010**). As this Bangladeshi study included children under the age of five years the use two sets of charts would be required which would also increase the risk of erroneous readings.

The WHO 2006 growth standards were not available at the time of the analysis of the baseline or midterm data, therefore the CDC2000 was applied for all growth analyses throughout the thesis. This PhD study looked into the trends in child health and growth in Bangladesh and any changes in the child growth over the duration of the field project (18-months in total). As the WHO 2006 record the child growth as compared to the optimal conditions it is useful for the quantification of how child growth deviates from the optimum. The CDC2000 reference charts instead assess the

growth at the time of the measurement, relative to a reference sample, and it is useful for monitoring changes in growth and anthropometric status over time.

3.4.2. Date of birth

The date of birth was recorded at each survey. The data was collected as a form of check that a correct child has been identified during the follow-up surveys. During the data checks the details were checked against each other from each survey. If different details for the date of birth was observed between the three surveys, as a rule of thumb, the date of birth which had been recorded as the same during two surveys (most often appeared to be the baseline and the final surveys) was accepted as the correct one (n=47). The details were always checked from the paper copy of the survey questionnaire.

3.5. Data distribution

Data distribution for haemoglobin, albumin, alpha-1-acidglycoprotein and c-reactive protein, as well as the growth z-scores (height-for-age, weight-for-age, and weight-for-height) were examined using the Explore function of the SPSS programme. All continuous data was also checked for normal distribution using SPSS histograms with normal curve and a function which lists any extreme values deviating from the normal distribution. Sample data was also plotted using stem and leaf plots to observe data distribution patterns. The distribution of residuals was checked using a normality plot function for all data under investigation to ensure the best quality data. Any deviations of the residuals from the straight line as well as data under the normal curve of the data histograms were examined using normal Q-Q and detrended Q-Q plots. The limits for exclusion of outliers were defined on the basis of the natural data distribution which provided the most normal data distribution without losing interesting extreme values. Any deviation from normality was also tested statistically using the Shapiro-Wilks test and Kolmogorow-Smirnoff statistic with Lilliefors significance, as well as Cox's test for skewness. A p-value of 0.05 was considered as a cut-off limit. For the Kolmogorow-Smirnoff statistic a p-value under 0.05 was considered as an indication of non-normally distributed data. For the Cox-test a p-

value under 0.05 indicated normal data distribution. Data transformation was applied to any data which was not normally distributed in order to improve the data distribution of continuous data. Details of any data transformation performed are provided below: Data that could not be normalised by any transformation methods were analysed using non-parametric tests.

3.5.1. Data transformations

Table 3.2 illustrates the data distribution and transformation methods applied if necessary (see Table 3.2, p.75). Haemoglobin data was normalised by excluding abnormally low haemoglobin values below 70g/L (only two samples were below 70g/L – around 40g/L). These two extremely low data values were not normal representatives of the data distribution in this sample. Despite them representing the interesting cases of severe anaemia their exclusion was considered as the best way to conserve the data (n=2) in terms of quality and quantity. Albumin data was non-normally distributed and required transformation. Inverse transformation (1/X) worked well and normalised the data with some exclusion of outliers. C-reactive protein data could not be normalised by log-transformation, inverse transformation (1/X), square or square root methods. C-reactive protein data was transformed into z-scores by calculating any deviations from the median c-reactive protein value, however this could not normalise the data either. Non-parametric tests were applied to the c-reactive protein data throughout the data analyses and no outlier exclusion was necessary. Alpha-1-acidglycoprotein data was non-normally distributed and 1 $\log_{10}$-transformation was applied to normalise the data. The data was transformed using the $\log_{10}$ (X+1) method. Pollution data required normalisation too. $\log_{10}$-transformation was used to normalise daytime continuous kitchen carbon monoxide data as well as particulate matter (less than 2.5μm) data. However living room pollution levels could not be normalised by any transformation methods and therefore untransformed data was analysed by non-parametric tests. More details of the analyses applied are provided in Chapter 7 (from p.197).The analytical framework by Mosley and Chen was applied in the data analyses in order to clarify the complex relationships of external determinants of child health and growth in the study sample. The background

to the Mosley-Chen framework was provided in Chapter 2 (p. 40). This model examined the child health and immune status in this Bangladeshi sample through external factors such as socio-demographic determinants and socio-economic status, as well as biological factors (maternal health) and injury (household fuel pollution) which were highlighted as key determinants of child health, growth and immunity. A list of statistical analyses including the justification for each test is provided in Table 3.3 (p.76).

3.5.2. Determination of household socio-economic status

Households were assigned an individual socio-economic status (SES) using the methods applied in the Bangladesh Demographic Health Survey 2004 using principal component analysis **(Ruthstein and Johnson, 2004)**. This method is based on asset information such as household ownership, income, number of items owned, household structure and material characteristics including the floor material as well as sanitation facilities **(BDHS, 2004)**. The total sample score was divided into quintiles ranging from SES1 (lowest) to 5 (highest). The main advantage of this method is its applicability across populations as this method takes into account simple household items (such as radio, television black and white or colour, fridge) and therefore is often referred to as a 'possession score'. This method can be used to categorise study samples into SES classes at a local level. Recent research suggests that the possession score is a better indicator of child nutritional status than the poverty index in Bangladesh when used together with maternal education **(Mohsena, Mascie-Taylor, Goto, 2010)**. However, one of its main limitations is that it does not accurately assess the level of literacy or education which both are significant determinants of wealth. Associates for Community and Population Research who were responsible for the data entry also supplied the calculated SES quintiles. (Please see earlier section of this chapter – (3.3.9. Study collaborators and their roles, p.61) for more details of the division of the work in this project). No significant differences were found between the national and the municipal SES classification in this sample. A binary SES variable (high and low SES) was created from the five SES quintiles in order to simplify the analysis ("low" SES represented the previous categories of SES classes

1-3 and "high" grouped the previously used SES classes 4 and 5). The SES quintiles were determined by Associates of Community and Population Research.

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Tables and figures

Figure 3.1: Traditional portable and fixed biomass stoves outside

Figure 3.2. Traditional fixed biomass stove inside a household

Figure 3.3: Interviewer team

Figure 3.4: Doctor performing medical diagnosis

Figure 3.5: MiniVol and T28 machines placed near stove in a household

Figure 3.6: MiniVol filters pre and post monitoring

Figure 3.7: Draeger carbon monoxide stain tube for monitoring personal carbon monoxide exposure

Table 3.1: Child height details which were excluded from the data analysis

Survey	Reasons for missing data *	Total children obtained	Corrections successful via manual data checks	Corrections failed (exclusions due to data looking strange)	Children, due to erroneous data recording	Included children with outliers
Baseline	30 missing	445	12	0	48	397
Midterm	15 not possible	421	75	12	56	365
Final	51 missing	407	6	1	53	354

*Reasons for missing data:
1. Not possible to obtain data
2. Data missing
3. Child absent
4. System error

Table 3.2: Data exclusion range for outliers

Variable	Data normalisation	Range of inclusion
Haemoglobin	Normal data distribution by excluding outliers	> 70g/L for data from all three surveys
Alpha-1-acidglycoprotein	Log_{10}-transformation	All cases included for all three surveys
Albumin	Inverse transformation (1/x) and exclusion of outliers	Baseline: exclude >0.08 Mid-term: exclude >0.20 Final: exclude >0.08
C-reactive protein	None of the normal transformation techniques normalised the data (including z-scores calculated using mean)	Non–parametric tests applied. All cases included
Height-for-age	Normal data distribution by excluding outliers	>-7SD and <+2SD
Weight-for-age	Normal data distribution by excluding outliers	>-7SD and <+2SD
Weight-for-height	Normal data distribution by excluding outliers – Chapter 5	>-6SD and <+2SD
	Normal data distribution by excluding outliers – Chapter 6	>-5SD and <+2SD

Table 3.3: Description of statistical analyses applied and the reasoning for each choice.

Data type	Choice of statistical analysis
Socio-demographic factors Cooking and fuel-use practices	Chi-square tests or multilevel chi-square
The selected biomarkers of immunity Growth z-scores Cross-sectional analyses	Continuous data Two tailed t-test for a simple comparison of a variable between two categorical groups e.g. municipality (socio-demographic determinants and cooking-and fuel-use practices. One-way-ANOVA: as above but used when continuous data test between three separate categories
Longitudinal analysis of the levels of immune markers and growth	Continuous data GLM repeated measures test was used to test for any changes in the levels of immune markers or growth z-scores for the same individual over the three field surveys. Paired t-test was used to compare just two sets of longitudinal data. Continuous data against socio-demographic determinants over three field surveys
Cross-sectional levels of immune markers and growth	Continuous dependent variable Linear regression was used to identify any predictors of patterns observed in the continuous dependent variable. Predictors could be continuous of categorical. Stepwise (only significant ones included) or enter (applied when some non-significant wanted to be included in order to improve the overall model.
Health categories Stove type Average income groups	Categorical data Used as categories in t-tests or one-way ANOVA. Binary logistic regression used to identify any predictors of the risk of a child falling into a particular health category (two categories compared at a one time).

Chapter 4: Methods - Assessment of child immunity using selected biomarkers.

4.1. Introduction

Chapter 2 provided a detailed description of potential health effects of chronic exposure to household fuel pollution (including more frequent and more severe respiratory infections, COPD, lung cancer, chronic bronchitis, eye irritation as well as adverse pregnancy outcomes). It has been suggested that smoke irritation disrupts the immune defence of the pulmonary mucus, making individuals chronically exposed to smoke more vulnerable to infections **(Smith and Mehta, 2003)**. Chronic exposure to particulate matter pollution is likely to be a cause of so-called 'silent' or sub-clinical chronic immune stimulation (as described in Chapter 2, Section 2.6.2, p.42). These types of infections are rarely linked with visible symptoms of an illness and often go unreported as these patients are rarely seen by a doctor.

Sub-clinical infections can be analysed by measuring the levels of selected acute-phase protein levels associated with general or specific causes of infection. An immune analysis of infection is a commonly used method to assess health, infection status and nutritional status (e.g. **Lunn *et al*, 2000; Sankaranaraynan *et al*, 2006**). C-reactive protein (or CRP) and α-1-antiglycoprotein (or AGP) are commonly used as general markers for infections and ill-health, together with haemoglobin. More detailed descriptions of these immune markers and proteins are given in Chapter 2, p.42. Nutritional status can also be affected by a systemic inflammation, as this can be highly energy demanding **(Yang *et al*, 2001)** and lead to poor nutrient absorption through an impaired transportation of nutrients to target tissues **(Stephensen, 1999)**. A reduction of the frequency and the severity of infections and improved nutrition have been shown to improve the linear growth in children in developing countries **(Guerrant *et al*, 1992)**. It may be that a reduction of household fuel pollution would reduce the silent immune-stimulation and improve the immune defences

against further infection, thus improving the general well-being and nutritional status of the children.

In this PhD the level of sub-clinical infections in children was assessed using commonly used markers of infections: c-reactive protein and α-1-antiglycoprotein. Haemoglobin was also measured for the assessment of iron status. Albumin levels were measured to obtain clinical information about the immune and nutritional status of children under the age of five years. This chapter will provide detailed information on blood sample collection from children and the techniques of immune analyses.

4.1.1. Collection of blood samples on filter paper

Blood samples were collected from children under five in the study sample in order to measure selected biomarkers of immunity. The purpose and the protocol of the blood collection were explained fully to mothers and their informed consent to participation was obtained. Two pairs of interviewers were trained to collect blood samples from children on filter-paper (Whatman 3, Whatman, UK) using a non-invasive finger-prick technique (see Figure 4.1, p.92). This method was considered as best suited for field-work in conditions where blood samples could not be immediately centrifuged and frozen due to the lack of appropriate facilities and electricity. Finger-pricking as a blood collection method was considered to be better suited for the use with small children than venipuncture.

The child's middle finger was cleaned with an alcohol wipe and pricked using an automatic sterile lancet (HemoCue, UK). The first drop was wiped off with a tissue in order to stimulate blood flow. The finger was also gently squeezed to improve the circulation. Milking of the finger was avoided as this would alter the composition of blood with an addition of extra tissue fluids. Approximately five drops of blood were dropped onto the filter paper labelled with the participant's unique identification number using a permanent marker. Only the blood drops where blood had been absorbed through the paper forming identical circles on both sides of the paper were accepted as good samples. One drop was scooped up using a

HemoCue microcuvette for a haemoglobin measurement. The microcuvette was placed in the HemoCue device which read the haemoglobin value within 30 seconds. If not enough blood was obtained for the filter paper and the haemoglobin measurements another finger was pricked with the mother's permission.

The filter papers were aerated for a few minutes to allow maximum drying before they were placed into labelled envelopes for the rest of the day until the data collection was completed for the day. At the end of the day the filter papers were removed from their envelopes and were placed on a net horizontally under an electric ceiling fan to dry for three hours. Once dry the filter papers were stored in air-tight plastic bags and were kept at room temperature until the return to the UK for laboratory analysis.

4.2. Laboratory analysis of blood on filter paper for biomarkers of immunity

Laboratory analysis of the selected biomarkers of immunity was carried out in the UK. Enzyme-linked-immune assay (ELISA) was applied for the analysis of c-reactive protein and alpha-1-acidglycoprotein and the standard immune-precipitation technique was used to measure the albumin levels in order to determine protein deficiency malnutrition in children (see the detailed descriptions of assays in Appendices 3a, 3b, 3c, p.284-292). A list of equipment used in the field and laboratory as well as the reagent used in blood spot analyses are provided in Tables 4.1., 4.2, and 4.3., p.92-94).

4.2.1. Liquidising blood on filter paper

Blood was eluded from the filter paper by punching a disc 6mm in diameter (containing 12.5µL of blood) and placed into 1.25mL of washing buffer (prepared following the DAKO method). The disc was allowed to soak in washing buffer for at least 24 hours, but preferably a minimum of 48 hours to ensure the maximal release of blood proteins into the washing buffer from the filter paper. This

provided a master blood solution of a 1:100 dilution which was suitable for use in the immune assays. Plasma was used for the immune analysis in the same way as the liquid whole blood samples. Liquid whole blood samples were diluted by 1:100 by adding 5μL of liquid whole blood plasma into 495μL of washing buffer. This master mix was then used for the ELISA and immune-precipitation analyses.

4.2.2. C-reactive protein ELISA

A NUNC Maxisoap microwell plate (Fisher DIS-971-010P) was coated with 100μL of rabbit anti-human antibody to c-reactive protein (DAKO A0073) diluted in the coating buffer (prepared following the DAKO method, Appendix 3a) to a final concentration of 10mg/L. The plate was wrapped in cling film and kept in a fridge overnight until the beginning of the assay. The assay standards (10μg/L, 8μg/L, 6μg/L, 5μg/L, 4μg/L, 2μg/L, 1μg/L and blank) made using human serum CRP calibrator (DAKO X0923) were used to calibrate the sample CRP concentrations.

The plate was washed three times manually using washing buffer. 100μL of blood samples (1:100 dilutions) and the standards were distributed in duplicate and the plate was wrapped in cling film for 2hours incubation at 27°C. The plate was washed three times using washing buffer before adding 100μL peroxidase-conjugated rabbit anti-human c-reactive protein (DAKO P0227) diluted by 1:4000 using washing buffer. The plate was wrapped in cling film for 2hrs incubation at 27°C. Four OPD tablets (DAKO S2045) were brought to an ambient temperature and then diluted into 12mL of deionised /distilled water which was covered with foil. Once dissolved 4.3μL of 35% hydrogen peroxide was added into the OPD solution. 100μL of OPD solution was distributed into each well and the plate was incubated in the dark for exactly 30 minutes.100μL of 0.5M sulphuric acid was added into each well to stop the colour development. The plate was read at 490nm using a linear curve with no blank subtraction.

4.2.3. Alpha-1-acid glycoprotein (AGP) ELISA

A NUNC Maxisoap microwell plate (Fisher DIS-971-010P) was coated with 100μL
of polyclonal antibody to alpha-1-acid-glycoprotein (Acris BP518) diluted 1:1000
using coating buffer (DAKO method – Appendix 3b). The plate was kept in a
fridge, wrapped in cling film overnight until the beginning of the assay. The
following assay standards (plasma concentrations) were used: 1.46g/L, 0.876g/L,
0.73g/L, 0.438g/L, 0.365g/L, 0.219g/L, 0.0912g/L using human serum calibrator
(DAKO X0908). The plate was washed three times manually using washing buffer.
100μL of 1:10000 dilution of both blood samples and the standards were distributed
in duplicate and the plate was wrapped in cling film for 2hrs incubation at 27°C.
The plate was shaken for ten seconds every 30min to enhance the antibody-antigen
interaction. After three washes with washing buffer 100μL of polyclonal antibody
to alpha-1-acid glycoprotein - HRP (Acris BP518-HRP) diluted by 1:500 in
washing buffer was added. The plate was wrapped in cling film for one hour
incubation at 27°C. OPD solution was used to facilitate the colour change as
described previously and the plate was incubated in the dark for exactly 15
minutes.100 μL of 0.5M sulphuric acid was added into each well to stop the colour
development. The plate was read at 490 nm using a linear curve with no blank
subtraction.

4.2.4. Albumin immune-precipitation

10μl of 1:100 dilutions of blood solution and albumin standards were distributed to
the Microplate Nunc MicroWell 96 well flat bottom plate (Fisher DIS-984-090M)
in duplicate. The standards of the following concentrations (44.6g/L, 41.813g/L,
33.45g/L, 27.87g/L, 23.89g/L, 6.99g/L, 13.94g/L 0g/L) were made from human
serum standard (DAKO X0908) stock using dilution buffer (DAKO S2005)
(Appendix 3c). 225μl of reaction buffer (DAKO S2007) was added to each well.
The plate was read using the following programme: (Incubate 1: incubation time
5mins/Temperature 37°C', Shake 1: Total time 10sec/ON time 10sec/Speed
900rpm, Measure 1: Stepping/Single/Filter 340nm, Pause 1, Shake 2: Total
10sec/ON time 10sec/Speed 900rpm, Measure 2: Stepping/Kinetic/Interval

30sec/Measure count 10 (total 5mins)/Filter 340nm). 75µL diluted (1:12.5) rabbit anti-human albumin (Dako, Q0328) was added to the reaction during the pause. The antibody binding rate was recorded

4.3. Comparison of filter paper as an alternative to the use of liquid whole blood as blood collection method in immune analysis – validation study.

4.3.1. Introduction

Filter paper as a blood collection method has been applied successfully in many epidemiological studies (**McDade, 2001**; **Panter-Brick** *et al*, **2001**; **McDade, Hawkley, Cacioppo, 2006**). The use of blood samples on filter paper eliminates the need to process and freeze the samples in the field. The use of finger-prick blood sampling is advantageous over veni-puncture due to its less invasive nature which is particularly important with child participants. Finger-prick blood samples can be obtained by untrained personnel unlike veni-puncture which requires a phlebotomists' training. Recent advances in the immune-serum assays (ELISA and immune-precipitation) means that a smaller amount of blood is required for the analysis. Immune-assays performed using whole blood on filter paper has been reported as accurate and reliable (**Mei** *et al*, **2001**; **McDade** *et al*, **2003**).

Sources of potential errors and unreliable and imprecise readings for immune assays have been reported (**Mei** *et al*, **2001**; **McDade and Shell-Duncan, 2002**). Unreliable results may be due to uneven distribution of blood on the filter paper due to smearing; blotting of blood drops twice on the same area of the filter paper; the binding of blood proteins on the matrix of the filter paper as well as an uneven spread of blood due to chromatographic effects (**McDade and Shell-Duncan, 2002**). Storage methods may also affect the blood constituency. It has been reported that blood spots on filter paper are stable for up to four weeks at ambient temperatures before any degradation in the protein and acute-phase proteins can be detected (**McDade and Shell-Duncan, 2002**). Humidity is likely to have an effect

on the chromatographic properties of the filter paper. A report has shown that in highly humid conditions blood proteins may migrate further on the filter paper and the blood concentration of the proteins may vary depending where the disc is punched. Temperature, and high temperatures in particular, may reduce the stability of blood proteins as well as alter their protein folding state (**Cook *et al*, 1998**). How humidity and temperature impact the stability of the blood protein when acting simultaneously is also unclear. It has also been proposed that the matrix differences (filter paper vs. liquid blood) may contribute to any differences in the serum protein concentrations (**Mei *et al*, 2001**). The presence of porous material may cause the serum proteins to bind to the paper matrix and this may lead to a poor detachment of the serum proteins from the filter paper disc during the laboratory analysis reducing the protein concentration. It is important to test for the effect of the filter paper matrix on the serum protein concentration using blood samples with a known concentration of the serum protein of interest in order to eliminate the influence of the matrix on the concentrations. The effect of environmental conditions such as high humidity and high temperature in blood protein analysis should also be considered.

4.3.2. Methodology for the validation study

Whole liquid blood was collected from 22 healthy adult UK volunteers using the filter paper method (5 drops) as well as in heparinised microtainer tubes (500mL).(please see Appendix 4 for further details of the methods applied). The levels of the selected biomarkers of immunity from the liquid blood (microtainer tubes) and filter paper blood sample from the same volunteer were examined. The procedure was also repeated on 17 randomly selected child participants from the Bangladeshi study sample. This was to pinpoint any differences in the relationship between blood spots and liquid blood samples collected under laboratory and field conditions in order to quantify the effect of the filter paper on blood protein levels.

4.3.3. Environmental effect on blood protein concentration obtained via filter paper

In Bangladesh the average humidity ranges between 65-100% throughout the year. This high humidity may affect the blood protein concentrations on the filter paper and impact the accuracy of blood protein analysis. In order to validate the use of filter paper for the collection of whole blood in this study and to measure the environmental effect on the filter paper matrix, high temperature and humidity on the blood protein levels, a validation study was set up. To examine the effect of temperature and humidity samples were exposed to a) high temperature (37°C), low humidity and b) high temperature (37°C), high humidity (65% humidity).

4.3.3.1. Exposing filter paper blood spots to environmental effect

The effect of the environment during blood spot collection in field conditions were examined using artificially set hot and humid conditions which aimed to replicate the environmental conditions experienced in the field in Bangladesh. Four blood spots on two separate filter papers (two drops per each filter paper) were collected from each volunteer participating in the validation study. One of the filter papers was treated as a control and dried in laboratory conditions for 22 hours before freezing. The other filter paper was taken into a thermal chamber immediately after the collection in order to expose the samples to the same conditions as in Bangladesh. The temperature of the thermal chamber was set to 37°C representing the typical temperatures during the monsoon in Bangladesh. Humidity was set for 65 °C to replicate the environmental conditions in Bangladesh. Two fans were setup in the thermal chamber to replicate the air flow in the room when drying the samples in Bangladesh. Two different conditions were set up to examine the effect of the duration of exposure to high temperatures and also the difference between high temperature and high temperature with high humidity. The blood spot conditions were as follows:

A) Short and long exposure to humidity

1. Short exposure: One of the blood spots exposed to a high humidity was kept in the thermal chamber for 22 hours before freezing.

2. Long exposure: The other blood spot was placed in an air-tight plastic bag after the 22 hours and left in the thermal chamber for another 66 hours before freezing. This will replicate the effect of a humid and hot climate on a long-term storage of blood spot samples in an air-tight storage space as in the field in Bangladesh.

B) Two week exposure to high temperature only versus high temperature and high humidity:

1. Control: Two drops were aerated in laboratory conditions at room temperature (21°C) overnight before freezing. Comparative analysis of inter-spot variation in the blood protein concentrations.

2. High temperature: One drop was exposed to high temperature (37°C) for two weeks to replicate the time-frame of fieldwork and storage conditions in Bangladesh.

3. High heat and humidity: One drop was exposed to high temperature (37°C) and high humidity (humidity range 65%) for two weeks after which the filter papers were stored at (-20°C) prior to their use in the immune assay (as described previously).

ELISA and immune-precipitation methods (as for the whole field study) were used to examine the effect of the filter paper matrix on the levels of the selected biomarkers via comparison between filter paper blood samples and whole liquid blood from veni-puncture. The effect of environmental exposures on the filter paper blood samples were examined in the same way.

4.4. Results of the validation study

Data distribution was examined using skewness, Kolmorogov and Shapiro-Wilks tests as well as normal Q-Q and detrended Q-Q plots. Details of the general tests to examine the data distribution were provided in Chapter 3 (section 3.5. Data distribution, p.66). The raw alpha-1-acidglycoprotein (or AGP) data from the UK adults' filter paper blood spot deviated from the normal distribution and required transformation. The raw data for AGP from the liquid whole blood were normally

distributed and required no further action prior to the analyses. Table 4.4 (p.95) shows the mean c-reactive protein, albumin and alpha-1-acidglycoprotein levels obtained from adult and child blood spots and venous blood samples.

4.4.1. Interspot variation for c-reactive protein using UK adult samples

Interspot variation of c-reactive protein levels were tested by comparing two blood spots taken for the study participants at the same time on the same filter paper. This test aimed to identify any deviation in the blood protein distribution on the filter paper in laboratory conditions. Spearman's correlation applied to the non-normally distributed c-reactive protein data indicated a very strong positive correlation of the blood protein levels between the two different blood spots (r=0.996, p<0.001, n=19). This was taken as an indication of very low probability for inter-blood spot variation for albumin and alpha-1-acidglycoprotein and therefore this test was not repeated for the named biomarkers of immunity.

4.4.2. Comparative analysis of serum vs. blood spot levels of c-reactive protein using UK adult samples

The mean CRP levels from blood spot and serum samples were as follows: data from 05/01/07 run: spot=1.443±1.608, serum=5.993 ±6.735 and data from 08/01/07 run: spot=0.108 ±0.135, serum= 0.412 ± 0.558 (Table 4.4, p.95). Spot and serum c-reactive levels were tested for correlation using data from two different ELISA runs on two different days (05/01/07 and 08/01/07). This was to ensure that any relationship observed was consistent over different working days to allow for intra-tester variation between working days. A high correlation was observed between the c-reactive levels from the blood spots and liquid plasma on both test days using Spearman's correlation (05/01/07 r= 0.741, p<0.001, n=20 and 08/01/07 r=0.832, n=16, p<0.001). The correlation was similar on different days. The relationship between the blood spot and venous c-reactive protein levels were assumed to be relatively stable on the basis of the correlation observed.

Figure 4.2 (p.96) illustrates the significant relationship between the spot and serum data for c-reactive protein (Spearman correlation r=0.879, p<0.05). Figure 4.3 (p.96) shows the distribution of the residuals of the mean difference between the serum and the spot data for c-reactive protein. An identification of a conversion factor was attempted in order to convert the levels of the selected biomarkers of immunity measured on the filter paper blood samples to the values found in the venous whole blood. The observed variation between the CRP residuals was relatively low therefore the calculation of a conversion factor was attempted using a ratio between serum spot levels and was considered as a very suitable method. A conversion factor of 4.661 was obtained for mean serum/spot CRP ratio. A multiplication of the c-reactive values from blood spots by a factor of 4.661 would provide an accurate estimate of the serum CRP levels. This data was applied in all further analyses involving the use of c-reactive protein data.

4.4.3. Comparative analysis of the levels of blood spot and serum albumin in UK adults

The mean adult albumin levels observed from the blood spots and serum samples were 12.7±1.4g/L and 29.8±5.3g/L (n=17) respectively (Table 4.4, p.95). The relationship between serum and spot albumin levels were plotted in a graph with a line of best fit and 95% confidence intervals (Figure 4.4, p.97). The spot and serum albumin levels were significantly correlated (r=0.109, p<0.05).Figure 4.5 (p.98) illustrates the distribution of residuals of the mean difference between the serum and spot data for albumin. A conversion factor of 2.23 for albumin was worked out by calculating the ratio between blood spot and serum levels for the UK adults. The calculated conversion factor was then applied for all analyses using child albumin data in this thesis. (All data provided in the latter chapters shows the converted serum levels of albumin in the study sample).

4.4.4. Comparative analysis of serum vs. blood spot levels of $\log_{10}$-alpha-1-acidglycoprotein levels using UK adult samples

Alpha-1-acidglycoprotein data was transformed using $\log_{10}$-transformation in order to normalise the data distribution. The mean $\log_{10}$-AGP levels from the blood were -0.385±0.117 (n=22) and -0.883±0.092 (n=17) respectively (Table 4.4, p.95). The relationship between the venous serum and blood spots from the UK adults was tested using Pearson's correlation test but no significant correlation (r=0.167, p=NS, n=17) was detected. Figure 4.6 (p.99) shows the data distribution of AGP serum versus spot data. The lack of any relationship is evident from the homogenous distribution of the data points. Hence the determination of a conversion value for alpha-1-acidglycoprotein from spot to serum levels could not be carried out.

4.4.5. Examination of blood spot to serum conversion factor using child data from Bangladesh

A sub-sample of children (n=17) from the child study sample in Bangladesh were included in this validation study which aimed to work out a conversion factor for calculation serum protein levels (for c-reactive protein and alpha-1-acidglycoprotein only) from the blood spots. Non-normal c-reactive data was analysed using non-parametric tests. Acid-1-alphaglycoprotein data (serum and blood spot) were normalised using $\log_{10}$-transformation using the criteria described in the methods section of Chapter 3 (section 3.5.Data distribution). The mean CRP and $\log_{10}$-transformed AGP levels from blood spots (n=13) and serum samples (n=17) are provided in Table 4.4 (p.95). No significant correlation between the child spot and serum data for c-reactive protein was observed (r=0.462, p=NS, n=13) (Figure 4.7, p.100). No significant relationship between the $\log_{10}$-AGP blood spot and serum data was found either (Pearson's correlation r=0.187, p=NS, n=13) (Figure 4.8. p.100). From the graphs it is evident that the data was not suitable for calculating a conversion factor using the ratio.

4.4.6. Environmental pilot examining the effect of heat and humidity on the levels of c-reactive protein and albumin

The effects of the environment on the levels of the selected biomarkers of immunity were examined (Table 4.5., p.101). Due to unsuccessful laboratory runs data suitable for analysis was available only for albumin (adequate sample size n=18). Only five samples produced readable results for the c-reactive protein and therefore the results were inconclusive. The c-reactive protein data was plotted only as a potential indicator of the effect that an extended heat exposure may have on the blood protein levels. Figure 4.9 (p.102) suggests a lack of effect on the c-reactive protein concentration within a blood spot as a result of 22 hour exposure or 66 hour exposure to high humidity (65%). Table 4.4 (p.95) provides the mean level of CRP for the control, short and long exposure to humidity. This test needed to be carried out with a larger sample size in order to draw any informative conclusion about the effect of extended exposure to humidity.

For albumin a comparison of the effect of heat only versus heat and humid conditions on the blood spots was possible (Figure 4.10, p.102). The mean levels of albumin measured in the control (frozen), heat only and heat and humidity were as follows: 28.9±4.8g/L and 27.5±3.3g/L and 19.3±4.8g/L (n=18) respectively (Table 4.5.,p.95). An exposure to high temperature only (37°C) showed no degradation of the albumin levels as compared with the samples stored in the freezer after a 22 hour exposure to room temperature (t=1.210, p=NS). However, significantly lower albumin concentrations were observed in the samples which had been exposed to high temperature and humidity (37°C and 65% humidity) for two weeks (t=6.693, p<0.01) when compared with the control. High temperature and humidity also caused a significantly lower albumin concentration than exposure to high temperature only (t=5.873, p<0.01). These data suggest that an exposure to heat increases the protein degradation but hot and humid conditions cause a faster rate of protein loss, at least in the case of albumin.

4.5. Summary of the validation study

The validation study aimed to identify any differences in the blood protein concentration of the whole liquid blood samples collected using veni-puncture and whole blood collected on filter paper using the finger-prick method. This validation was carried out using adult UK data collected in laboratory conditions. The validation study was repeated with samples collected during the midterm survey in Bangladeshi under fieldwork conditions. The main purpose of the work was to calculate a conversion factor which could be used to estimate the venous blood protein concentration based upon the finger-prick samples on the filter paper. The second aim was to examine the environmental effect on the loss of blood protein that may be associated with the use of filter paper as a collection method for finger-prick blood samples.

The inter-spot variation of c-reactive protein levels showed a very strong correlation between the two blood spots collected during the same blood collection procedure. This indicates the c-reactive protein concentration measurement variability between different blood spots is very low as long as the blood spots are collected at the same time. Intra-spot variation of the blood protein levels was suggested as a potential source of error by McDade and Shell-Duncan (**McDade and Shell-Duncan, 2002).** Intra-spot variation was not tested in this study due to the small size of blood spots collected for the study. Intra-spot variation may be overcome by consistent sampling method; hence the punching a disc from the centre of each blood spot in the same manner, in order to minimise any differences in the protein concentration due to the chromatographic effect.

The blood protein concentrations collection using serum and finger-prick blood samples on filter paper were highly correlated for both albumin and c-reactive protein. However, blood protein concentrations were consistently lower in samples collected using the filter paper technique. The UK adult data showed an almost five times (4.661x) higher concentration of c-reactive protein in the whole liquid blood collected using veni-puncture than dried filter paper blood spots. The levels of

albumin were at least twice (2.23x) as high in the venous blood samples as in the dried filter paper blood spots in the UK adults. No conversion factor could be calculated for the alpha-1-acidglycoprotein due to a high variation between the venous versus filter paper sample concentrations of AGP. The data highlights the importance of calculating the conversion factor individually for each blood protein under investigation. The use of a universal conversion rate to calculate the whole liquid blood concentrations from the filter paper blood samples could lead to large inaccuracies.

The attempt to calculate the conversion rate from whole liquid blood samples and filter paper samples both collected using the finger-prick technique from children in Bangladesh was not successful. No conversion rate could be calculated. This could be due to the environmental effect (high temperate and high humidity) during the sample collection and the long storage period in Bangladesh which may have altered the blood protein concentrations in the whole liquid blood as well as the filter paper samples. This test should be repeated with a larger sample size.

The validation study also examined the effect of the environmental conditions on the filter paper blood concentrations as the filter paper technique is most commonly used in the areas of the world where laboratory facilities are not available. High temperature with humidity was identified as a cause of considerable protein degradation whereas high temperature only had no significant effect on the blood protein concentrations in this study.

The calculated conversion rates (4.661 for c-reactive protein and 2.23 for albumin) were applied to the data collected during the field work for this PhD work. However, as no conversion value for the alpha-1-acidglycoprotein could be calculated the raw data for alpha-1-acidglycoprotein levels were used for all statistical analyses.

Table and figures

Figure 4.1: Collecting blood on filter-paper using finger-prick technique

Table 4.1: List of equipment used in finger-prick blood sampling in the field

Air-tight plastic bags
Alcohol wipe
Automatic sterile lancet.
Centrifuge
Drying rack or a net
Filter-paper (Whatman 3, Whatman, UK)
FloTop scoop
Fridge
HemoCue device
HemoCue microcuvette
Heparinised microtainer tubes
Ice-cool box
Labelled envelopes
Permanent marker
Tissue

Table 4.2: List of equipment used for laboratory analyses of biomarkers of immunity

Aluminium foil.
Centrifuge
Centrifuge with a rotor of 10 cm, rpm is 3500)
Cling film
Disc puncher (diameter of disc was 6mm)
EDTA vacutainer tube with syringe
Fridge
Hematorotor
Humidity chamber with 70-80% humidity and (37°C) temperature settings
Incubator
Magnetic stirrer plate
Master mix tubes
Measuring cylinders
Multiscan photometric reader
NUNC maxisoap microwell plate (Fisher DIS-971-010P)
pH meter
Pipettes (1mL, 250μl, 20μl and 10μl
Soft squeezer bottle for washing ELISA plates
Stirrer bar
Stretchy tube sealer material
Weighing scales with three decimal place accuracy

Table 4.3: List of reagents used in the laboratory analyses of biomarkers of immunity

Coating buffer (prepared using DAKO method: 0.01M Phosphate buffer (PBS buffer), 0.15M NaCl, pH 7.2).
Deionised /distilled water
Dilution buffer (DAKO S2005)
Distilled water
Distilled water
Dry ice.
Human serum calibrator (DAKO X0923) suitable for calibrating CRP and AGP
Hydrogen peroxide 35 Mol
Microplate Nunc MicroWell 96 well flat bottom plate (Fisher DIS-984-090M)
Sodium chloride (NaCl)
OPD tablets (DAKO S2045)
Peroxidase-conjugated rabbit anti-human c-reactive protein (DAKO P0227
Polyclonal antibody to alpha-1-acid glycoprotein - HRP (Acris BP518-HRP)
Polyclonal antibody to alpha-1-acid-glycoprotein (Acris BP518)
Rabbit Anti-human Albumin (Dako, Q0328)
Rabbit anti-human antibody to C-reactive protein (DAKO A0073)
Reaction Buffer (DAKO S2007)
Saline solution (0.86g of NaCl into 100mL of deionised water)
Sodium chloride (NaCl)
Sodium phosphate dibasic anhydrous (Na_2HPO_4)
Sodium phosphate dibasic anhydrous (Na_2HPO_4)
Sodium phosphate monobasic monohydrate (NaH_2PO_4-H_2O)
Sodium phosphate monobasic monohydrate (NaH_2PO_4-H_2O)
Sulphuric acid0.5M
Tween20
Washing buffer (prepared using DAKO method: 0.01M Phosphate buffer (PBS buffer), 0.50M NaCl, Tween 20, pH 7.2).

Table 4.4: Concentration of the selected biomarkers of immunity from filter paper blood samples and venous whole blood

Sample	Collection method	Mean CRP±SD (n)	Mean AGP±SD (n)	Mean Albumin±SD (n)
Adult	Blood spot	1.443±1.608 (20)	-0.385±0.117 (22)	12.7±1.4 (n=17)
	Venous serum	5.993±6.735 (20)	-0.883±0.092 (17)	29.8±5.3 (n=17)
Child	Blood spot	1.125±1.305 (13)	-0.167±0.088 (13)	n/a
	Venous serum	5.742±5.004 (18)	-0.668±0.120 (18)	n/a
Conversion factor		4.661	n/a	2.23

Figure 4.2: Relationship between filter paper and venous blood levels of c-reactive protein

C-reactive protein spot versus serum data for UK adult validaton

R^2 Linear = 0.879
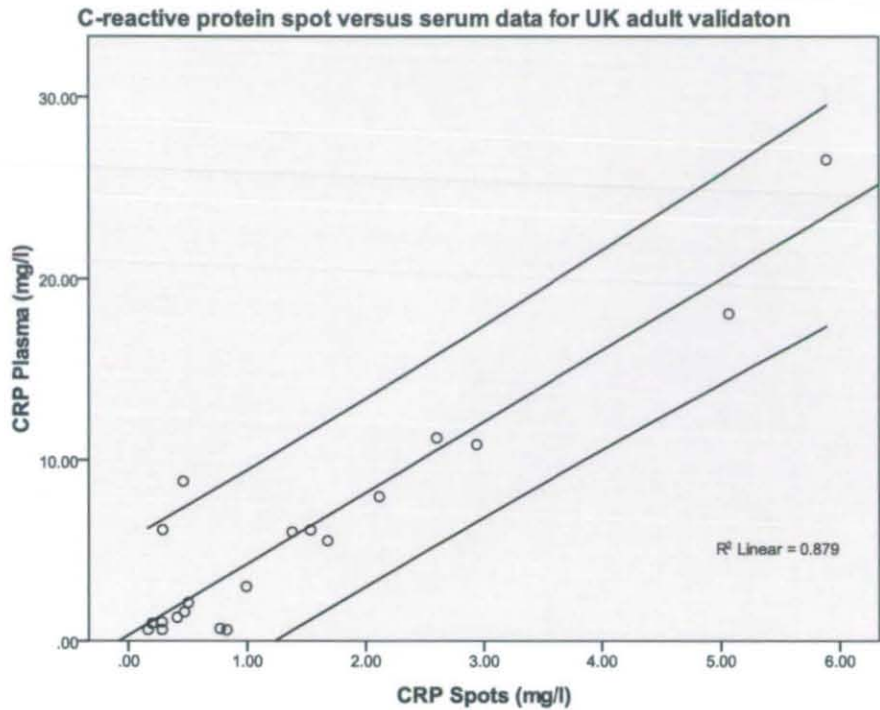
Figure 4.3: Residuals of mean difference in the c-reactive protein levels between filter paper and venous blood samples
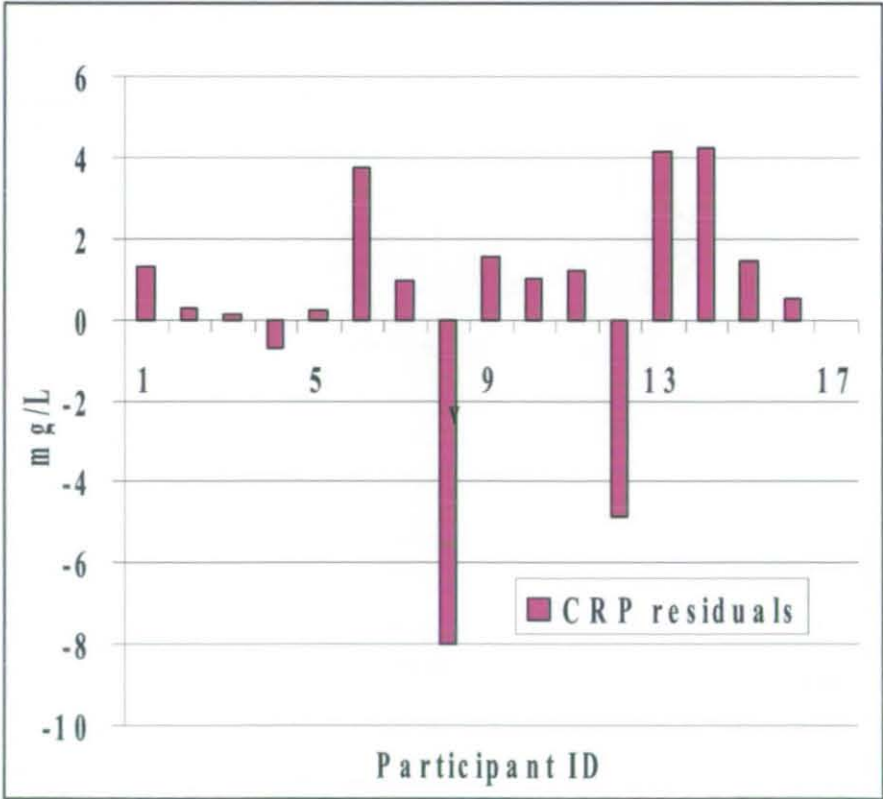
Figure 4.4: Relationship between albumin levels from filter paper and venous blood

Albumin spot versus serum data for Uk adult validation study

R^2 Linear = 0.109
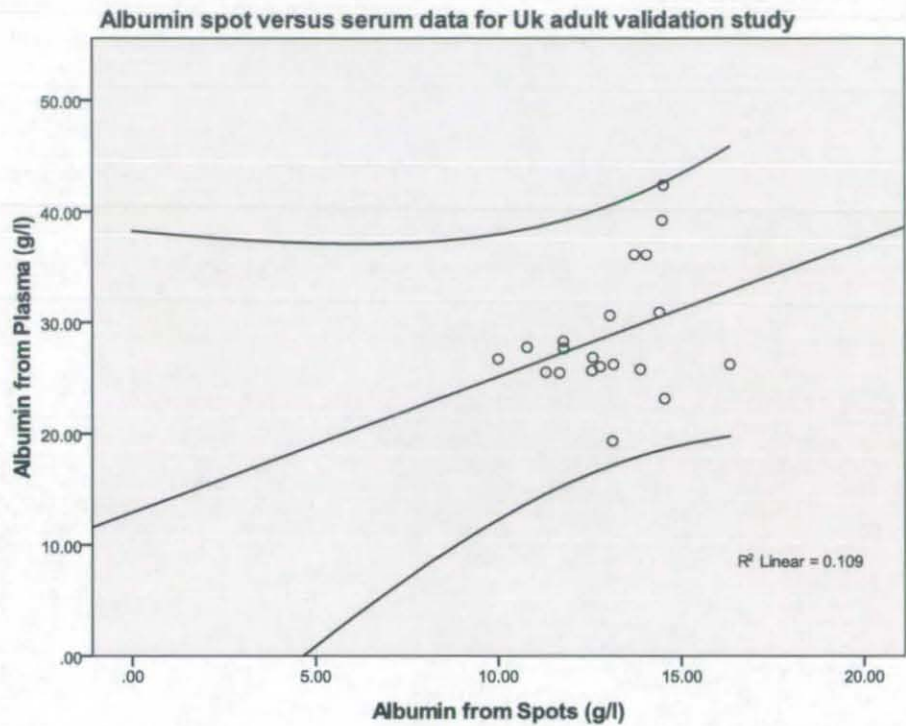
Figure 4.5: Residuals of the mean difference of albumin levels between filter paper and venous blood samples
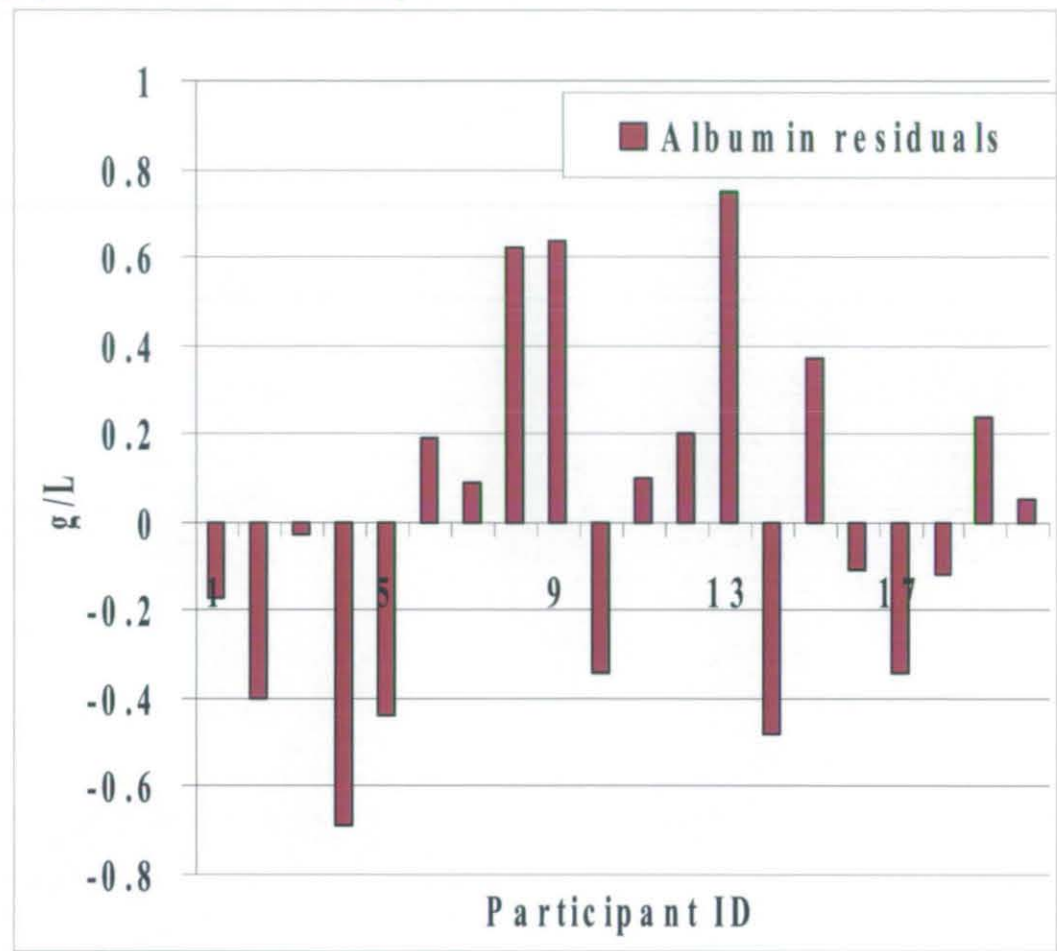
Figure 4.6: Relationship between filter paper and venous blood for alpha-1-acidglycoprotein levels –UK adults

AGP spot versus serum data for UK adult validation study
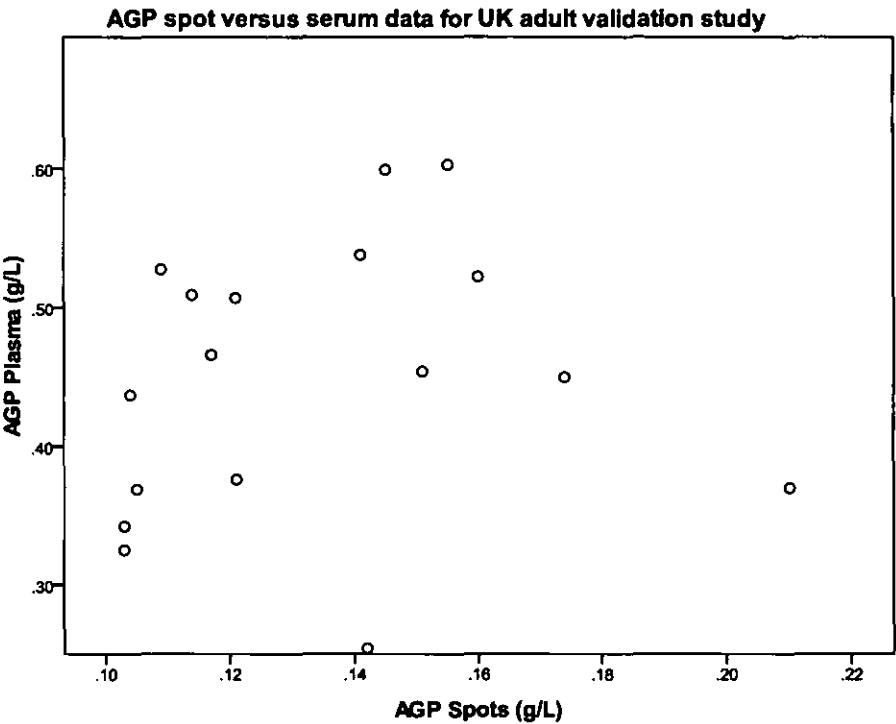
Figure 4.7: Relationship between c-reactive protein levels from filter paper and venous blood from Bangladeshi child validation study

C-reactive protein spot versus serum Bangladeshi child validation study
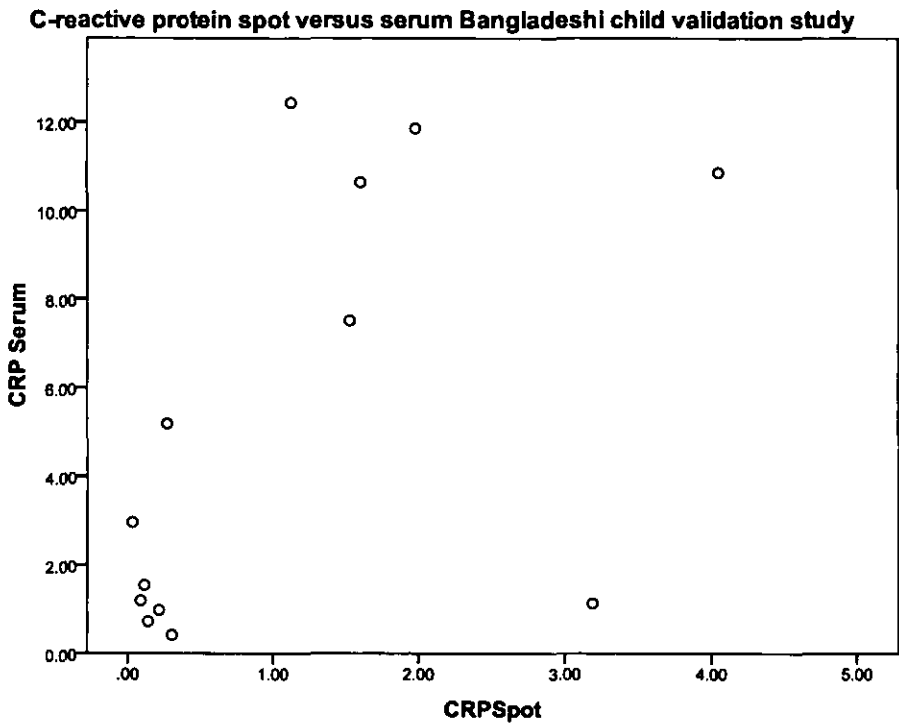
Figure 4.8: Relationship between filter paper and venous blood alpha-1pacidglycoprotein levels -Bangladeshi child validation study

AGP spot versus serum values for Bangladeshi child validation study
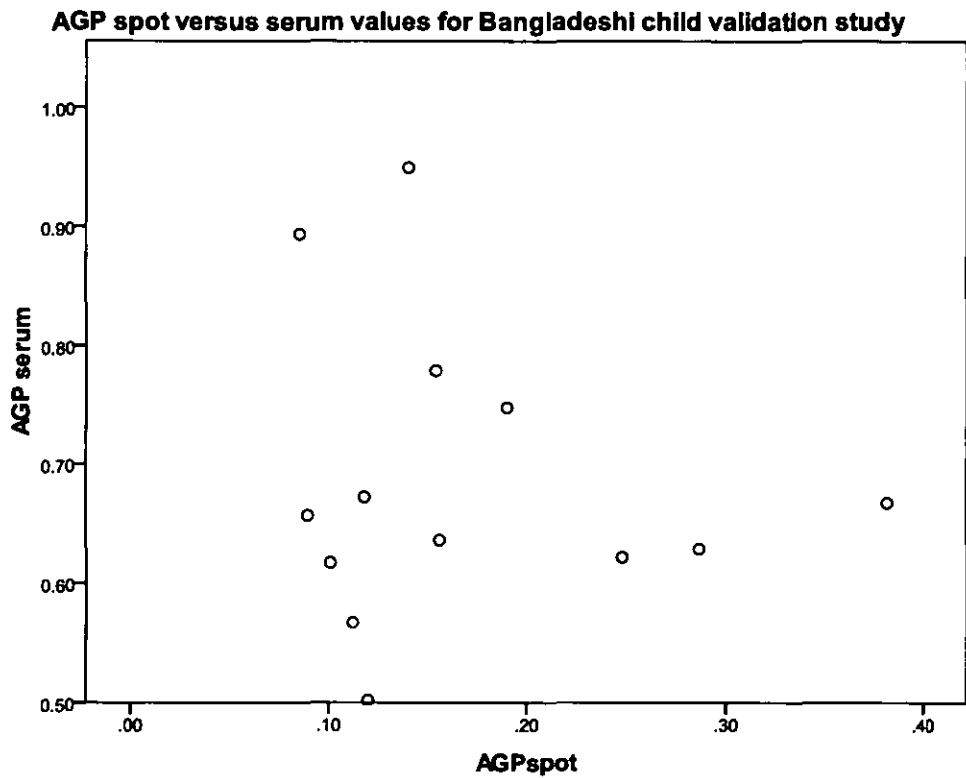
Table 4.5: Validation of the effect of environmental factors on the levels of the selected biomarkers of immunity

Environmental condition	Mean CRP (mg/L) ± SD (n=5)		Mean Albumin (g/L) ± SD (n=18)	
	Frozen (control)	1.449±1.532	Frozen (control)	28.9±4.8
	Frozen+heat for 1 day	1.162±1.237	Frozen+heat only	27.5±3.3
	Frozen+heat for 3 days	0.976±1.021	Frozen+heat with humidity	19.3±4.8
	Paired t-test of environmental conditions		Paired t-test of environmental conditions	
	Frozen vs. frozen+heat 1 day	n/a	Frozen vs. frozen+heat	t=1.210, p=NS
	Frozen vs. frozen+heat 3 days		Frozen vs. frozen+heat+humidity	t=6.693, p<0.01
	Frozen+heat 1 day vs.frozen+heat 3 days		Frozen+heat vs.frozen+heat+humidity	t=5.873, p<0.01

Figure 4.9: The effect of environmental factors on the levels of c-reactive protein on filter paper
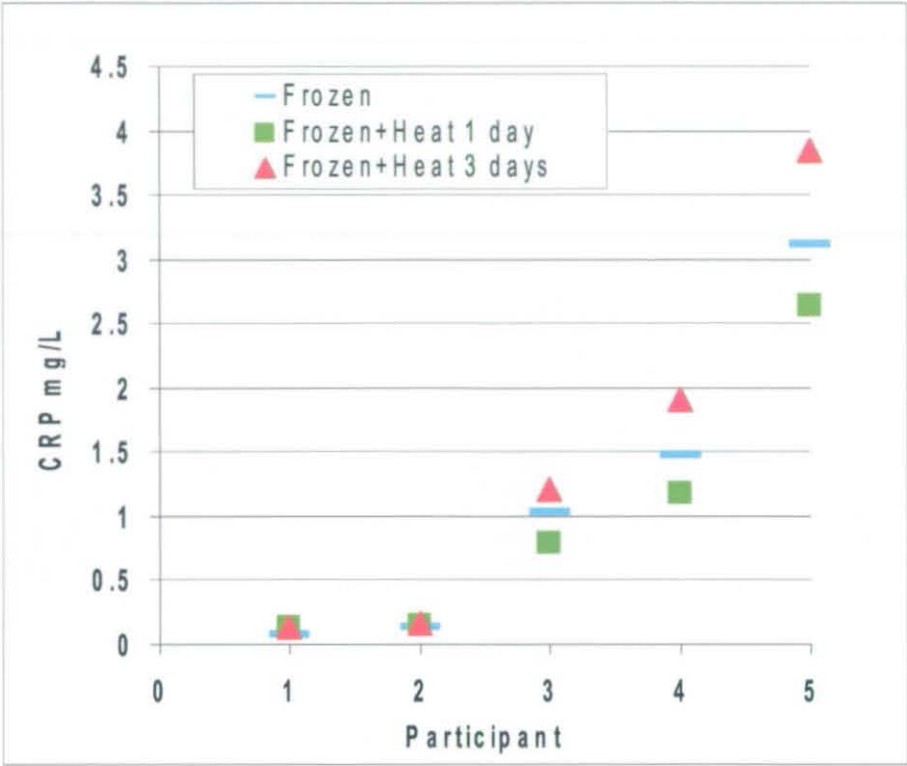
Figure 4.10: Effect of environmental factors on the levels of albumin on filter paper.
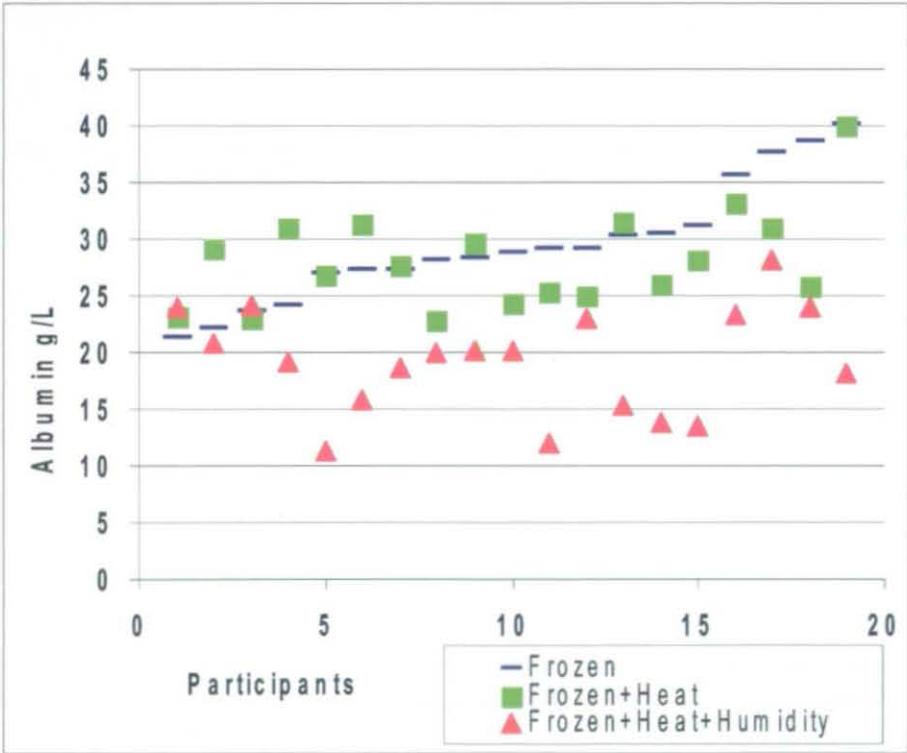
Chapter 5: Sample descriptives.

5.1. Introduction

The mechanisms which determine the extent of household fuel pollution exposure may be greatly influenced by the household structure and building materials, the type of fuels used, as well as the cooking practices and socio-economic factors such as income and occupation. In order to study the potential impact of household fuel pollution on health it is essential to identify the underlying characteristics in a sample. This chapter will provide an overview of the baseline characteristics of the study sample and looks at the potential health hazards of indoor air pollution from cooking fuels in Bangladesh.

This study was carried out in two municipalities (Saidpur and Parpatipur) in Northern Bangladesh. Saidpur (Figure 5.1, p.123) represented an urbanised settlement with high population density, whereas Parpatipur was a rural, less dense settlement with more open air and space around individual households (Figure 5.2, p.124).

In this chapter socio-demographic characteristics of households will be described with a view of identifying the potential determinants of household fuel pollution exposure in this sample. Child health status will be described using maternally-reported health data, medical diagnoses and biochemical indicators of immune status. The health status of the mothers will be described in brief too. The chapter describes the study sample using data from the baseline study only. The information provided in this chapter will help to determine suitable methods of analyses to study the impact of indoor air pollution within specific population settings for further chapters. Longitudinal analyses will be provided in the chapters dealing with child health and household fuel pollution (Chapter 6 and 7 respectively).

5.1.1 Sample sizes of analysis

The total number of households that were included in the study was n=625. The sample included five different wards in the municipalities (two in Saidpur and three in Parpatipur) using the selection criteria described in the Chapter 3 (section 3.2.1. p.51). As many households as possible from each ward were recruited for this study. Hence not all households had children under the age of five present. The recruitment of households in larger clumps or wards aimed to maximise the efficiency and the acceptance of the delivery of the planned intervention (further details in Chapter 7) as all households within the area were taking part in the intervention. Children under the age of five were present in 408 out of 625 households (65.3%). All subsequent analyses include only those households where children under the age of five years were present (n=408). This will strengthen the analyses socio-demographic characteristics which may have a direct or indirect effect on the health of children and women in this Bangladeshi community.

5.2. Household and socio-demographic variables

5.2.1. Household characteristics

The study sample was ethnically mixed: 59.3% of the households were Bangla-speaking families and 40.7% were Urdu-speaking Bihari migrants who had settled in the area after 1947 as a result of the union between Bangladesh (East Bengal) and Pakistan into East and West Pakistan (Chapter 1, section 1.2, p.2). Islam was the dominant religion practiced (Muslims: 91.7%) but some Hindus lived in the community (8.3%). The mean age of the household mothers was 25.8years ± 8.1SD. The mean age was not significantly different in the two municipalities (Saidpur: 25.9 ± 8.3SD years, Parpatipur: 25.7 ± 7.8SD years, t=0.242, p=NS). The age of the mothers was obtained verbally during the health interview. The majority of households had a male head, only 2.1% of the household were female-led. All families included in the analyses had at least one child under the age of five years. About a third of the families within this study sample had two children and 4.5% of the households had three or more children under the age of five years (Table 5.1, p.124). Slight variations in the number of children between the wards within Saidpur and Parpatipur were found, but these were not significant.

5.2.2. Education and literacy level in the households

The education level of the household members was low. Almost half of the household heads (51.5%) did not have formal education and 48.5% had some. 23.5% of household heads had either completed or partially completed primary school, 16.9% had partially or fully completed secondary school education. Only 5.2% of the household heads had attended a college/university. Over half of the respondents (mothers) had not received any formal education (52.0%). 48.0% of the respondents had received some education (primary: 30.7%, secondary: 16.7%, college: 0.7%). Only 24.8% of the respondents could read and/or write, 57.1% of the respondents were illiterate (the information was missing for 18.1% of the respondents).

5.2.3. Education and socio-economic status

Women and the heads of households from low SES households were significantly more likely to be illiterate than those from higher SES households (X^2 = 12.814, $p<0.001$, X^2 = 9.079, $p<0.003$, respectively). The households were divided into national socio-economic status (SES) quintiles which were calculated by the Associates of Community and Population Research using the methods as described by Ruthstein and Johnson (**Rutstein and Johnson, 2004**) in Chapter 3 (section 3.5.2, p.68). The information applied included household assets such as household ownership, income, number of items owned, household structure and material characteristics including the floor material as well as sanitation facilities. Similarly, municipal SES quintiles were formed using the same categories, on the basis of items owned by a household, occupation opportunities and the range of salaries which were available for the local occupants. No significant differences were found between the national and the municipal SES classification in this sample, therefore the national SES classification was used in the analysis. A binary SES variable (high and low SES) was created from the five SES quintiles in order to simplify the analysis (low SES was formed from SES classes 1-3 and high SES from classes 4 and 5). On the basis of socio-demographic characteristics this division was natural.

5.2.4. Household occupation

The most common occupation in Parpatipur was a hawker or petty trader (39.3%).
In Saidpur most primary household earners worked as skilled day labourers
(31.3%), which was also the second most common occupation in Parpatipur
(18.0%). Hawking or petty trading employed a large proportion of household
earners (29.6%), as well as rickshaw/car/van drivers (16.5%) in Saidpur (Table 5.2,
p.125). Only a few individuals worked at their own store, or as a household helper
or government employee. Railway work and border business provided a significant
amount of employment in Parpatipur. Only a small proportion of mothers were
employed outside the home, they were mostly child carers and worked as house-
wives at home.

5.2.5. Average monthly income

Average income in this study sample was determined using cash income from
wages and excluded in-kind income. The average monthly income was 3551 ±
2226SD Bangladeshi Taka (TK) (£37 UK Stirling). Due to non-normal data
distribution, the average income data was divided into three groups of equal sizes
(33%): low, medium and high income using TK2500 and TK3667 as cut-off points
(Table 5.2, p.125). The percentage of households within the low income group was
significantly higher in Saidpur (42.6%) than in Parpatipur (28.1%), and more
households in Parpatipur (medium: 31.5% and high: 40.3%) belonged to the higher
income group than in Saidpur (medium: 29.6% and high: 27.8 %) (X^2= 10.747,
p<0.01) (Table 5.4, p.127).

5.2.5.1. Average monthly income and socio-economic status (SES)

Socio-economic status varied significantly between Saidpur and Parpatipur
(X^2=7.313, p<0.01) (Table 5.2, p.125): more households in Parpatipur (low: 68.0%,
high: 32.0%) belonged to the low SES than in Saidpur (low: 54.8%, high: 45.2%).
Socio-economic status was significantly associated with income (X^2=45.432,
p<0.001) with high income being more often within the high SES group (high:
52.2% and low: 21.7%), whereas low and medium income households were more
commonly found among low SES families (Table 5.4, p.127). Due to a lower

average income in Saidpur the SES status alone could not explain the differences in the income between the municipalities. An exclusion of in-kind income in the analyses may explain the unexpected relationship between SES and average income in this sample.

5.2.5.2. Average monthly income and ethnicity

The study sample was ethnically diverse: Bangla: 59.3% and Bihari: 40.7%. Parpatipur was predominantly Bangla (Bangla: 97.2% and Bihari: 2.8%) whereas Saidpur had a majority of the Bihari ethnicity (Bangla: 27.3%, Bihari: 72.7%) (Table 5.2, p.125). The observed ethnic difference may influence the average monthly income of households and explain the higher average income in the rural Parpatipur. A larger proportion of Bangla-families belonged to the high income earning group (38.8%) than the Bihari families (26.5%) (X^2=17.259, p<0.001) (Table 5.4, p.127). When the ethnic effect on income was tested within Saidpur only, no differences were found (X^2=0.996, p=NS n=230). Ethnicity alone therefore could not explain the higher average monthly income in Parpatipur.

5.2.5.3. Average monthly income, education and literacy

The municipalities of Saidpur and Parpatipur differed significantly in the rates of household heads who had no formal education versus those who had attended school (51.9% vs. 41.6%, X^2=12.384, p<0.001, n=408) (Table 5.2, p.125). Also a significantly high proportion of mothers were educated in Parpatipur than in Saidpur (55.1% vs. 42.6%, X^2=6.228, p<0.05, n=408). Uneducated mothers were more likely to be found in low income families (43.4%) than medium or high income households (30.7% and 25.9% respectively) in both towns (educated mother income: low = 28.6%, medium= 30.1%, high= 41.3%) (X^2=13.410, p<0.05, n=408) (Table 5.4, p.127). A better maternal education level is likely to improve the occupation opportunities in the communities and may explain the higher average monthly household earnings. Approximately two thirds of the household mothers were literate in Parpatipur (72.7%) and Saidpur (67.7%) and the observed difference was not significant (X^2= 0.770, p=NS, n=334) (Table 5.2, p.125). No association was found between maternal literacy and average monthly income (X^2=2.941, p=NS, n=334) (Table 5.4, p.127).

5.2.5.4. Regression analysis of average income

Binary logistic regression (method: forward likelihood ratio) was applied in the analysis of predictors of average monthly income. The criteria used for the regression analysis are described below:

Variables selected for the analysis were municipality, SES, ethnicity and education of a mother as these variables showed a significant influence on the average income (three groups) in the univariate analysis (see table 5.4, p.127). Collinearity was avoided using tolerance tests and VIF statistics: the level of tolerance less than 0.8 caused an exclusion of a variable from the regression analysis. Hosmer and Lemeshow tests (p=NS was accepted) and goodness of fit techniques were also applied. A significant correlation was also an indication of suitability for the regression analysis. The appropriate maximum number of predictors was calculated using the formula N>=50 + 8m (where m is the number of predictors) **(Harris, 1975)**. The regression analysis aimed to find a combination of predictors which explained the variance observed in the dependent variable the best. Table 5.5 lists the variables and their sub-groups applied in the analysis (Table 5.5. p.128).

Forward LR binary regression on binary average income showed that SES and ethnicity were the best predictors of the average monthly income grouping in this Bangladeshi sample. SES and ethnicity overrode the effect of municipality and education. Ethnicity was highly collinear with municipality however the effect of the ethnicity was stronger on its own in predicting average income than municipality (Table 5.6, p.128). Model 1 shows that households from the high SES group were 4.072 times (95%CI: 2.669 – 6.211) more likely to have a higher average monthly income than those families belonging to the low SES group (reference) (Wald: 42.475, p<0.001). Model 2 shows that households belonging to the higher SES group (Wald: 51.493, p<0.001, OR: 3.752, 95% CI: 3.567 – 9.276) and of Bangla ethnicity (Wald: 18.278, p<0.001) were most likely to belong to a higher income earning group (Bangla reference, Bihar: OR: 0.345, CI: 0.212 – 0.562) (Wald: 21.440, p<0.001).

5.3. Household Cooking practices

5.3.1. Cooking frequency patterns

During the baseline survey households were asked to report their typical cooking practices in the dry and wet seasons to see whether seasonal climates had an impact on the reported cooking behaviour. During the dry season households reported cooking normally more than once a day (once: 40.7%, twice: 38.2% and three times: 21.1%) (Figure 5.3, p.129). The cooking pattern reported during the baseline survey for the monsoon season was very similar. Cooking frequency varied very significantly between the municipalities during the baseline survey (dry) ($X^2=$ 79.618, p<0.001 n=408) (Table 5.2, p.125). Households which cooked only once a day were a lot more common in Parpatipur than in Saidpur (62.5% vs. 21.7%). In Saidpur households were more likely to cook more than once a day (two or three times a day) than in Parpatipur. Any changes in the cooking practices due to seasonality could not be tested within a single municipality due to the small size of sub-groups.

5.3.1.1. Number of dishes cooked

An interesting pattern was observed in how many dishes the households cooked on average for breakfast, lunch and dinner (Table 5.7, p.129). For breakfast the majority of households did not cook anything. In these houses the breakfast most likely consisted of the leftovers of the dinner from the night before or just rice with water. 39.2% of the households cooked two dishes for breakfast (only a few houses reported cooking just a single dish). It seemed that the majority of the households prepared a large lunch which consisted of three or more dishes (65.8%). Only a very small number of households prepared nothing for lunch or just cooked one dish. The majority of the households did not cook anything for dinner (65.4%), and most likely relied on leftovers from lunch. 12.0% of the houses cooked just one dish for dinner.

The different municipalities appeared to have quite different cooking practices (Table 5.8.,p.130). In Parpatipur a large proportion of families did not cook anything for breakfast or dinner (69.7% and 81.5% respectively). In Saidpur

approximately a quarter of families did not cook breakfast and about 50% did not cook dinner. Due to the nature of this data it was not possible to test for the difference in the number of dishes cooked between the municipalities statistically.

5.3.1.2. Length of time spent cooking by households

Table 5.9 illustrates the length of time families spent cooking breakfast, lunch and dinner in the study sample. As said previously the majority of households did not use their stove to cook breakfast (44.6%) or dinner (65.4%) (Table 5.9., p.130). The largest number of households spent one to two hours cooking lunch each day (69.4%). This pattern closely matches the number of dishes reported for each meal. All households used a lid to cover the pots during cooking. This practice may shorten the cooking times and reduce household fuel pollution exposure.

5.3.2. Household Fuel Choice

The most common fuel choice within households varied. Almost half of the households used wood (45.6%), but also golden (a compressed form of rice husk residues) (17.9%), dry leaves (3.2%) coconut fronds (0.3%), rice husks (1.0%), dung cakes (13.0%), saw dust (4.7%), biogas (0.3%), bamboo (13.5%) jute straw (0.3%) and others (unspecified – 0.5%) were used. Significant differences were observed in the most common fuel choice between high and low socio-economic classes in this sample $X^2= 12.747$, $p<0.01$, n=405 (Figure 5.4, p132). To simplify the analysis fuels were grouped into four categories: wood, golden, plant-derived fuels and dung (excluding others n=3).

5.3.2.1. Cooking fuels in Saidpur and Parpatipur

The choice of cooking fuel varied between the municipalities but wood was the most common choice of fuel in both areas (Saidpur: 49.6%, Parpatipur: 40.4%) (Figure 5.5, p.131). In Saidpur, approximately one third of the households used golden as their main fuel, whereas in Parpatipur no households reported the use of golden (Table 5.3, p.126). The use of dung was very common in Parpatipur (27.09%), whereas in Saidpur only a few households relied on dung as their main fuel ($X^2=47.865$, $p<0.001$, n=332, excluding golden and others). Coconut husks and

other plant-derived fuels were used less in Saidpur than in Parpatipur. No
association was seen between SES status and fuel-choice when tested within each
municipality (Saidpur and Parpatipur: X^2=5.774, p=NS, n=228, X^2=5.111, p=NS
n=177). Most households purchased their household fuel (81.1%) from the local
market. Almost two thirds of the households claimed they used dry fuel only
(63.5%), the rest stated that they used damp, wet or green fuel. Fuels were
commonly dried in the sun (86.0%), or by hanging it near the stove.

5.3.2.2. Ethnicity and fuel choice

The effect of ethnicity on fuel choice was examined within Saidpur only, as the
sample of this municipality was heterogeneous (Table 5.3, p.126). Significant
differences in fuel choice were found between Bangla and Bihari families in
Saidpur (X^2=9.339, p<0.01, n=223, excluding dung due to small sample size)
(Table 5.10, p.132). Wood was the main choice of fuel in both ethnic groups but the
percentage of households where golden was used instead of coconut or other plant-
derived fuel (leaves, jute, thatch etc) was much higher among Bihari. The use of
golden and coconut and plant-derived fuels were equally prevalent among the
Bangla in Saidpur.

5.3.2.3. Education, literacy and household fuel choice

The effect of education of the household head and the respondent on the fuel-choice
was examined. Significant differences in the fuel-type were seen in households of
educated and uneducated household heads (X^2=11.268, p<0.01, n=405). When the
impact of education (household head) on the fuel-use was studied for the
municipalities using multidimensional chi-square, no significant association was
found (Saidpur: X^2=1.006, p=NS, n=150 and Parpatipur: X^2=1.444, p=NS, n=129,
excluding dung and golden, due to their uneven use across municipalities). The
education level or the literacy of the mother did not significantly affect the fuel
choice (X^2=4.468, p=NS, n=405 and X^2=2.658, p=NS, n=333).

5.3.2.4. The average monthly income and the fuel choice

The average monthly income was associated with fuel-type when using binary
values of high and low with a median (TK3000) as a cut-off. When the monthly

income was grouped into low, medium and high income groups no significant association was found. No significant association was found between the binary income groups and the fuel type within municipalities either (three income groups X^2= 8.347, p=NS, n=405, binary income groups: X^2= 6.861, p=NS, n=405).

5.3.3. Household stove usage

The traditional biomass stove was very commonly used in this sample (Figure 5.6 and Table 5.11, p.132). About half of the households used portable biomass stoves (one or two openings for the cooking pot) and the rest used fixed biomass stoves (one or two openings) during the baseline survey (the term 'mouth of a stove' refers to the point on the top of the stove where a pot rests during cooking). The fixed stoves were built *in situ* and could not be moved. Only one household used an LPG stove. Stoves with chimneys were not used by households in this community. No municipal difference in stove type was seen (X^2=0.250, p=NS, n=407) (Table 5.2, p.125). Within Saidpur only ethnicity was not significantly associated with the stove type either (X^2=0.640, p=NS, n=407). The level of maternal education, education of the household head, or literacy were not associated with stove type (X^2=0.04, p=NS, n=407, X^2=3.151, p=NS, n=407, X^2=1.166, p=NS, n=334, respectively). Cooking on a raised platform to improve the ventilation to reduce immediate pollution exposure by the cook was rare in this community: 99.0% (n=408) of the households reported no use of a raised platform. Stove-use behaviour and its potential effect on the exposure to household pollution will be looked at in more detail in Chapter 7.

5.3.3.1. Socio-economic status, average income and the stove choice

Cooking on a fixed stove was significantly more common among high SES households (fixed: 60%, portable: 40%), whereas low SES households used both types of stove in equal proportions (X^2 = 5.446, p=0.05, n=407) (Figure 5.7, p.133). This pattern was observed within Saidpur but not in Parpatipur (X^2 = 5.938, p<0.05, n=230, X^2 = 0.726, p=NS, n=177, respectively). Reported stove use by households during the monsoon showed accentuation of the observed pattern (X^2 = 11.415, p<0.001, n=406). The stove choice was significantly associated with the average income groups (X^2= 7.445, p<0.05, n=407). Medium and high income households

were more likely to cook on a fixed stove rather than on a portable model. The use of multidimensional chi-square found no significant differences in the stove use as related to the income group across municipalities (Saidpur: X^2= 4.388, p=NS, n=230 and Parpatipur: X^2= 2.960, p=NS, n=177). Stove choice is likely to be more effected by the nature of the settlement than the average income in this community.

5.3.3.2. Fuel use and stove type

Fuel type was significantly associated with the stove type (X^2=15.534, p<0.001, n=405). Wood use was significantly associated with the use of fixed stoves, whereas a portable stove was more commonly used in households which used golden for fuel (Figure 5.8, p.134). The use of dung and plant-derived fuel were equally prevalent among households using the two stove types across the sample (Table 5.12, p.134). The described differences were significant in Saidpur (X^2 = 15.377, p<0.005, n=228) but not in Parpatipur (X^2 = 0.868, p=NS, n=177).

5.3.4. Cooking location

Interviews showed that cooking was done either indoors in the living space, indoors in a separate kitchen room, in a separate kitchen building, outdoors in the open air or outdoors on a two-walled veranda just outside the house. A binary variable was derived comprising indoor stoves and separate kitchen in the indoor group and outside stoves and stoves on veranda formed the outdoor group.

5.3.4.1. Cooking location and municipality

Cooking indoors was common in Saidpur (31.3%) and in Parpatipur households preferred cooking in the open air (58.4%) or on two-walled verandas (24.7%). Indoor cooking was more prevalent in Saidpur (excluding households which cooked in the open air, X2 = 33.985, p<0.001, n=217). Cooking location is likely to reflect the nature of the municipality. The proportion of the households cooking indoors or outdoors was not significantly different between the three monthly income groups (X^2=0.773, p=NS, n=408).

5.3.4.2. Cooking location and SES

Significant differences in the cooking location between different socio-economic classes were observed. Figure 5.9 (p.135) shows the cooking location significantly differed for the low and high socio-economic classes in the total sample (X^2 = 18.883, p<0.001, n=408). Most households (in both high and low socio-economic classes) reported cooking outdoors (low=54.3%). The low SES households were more likely to cook outdoors than the higher SES households (54.3% versus 35.4% respectively). Cooking indoors in the living space, in a kitchen or in a separate kitchen building was practiced more in the higher SES households (SES (high) =44.7%, SES (low) =27.5%). However when cooking in the open air (in order to reduce the municipality effect) was removed from the analysis, no differences were found (X^2 = 4.769 p=NS, n=217). The same result was obtained when using four groups of cooking locations (X^2 = 8.068 p<0.00, n=408). When looking at cooking practices across municipalities using multidimensional chi-square, low SES households were significantly more likely to cook outdoors in both municipalities (Saidpur: X^2 = 4.020 p<0.05, n=230, Parpatipur: X^2 = 7.326 p<0.05, n=178, using binary variable of cooking location).

5.3.4.3. Education and cooking location

The maternal education may influence the decision made about the household cooking practices and therefore it may influence the extent of exposure to the cooking fuel pollutants. Education did not show a significant association with the cooking location when using 4 groups and 2 groups (indoors/outdoors) of cooking locations (X^2 = 1.571, p=NS, n=408, X^2 = 0.113, p=NS, n=408) (Table 5.13. p.135). However the cooking location was significantly associated with the SES status both within the educated as well as uneducated groups. In both education groups the low SES households cooked more outdoors and the high SES households were associated with indoor cooking (uneducated: X^2 = 9.796, p<0.05, n=210 and educated: X^2 = 9.647, p<0.05, n=198 using multidimensional chi-square). When the above was carried out within a single municipality (within Saidpur or Parpatipur) no differences in the cooking location between the SES groups within educated or uneducated households were found.

5.3.4.4. Cooking location and stove choice

Cooking location was considered to be a likely reason for choosing a certain stove type. The data showed that a higher proportion of households cooking in the open air cooked using a portable stove ($X^2= 11.419$, p<0.001, n=407) (Table 5.14, p.135). A fixed stove was mainly used in those household who cooked indoors, in a separate kitchen building or on the outside veranda. An examination at municipality levels showed that a portable stove was used by a quarter of households for indoors (25.2%) as well as for the outdoors cooking (23.0%) in Saidpur (Table 5.15, p.136). A fixed stove instead was used more indoors (37.0%) than outdoors (14.0%) ($X^2= 8.890$, p<0.005). In Parpatipur the use of portable stoves was more commonly used for cooking outdoors (30.5%) than indoors (15.3%), but fixed stoves were used indoors and outdoors at equal proportions. However, the described differences were just significant in Parpatipur ($X^2= 3.856$, p=0.05).

5.3.4.5 Regression analysis of stove choice

Binary logistic regression (method: forward likelihood ratio) was applied in the analysis of predictors of the stove choice among households in this sample. Criteria used for the regression analysis was as described earlier in this chapter. The regression analysis aimed to find a combination of predictors which best explain the variance observed in the dependent variables. Table 5.16 (p.136) lists the variables and their sub-groups applied in the analysis. Forward likelihood ratio analysis of binary stove choice excluded SES and municipality from the regression equation when cooking location (inside versus outside) and fuel choice were included, hence the effect of SES and municipality were overridden by fuel choice and cooking location.

Cooking location alone was a significant predictor of household stove type (Wald, 10.107, p<0.001) (Table 5.17, p.138): households cooking outdoors (reference: indoor) were 0.508 times more likely to use a portable stove (reference) (OR; 0.508, 95% CI: 0.342 – 0.755, Wald: 11.212, p<0.001). Household stove type could be better predicted by household cooking location and the choice of fuel. The use of golden as a fuel (reference: wood) was a significant predictor of the use of a portable stove for cooking (OR=0.325, 95% CI: 0.183 – 0.577) whereas other fuels

did not significantly predict the stove choice. The use of portable stoves was 2.117 times more likely in households which cooked outdoors and used golden (Wald: 17.870, p<0.001, OR= 2.117).

5.3.5. Household structure and ventilation

Materials used for houses were brick, thatch, mud, bamboo, clay and corrugated iron (Table 5.18, p.138). Corrugated iron was the most common material for roofs. About half of the households had walls made out of bamboo and the use of brick was also common (23.3%). Clay was the most common material for floors and was also used for walls in just over 10 percent of the households. About a fifth of the households had brick floors.

Approximately half of the households reported having some holes or cracks in the household structure to aid in ventilation (Table 5.19, p.138). No information on ventilation methods was available for almost half of the households. A small percentage of households reported no use of any method of ventilation in the house. 50.0% of the households reported not having any windows in the kitchen, only 4.5% of the households had one window and 0.4% of the households had two windows in their cooking space. Information was missing for 45.1% of the households for an unknown reason.

5.4. Cross-sectional introduction to child health and growth (baseline survey)

This study included three separate field-surveys and the involvement of a total of 568 children under the age of five years from 408 households. Most of these children were surveyed three times throughout the study but some were only present for one or two of the three surveys. The three separate surveys will be referred to as 'baseline', 'midterm' and 'final' (Table 5.20, p.139). Chapters 6 and 7 will examine the longitudinal health data obtained from the three surveys. This section will describe the child health and growth status from the baseline survey only.

5.4.1. Child health status

The health status of the children was determined in three ways: firstly by asking mothers to report the health of the child up to two weeks prior to the survey in terms of the presence of blocked and runny nose, coughs, fever, asthma and diarrhoea. Fieldworkers asked mothers about some specific health symptoms related to respiratory tract infections such as breathing difficulties and fast breathing, wheezing as well as lower chest in-drawing (Table 5.21, p.139). All children who were reported as having suffered from any of the symptoms described above (apart from a blocked/runny nose) were asked to undergo a general medical examination which aimed at diagnosing the presence of respiratory infections. During this examination the respiratory rate and the body temperature were measured, and the respiratory health was examined by chest auscultation. The third health assessment was via analysis of biochemical indicators of immune status (c - reactive protein, α-1-antiglycoprotein and albumin) from finger-prick blood samples.

Data on the child health was therefore obtained at three different levels:

        a) General health status, reported by the mother

        b) Medical examination of those children who had had symptoms of illness two weeks prior to the survey

        c) Immune status through the use of selected biomarkers of immunity.

In the baseline survey the health status was obtained for n=473 children under the age of five years via maternal interviews (Table 5.20, p.139). Over half of the children (n=263, 55.6%) were seen by a medical practitioner on the basis of the health status report provided by their mother (these children had suffered from an illness up to two weeks prior to the survey), and 44.4% of the children were well and healthy according to the mother. 38.8% of the medical examinations (102 cases of n=263) reported a respiratory tract infection, which was 21.6% of the total sample size.

5.4.1.1. Health status report through maternal interviews

The most frequently reported health symptoms among the children were a runny nose, cough and fever (Table 5.21, p.139). Over a quarter of the children had

suffered from a cough and over a third had experienced a raised temperature, according to their mother. About 10 percent of children were reported as having wheezing and whistling of breath. Only 7.0% of the children had had or still had diarrhoea in the baseline survey.

5.3% of the children were reported as having a cough with wheeze and an additional 2.1% had a cough with fast breathing. Fever and cough were reported in almost a quarter of the cases. Fever with wheeze or fever with cough and wheeze were reported for almost a fifth of the children. No children were reported as having a cough as well as lower chest in-drawing in this sample. 4.7% of the children had diarrhoea with fever (which accounts for almost seventy percent of all cases of diarrhoea reported). Some children with diarrhoea also had a cough (2.5% of n=473). Table 5.22 (p.140) lists all the combinations of illnesses related to cough and fever, as they are the most relevant to this study.

5.4.1.2. Medical diagnosis

Over half (55.6% of n=473) of the children under the age of five identified in the study households were referred to a doctor for a medical diagnosis (on the basis of their health status reported by their mother). 44.9% of the 263 children who had a medical diagnosis had a raised temperature (>37.0°C) as measured by the doctor. This represents 24.8% of the total 473 children. The breathing rate was assessed by the doctor by counting the number of breaths per one minute. The age-specific breathing rate was calculated as described in Chapter 3 (section 3.3.4.2, p.56). Only five children were diagnosed with fast breathing rates and only one was less than one year of age. 12.2% of the 263 children who had been referred to a doctor were diagnosed with chest in-drawing. 16.0% of the children seen by the medical doctors were receiving some form of medication at the time of the survey. Chest auscultation was used by doctors to define the severity of a respiratory infection (normal, mild, moderate or severe). Moderate and severe respiratory infection was analysed with having chest sounds (rattling of phlegm in the bronchi and bronchioles) and diminished breathing sounds. 21.6% of all children (n=473) were diagnosed with moderate or severe respiratory tract infection during the medical check-up in the baseline survey (Table 5.20, p.139).

5.4.2. Anaemia prevalence and the immune status among children

The levels of selected biomarkers of immunity and the prevalence of anaemia among children were measured from finger-prick blood samples collected in the field. The precise methods applied in the blood collection and laboratory analyses are provided in Chapter 3. Anaemia was measured using a quick haemoglobin test in the field. The immune status was measured using albumin (a negative acute-phase protein) and c-reactive protein (or CRP) and alpha-1-acidglycoprotein (or AGP) (positive acute-phase proteins). The levels of negative acute phase proteins such as albumin become depleted as a result of an immune response or dietary deprivation. Elevated levels of the two positive acute phase proteins were associated with the presence of an infection or an immune response. The mean haemoglobin measured among the children was $105.3 \pm 13.1g/L$ (n=414 excluding one outlier<70g/L). 70g/L was used as an exclusion cut-off in order to obtain a normally distributed data – only one sample below 70g/L was identified. 63.7% of the children for whom haemoglobin data was available (n= 421, Table 5.20, p.139) were classified as anaemic using the World Health Organisation's cut-off point of 110g/L for children aged 6 to 59 months **(WHO, 1992b)**. The haemoglobin status was considerable worse in Saidpur than Parpatipur (Saidpur Hb=$103.4g/L \pm 13.5SD$, n=227, and Parpatipur Hb= 107.6 ± 12.2, n=187, t=-3.313, p<0.005). Univariate analysis (controlling for age) showed no sex differences in the haemoglobin levels (male: $106.0g/L \pm 13.9SD$ (n=211), female: $104.4g/L \pm 12.1SD$ (n=201), F=1.337, p=NS).

Albumin depletion (93.0%) was highly prevalent (cut-off point: <35g/L) **(Spencer and Price, 1977)** but no municipal differences in the prevalence of albumin depletion was found (X^2= 0.509, p=NS, n=400).16.5% of the children had elevated CRP levels indicating the presence of an active infection at the time of the survey (CRP >5mg/L = infection). The prevalence of elevated CRP levels among young children was significantly higher in Parpatipur (59.1%) than in Saidpur (40.9%) (X^2= 6.710, p<0.05, n=399). Elevated alpha-1-acidglycoprotein (AGP) levels were detected in 29.0% of the children (0.8g/L as a cut-off). No differences in the prevalence of AGP elevation among children was found between the municipalities (X^2= 0.104, p=NS, n=397).

5.4.3. Growth status

The anthropometric z-scores (height-for-age or HAZ, weight-for-age or WAZ, and weight-for-height or WHZ) were calculated using measures of height and weight using the Centers for Disease Control and Prevention (CDC) 2000 reference growth charts (National Center for Health Statistics, US). Growth z-scores outside the range of -7 and above +2 standard deviations (for height-for-age and weight-for-age) and -6 and above +2 standard deviations (weight-for-height) were considered as abnormal and were excluded from the analysis. The determined data ranges were to maximise the sample size and to maintain normal data distribution. The total numbers of children from the baseline survey included in the growth z-score analysis are shown in Table 5.23, p.141).

The mean height-for-age z-score for the children in this Bangladeshi sample was -1.805±1.256SD (Table 5.23, p.141), and 43.8% of the children were stunted. The mean weight-for-age (WAZ) was -2.456±1.704SD and 66.4% of the children were underweight. The mean weight-for-height z-score was very low:-1.653±1.239SD. Approximately 38.4% of children were wasted. Table 5.24, p.142 shows a breakdown of the nutritional indices and the combinations of indices. Only 19 children were stunted only (HAZ<-2SD). 31 children fell into the category of a low weight-for-age z-score (<2SD) with normal height-for-age and weight-for-height z-scores (>-2SD). 58 children were categorised as having a poor growth due to low height-for-age, weight-for-age as well as weight-for-height (Table 5.24, p.142).

Municipal differences in the severity of poor growth were detected. Children from Saidpur had lower height-for-age, weight-for-age and weight-for-height (t=-3.818, p<0.001, t=-3.932, p<0.01, and t=-2.619, p<0.01 respectively) (Table 5.23, p.141). Child growth faltering was not strongly associated with sex in this sample: only the weight-for-height z-score was significantly poorer among females than in the male children (WHZ for female: -1.793±1.793, and male: -1.523±1.124, t=2.250, p<0.01). Only height-for-for age was significantly associated with child age (R=0.150, F=16.394, p<0.001,B for age: 0.015, t=-4.049) and indicated a poorer height-for-age status among older children in this study sample.

The data indicated poor growth and nutritional status of children under the age of five in this study sample. Chapter 6 will describe factors which may explain the patterns observed in the child growth and nutritional status with a particular attention to household fuel pollution and the prevalence of respiratory infections.

5.5. Summary

This chapter examined general socio-demographic characteristics such as socio-economic, educational and occupational diversity of the households across this Bangladeshi study sample. Household cooking- and fuel use practices were also described.

The study sample included people of Bangla and Bihari ethnicity. Household members were poorly educated and cooked on basic chimneyless mud stoves. Cooking was commonly done indoors, in a separate kitchen building, outdoors or on a veranda. An examination of household occupations revealed a hawker or petty trader as the most common occupation in Parpatipur, whereas in Saidpur most primary household earners worked as skilled day labourers.

Low SES household mothers and heads were less educated than those from a high SES class. Household mothers were significantly more educated in Parpatipur than in Saidpur. Households classed as 'low' for their average monthly income were more common in Saidpur than in Parpatipur. Forward likelihood ratio analysis showed that the average income (grouped as low, medium, high) was best predicted by SES status and ethnicity: Bangla families of high SES were most likely to have the highest average income. The poorest households were low SES Bihari families.

Households commonly cooked once a day in Parpatipur whereas in Saidpur cooking was done more than once a day (twice or three times). Overall, wood was the most commonly used fuel. Regression analysis showed that a portable stove was a significant predictor of outdoor cooking and the use of golden as a fuel for cooking (Wald: 17.870, $p < 0.01$). Ventilation was not common in this community. Indoor cooking was very common among the households of Bihari ethnicity.

A high prevalence of respiratory infections, poor immunity (using selected biomarkers of immunity) and growth retardation were observed among the children. No gender or municipal differences of the levels of haemoglobin and the selected biomarkers of immunity were observed at the baseline survey. C-reactive protein levels were more elevated in Parpatipur indicating poorer health. The following chapters will describe the longitudinal child health and its predictors as well as aim to identify the predictors of household fuel pollution in households with different building structures and behaviours.

Tables and figures

Figure 5.1: Views of semi-urbanised Saidpur
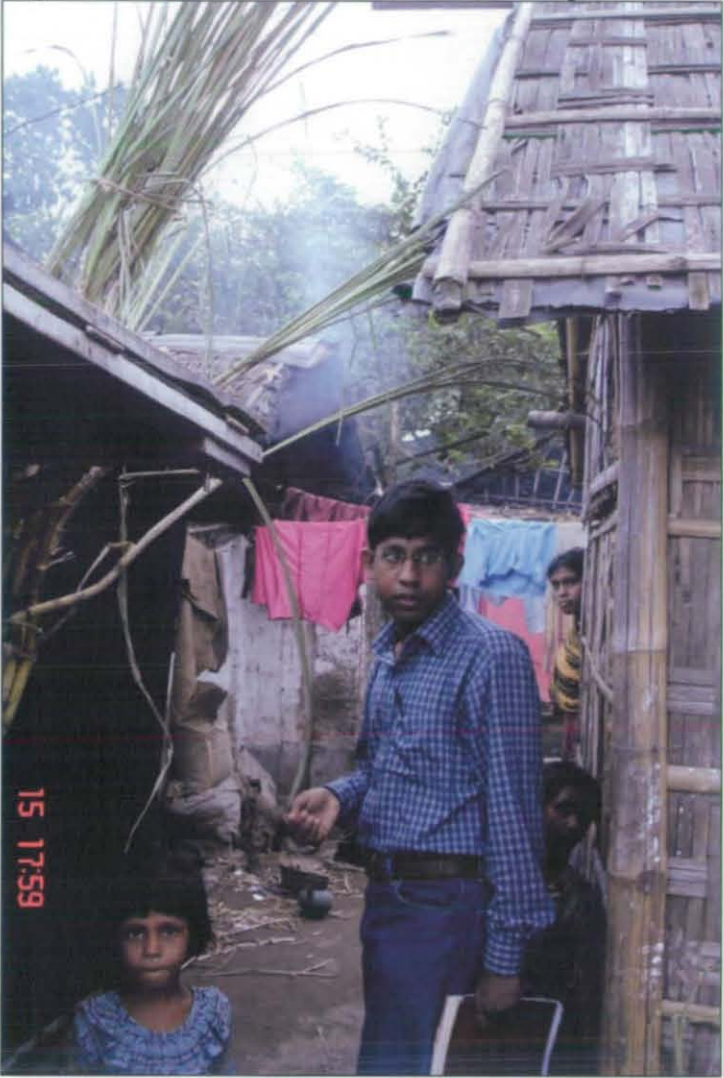
Figure 5.2: Views of rural Parpatipur

Table 5.1: Number of children within each household, for those households with at least one child under the age of 5 years within Saidpur, Parpatipur and the total sample

Sample	1 child	2 children	3 children	4 or more children
Saidpur	100.0% (n=230)	38.3% (n=88)	3.0% (n=7)	1.7% (n=4)
Parpatipur	100.0% (n=178)	30.3% (n=54)	3.9% (n=7)	0% (n=0)
Total	100.0% (n=408)	34.8% (n=142)	3.4% (n=14)	1.1% (n=4)

Table 5.2: Study sample socio-demographic descriptives by municipality

Grouping	Sub-grouping	Saidpur (n=230)	Parpatipur (n=178)	Statistics
Occupation	Hawker/petty trader	16.5(38)	39.3 (70)	NA
	Skilled day labourer	31.3 (72)	18.0 (32)	
Average income	Low <TK2666	42.6 (98)	28.1 (50)	X^2=10.747, p<0.05, n=408
	Medium TK2666<x<TK3666	29.6 (68)	31.5 (56)	
	High>TK 3666	27.8 (64)	40.4 (72)	
SES	Low	54.8 (126)	68.0 (121)	X^2= 7.313, p<0.05, n=408).
	High	45.2 (104)	32.0 (57)	
Ethnicity	Bangla	27.3 (63)	97.3 (173)	NA
	Bihari	72.7 (167)	2.7 (5)	
Education (household head)	Uneducated	59.1 (136)	41.6 (96)	X^2= 12.384 p<0.05, n=408).
	Educated	40.9 (94)	58.4 (104)	
Education (mother)	Uneducated	57.4 (132)	44.9 (80)	X^2= 6.228, p<0.05, n=408).
	Educated	42.6 (98)	55.1 (98)	
Literacy (mothers)	Illiterate	32.1 (62)	27.7 (39)	X^2= 0.770, p=NS, n=334).
	Literate	67.9 (131)	72.7 (102)	

Table 5.3: Study sample cooking and fuel-use practices by municipality

Grouping	Sub-grouping	Saidpur % (n=230)	Parpatipur (n=178)	Statistics
	Once	21.7 (50)	65.2 (116)	
	Twice	48.7 (112)	24.7 (44)	
Cooking frequency	Three times	29.6 (68)	10.1 (18)	(X^2= 79.618, p<0.001 n=408).
	Wood	49.6 (114)	40.4 (72)	
	Golden	31.7 (73)	0 (0)	
	Plant-derived fuels	23.9 (16)	32 (57)	X^2=47.865, p<0.001, n=332,
Fuel type	dung	2.2 (5)	27 (48)	excluding *golden* and others
	Saidpur only (n=230)	**Bangla**	**Bihari**	
	Wood	56.7 (38)	48.7 (77)	X^2=9.339, p<0.01, n=223,
	Golden	19.4 (60)	38.5 (13)	excluding dung
Fuel type	Plant-derived fuels	23.9 (16)	12.8 (20)	
	Portable	48.3 (111)	45.5 (81)	
	Fixed	51.7 (119)	53.9 (96)	X^2=0.250, p=NS, n=407,
	Fixed with chimney	0	0	Excluding biomass stoves with chimney and non-biomass
Stove choice	Other (no biomass or other)	0	0.6 (1)	stoves
	Indoors	31.3 (72)	6.7 (12)	
	Separate kitchen building	17.0 (39)	10.1 (18)	
	Open air	37.8 (87)	58.4 (104)	
Cooking location	Veranda with 2 low walls	13.9 (32)	24.7 (44)	X^2=48.157, p<0.001
	Indoors	62.2 (143)	41.6 (74)	
Cooking location	Outdoors	37.8 (87)	58.4 (104)	X^2 = 17.104, p<0.001, n=408

Table 5.4: Average income and sample parameters

Variable	Location	Average income groups (% of households)			Statistics	
		Low <TK2400 %	Medium TK2400<X<3600 %	High >3600 %	X^2	p-value
Municipality	Saidpur	42.6	29.6	27.8	10.747	0.001
	Parpatipur	28.1	31.5	40.4		
SES status	High	21.7	26.1	52.2	45.432	0.001
	Low	45.7	33.2	21.1		
Ethnicity	Bangla	31.8	30.2	38.0	7.180	0.05
	Bihari	42.8	30.7	26.5		
Education (household head)	No	42.9	28.1	29.0	8.305	0.05
	Some	29.3	32.8	37.9		
Education (Respondent)	No	43.4	30.7	25.9	13.410	0.05
	Some	28.6	30.1	41.3		
Literacy	Illiterate	31.7	34.7	33.7		NS
	Literate	41.6	29.6	28.8	2.941	

Table 5.5: Sub-groups of variables included in the forward LR regression analysis of the predictors binary average monthly income

Education maternal	%	Ethnicity	%	Municipality	%	SES	%
No	52.1 (212)	Bangla	59.5 (242)	Saidpur	56.5 (230)	Low (SES1-3)	60.7 (247)
Yes	47.9 (196)	Bihar	40.5(166)	Parpatipur	43.5 (178)	High (SES4-5)	39.3 (161)

Table 5.6: Binary logistic regression with forward LR model of the predictors of average incomes

Model	Variable	Category	B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I	
									Lower	Upper
1	SES	Low (reference)						1.000		
		High	1.404	0.215	42.475	1	0.001	4.072	2.669	6.211
	Constant		-0.988	0.143	47.687	1	0.001	0.372		
2	SES	Low (reference)						1.000		
		High	1.750	0.244	51.493	1	0.001	5.752	3.567	9.276
	Ethnicity	Bangla (reference)								
		Bihar	-1.064	0.249	18.278	1	0.0001	0.345	0.212	0.562
	Constant		-0.714	0.154	21.440	1	0.001	0.490		

Figure 5.3: Cooking frequency in municipalities (X^2=79.618, p<0.001)
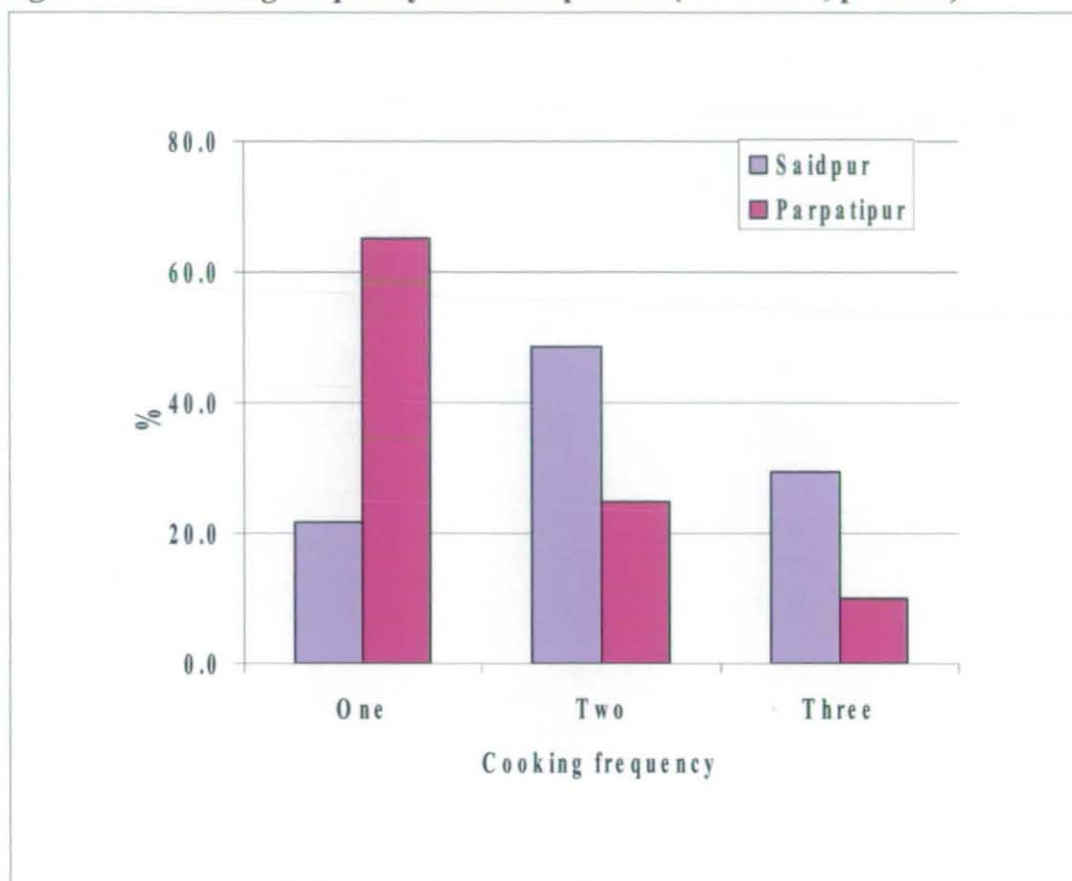
Table 5.7: Number of dishes cooked daily

Number of dishes	Breakfast % (n)	Lunch % (n)	Dinner % (n)
None	44.6 (183)	9.8 (40)	65.4 (267)
One	4.9 (20)	0.5 (2)	12.0 (49)
Two	39.2 (160)	27.9 (114)	17.9 (73)
Three or more	11.0 (45)	65.8 (252)	4.7 (19)
Total	100 (408)	100 (408)	100 (408)

Table 5.8: Municipal differences in the number of dishes cooked daily

n	Municipality					
	Saidpur			Parpatipur		
	Breakfast % (n)	Lunch % (n)	Dinner % (n)	Breakfast % (n)	Lunch % (n)	Dinner % (n)
None	25.7 (59)	13.5 (31)	53.0 (122)	69.7 (124)	5.1 (9)	81.5 (145)
One	5.2 (12)	0.4 (1)	14.3 (33)	4.5 (8)	0.6 (1)	9.0 (16)
Two	55.2 (127)	26.5 (61)	24.8 (57)	18.5 (33)	29.6 (53)	9.0 (16)
Three or more	13.9 (32)	59.5 (137)	7.8 (18)	7.4 (12)	64.7 (115)	0.6 (1)

Table 5.9: Duration of cooking at meal times

Duration of cooking	Breakfast % (n)	Lunch % (n)	Dinner % (n)
None	44.6 (182)	9.8 (40)	65.4 (267)
<1 Hour	25.7 (105)	2.7 (11)	15.4 (63)
1 -2 Hours	28.4 (116)	69.2 (283)	17.4 (71)
3-5 Hours	1.2 (5)	17.9 (73)	1.7 (7)
>5 Hours	N/A	0.2 (1)	N/A
Total	100 (408)	100 (408)	100 (408)

Figure 5.4: Fuel use and SES (X^2=12.747, p<0.001, n=405 excl. others)
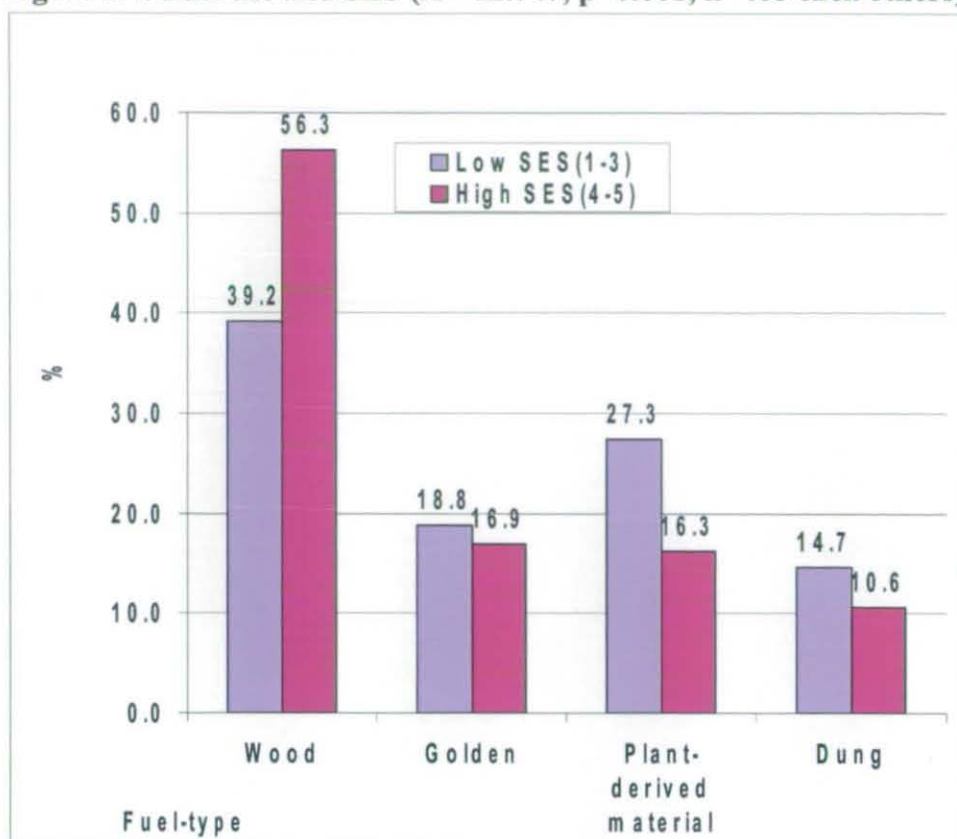
Figure 5.5: Fuel use in Saidpur and Parpatipur
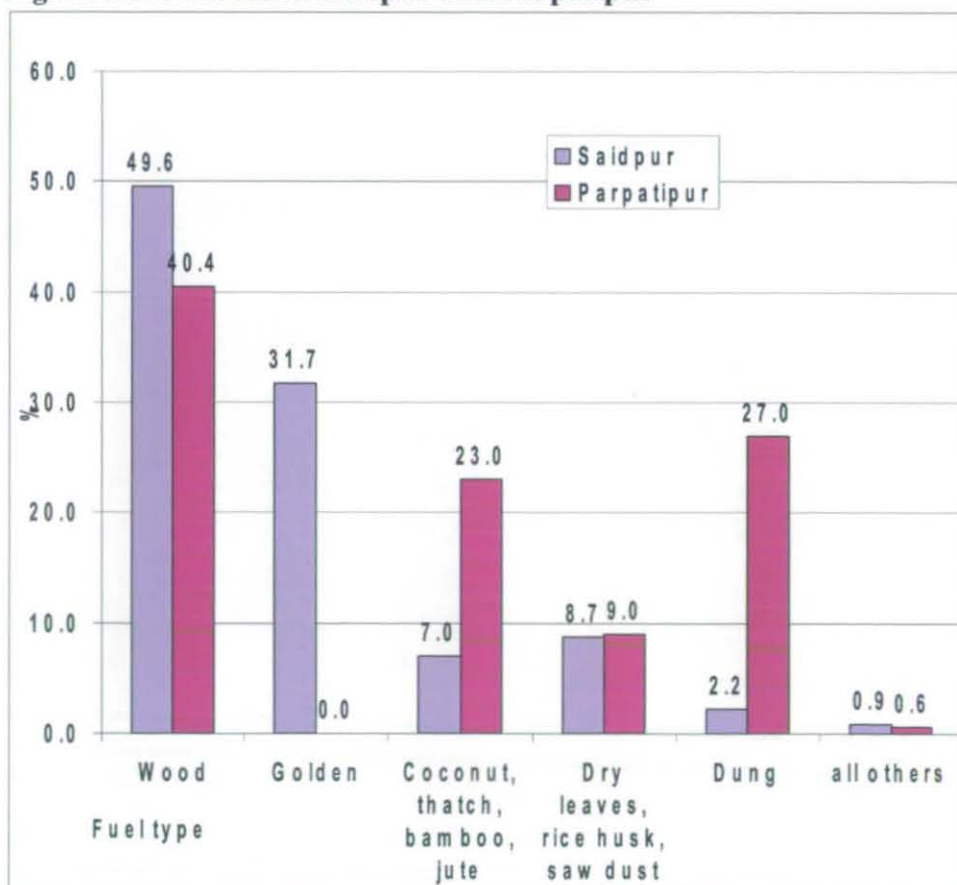
Table 5.10: Fuel type and ethnicity (X^2=9.339, p<0.01, n=223)

Ethnicity	Wood % (n)	Golden % (n)	Plant-derived fuel % (n)
Bangla	56.7 (38)	19.4 (13)	23.9 (16)
Bihari	48.7 (76)	38.5 (60)	12.8 (20)

Table 5.11: Description of stove types

Stove type	% (n)
Portable (biomass)	47.1 (192)
Fixed (biomass)	52.7 (215)
Fixed with chimney (biomass)	0
Other (non-biomass or other)	0.2 (1)

Figure5. 6: Portable and fixed chimneyless biomass stoves
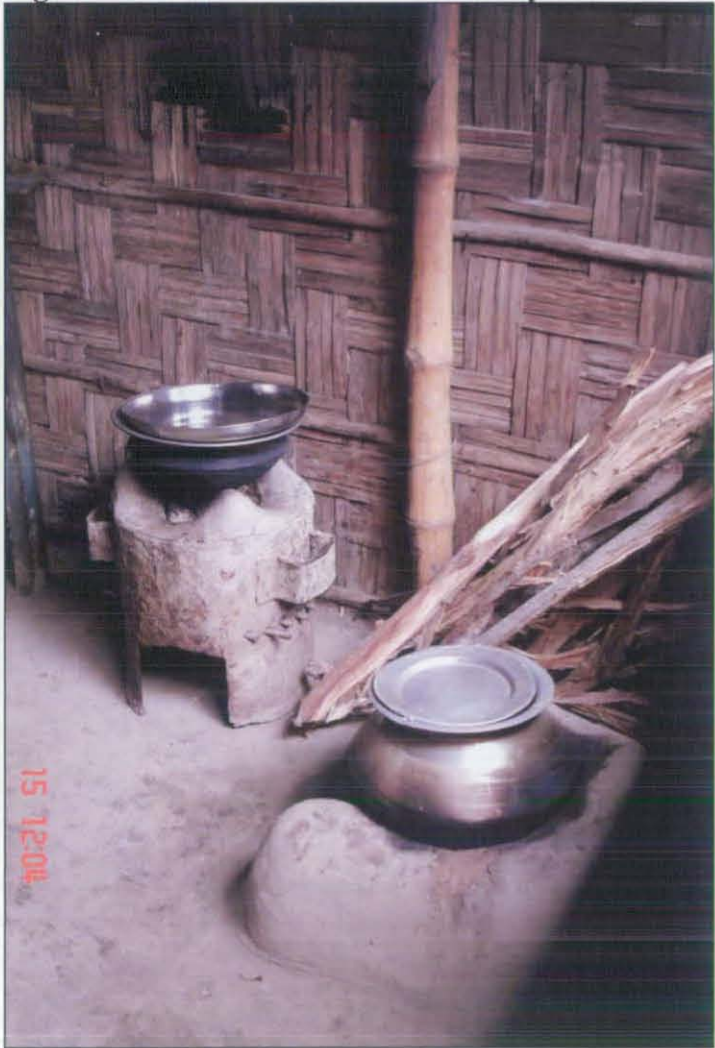
Figure 5.7: Stove choice within high and low SES (X^2=11.415, p<0.001, n=405)
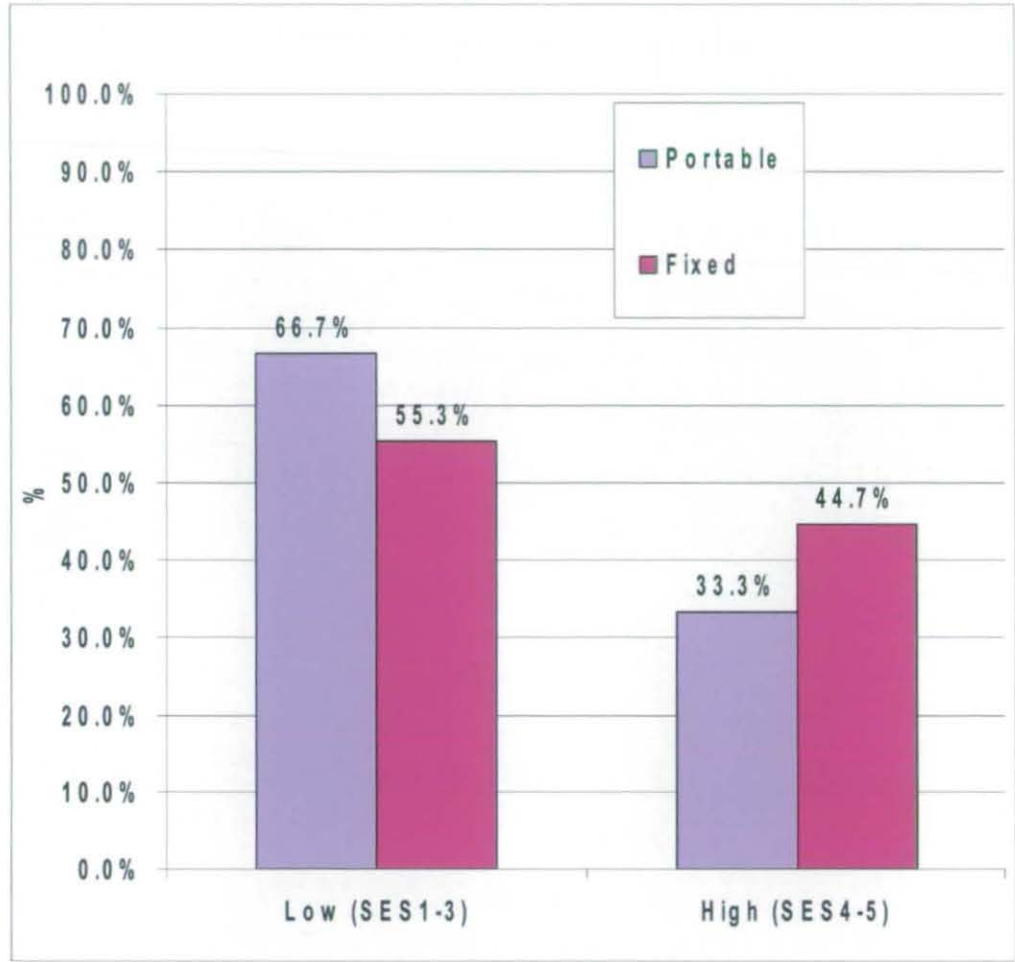
Figure 5.8: Stove type and fuel choice ($X^2=15.534$, p<0.005, n=407)
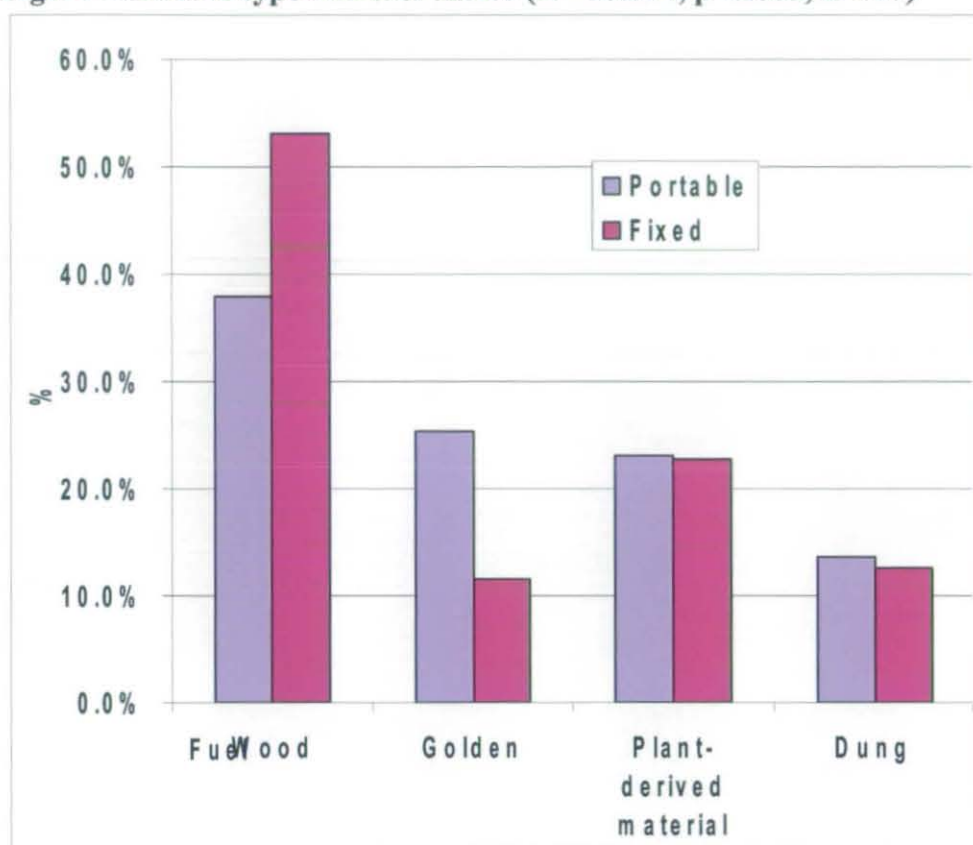
Table 5.12: Fuel choice and stove type in municipalities

Variable		Fuel type				Statistics	
Sample	Stove type	Wood % (n)	Golden % (n)	Plant-derived material % (n)	Dung % (n)	X^2	p-value
Saidpur	Portable	39.6(42)	45.3 (48)	15.1 (16)	-	15.080	0.001
	Fixed	61.5 (72)	21.4 (25)	17.1 (20)	-		
Parpatipur	Portable	37.0 (30)	34.6 (28)	-	28.4 (23)	0.836	NS
	Fixed	43.8 (42)	30.2 (29)	-	26.0 (25)		
Total	Portable	37.9 (72)	25.3 (48)	23.2 (44)	13.7 (26)	15.534	0.005
	Fixed	53.0 (114)	11.6 (25)	22.8 (49)	12.6 (27)		

Figure 5.9: Cooking location in high and low SES groups (X^2=18.883, p<0.01, n=408)
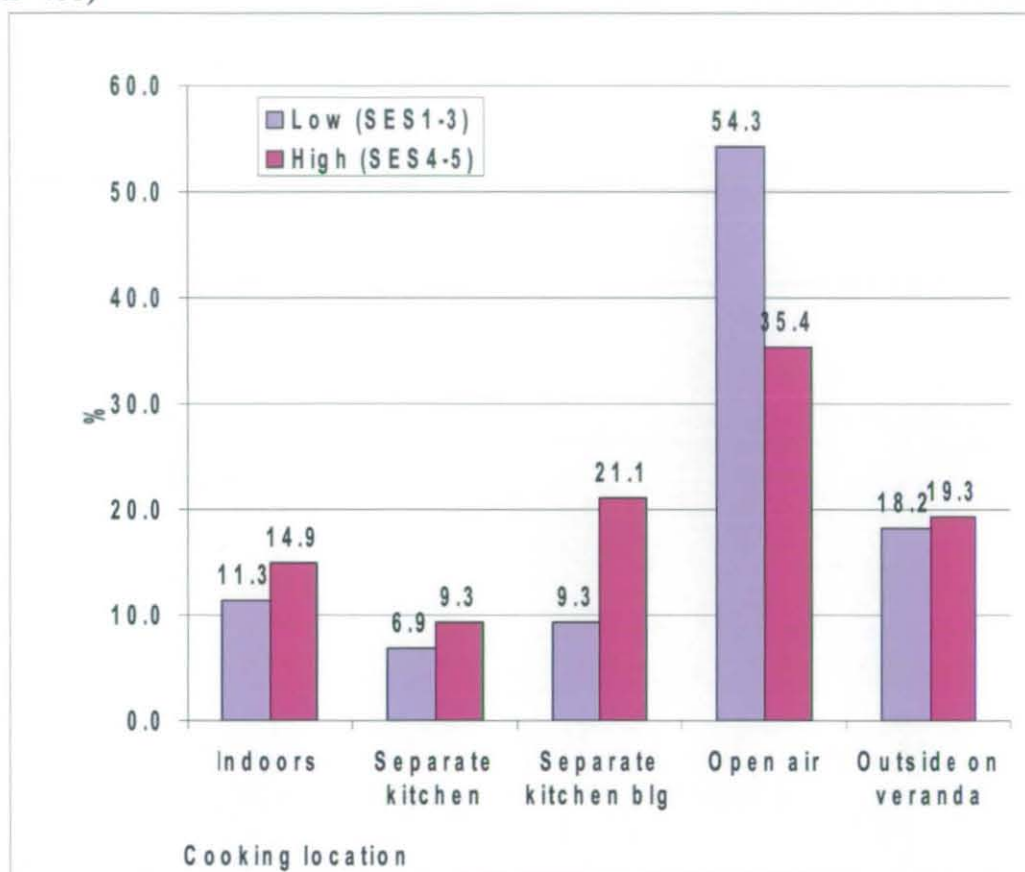
Table 5.13: Education and cooking location in low SES (1-3) and high SES (4-5)

	Educated % (n=198)		Uneducated % (n=210)	
Cooking location	Low SES	High SES	Low SES	High SES
Indoors	45.7 (48)	63.4 (59)	45.8 (65)	66.2 (45)
Outdoors	54.3 (57)	36.6 (34)	54.2 (77)	33.8 (23)
Statistics	X^2=6.240, p<0.05		X^2=7.673, p<0.001	

Table 5.14: Cooking location and stove type (X^2=11.419, p<0.01, n=407)

	Stove	
Cooking location	Portable % (n)	Fixed % (n)
Indoors	38.1 (32)	61.9 (52)
Separate kitchen building	39.3 (22)	60.7 (34)
Open air	56.0 (107)	44.0 (84)
Veranda -2walls	40.8 (31)	59.2 (45)

Table 5.15: Cooking location and stove type with municipalities

Municipality	Stove	Cooking location		Total	Statistics
		Indoors % (n)	Outdoors % (n)	% (n)	
Saidpur	Portable	25.2 (58)	23.0 (53)	48.2% (n=111)	X^2= 8.890, p<0.005
	Fixed	37.0 (85)	14.0 (34)	51.7% (n=119)	
Parpatipur	Portable	15.3 (27)	30.5 (54)	45.8% (n=81)	X^2= 3.856, p=0.05
	Fixed	26.0 (46)	28.6 (50)	54.8% (n=96)	

Table 5.16: Sub-group sizes of variables included in the binary logistic regression of stove choice in the sample (excluding dung and plant-derived fuels)

Fuel	% (n)	Cooking location	% (n)	Municipality	% (n)	SES	% (n)
Wood	45.9 (186)	Indoors (incl. veranda)	53.1 (215)	Saidpur	56.3 (228)	Low	60.5 (245)
Golden	18.0 (73)	Outdoors	46.9 (190)	Parpatipur	43.1 (177)	High	39.5 (160)

Table 5.17: Binary logistic regression of the predictors of stove type

Model	Variable	Category	B	S.E.	Wald	df	p-value	Exp(B)	95% C.I. Lower	Upper
1	Cooking location	Indoors (reference)						1.000		
		Outdoors	-0.677	0.202	11.212	1	0.001	0.508	0.342	0.755
	Constant		0.444	0.140	10.107	1	0.001	1.560		
2	Cooking location	Indoors (reference)						1.000		
		Outdoors	-0.700	0.210	11.137	1	0.001	0.497	0.329	0.749
	Fuel type	Reference: wood			14.864	3	0.002	1.000		
		Golden	-1.125	0.293	14.730	1	0.001	0.325	0.183	0.577
		Plant-derived	-0.216	.263	0.677	1	NS	0.805	0.481	1.349
		Dung	-0.328	.319	1.054	1	NS	0.721	0.386	1.347
	Constant		0.750	.177	17.870	1	0.001	2.117		

Table 5.18: Use of materials for household roofs, walls and flooring

Building material	Roof % (n)	Wall % (n)	Floor % (n)
Thatch	2.7 (11)	1.7 (7)	-
Wood	-	0.2 (1)	-
Bamboo	5.4 (22)	49.5 (202)	-
Corrugated iron	84.1 (343)	13.7 (56)	1.2 (5)
Bricks	7.6 (31)	23.3 (95)	20.6 (84)
Clay	-	11.5 (47)	78.2 (319)
Other	0.2 (1)	-	-
Total		408 households	

Table 5.19: Household ventilation methods

Quantity	Holes/Cracks % (n)	Windows % (n)	Doors % (n)
No ventilation	4.7 (19)	50.0 (204)	14.5 (59)
Some	49.4 (202)	4.9 (20)	40.4 (165)
Other	0.7 (3)	-	-
N/A	45.1 (184)	45.1 (184)	45.1 (184)
Total	408 households		

Table 5.20: Number of children included in the three surveys without excluding outliers.

Survey	Date of birth	Maternally reported health % (n)	Anthropometry % (n)	Haemoglobin % (n)	Blood sample % (n)	Medical diagnosis % (n)	RTI % (n)
Baseline	473	100 (473)	94.1 (445)	89.0 (421)	89.0 (421)	55.6 (263)	21.6 (102)
Midterm	440	100 (422)	95.9 (422)	88.2 (388)	88.2 (388)	53.4 (226)	5.9 (25)
Final	458	100 (458)	88.9 (407)	(84.5 (387)	84.7 (388)	56.6 (259)	16.2 (74)

Table 5.21: Maternally reported health symptoms in children up to two weeks prior to the survey

Symptom	% of total (n=473)
Runny nose	24.1 (114)
Sore throat	1.7 (8)
Ear discharge	1.3 (6)
Fever	37.6 (178)
Cough	25.6 (121)
Asthma	2.1 (10)
Wheezing/Whistling of breath	9.1 (43)
Fast breathing	3.6 (17)
Unable to breastfeed	1.5 (7)
Lower chest in drawing	2.7 (13)
Diarrhoea	7.0 (33)

Table 5.22: Maternally reported health symptom combinations (baseline survey)

Symptoms (or combination of symptoms)	% of total (n=473)
Cough and Fever	23.3 (110)
Cough and asthma	1.1 (5)
Cough and fast breathing	2.1 (10)
Cough and wheezing	5.3 (25)
Cough and ear discharge	0.8 (4)
Cough and diarrhoea	2.5 (14)
Cough and fever with ear discharge	0.6 (3)
Cough and fever with wheezing	4.7 (22)
Cough and fever with fast breathing	2.1 (10)
Cough and fever with asthma	1.1 (5)
Cough and fever with diarrhoea	2.7 (13)
Cough and lower chest in-drawing	0 (0)
Fever and diarrhoea	4.7 (22)
Fever with diarrhoea and fast breathing	1.1 (5)

Table 5.23: Growth status of children during the baseline survey

Growth z-score	Mean ± SD (n)	Municipality			Sex			Age	
		Saidpur	Parpatipur	t-test	Male	Female	t-test	Mean±SD (n)	Univariate
Height-for-age (HAZ)	-1.805±1.256 (438)	-2.012±1.264 (239)	-1.558±1.204 (199)	t=-3.818, p<0.001	-1.877±1.232 (223)	-1.732±1.279 (215)	t=-1.207, p=NS	-1.806±1.256 (438)	F=16.394, p<0.01
Weight-for-age (WAZ)	-2.456±1.704 (437)	-2.690±1.417 (240)	-2.173±1.296 (195)	t=-3.932, p<0.001	-2.332±1.181 (222)	-2.591±1.566 (213)	t=-1.953, p=NS	-2.456±1.704 (437)	F=0.001, p=NS
Weight-for-height (WHZ)	-1.653±1.239 (424)	-1.795±1.234 (232)	-1.481±1.226 (195)	t=-2.619 p<0.001	-1.523±1.124 (221)	-1.793±1.340 (203)	t=2.250, p<0.05	-1.653±1.239 (424)	F=0.004, p=NS

Table 5.24: Relationship between poor (<-2SD) z-scores among children under the age of five years.

Low z-score	n
HAZ only	19
WAZ only	31
WHZ only	16
HAZ*WAZ only	88
HAZ*WHZ only	0
WAZ*WHZ only	75
HAZ*WAZ*WHZ	58

Chapter 6: Child health and immunity.

6.1. Introduction

Chapter 5 described housing, socio-demographic characteristics as well as common cooking and fuel practices recorded for this Bangladeshi study sample. The baseline data indicated a high prevalence of poor growth and immunity (using c-reactive protein, albumin and alpha-1-acidglycoprotein as biochemical indicators) among children under the age of five years. Abundant cases of moderate or severe respiratory infections and anaemia were reported (21.6%).

Chapter 6 examines any trends in child growth and immunity using longitudinal data from the three field surveys (baseline, midterm and final) for height-for-age, weight-for-age and weight-for-height z-scores, haemoglobin, albumin, alpha-1-acidglycoprotein and c-reactive protein. This chapter examines the role of household fuel pollution as a potential cause of respiratory infections and ill-health in this community using data from maternally reported child health, medical diagnoses of respiratory tract infections and the levels of biomarkers of immunity (as listed above). The effect of cooking and fuel use practices on child growth and immune status as well as the prevalence of respiratory infections are examined.

This chapter tests the three hypotheses as listed below:
1.   Child growth over 18 months will be associated with cooking and fuel use practices in this Bangladeshi sample
2.   Child immune status measured using biomarkers of immunity over 18 months will be associated with cooking and fuel use practices in this Bangladeshi sample
3.   The presence or absence of respiratory tract infections over the 18 months will be associated with cooking and fuel-use practices in this Bangladeshi sample.

The hypotheses were tested using general linear model (or GLM) repeated measures analysis to identify single determinants of child growth (height-for-age, weight-for-

age and weight-for-height z-scores, the change scores for the growth z-scores listed), child immunity (haemoglobin and the selected biomarkers of immunity) and the presence of respiratory infections. The GLM multivariate analysis and regression were used to identify a combination of the determinants of each item hypothesised.

6.2. Child health and immunity across the three field surveys

Child health data was collected across the 18-month field study three times: during the baseline, mid-term, and the final surveys. Table 5.20 (p.139) in Chapter 5 listed the numbers of children from whom different types of data were collected during each survey.

Child health status reports by mothers were obtained (baseline n=473, midterm n=440 and final n=458). The baseline and the final surveys targeted all n=625 households (with or without children under the age of five). The mid-term survey targeted only those households where children under the age of five were previously identified. A lower sample size for the mid-term survey was due to emigration, voluntary withdrawal from the study or incorrect identification of a child or children. A larger sample size at the final survey was due to an inclusion of all new born children. The analyses of this chapter only included children present in all three field surveys (n=321). Only the youngest child from each household (n=213) was included in the analyses in order to avoid biased results due to a household effect as a result of an inclusion of more than one child from the same household. All analyses included age as a covariate in order to exclude the age-effect on the results.

6.2.1. Child health reports by mothers

Over half of the children had experienced an illness up to two weeks prior to the survey according to their mother (baseline: 56.7%, n=157, midterm: 59.2%, n=129, final: 64.6%, n=137). Figure 6.1 (p.170) shows that fever and cough were the most commonly reported symptoms in children in all surveys. The prevalence of a cough during the midterm survey was approximately 10% lower (43.7%) than during the

baseline or final surveys (53.5% and 53.1% respectively). The prevalence of diarrhoea (11.7%, n=25) and the inability to breastfeed (21.6%, n=46) appeared much higher during the midterm than at the baseline or the final survey (diarrhoea baseline: 8.5% (n=18), final: 4.7%, (n=10) and inability to breastfeed baseline: 15.5% (n=33), final 13.6% (n=29)) which could reflect the timing of the midterm survey at the beginning of the monsoon season.

Table 6.1 (p.171) lists combinations of different reported illnesses experienced by children in this sample. A cough was commonly linked with fever in this sample (baseline: 23.5%, midterm: 28.2%, final: 28.6%). Ear discharge as an indicator of a potential ear infection (*otitis media*) was fairly uncommon in this sample and no cases were detected during the final survey. A cough was also linked with wheezing (with or without fever) and was experienced by approximately twice as many children as cough with diarrhoea throughout the field work. The prevalence of a cough with diarrhoea appeared to decrease throughout the fieldwork.

The data collection trips did not follow strictly the seasonal changes in Bangladesh. Bangladesh has three main seasons; dry season between October and March, the rainy monsoon season between July and September as well as hot and humid pre-monsoon period from April to June. The first sample collection trip took place in early October in 2005 which was fairly rainy as the monsoon rains had not ceased by the start of the fieldwork. The second fieldtrip was aimed to coincide with monsoon rains in late June 2006, however the monsoon was late that year. Even though some heavy rains were experienced with high humidity the season could be not classified as the rainy season. The last survey took place in late February 2007 when the weather was typical of dry season conditions in Bangladesh with the lack of rains and lower temperature and humidity. Due to the inconsistent weather patterns experienced during the fieldwork this data cannot unfortunately be used strictly to study seasonal effects on child health and growth due to household fuel pollution.

6.2.2. Medical diagnoses of children

Table 6.2 illustrates the percentage of children (taking the youngest only from each household) who did not require medical diagnosis (healthy), those who were seen by a doctor and were diagnosed as unwell with an illness other than respiratory infection, and finally those unwell with a respiratory tract infection (RTI). During the baseline survey 62.4% of the children were referred for medical diagnosis (respiratory tract infection or other illness) (Table 6.2, p.172). The percentage of children seen by a doctor during the mid-term and the final survey were similar (60.3% and 61.4%). The prevalence of respiratory infections was significantly higher during the baseline than the mid-term survey (24.8% versus 7.4%) (X^2=38.162, p<0.001) but was not significantly different between the baseline and the final surveys (24.8% and 17.3% respectively) (X^2=2.720, p=NS).

Other illnesses diagnosed by medical doctors included health problems such as diarrhoea, scabies, skin infections, ascariasis (as reported by mothers on the basis of earlier medical diagnosis). However minor colds, upper respiratory tract infections and coughs contributed about 85% of all ill-health children in this group. A significant increase in the prevalence of other illnesses (no RTI) was observed between the baseline (37.6%) and mid-term (53.0%) surveys (X^2=10.447, p<0.005) but not between the baseline and final surveys (44.1%) (X^2=3.763, p=NS).

6.2.3. Trends in child immune status across the field surveys

At baseline, 54.7% of all children were anaemic with mean haemoglobin levels of 105.6 ± 12.8g/L, n=179 (using 110g/L as a cut-off (**WHO, 1992b**)). No significant change in haemoglobin was detected throughout the fieldwork using repeated measures ANOVA (F=0.611, p=NS) when age was included as a covariate (Table 6.3, p.173). When age was excluded a significant improvement of haemoglobin was detected between the baseline and the final surveys but not between the midterm and the final surveys (baseline vs. final p<0.01). The mean $\log_{10}$-α-1-acidglycoprotein (AGP) levels for each of the surveys were as follows: baseline: -0.748 ± 0.180g/L;

mid-term:-0.855 ± 0.184g/L; final: -0.675 ± 0.157g/L (Table 6.3, p.173). No changes in the child infection status were detected using this biomarker of infection (F=0.701, p=NS) (age included as a covariate). An exclusion of the age covariate showed a significant change in AGP status among children during the fieldwork (F=8.484, p<0.01). A drop in the AGP levels between the baseline and the midterm surveys indicated an improvement in the immune status (baseline vs. midterm, p<0.01) but the AGP levels were again elevated by the final survey (midterm vs. final, p<0.01), (baseline vs. final, p<0.05). Albumin levels were measured as a negative acute-phase protein as an indicator of the children's immune status. Mean inversely transformed (1/x) albumin levels for the baseline were 0.041 ± 0.009 g/L, midterm: 0.050±0.009g/L and final survey: 0.035±0.008g/L (n=103) (Table 6.3, p.173). The albumin levels significantly improved throughout the fieldwork (F=4.042, p<0.01, baseline vs. final p<0.01) indicating an improved nutritional status of children by the end of the survey. The albumin levels however dropped during the midterm survey (baseline vs. midterm, p<0.01). C-reactive protein (CRP) was used to detect the presence of an infection at the time of the survey as a positive acute phase protein. The mean c-reactive protein levels were as follows: baseline: 0.547 ± 0.758g/L, midterm: 1.173 ± 2.041g/L, final: 0.348 ± 0.605g/L (Table 6.3, p.173). C-reactive protein levels became lower throughout the work indicating an improved child health status (Kendall's test X^2=21.900, p<0.001). An elevation in the CRP levels was observed during the midterm survey, indicating a higher infection status and poorer immunity of the children at the time of the midterm survey.

6.3. Longitudinal trends in child growth

Growth z-scores outside the range of -7 and above +2 standard deviations (for height-for-age and weight-for-age) and -5 and above +2 standard deviations (weight-for-height) were considered as abnormal and were excluded from the analysis. Child growth was examined using height and weight for children who took part in all three field surveys (for growth z-scores n=188). Only the youngest child was included.

Table 6.3 shows a significant decline in mean height-for-age z-scores from baseline to midterm surveys (-1.840 ±1.144 and -2.071±1.313 respectively, F=3.115, p<0.05 post-hoc). No significant change in the mean level of z-score was observed after the midterm survey (midterm vs. final mean: -2.071±1.313 versus -1.979±1.155, post-hoc: p=NS or baseline vs. final p=NS). Low weight-for-age z-scores were observed during all the surveys: baseline: -2.557±1.338SDg/L, midterm: -2.555±1.450SD and final: -2.269±1.244SD (Table 6.3, p.173). A significant improvement in the weight-for-age status from the baseline to final survey was seen and from midterm to final (F=8.500, p<0.01, post-hocs: baseline vs. final p<0.01, midterm vs. final p<0.01). The mean weight-for-height z-scores were as follows: baseline (-1.693±1.247SD), midterm (-1.690±1.484SD) and the final (-1.394±1.054SD) surveys (Table 6.3, p.173). Mean weight-for-height status increased significantly from the baseline to final (post-hoc; p<0.01) and from midterm to final (post-hoc p<0.01). Children who provided health and growth data during all three field surveys were not significantly different on the basis of their health and growth status (age, haemoglobin, log_{10}-transformed alpha-1-acidglycoprotein, inversely transformed albumin, c-reactive protein, height-for-age, weight-for-age or weight-for-height z-scores) from those children were only provided data on the baseline, midterm, final or any two of the field surveys (age was included as a covariate in the analysis) (p=NS) (Table 6.4, p.174).

6.4. Longitudinal analysis of child health and growth status against socio-demographic factors and cooking and fuel use practices

Child growth z-scores (height-for-age, weight-for-age and weight-for height), the levels of the selected biomarkers of immunity and the prevalence of respiratory tract infections were tested for an association with socio-demographic factors and cooking and fuel use practices. The child growth z-scores (height-for-age, weight-for-age and weight-for-height) were used to calculate the change in the growth status between the baseline and the final surveys by deducting the baseline survey z-score from the final z-score. The normal data distribution was obtained by excluding outliers when the z-

score change was less than -1.6 or more than 1.4. A period of 18 months was
expected to be more informative than a period of 9 months (due to the slow-
responding nature of the growth z-scores) in determining the effect of socio-
demographic determinants and household cooking behaviour on child growth.
Therefore the change scores for growth z-scores were examined between the baseline
and the final surveys. Age and the baseline growth z-score were included in the
univariate model of analysis in order to see how much those factors contributed to the
observed pattern together with the socio-demographic factor being tested.

General linear model (GLM) repeated measures were used as preliminary tests to
establish variables which may have an effect individually on health status and the
growth pattern of children before conducting regression. Table 6.5 (p.175) provides a
summary of socio-demographic determinants and cooking and fuel use practices
which were found to influence child growth (actual z-scores and changes in z-scores)
and health (in terms of the levels of the selected biomarkers of immunity) among this
Bangladeshi sample. Tables 6.6 (p.176) and 6.7 (p.177) provide details of subgroup
sizes observed for cooking and fuel use practices observed in the study sample and
how these groups were pooled for longitudinal analyses. Household fuel choices were
divided into three groups: wood (1), golden (2), and dung (3). The actual detailed
description of the effect of socio-demographic variables as well as cooking and fuel
use practices on child health and growth measures are provided in Tables 6.8 to 6.17
(p.178-187). Longitudinal GLM was used for the bivariate analyses for height-for-
age, weight-for-age, weight-for-height as well as the change scores for the listed z-
scores, haemoglobin, $\log_{10}$-AGP and albumin. Child immune status using c-reactive
protein was examined using cross-sectional data applied in non-parametric Mann-
Whitney or Kruskal-Wallis tests. The measured impact of significant variables
identified using bivariate analyses were examined using the multivariate GLM and
regression analysis later in this chapter. The methods applied in the regression
analyses are provided below:

Regression analysis was carried out in order to examine the relationships between haemoglobin, the selected biomarkers of immunity and the growth parameters with socio-demographic factors and factors which may have an influence on the experienced household fuel pollution exposure (Table 6.18 (p.188) - sub-grouping and reference categories within each variable provided). This helped to determine the power of each potential parameter on the immunity and the growth of these Bangladeshi children. A standard regression model with method=enter was used in all applications. Variables chosen for each regression were considered as potentially significant determinants of household fuel pollution exposure of young children directly (as using repeated measures GLM as described in earlier sections of this chapter). The appropriate maximum number of predictors was calculated using the formula $N >= 50 + 8m$ (where m is the number of predictors) (**Harris, 1975**). Normal data distribution, evidence for outliers and the distribution of residuals were checked for a linear relationship using normal p-p plot for residuals. A scatter plot was used to examine data for any evidence of homoscedasticity. Potential collinearity was avoided by using the tolerance test and VIF statistics and factors producing a tolerance of 0.8 or less were excluded from the equation. The regression analysis aimed to find a combination of predictors which explain the variance observed in the dependent variable the best. Child age was included in all regression equations as it is an important biological determinant of child health status, immunity, growth and behaviour. Age may have an influence on the levels of selected biomarkers of immunity and child growth z-scores, as well as cooking and fuel use behaviour, even though it was not found to have an effect on variables using a general linear model with repeated measures test or univariate analysis of variance (as described previously). Variables related to cooking and fuel-use behaviour were applied in the regression analysis using groupings from longitudinal analysis (as listed in Table 6.18, p.188). The predictors of haemoglobin, albumin, log_{10}-transformed alpha-1-acidglycoprotein levels as well as growth z-scores and change scores of growth were applied in the regression analysis using cross-sectional data from the baseline and the final surveys. Table 6.5 (p.175) lists the significant determinants of child health and growth measures examined using bivariate methods. Only the child health and growth

measures which were significantly associated with socio-demographic and behavioural determinants, according to the bivariate analyses described previously, were further examined using linear regression. The effect of SES and maternal education in the regression was attempted in order to see their indirect effect on the dependent variable being tested. As described previously in the Mosley and Chen framework for child health and nutritional analyses these factors have been identified as important influences of child health and nutrition **(Mosley and Chen, 1987).** In the regression analyses the effect of SES and maternal education were only included if their effect improved the model.

The description of the preliminary analyses of child growth, immune status and the presence of respiratory tract infection in terms of socio-demographic determinants and cooking and fuel use practices will be provided under the three main headings stating the proposed hypotheses tested in this chapter. Longitudinal GLM will then be used to carry out multivariate analyses in order to identify the underlying determinants of the patterns observed in the child growth, health and immunity in this Bangladeshi sample. Cross-sectional regression analyses will examine the relative contribution of the sum of factors on each child variable tested.

6.5. The association between child growth over 18 months and fuel use practices

Chapter 5 described diverse cooking practices in this Bangladeshi community. Cooking locations were indoors (1), indoors in a separate kitchen building (2), outdoors (3) or on veranda (4). In order to simplify the longitudinal (three field trips) analyses of child growth and immunity in terms of cooking location this variable was regrouped into indoor (consisting of cooking indoors or in a separate kitchen building) and outdoor (indicating outdoors or on veranda) cooking. Households in this Bangladeshi sample cooked once, twice or three or more times. At the baseline 85.1% of children stayed by the stove during cooking. The percentage of children

present during cooking had dropped down to 69.1% by the time of the midterm and down to 42.6% during the final surveys.

Table 6.5 (p.175) describes the summary of the results of the bivariate analyses of the effects of socio-demographic determinants (such as municipality, SES, average income, ethnicity, maternal education) and cooking fuel practices (child's presence by the stove during cooking, cooking location, fuel type, cooking frequency and stove type) on longitudinal data on child growth including z-scores for height-for-age, weight-for-age, weight-for-height as well as change scores (baseline to final) for the z-scores listed above. Only ethnicity was shown to have some effect on the longitudinal child growth measures in this Bangladesh child sample (Table 6.11, p.181). The Bihari children appeared more severely stunted throughout the fieldwork (baseline stunting: Bangla: -1.587 ± 1.103 and Bihari: -2.192±1.115). However, the observed longitudinal ethnic difference was just insignificant (F=2.952, p<0.055). The Bangla children were significantly less underweight than the Bihari children throughout the fieldwork (baseline weight-for-age for Bangla: -2.314 ± 1.394 and Bihari: -2.883 ±1.192, F=9.510, p<0.01). When the three growth parameters were tested between the Bangla and Bihari in Saidpur only, the pattern remained as described above. The effect of ethnicity on weight-for-height z-scores could not unfortunately be tested in this study due to small subgroup sizes. No association was found between household cooking frequency, cooking location, fuel choice and the child growth measures (height-for-age, the weight-for-age or the weight-for-height). The cooking location was shown to be heavily associated with the SES and municipality and therefore an interaction with these variables may produce the result above. The interaction will be examined in the regression analysis later in this chapter.

No ethnic effect was seen on the change scores for height-for-age, weight-for-age and weight-for-height using bivariate longitudinal analyses. Cooking frequency and fuel type instead were identified as having an effect on the change score for weight-for-age (Table 6.17, p.187). An overall improvement in weight-for-age was described earlier however the improvement was significantly larger in children from households

where golden (0.341±0.524) and dung (0.398±0.444) were used for fuel instead of wood (0.159±0.491) ($F=3.087$, $p=0.05$) (Table 6.17, p.187). A significant age-effect was also detected, but baseline weight-for-age z-score had no effect on the change score. Cooking frequency was also shown to affect the change score for weight-for-age significantly ($F=5.779$, $p<0.05$) (Table 6.17, p.187). The greatest improvement in the weight-for-age z-score was seen among children from households which cooked only once (0.403±0.407) a day rather two or three times a day (0.211±0.477) (significant age-effect was seen too $F=5.055$, $p<0.05$, but not baseline WAZ effect). No significant association between the socio-demographic determinants or cooking and fuel-use and the change score for height-for-age or weight-for-height change score could be detected in this study using bivariate analyses.

6.6. Longitudinal GLM multivariate analysis of the effect of cooking practices on child growth

GLM repeated measures test was carried out to investigate the joint effect of cooking and fuel use practices on child immune status using haemoglobin and the selected biomarkers of immunity as indicators. Age and the socio-demographic determinants which were identified as having a significant effect on the health measure of interest were included in the model as a covariate. The model tested the effect of the presence of a child by the stove during cooking, cooking location, fuel type, cooking frequency and stove type.

Height for-age: Ethnicity was shown to have a significant effect on the longitudinal data on height-for-age z-scores so it was included in the analysis as a covariate, together with age. Inclusion of the presence of a child by the stove during cooking lead to a very small group size hence this variable of cooking practice was excluded from the analysis. An inclusion of cooking frequency and fuel type had the same effect (led to small subgroup sizes) hence they were excluded. The tested model therefore consisted of stove type and cooking location with age and ethnicity as covariates. Significant patterning of the longitudinal height-for-age z-score data was

observed (F=7.413, p<0.01, n=166). But only age was shown as a significant predictor of the patterning observed (F=16.299, p<0.01). Cooking location, stove type, or ethnicity were not associated with the patterning observed in the height-for-age data (F=0.308, F=1.051 and F=.0.465, p=NS respectively).

Weight-for-age: The preliminary analyses of singular predictors of the patterning observed in the weight-for-age z-scores showed an association between cooking frequency and fuel. These two variables were included in the general linear model repeated measures test as covariates together with child age. Fuel type was further excluded due to small subgroup sizes within. No significant changes in weight-for-age z-scores were seen over the three field surveys using this model (F=0.957, p=NS). None of the variables or covariates included in the model showed any association with the weight-for-age z-scores in this study sample. An exclusion of cooking frequency improved the results. Weight-for-age z-scores data changed significantly between surveys (F=8.642, p<0.01, n=176). However, age was still the only significant predictor of the patterning observed in the weight-for-age status (F=5.787, p<0.05). Cooking location and stove type were not associated with weight-for-age (F=1.212 and F=2.062, p=NS respectively).

Weight-for-height: The effect of cooking practices on weight-for-height was examined. Cooking location and stove type were included in the model (the presence of a child by the stove during cooking, cooking frequency and fuel type was excluded due to small sample sizes). Age was included as a covariate in the GLM repeated measures test. A significant patterning of the weight-for-height status was detected (F=7.585, p<0.01, n=132). The patterning was only associated with child age (F=4.183, p<0.05). No associations of the weight-for-height z-score data with cooking location or stove types could be seen (F=0.083 and F=1.679, p=NS respectively).

Change score for weight-for-age: The effect of cooking and fuel use practices on change score for weight-for-age were examined using univariate analysis of change

score between baseline and final surveys. The baseline weight-for-age data and age were included in the analysis as covariates together with cooking frequency which was shown to be significant in the preliminary analyses. The presence of a child by the stove during cooking was excluded due to small and uneven group sizes. Cooking practices did significantly shape the change score for weight-for-age in this study ($F=34.893$, $p<0.01$, $n=105$). Age and cooking frequency (included as covariates in the model) were significantly associated with the change score ($F=6.441$ and $F=6.553$, $p<0.05$ respectively). Other factors such as stove type and cooking location had no significant effect on the change score for weight-for-age among these Bangladeshi children.

Summary: The longitudinal multivariate GLM showed that the patterns observed in the child growth z-scores in this Bangladeshi sample were influenced by child age only. The change score for weight-for-age z-score was influenced by child age as well as cooking frequency in this study. This study has shown that child growth is not influenced by cooking practices in this Bangladeshi sample.

6.7. Linear regression of stunting and underweight (height-for-age and weight-for-age z-scores)

GLM repeated measures test showed significant ethnic effect on the growth z-scores of children in this Bangladeshi sample (Table 6.11, p.181). The effect of socio-demographic determinants and cooking and fuel-use behaviour were examined using regression. Child age was included in all regression analyses.

6.7.1. Height-for-age

In the analyses of height-for-age data at baseline maternal education and cooking frequency were highly collinear therefore only the predictor with the strongest individual effect was kept in the regression model (maternal education). Municipality was excluded due to its highly insignificant role in the model and due to its high collinearity with ethnicity (which was a strong predictor of stunting in this model).

Age was included in the equation despite its insignificant effect on the model. SES and cooking location were highly collinear therefore only the SES which had the stronger impact on the baseline stunting was included. (Table 6.21, p.191). Ethnicity was the strongest predictor of the degree of stunting: Bihari ethnicity predicted more severe stunting (t=-3.532, p<0.001). Older children were more likely to be more stunted than their younger counterparts, despite the insignificant age effect in this model (t=-1.306, p=NS). High SES and an educated mother signalled a better height-for-age growth z-score (t=1.810, p<0.079 and t=1.351, p=NS). Both SES and maternal education improved the regression model; therefore they were included in the model, despite their insignificant predictive power. Some degree of collinearity was observed between SES and maternal education however it was within acceptable limits (normal data distribution, linear residual scatter plot).

For the final survey the model predicting the height-for-age status differed from the regression model obtained for the baseline measures. The final survey height-for-age status was best explained by age, maternal education, the presence of a child by the stove during cooking and cooking frequency (R=0.342, R^2=0.117, F=2.889, p<0.001, n=97) (Table 6.21, p.191). Older children (t=2.285, p<0.001) whose mothers had received some education (t=2.160, p<0.05) were more likely to have better height-for-age status than young babies born to uneducated mothers. The effect of age on the degree of stunting contradicts the baseline model. An inclusion of the presence of a child by the stove during cooking improved the model, however its effect was insignificant. Cooking frequency was on the borderline of significance and indicated that children from households where cooking was done several times a day suffered from more severe stunting.

6.7.1. Weight-for-age z-score

The best model to explain the baseline pattern in the weight-for-age change included age, ethnicity, SES and stove type (R=0.261, R^2=0.068, F=3.150, p<0.001, n=167) (Table 6.22, p.191). Ethnicity was the strongest predictor of the weight-for-age z-

score during the baseline (t-3.279, p<0.01). An inclusion of age, SES and stove type improved the overall model (t=0.139, p=NS, t=1.367, p=NS and t=-1.253, p=NS respectively) despite their insignificant individual role in this regression model. Being of Bihari origin predicted a much worse weight-for-age status (t=3.279, p<0.001). The Bihari ethnicity lowered the weight-for-age z-score by 0.245. The use of a portable stove (or a mixed use of fixed and portable stoves) appeared to worsen the weight-for-age z-score in these Bangladesh children (t=-1.253, p=NS). An older age predicted a better weight-for-age z-score and a better weight-for-age was likely to be predicted by a high SES status, despite the effect of these predictors being insignificant in the model (age t=0.139, p=NS and SES t= 1.367, p=NS) (Table 6.22, p.191). No significant model to predict the patterns observed in the final survey data for weight-for-age could be identified. No model could be identified for the weight-for-height data from the baseline or the final surveys either.

6.8. Linear regression of change in child growth z-scores

GLM repeated measures test showed a significant effect of cooking frequency and the choice of fuel used by a household on the change in the weight-for-age z-score. No other change scores of growth z-scores were significantly associated with any of the socio-demographic determinants or cooking and fuel use behaviour described for this Bangladeshi sample, hence they were not examined using regression. The predictive power of cooking frequency and fuel type will be examined in relation to the weight-for-age change score (change between the baseline and the final surveys) (Table 6.23, p.192). The baseline weight-for-age z-score was included in the regression to allow for different starting points and regression to the mean. The inclusion of fuel type in the analysis reduced the sample size to n=45 which is too low for any regression analysis to be carried out and so it was excluded from all regression analysis. Fuel type however was significantly associated with the stove type (as described in Chapter 5, section 5.3.3.2, p.113) so the effect of stove type on the change score for weight-for-age was examined in this study. Age was also included in the regression analysis. The effect of municipality was strong but its high collinearity with cooking

frequency led to its exclusion. An inclusion of cooking frequency was essential for the regression model. The best model to explain any trends in the weight-for-age change score included age, cooking frequency and the presence of a child by the stove during cooking. (R=0.391, R^2=0.153, F=3.886, p<0.05, n=91) (Table 6.23, p.192). Older age predicted a more severe degree of being underweight among these Bangladeshi children (t=-2.386 p<0.05). Cooking more than once per day indicated a worsening of the weight-for-age z-score throughout the survey (t=-2.096, p<0.05). The presence of a child by the stove improved the regression model even though its independent effect was insignificant. Children who stayed away from the stove during cooking had a more positive weight-for-age change score indicating an improvement in the weight-for-age z-score throughout the field work (t=0.866, p=NS). The baseline weight-for-age z-score was a strong predictor of the change score for weight-for-age. Those children who had a better weight-for-age z-score were more likely to grow less relative to the children with a poorer weight-for-age status at the baseline (t=-2.966, p<0.05).

6.9. An association of child immune status over 18 months and fuel-use practices in this Bangladeshi sample.

The immune status of the Bangladeshi children examined was described in terms of socio-demographic determinants and cooking and fuel use practises using haemoglobin, log_{10}-transformed alpha-1-acidglycoprotein, inversely transformed albumin and c-reactive protein levels as indicators. Multivariate analysis using repeated measures tests showed a significant municipality effect on log-transformed alpha-1-acidglycoprotein levels of children (F=4.331, p<0.05) (Table 6.5 and 6.8, p.175 and 178). Children from Saidpur had significantly higher levels of log_{10}-α-1-acidglycoprotein than those from Parpatipur (at the baseline and the final surveys, post-hocs p<0.05 for both) indicating a poorer health status among children in Saidpur. Interestingly a lower infection status (hence better immunity) was detected in Parpatipur during the midterm survey (post hocs p<0.05). No significant municipality effect on the longitudinal inversely transformed albumin levels was seen

(F=2.108, p=NS). A Mann-Whitney test showed significantly higher levels of CRP among children in Parpatipur during the baseline (Table 6.8, p.178). This difference however disappeared by the time of the mid-term survey (baseline Z= -3.441, p<0.001, midterm Z=-0.041, p=NS and final Z-0.133, p=NS). Socio-economic status, average income, maternal education, the presence of a child by the stove during cooking and cooking location were not associated with longitudinal data on the levels of the biomarkers of immune status using bivariate analyses.

Chapter 5 described a common use of chimneyless fixed or portable biomass stoves in this Bangladesh sample. The chapter identified the fuel type and cooking location as significant determinants of the household stove type (SES and municipality effect were overridden by the fuel type and cooking location) (see Chapter 5, section 5.3.4.2, p.114). Households using fixed stoves were more likely to cook outdoors and use dung or golden as a fuel. A significant influence of stove type was seen on the longitudinal levels of log-transformed alpha-1-acidglycoprotein levels (F= 6.894, p<0.01) (Table 6.13, p185). The levels were significantly more elevated in children from households using a fixed stove rather than a portable stove type or practicing a mixed use of stove (fixed or portable) throughout the year (F=6.894, p=0.001). The impact of a stove type on haemoglobin was almost significant (F=2.496, p<0.086). The haemoglobin levels of children from households using a fixed stove appeared to be the lowest. No significant effect of stove type was found in inversely transformed albumin among children from households where portable or fixed biomass stoves were used (F=0.356, p=NS). No significant effect of the stove type was seen in the c-reactive levels using cross-sectional analyses (Mann-Whitney z-scores for the baseline: Z=-0.386, midterm: Z=-0.679 and final: Z=-0.864, p=NS respectively). The effect of fuel type on longitudinal haemoglobin levels was close to significant (F=2.262, p<0.066). The haemoglobin levels among children from households burning golden as a fuel appeared lower during the baseline and the midterm survey relative to children from households using wood or dung for cooking. The difference had disappeared by the time of the final survey. The just insignificant result may indicate an interaction of a fuel type and another socio-demographic factor or

behavioural pattern (such as SES or cooking location or the presence of a child by the stove during cooking) as a potential influence of haemoglobin and child health in this Bangladeshi community. This needs to be investigated further. Fuel type had no effect on the patterns of c-reactive protein, inversely transformed albumin or $\log_{10}$-transformed alpha-1-acidglycoprotein. Cooking frequency or cooking location were not significantly associated with the levels of haemoglobin or the selected biomarkers of immunity.

6.10. Longitudinal GLM multivariate analysis of the effect of cooking practices on child immune status

The effect of cooking practices on the longitudinal data on haemoglobin, $\log_{10}$-transformed alpha-1-acidglycoprotein and inversely transformed albumin levels were tested using a general linear model. The cooking practices were identified as cooking location and the presence of a child by the stove during cooking. Ethnicity, stove type and child's age were included as covariates as their singular effect was identified in the bivariate analyses earlier in this chapter (Table 6.5, p.175). Cooking frequency and fuel type was excluded from the analysis altogether due to a small subgroup sizes. The effect of the baseline growth z-scores on child immune status (using the listed markers) was examined by running the GLM analysis with and then without the baseline growth z-scores.

6.10.1. Haemoglobin

The model showed no significant patterning of the haemoglobin data linked with the cooking location and stove type (F=1.555, p=NS). The analysis showed a significant association between longitudinal haemoglobin levels and the stove type as well as ethnicity (they had been identified as significant determinants before and were added as covariates in the analysis) (F=3.718, $p<0.05$ and F=3.716, $p<0.05$). No associations between the cooking location and the presence of a child by the stove were detected.

An inclusion of the baseline height-for-age, weight-for-age and weight-for-height z-score data showed that child growth z-scores had no effect on the patterning observed in the haemoglobin levels (F=0.711, p=NS) and the observed effect of ethnicity and stove type disappeared too (F=2.874 and F=2.996, p=NS).

6.10.2. Log_{10}-alpha-1-acidglycoprotein

GLM repeated measures test was performed using the same criteria as described above. Municipality, stove type and child age were included as covariates in the model. The variables tested in this model were the presence of a child by the stove during cooking and cooking location. The patterning of the log_{10}-AGP could not be associated with the cooking location or the presence of a child by the stove (together with the listed covariates) (F=2.856, p=NS). Stove type and municipality (covariates) were shown to have significant effects on the log_{10}-AGP levels (F=4.581, p<0.05 and F=3.414, p<0.05). The effect of municipality was just insignificant (F=2.670, p=NS). When the baseline growth z-score data was included in the model a significant patterning in the LOG-AGP levels was seen (F=4.371, p<0.05). Stove type and municipality as a covariate was identified as a significant determinant of the patterns observed in the log_{10}-AGP (F=3.780, p<0.05 and F=4.123, p<0.05).

6.10.3. Albumin (1/X)

Only child age was included as a covariate in the model as preliminary bivariate analyses did not identify any significant determinants of the inversely transformed albumin levels. The variables of interest were the presence of a child by the stove during cooking and cooking location and stove type, however their subgroup sizes were too small for any analyses. Hence, they were excluded from the analysis. A significant patterning of the albumin levels were seen (F=15.327, p<0.01). The model therefore tested the effect of cooking location on the inversely transformed albumin levels with age as a covariate. The mean levels were as follows: baseline: 0.040±0.001, midterm: 0.051±0.002 and the final: 0.036±0.001. A significant age effect was seen (F=4.050, p<0.01) but no other determinants of the observed

patterning observed were identified. The inclusion of the baseline growth z-scores had no effect on the model.

The GLM was not carried out on c-reactive data due to its non-normal data distribution.

Summary: The longitudinal multivariate GLM performed showed a significant effect of cooking practices on the biomarkers of child immune status. The GLM model identified a significant effect of the stove type and ethnicity on the levels of haemoglobin (which however disappeared by an inclusion of baseline growth z-score data). Log_{10}-transformed alpha-1-acidglycoprotein levels were significantly influenced by municipality and stove type, and this association was only strengthened by the inclusion of the baseline z-score data. Inversely transformed albumin only showed an age-effect in this study.

6.11. Regression analysis of the predictors of child immune status

6.11.1. Haemoglobin regression

Baseline: Socio-demographic factors and cooking behaviour were examined in relation to haemoglobin during the baseline and the final surveys. Repeated measures GLM showed a significant effect of ethnicity, fuel type and stove type on the longitudinal levels of haemoglobin in this study. The sample size for the data with three types of fuel was n=83, hence borderline in terms of a large enough sample size for the analysis. The inclusion of fuel type did not add anything to the model and therefore it was discarded from the analysis, despite its significant effect in the bivariate analysis. The best model to explain the haemoglobin levels during the baseline survey included age, ethnicity, stove type and the presence of a child by the stove during cooking (R=0.558, R^2=0.312, F=18.127, p<0.001, n=167) (Table 6.22, p.191). No gender was included in the model due to its insignificant influence on haemoglobin in this analysis. Haemoglobin levels improved with age (t=6.517, p<0.001). Child haemoglobin improved by 0.440g/L per month of age. The presence of a child was not a significant predictor in the model, however its inclusion

improved the overall model. Bihari ethnicity predicted a lower haemoglobin (t=-3.009, p<0.001). The use of a portable stove (or switching between portable and fixed stoves throughout the year) predicted better haemoglobin levels (t=3.961, p<0.0010). Neither the cooking location nor the cooking frequency improved the regression model so they were excluded from the final regression equation.

For the final survey the regression analysis showed that the haemoglobin levels were best predicted by the child's age, maternal education, stove type and the presence of a child during cooking (R=0.397, R^2= 0.158, F=6.556, p<0.001, n=145) (Table 6.22, p.191). Ethnicity was excluded due to its highly insignificant effect on the model. Gender-effect was excluded due to its highly insignificant effect on the model. According to the model haemoglobin levels improved with increasing age (t=3.928, p<0.001) and better maternal education (t=2.396, p<0.05). An inclusion of stove type and the presence of a child by the stove during cooking improved the model, despite their individually insignificant effect on the model. The child's absence from the stove during cooking was associated with better haemoglobin levels.

This regression analysis showed clearly that age was a very strong predictor of haemoglobin levels of children in this study, however the evidence for stove type and the presence of a child by the stove during cooking is equivocal.

6.11.2. Log_{10}-alpha-1-acid glycoprotein regression

At the baseline survey, municipality and stove type were significantly associated with the log_{10}-transformed alpha-1-acidglycoprotein levels using bivariate analysis (Table 6.5, p.175). The regression model that best explained the patterns observed in the log-transformed alpha-1-acidglycoprotein levels included child age, maternal education, municipality and the presence of a child by the stove during cooking (R=0.290, R^2=0.084, F=3.465, p<0.001, n=154) (Table 6.23, p.192). In this model lower log_{10}-transformed alpha-1-acidglycoprotein indicated better health and an elevation in the alpha-1-acidglycoprotein levels indicated an infection and poor health. An educated mother (t=-2.558, p<0.05) and residence in the municipality of Parpatipur (t=-1.829,

p<0.069 – close to being significant) predicted lower levels of $\log_{10}$-transformed alpha-1-acidglycoprotein levels indicating better child health. The inclusion of child age and the presence of a child by the stove during cooking improved the model despite their insignificant predictive nature. Lower AGP was associated with older age and staying away from the stove during cooking. SES and income were excluded due to their lack of impact on the model and their highly insignificant predictive nature. Cooking location, cooking frequency and ethnicity were highly collinear with municipality and therefore excluded. No gender effect was seen on log-transformed AGP either, so sex was excluded from the analysis.

In the final survey the best model to explain the log-transformed alpha-1-acidglycoprotein data was quite different from the predictive model for the baseline survey (n=109) (Table 6.23, p.192). No gender effect was seen on the log-transformed AGP either, so sex was excluded from the analysis. The use of a fixed stove throughout the study was related to lower $\log_{10}$-transformed alpha-1-acidglycoprotein levels and better health (t=2.076, p<0.05). The use of a portable stove or the mixed use of fixed and portable stove indicated poorer health and elevated $\log_{10}$-transformed alpha-1-acidglycoprotein levels. Older age was likely to predict lower infection status and better health (t=-1.221, p=NS), however the effect was insignificant. Cooking indoors improved the health and cooking only once a day predicted poorer health through elevated levels of $\log_{10}$-transformed alpha-1-acidglycoprotein (t=-0.496, p=NS). However the effects of cooking location and the frequency of cooking were insignificant but they improved the overall model.

6.12. An association between the presence or absence of respiratory tract infections over 18 months and cooking and fuel-use practices

In this study all children who were reported as having cough, fever, discharge, asthma, wheezing and whistling of breath, fast breathing, lower chest in-drawing, diarrhoea or unable to breastfeed in the two weeks prior to the survey were examined by a doctor. Children who were healthy or with runny nose or sore throat according to

their mother were not seen by a doctor and were categorised as healthy children. Tests were conducted to examine whether children within the three health groups had elevated levels of biomarkers of infection or lower haemoglobin levels.

6.13. Longitudinal data on the levels of biomarkers of immunity between baseline child health groups (healthy, other illness, respiratory infection (RTI)

The levels of biomarkers of immune status were tested against child health groups using the health status from the baseline survey (due to small subgroup sizes when using longitudinal health status data). Haemoglobin from the three field surveys did not vary significantly between children who were seen by a doctor and those who were not (healthy vs. unwell excluding RTI) using the baseline health status groupings (age was included as a covariate) (F=0.711, p=NS) (Table 6.24, p.193). No significant differences were seen in the longitudinal inversely transformed albumin levels between healthy and unwell (excluding RTI) groups (F=0.146, p=NS, but a significant age-effect was seen F=4.262, p<0.05). Neither did the $\log_{10}$-transformed alpha-1-acidglycoprotein levels vary between the two groups (F=1.467, p=NS, no age effect was seen either). The child growth z-scores did not vary between the healthy and unwell (excluding RTI) children in this study (height-for-age: F=1.196, p=NS, age-effect 15.035, p<0.01, weight-for-age: F=0.043, p=NS, age-effect 5.804, p<0.01 and weight-for-height: F=1.628, p=NS, no age-effect). C-reactive protein levels were not significantly associated with the children's need for medical attention during any of the surveys (Mann-Whitney Z=-0.710, p=NS, Z=-0.985, p=NS, Z=-1.778, p=NS) (age as a covariate).

A comparative analysis of the levels of immune markers in children who were healthy at the time of the baseline survey and those with a respiratory tract infection were examined for any patterning related to the immune status of the children (healthy vs. RTI only). No significantly different levels of longitudinal haemoglobin, inversely transformed albumin (1/X albumin) or $\log_{10}$-transformed alpha-1-

acidglycoprotein were observed in children who were healthy (no medical check needed) at the baseline survey and those with a medically diagnosed RTI. The same was repeated for children who were unwell (no RTI) and the children with RTI (Unwell other vs. RTI). No differences in the immune status of children with RTI and those who had an illness other than an RTI using the levels of haemoglobin, inversely transformed albumin and log_{10}-transformed alpha-1-acidglycoprotein as described. Neither of the growth z-scores showed any patterning on the basis of the ailment grouping (RTI or other) at the baseline study.

6.14. Longitudinal analysis of socio-economic determinants and cooking practices within child health groups

The effects of socio-economic determinants and cooking practices on the child health status (healthy, other illness (excluding RTI) and RTI) were examined using longitudinal groups of child health as listed in Table 6.25 (p.194). Due to very small subgroup sizes of the longitudinal child health status data it was only possible to carry out very simple tests. Hence, Chi-square tests were carried out. Healthy children were more common in Parpatipur than in Saidpur (62.5% and 37.5% respectively) (X^2=9.296, p<0.01). Bangla children were more commonly found within the healthy group than the Bihari children in this study (67.5% and 32.5% respectively) (X^2=3.899, p<0.05). Children of educated mothers were more commonly classified as healthy (educated: 62.5% versus 37.5%) (X^2=7.244, p<0.01). No effect of average income, SES, cooking location or stove type (longitudinal groups) between healthy children and children with an illness other than RTI could be detected (X^2=0.524, p=NS, X^2=1.119, p=NS, X^2=0.237, p=NS, X^2=1.628, p=NS) (Table 6.25, p.194). The effect of the presence of a child by the stove or cooking location could not be tested longitudinally due to small subgroup sizes. SES municipality, maternal education, literacy, average income or ethnicity were not associated with the health groupings (healthy vs. RTI) (using chi-square test). Healthy versus RTI could not be tested longitudinally for cooking practices as the subgroup sizes were under n=10 for some of the categories.

6.15. Predictors of the risk factors for respiratory tract infection (logistic regression) using the baseline healthy versus RTI grouping

Logistic regression analysis tested the best model to explain the patterns in child health status (healthy versus RTI) in this community. Due to a small sample size the regression of healthy versus unwell (excluding RTI) could not be carried out. The aim was to identify any differences in the socio-demographic background of household cooking and fuel-use behaviour which would explain the higher risk of getting an RTI. The binary logistic regression using a forward stepwise model was applied. Age was included in all binary logistic regression analyses as age was considered as a likely risk factor for RTI (younger children are at highest risk). All background variables and their categories were as listed in Table 6.26 (p.195).

The baseline data on child health groups (healthy) and those with medically diagnosed RTI (RTI) were examined in relation to longitudinal socio-demographic factors and cooking and fuel-use behaviour. The model identified the presence of a child by the stove during cooking as the only risk factor for RTI (Wald= 7.580, $p<0.01$, n=61) (Table 6.27, p.196). The risk of RTI was 3.89 times (OR=3.890, 95% CI: 1.479 - 10.309) more likely among children who stayed by the stove during cooking than children who stayed away (OR= 0.257 times (95% CI: 0.097 – 0.676) as likely to develop an RTI as the reference category= being present by the stove during cooking OR=1.000). No significant age effect or any socio-demographic determinants were identified which could influence the risk of having an RTI in this Bangladeshi community.

No significant predictors of child health status (healthy versus RTI) could be identified using the child health groupings from the final survey against the longitudinal socio-demographic determinants and cooking and fuel-use behaviour (Table 6.24, p.193). The small sample size (n=61) hindered the analysis too.

Summary: The analysis of the data on child health status associated the presence of a child by the stove during cooking as main risk factor for RTI among children in this study. Chi-square tests also brought to light the increased risk of RTI when living in semi-urban Saidpur and Bihari ethnicity, especially if the mother of a household was uneducated. Cooking practices may have some effect on the child health in this Bangladeshi study, however the association is quite weak with the data available. More complex analyses were limited by the small sample size.

6.16. Summary

The chapter examined any associations between child growth, the immune status of children and the prevalence of respiratory tract infections and socio-economic factors such as household cooking and fuel use behaviours over a period of 18 months. Approximately half of the mothers reported their child or children of having suffered from fever and cough throughout the fieldwork. Medical diagnoses revealed that moderate or severe respiratory infections accounted for over 20% of all ill-health in this sample, which is similar to the levels previously reported (**BDHS, 2005**). 85% of the other illnesses reported in medical examinations were respiratory related i.e. upper respiratory tract infections and minor colds; other conditions reported were skin infections, ascariasis, and diarrhoea. Children under the age of five years were commonly anaemic. No association was seen between child health status and the levels of haemoglobin or the selected biomarkers of immunity.

Child growth status was poor. An improvement was seen in terms of the prevalence of underweight (weight-for-age) and wasting throughout the survey, however the height-for-age status worsened. Older children whose mothers had received some education were more likely to have better height-for-age status than young babies born to uneducated mothers. The degree of stunting and being underweight were best predicted by low SES status, Bihari ethnicity and the presence of a child by the stove during cooking, together with poor maternal education and low cooking frequency. Longitudinal multivariate analysis showed that cooking practices were not associated

with the patterns observed within the child growth z-scores in this Bangladeshi sample. The child growth z-scores were only associated with child age with an improved growth in older children.

An examination of longitudinal child immune status using haemoglobin, $\log_{10}$-alpha-1-acidglycoprotein and inversely transformed albumin levels showed that the Bihari children whose mothers were uneducated and cooked on a fixed stove were the worst off in terms of anaemia. Longitudinal multivariate GLM showed a significant effect of ethnicity and stove type on the levels of the selected biomarkers of immunity in this study, which confirms the findings of the preliminary data analysis. Logistic regression analysis of the risk factors of developing a respiratory tract infections (versus healthy) among children identified the presence of a child by the stove during cooking and child age as the most important predictors of RTI

The chapter highlighted that child growth was not influenced by cooking practices in this study, whereas the use of a fixed stove significantly worsened the immune status of young children in this study. The RTI were more common among very young children who were present by the stove during cooking. The evident lack of effect of the SES status or average monthly income on child health and growth among these Bangladeshi children may be due a high prevalence of child growth retardation, irrespective of socio-economic status. Municipality was a weak determinant of the child health, growth and immunity in this sample, despite the fairly different nature of the settings in Saidpur and Parpatipur. Cooking indoors on a fixed stove is likely to expose children to higher levels of daily cooking fuel pollution which affects the health and growth of children. Chapter 7 will examine in detail measured household fuel pollution levels and aims to identify determinants of the extent of household fuel pollution exposure.

Tables and figures

Figure 6.1: Health symptoms reported by mothers across the field surveys
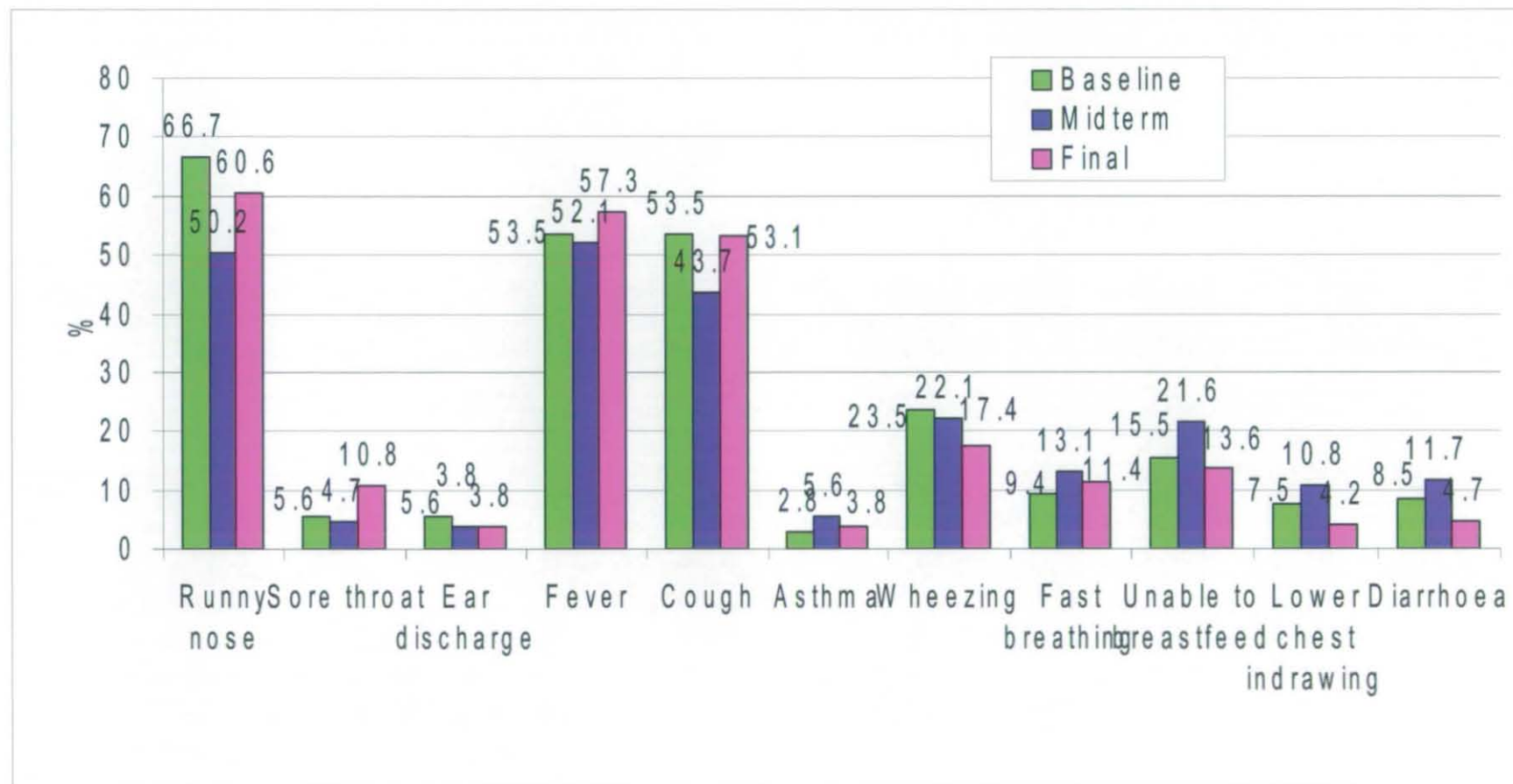
Table 6.1: Combination of health symptoms reported by mothers across all the surveys (using questions listed in Appendix 1)

Health symptom	Baseline % (n)	Midterm % (n)	Final % (n)
Cough and Fever	23.5 (50)	28.2 (60)	28.6 (61)
Cough and asthma	0.9 (2)	4.2 (9)	2.3 (5)
Cough and fast breathing	1.9 (4)	8.0 (17)	2.3 (5)
Cough and wheezing	5.2 (11)	10.3 (22)	5.2 (11)
Cough and lower chest in-drawing	2.3 (5)	5.6 (12)	0.9 (2)
Cough and ear discharge	0.5 (1)	1.9 (4)	0
Cough and diarrhoea	2.8 (6)	3.3 (7)	1.4 (3)
Cough and fever with ear discharge	0	1.9 (4)	0
Cough and fever with wheezing	4.7 (10)	9.4 (20)	4.2 (9)
Cough and fever with fast breathing	1.9 (4)	7.0 (15)	2.3 (5)
Cough and fever with asthma	0.9 (2)	3.3 (7)	2.3 (5)
Cough and fever with diarrhoea	2.8 (6)	2.8 (6)	1.4 (3)
Cough and fever with lower chest in-drawing	1.9 (4)	5.2 (11)	0.9 (2)
Fever and diarrhoea	4.2 (9)	3.8 (8)	1.9 (4)
Fever with diarrhoea and fast breathing	0.9 (2)	0.5 (1)	0

Table 6.2: Child health groups across the field surveys (youngest child only)

Survey (n)	Healthy % (n)	Medically examined	
		Unwell with other illness % (n)	Respiratory tract infection (RTI) % (n)
Baseline (202)	37.6 (76)	37.6 (76)	24.8 (50)
Midterm (202)	39.6 (80)	53.0 (107)	7.4 (15)
Final (202)	38.6 (78)	44.1 (89)	17.3 (35)

Table 6.3: The levels of selected immune markers and the growth parameters in children across the field surveys using repeated measures ANOVA

Variable (n)	Baseline mean±SD	Midterm mean±SD	Final mean±SD	Statistical method
Haemoglobin (g/L) (155)	105.5 ±13.1	106.2 ±12.9	111.6 ±13.8	F=0.611, p=NS, age as covariate (an exclusion of age detected significant improvement in the Hb between the baseline and the final surveys, F=7.347, p<0.01
Log_{10}-α-1-acidglycoprotein (g/L) (118)	-0.748±0.180	-0.855±0.184	-0.675±0.157	F=0.701, p=NS, age as covariate (an exclusion of age as detected significant differences F=8.484, p<0.012, post hocs: baseline vs. midterm p<0.001, midterm vs. final p<0.001, baseline vs. final p<0.001
Albumin (g/L) (1/X) (103)	0.041± 0.009	0.050 ± 0.009	0.035±0.008	F=4.042, p<0.01 age as covariate. Post hocs: baseline vs. midterm p<0.01, midterm vs final p<0.01, baseline vs. final p<0.01
C-reactive protein (mg/L) (140) (non parametric)	0.547 ± 0.758 Kendall's rank: 1.97	1.173 ± 2.041 Kendall's rank: 2.29	0.348 ± 0.605 Kendall's rank: 1.74	Kendall's test X^2=21.900, p<0.001
Height-for-age (SD) (167)	-1.840 ±1.144	-2.071±1.313	-1.979±1.155	F=3.115, p<0.05, baseline vs. midterm p<0.05, midterm vs. final p=PN, baseline vs. final p=NS
Weight-for-age (SD) (178)	-2.557 ± 1.338	-2.555 ±1.450	-2.269±1.244	F=8.500, p<0.01, baseline vs. midterm p=NS, midterm vs. final p<0.01, Baseline vs. final p<0.01
Weight-for-height (SD) (134)	-1.693 ± 1.247	-1.680 ±1.485	-1.394±1.054	F=7545, p<0.01, baseline vs.midterm p=NS, midterm vs.final<0.01, baseline vs. final p<0.01

Table 6.4: Comparison of child health and immune status between children from all three surveys and those included in the baseline, midterm or final surveys (non-three)

Variable	Children all three surveys Mean±SD (n)	Children from baseline, midterm or final surveys Mean±SD (n)	Statistics
Age	27.5±15.8 (247)	25.4±14.4 (59)	F=0.915, p=NS
Haemoglobin	104.1±13.3 (226)	104.8±12.0	F=0.731, p=NS, Age F=48.300, p<0.01
Log10-α-acidglycoprotein	-0.749±0.171 (218)	-0.729±0.185 (53)	F=0.490, p=NS, Age F=0.979, p=NS
(inv.trans.) Albumin	0.041±0.010 (225)	0.045±0.009 (55)	F=3.072, p=NS, Age F=12.446, p<0.01
C-reactive protein (non-parametric)	Mean rank: 142.3 (n=226), sum of ranks: 32125.50	Mean rank: 141.4 (n=57), sum of ranks: 8060.50	M-W=6407.500, Z=-0.061, p=NS,
Height-for-age	-1.783±1.213 (242)	-1.633±1.397 (59)	F=0.407, p=NS, Age F=9.178, p<0.05
Weight-for-age	-2.474±1.414 (239)	-2.560±1.504 (58)	F=0.201, p=NS, Age F=0.554, p=NS
Weight-for-height	-1.598±1.250 (232)	-1.777±1.193 (56)	F=1.182, p=NS, Age F=2.684, p=NS

Table 6.5: Summary of the bivariate analysis: + indicates a significant variable; x means non-significant determinant

Variable	Municipality	SES	Income (Binary)	Ethnicity	Education (maternal)	Child presence by stove	Cooking location	Fuel type	Cooking frequency	Stove type
Haemoglobin	x	x	x	+	x	x	x	+	x	+
Log$_{10}$-alpha-1-acidglycoprotein	+	x	x	x	x	x	x	x	x	+
Albumin	x	x	x	x	x	x	x	x	x	x
C-reactive protein	+	+	x	+	x	x	+	x	x	x
Height-for-age	x	x	x	+	x	x	x	x	x	x
Weight-for-age	x	x	x	+	x	x	x	x	x	x
Weight-for-height	x	x	x	n/a	x	x	x	x	x	x
Height-for-age change score	x	x	x	x	x	x	x	x	x	x
Weight-for-age change score	x	x	x	x	x	x	x	+	+	x
Weight-for-height change score	x	x	x	x	x	x	x	+	x	x

Table 6.6: Variable subgroups for cooking and fuel use behaviour for each of the three field surveys

Categories	n	Group details			
Cooking frequency	3	Once	Twice	Three or more	
Presence of a child by the stove during cooking	2	Yes	No	███████	████
Fuel type	4	Wood	Golden	Coconut	Dung
Cooking location	2	Indoors	Outdoors	███████	████
Stove choice	2	Portable	Fixed	███████	████

Table 6.7: Cooking and fuel-use behaviour groups over the longitudinal study

Variable	Subgroups	n	Regrouping	n	Justification
Stove choice	Fixed	50	Fixed	50	Cooking on a fixed stove indoors may be unhealthy
	Portable	26	Others (switchers)	104	Changeable cooking behaviour cannot be tracked
	Switchers	78			
Child present by stove during cooking	Yes	63	Yes	63	Children exposed to harmful smoke
	No	15	Switchers Yes to No	91	Healthier behaviour introduced after the baseline
	Switchers Yes to No	82			
	Switchers No to Yes	9			
Cooking location	All indoors	68	All indoors	68	Unhealthy behaviour (may be a sign of wealth)
	All outdoors	49	Others	101	Cooking outdoors healthier but could be a sign of poverty.
	Switchers IN to OUT	21			
	Switchers Out to IN	31			
Cooking frequency	All once	48	All once	48	Poorer SES maybe, poorer health?
	all two	45	More	64	Better health and nutrition
	all three	19	Switchers	57	Mixed behaviour - may cloud the analyses
	Switchers	57			
Fuel type	Wood	36	Wood	36	
	Golden	22	Golden	22	
	Coconut	15	Dung	15	
	Dung	16			
	Switchers	62			

Table 6.8: Municipal effect on growth, haemoglobin and the selected biomarkers of immunity

Variable	Subgroup (n)	Baseline mean±SD	Mid-term mean±SD	Final mean±SD	Statistics
Haemoglobin	Saidpur (82)	103.3±13.7	103.5 ±13.4	110.9 ±14.3	F=2.208, p=NS
	Parpatipur (73)	108.0 ±12.0	109.3 ±11.6	112.3 ±13.3	
Log_{10}-acidglyco-protein	Saidpur (65)	-0.723 ± 0.166	-0.875 ± 0.146	0.700 ±0.158	F=4.331, p<0.05)post hocs comparison of the municipal Log_{10}-AGp averages at baseline, midterm, final p<0.01)
	Parpatipur (53)	-0.778 ±0.193	-0.829 ± 0.222	-0.664 ± 0.152	
(inv.trans.) Albumin	Saidpur (47)	0.039 ± 0.009	0.050 ± 0.008	0.033 ± 0.008	F=2.108, p=NS
	Parpatipur (55)	0.042 ± 0.010	0.049 ± 0.011	0.036 ± 0.009	
C-reactive protein (non-parametric) CROSS-SECTIONAL)	Saidpur	Mean: 124.10 (n=119), Sum:18743.00	Mean: 124.84 (n=144), Sum:17977.00	Mean: 128.54 (n=144), Sum:18509.50	Baseline: M-W=7080.000,Z=-3.444, p<0.01 Midterm M-W=7537.000, Z=-0.041, p=NS Final= M-W=7914.5000,Z=-0.133, p=NS *M-W= Mann-Whitney test
	Parpatipur	Mean: 157.50 (n=157), Sum:19483.00	Mean: 125.22 (n=105), Sum:13148.00	Mean: 127.30 (n=111), Sum:14130.50	
Height-for-age	Saidpur (92)	-1.989 ± 1.043	-2.339 ± 1.285	-2.114 ± 1.116	F=0.313, p=NS
	Parpatipur (75)	-1.659 ± 1.241	-1.742 ± 1.280	-1.777 ± 1.176	
Weight-for-age	Saidpur (101)	-2.812 ± 1.241	-2.830 ± 1.392	-2.470 ± 1.135	F=0.474, p=NS
	Parpatipur (77)	-2.223 ± 1.395	-2.196 ± 1.455	-2.005 ± 1.337	
Weight-for-height	Saidpur (78)	-1.855 ± 1.223	-1.695 ± 1.465	-1.377 ± 0.932	F=2.958, p=NS
	Parpatipur (56)	-1.468 ± 1.254	-1.661 ± 1.525	-1.417 ± 1.211	

Table 6.9: SES effect on the growth parameters, haemoglobin and the selected biomarkers of immunity (age included as a covariate)

Variable	SES (n)	Baseline mean±SD	Mid-term mean±SD	Final mean±SD	Statistics
Haemoglobin	Low (SES1-3) (95)	104.5 ± 12.2	106.1 ± 12.4	109.9 ± 14.0	F=2.401, p=NS
	High (SES4-5) (60)	107.1 ± 14.5	106.4 ± 13.8	114.1 ± 13.1	
Log_{10}-α-acidglycoprotein	Low (SES1-3) (75)	-0.735 ± 0.185	-0.857 ± 0.186	-0.655 ± 0.146	F=1.005, p=NS
	High (SES4-5) (43)	-0.773 ± 0.173	-0.850 ± 0.184	-0.710 ± 0.171	
(inv.trans.)Albumin	Low (SES1-3) (61)	0.042 ± 0.010	0.049 ± 0.010	0.035 ± 0.009	F=0.557, p=NS
	High (SES4-5) (41)	0.039 ± 0.008	0.050 ± 0.010	0.034 ± 0.007	
C-reactive protein (non-parametric) (CROSS-SECTIONAL)	Low (SES1-3)	Mean: 146.72 (n=163), Sum=23915.00	Mean: 129.83 (n=144), Sum=12430.00	Mean: 138.31 (n=152), Sum=21023.00	Baseline: M-W=7870.00, Z=-2.054, p=NS Midterm: M-W=6865.000, Z=-1.238, p=NS, Final: M-W=6261.00, Z=-2.712, p<0.01
	High (SES4-5)	Mean: 126.62 (n=113), Sum=14311.00	Mean: 118.38 (n=105), Sum=12430.00	Mean: 112.79 (n=103), Sum=11617.00	
Height-for-age	Low (SES1-3) (94)	-1.929 ± 1.180	-2.189 ± 1.326	-2.087 ± 1.164	F=0.071, p=NS
	High (SES4-5) (73)	-1.726 ± 1.093	-1.917 ± 1.290	-1.841 ± 1.136	
Weight-for-age	Low (SES1-3) (178)	-2.622 ± 1.441	-2.619± 1.515	-2.362 ± 1.294	F=0.113, p=NS
	High (SES4-5) (76)	-2.470 ± 1.190	-2.470 ± 1.364	-2.144 ± 1.172	
Weight-for-height	Low (SES1-3) (72)	-1.649 ± 1.408	-1.531 ± 1.506	-1.359 ± 1.176	F=0.207, p=NS
	High (SES4-5) (59)	-1.701± 1.025	-1.666 ± 1.211	-1.363 ± 0.856	

Table 6.10: Average monthly income and the growth parameters, haemoglobin and the selected biomarkers of immunity (repeated measures ANOVA)

Variable	Average. Income (n)	Baseline mean±SD	Mid-term mean±SD	Final mean±SD	Statistics
Haemoglobin	Low<TK3000 (95)	104.4 ± 13.3	105.3 ± 13.1	110.2 ± 13.6	F=0.204, p=NS
	High>TK3000 (50)	107.3 ± 12.8	107.7 ± 12.4	113.8 ± 13.9	
Log_{10}-α-acidglycoprotein	Low<TK3000 (79)	-0.728 ± 0.177	-0.854 ± 0.183	-0.663 ± 0.157	F=0.934, p=NS
	High>TK3000 (39)	-0.788 ± 0.180	-0.856 ± 0.190	-0.698 ± 0.158	
(inv.trans.) Albumin	Low<TK3000 (63)	0.040 ± 0.010	0.050 ± 0.010	0.035 ± 0.008	F=0.531, p=NS
	High>TK3000 (39)	0.041 ± 0.008	0.049 ± 0.007	0.034 ± 0.009	
C-reactive protein (non-parametric) (CROSS-SECTIONAL)	Low<TK3000	Mean:137.15 (n=165), sum: 22630.00	Mean:130.15 (n=146), sum: 19002.00	Mean:131.84 (n=155), sum: 20435.50	Baseline: M-W=8935.00, Z=-0.340, p=NS Midterm: M-W=6767.00, Z=-1.344, p=NS Final: M-W=7154.500, Z=-1.030, p=NS
	High>TK3000	Mean:140.50 (n=111), sum: 15596.00	Mean:117.70 (n=103), sum: 12123.00	Mean:122.05 (n=100), sum: 12204.50	
Height-for-age	Low<TK3000 (102)	-1.872 ± 1.376	-2.185 ± 1.401	-2.099 ± 1.181	
	High>TK3000 (65)	-1.792 ± 1.162	-1.892 ± 1.150	-1.791 ± 1.095	F=1.187, p=NS
Weight-for-age	Low<TK3000 (108)	-2.608 ± 1.381	-2.597 ± 1.370	-2.340 ± 1.204	F=0.101, p=NS
	High>TK3000 (70)	-2.478 ± 1.275	-2.491 ± 1.574	-2.159 ± 1.306	
Weight-for-height	Low<TK3000 (83)	-1.666 ± 1.317	-1.589 ± 1.313	-1.336 ± 1.088	
	High>TK3000 (48)	-1.694 ± 1.126	-1.595 ± 1.497	-1.402 ± 0.982	F=0.053, p=NS

Table 6.11: Ethnicity effect on growth, haemoglobin and the selected biomarkers of immunity using repeated measures ANOVA

Variable	Ethnicity (n)	Baseline mean±SD	Mid-term mean±SD	Final mean±SD	Statistics
Haemoglobin	Bangla (97)	106.9 ± 11.9	107.0 ± 11.9	110.4 ± 14.0	F=3.944, p<0.05, Post hocs: baseline and final surveys (p<0.01) but no ethnic effect (p=NS)
	Bihari (58)	103.2 ± 14.7	104.8 ± 14.4	112.9 ± 13.5	
Log_{10}-α-acidglycoprotein	Bangla (73)	-0.776 ± 0.179	-0.851 ± 0.197	-0.668 ± 0.143	F=1.115, p=NS
	Bihari (45)	-0.718 ± 0.179	-0.861 ± 0.165	-0.686 ± 0.179	
(inv.trans.)Albumin	Bangla (68)	0.042 ± 0.010	0.049 ± 0.010	0.035 ± 0.008	F=1.930 p=NS
	Bihari (34)	0.038 ± 0.008	0.051 ± 0.008	0.034 ± 0.009	
C-reactive protein (non-parametric) (CROSS-SECTIONAL)	Bangla	Mean:154.73 (n=160), sum:24757.50	Mean:127.71 (n=142), sum:18135.00	Mean:125.53 (n=154), sum:19331.50	Baseline: M-W=6682.500, Z=-3.968, p<0.01 Midterm: M-W=7212.00, Z=--0.684, p=NS Final: M-W=7396.50, Z=--0.661, p=NS
	Bihari	Mean:1116.11 (n=116), sum:13468.50	Mean: 121.40, (n=107), sum: 12990.00	Mean:131.77 (n=101), sum:13308.50	
Height-for-age	Bangla (97)	-1.587 ± 1.103	-1.807 ± 1.323	-1.788 ± 1.126	F=2.952, <0.055
	Bihari (70)	-2.192 ± 1.115	-2.436 ± 1.218	-1.244 ± 1.150	
Weight-for-age	Bangla (102)	-2.314 ± 1.394	-2.361 ± 1.553	-2.151 ± 1.334	F=9.510, p<0.01
	Bihari (76)	-2.883 ± 1.192	-2.816 ± 1.262	-2.427 ± 1.102	
Weight-for-height (WHZ)	Bangla	Not enough data available for analysis			
	Bihari				

Table 6.12: Effect of maternal education on child growth and immunity using repeated measures ANOVA

Variable	Education (N)	Baseline mean±SD	Mid-term mean±SD	Final mean±SD	Statistics
Haemoglobin	No (76)	104.7 ± 12.4	104.6 ± 12.6	110.5 ± 12.4	F=0.250, p=NS
	Some (79)	106.3 ± 13.8	107.7 ± 13.1	112.5 ± 15.3	
Log_{10}-α-acidglycoprotein	No (65)	-0.732 ± 0.186	-0.819 ± 0.160	-0.678 ± 0.149	F=1.891, p=NS
	Some (53)	-0.768 ± 0.171	-0.898 ± 0.204	-0.671 ± 0.169	
(inv.trans.) Albumin	No (48)	0.042 ± 0.010	0.049 ± 0.010	0.033 ± 0.010	F=0.964, p=NS
	Some (54)	0.040 ± 0.009	0.050 ± 0.009	0.036 ± 0.009	
C-reactive protein (non-parametric) (CROSS-SECTIONAL)	No	Mean: 141.53 (n=146), sum:20664.00	Mean: 130.82 (n=131), sum:17137.00	Mean: 134.43 (n=135), sum:18148.00	Baseline: M-W:9047.00, Z=-0.668, p=NS Midterm: M-W:6967.00, Z:-1.343, p=NS Final: M-W: 7232.00, Z: -1.477, p=NS
	Some	Mean: 135.09 (n=130), sum:17562.00	Mean: 118.54 (n=118), sum:13988.00	Mean: 120.77 (n=120), sum:14492.00	
Height-for-age	No (90)	-2013 ± 1.064	-2.099 ± 1.243	-2.145± 1.108	F=1.814, p=NS
	Some (77)	-1.639 ± 1.207	-2.037 ± 1.399	-1.786 ± 1.185	
Weight-for-age	No (98)	-2.712 ± 1.327	-2.599 ±1.397	-2.326 ± 1.141	F=1.150, p=NS
	Some (80)	-2.368 ± 1.336	-2.502 ± 1.519	-2.199 ± 1.365	
Weight-for-height	No (76)	-1.668 ± 1.240	-1.637 ± 1.426	-1.286 ± 1.010	F=1.120, p=NS
	Some (55)	-1.679 ± 1.265	-1.529 ± 1.318	-1.465 ± 1.082	

Table 6.13: The effect of stove type on child growth and immunity using repeated measures ANOVA

Variable	Subgroup (n)	Baseline mean±SD	Mid-term mean±SD	Final mean±SD	Statistics
Haemoglobin	Fixed (41)	99.4±12.9	101.5±13.4	109.6±13.5	F=2.496, p<0.086
	Portable/mix (110)	107.9±12.6	107.9±12.5	112.4±13.9	
Log_{10}-α-acidglycoprotein	Fixed (30)	-0.709±0.157	-0.800±0.186	-0.746±0.133	F=6.894, p=0.001
	Portable/mix (86)	-0.752±0.177	-0.872±0.182	-0.652±0.160	
(inv.trans.) Albumin	Fixed (27)	0.042±0.008	0.050±0.010	0.037±0.009	F=0.356, p=NS
	Portable/mix (75)	0.040±0.009	0.051±0.019	0.034±0.010	
C-reactive protein (non-parametric) (CROSS-SECTIONAL)	Fixed	Mean: 134.84 (n=70), sum:9438.50	Mean: 119.14 (n=71), sum:8459.00	Mean: 134.42 (n=71), sum:9544.00	Baseline: M-W=6953.500, Z=-0.386, p=NS, Midterm: M-W=5903.00, Z=-0.679, p=NS, Final: M-W=6076.00, Z=-0.864, p=NS
	Portable/mix	Mean: 139.08 (n=205), sum:2811.50	Mean: 125.96 (n=176), sum:22169.00	Mean: 125.52 (n=184), sum:23096.00	
Height-for-age	Fixed (47)	-1.766±1.306	-2.206±1.543	-1.882±1.221	F=0.266, p=NS
	Portable/mix (119)	-1.873±1.083	-2.026±1.216	-2.021±1.135	
Weight-for-age	Fixed (53)	-2.600±1.134	-2.551±1.429	-2.208±1.120	F=0.262, p=NS
	Portable/mix (124)	-2.539±1.475	-2.570±1.475	-2.327±1.330	
Weight-for-height	Fixed (37)	-1.539±1.277	-1.662±1.158	-1.435±0.838	F=0.256, p=NS

Table 6.14: The effect of cooking frequency on child health and growth using repeated measures ANOVA

Variable (n)	Present (n)	Baseline mean±SD	Mid-term mean±SD	Final mean±SD	Statistics
Haemoglobin (102)	Once (n=45)	107.7±10.5	107.4±12.1	110.3±14.7	F=0.671, p=NS
	More than once (57)	104.6±13.8	104.6±12.5	110.9±12.3	
Log_{10}-α-acidglycoprotein (75)	Once (35)	-0.752±0.177	-0.829±0.217	-0.665±0.138	F=1.337, p=NS
	More than once (40)	-0.736±0.176	-0.899±0.159	-0.673±0.161	
(inv.trans.) Albumin (67)	Once (35)	0.042±0.009	0.050±0.011	0.035±0.009	F=0.193, p=NS
	More than once (32)	0.040±0.0108	0.050±0.006	0.036±0.011	
C-reactive protein (non-parametric) (CROSS-SECTIONAL)	Once	Mean: 90.92 (n=77), sum:7001.00	Mean:83.46 (n=68), sum:5675.00	Mean:84.34 (n=74), sum:6241.00	Baseline: M-W=3779.00, Z=-0.321, p=NS Midterm: M-W=3131.00, Z=-0.333, p=NS, Final: M-W=3268.00, Z=-0.324, p=NS
	More than once	Mean: 88.42 (n=101), sum:8930.00	Mean:80.96 (n=95), sum:7691.00	Mean:81.91 (n=91), sum:7454.00	
Height-for-age (105)	Once (49)	-1.831±1.207	-1.853±1.246	-1.819±1.327	F=0.503, p=NS
	More than once (56)	-1.909±1.109	-2.901±1.309	-1.980±1.131	
Weight-for-age (117)	Once (53)	-2.403±1.477	-2.413±1.528	-2.037±1.408	F=0.020, p=NS
	More than once (64)	-1.783±1.243	-2.773±1.378	-2.442±1.274	
Weight-for-height (80)	Once (34)	-1.479±1.423	-1.669±1.157	-1.277±1.274	F=1.884, p=NS
	More than once (46)	-1.883±1.133	-1.416±0.994	-1.980±1.131	

Table 6.15: The effect of a child's presence near the stove during cooking on growth parameters, haemoglobin and the selected biomarkers of immunity using repeated measures ANOVA

Variable (n)	Present (n)	Baseline mean±SD	Mid-term mean±SD	Final mean±SD	Statistics
Haemoglobin (130)	Yes (55)	103.9±13.3	103.4±12.6	108.6±13.0	F=0.927, p=NS
	No (75)	105.3±13.6	107.4±13.7	112.6±14.6	
Log_{10}-α-acidglycoprotein (104)	Yes (50)	-0.720±0.169	-0.845±0.181	-0.657±0.142	F=0.816, p=NS
	No (54)	-0.775±0.160	-0.853±0.176	-0.701±0.168	
(inv.trans.) Albumin (85)	Yes (36)	0.041±0.009	0.050±0.011	0.035±0.011	F=0.579, p=NS
	No (49)	0.041±0.008	0.049±0.008	0.035±0.009	
C-reactive protein (non-parametric) (CROSS-SECTIONAL)	Yes	Mean: 122.28 (n=115), sum:14062.00	Mean:116.49 (n=100), sum:11649.00	Mean:107.49 (n=111), sum:11931.00	Baseline: M-W=6983.00, Z=-0.381, p=NS Midterm: M-W=4901.00, Z=-1.866, p=NS, Final: M-W=5715.00, Z=-0.931, p=NS
	No	Mean:118.86 (n=125), sum:14858.00	Mean:100.62 (n=115), sum:11571.00	Mean:115.51 (n=111), sum:12822.00	
Height-for-age (143)	Yes (62)	-1.836±0.979	-2.246±1.276	-2.047±1.153	F=2.383, p=NS
	No (81)	-1.948±1.129	-1.976±1.251	-1.978±1.152	
Weight-for-age (156)	Yes (69)	-2.577±1.265	-2.650±1.550	-2.237±1.265	F=0.628, p=NS
	No (87)	-2.581±1.290	-2.551±1.317	-2.355±1.130	
Weight-for-height (116)	Yes (47)	-1.794±1.265	-1.672±1.495	-1.270±1.108	F=1.926, p=NS
	No (69)	-1.572±1.212	-1.599±1.394	-1.432±1.001	

Table 6.16: The effect of cooking location on child growth and immunity

Variable (n)	Present (n)	Baseline mean±SD	Mid-term mean±SD	Final mean±SD	Statistics
Haemoglobin (151)	Indoors (60)	104.9 ± 14.4	105.4 ± 14.3	112.4 ± 12.9	F=0.799, p=NS
	Outdoors/mix (91)	105.8 ± 12.5	106.6 ± 12.1	111.2 ± 14.4	
Log_{10}-α-acidglycoprotein (116)	Indoors (51)	-0.735 ± 0.176	-0.850 ± 0.174	-0.710 ± 0.156	F=1.993, p=NS
	Outdoors/mix (65)	-0.746 ± 0.171	-0.856 ± 0.194	-0.646 ± 0.155	
(inv.trans.)Albumin (102)	Indoors (38)	0.040 ± 0.009	0.055 ± 0.024	0.037 ± 0.009	F=2.040, p=NS
	Outdoors/mix (64)	0.041 ± 0.008	0.048 ± 0.009	0.034 ± 0.010	
C-reactive protein (non-parametric) (CROSS-SECTIONAL)	Indoors	Mean: 134.78 (n=112), sum:15095.50	Mean:108.21 (n=100), sum:10821.00	Mean: 129.04 (n=100), sum:12904.00	Baseline: M-W=8767.50, Z=-0.556, p=NS Midterm: M-W=5771.00, Z=-2.865, p<0.01, Final: M-W=7646.00, Z=-0.181, p=NS
	Outdoors/mix	Mean:140.21 (n=163), sum:22854.50	Mean:134.74, (n=147), sum:198707.00	Mean:127.33 (n=155), sum:19736.00	
Height-for-age (166)	Indoors (70)	-1.769 ± 1.065	-2.056 ±1.381	-1.842 ± 1.205	F=0.923, p=NS
	Outdoors/mix (96)	-1.897 ± 1.206	-2.092 ± 1.271	-2.084 ± 1.117	
Weight-for-age (177)	Indoors (75)	-2.600 ± 1.134	-2.551±1.429	-2.208 ± 1.120	F=0.644, p=NS
	Outdoors/mix (102)	-2.539 ± 1.475	-2.570 ± 1.475	-2.327 ± 1.330	
Weight-for-height (130)	Indoors (59)	-1.665 ± 1.000	-1.537 ±1.178	-1.285 ± 0.832	F=0.241, p=NS
	Outdoors/mix (71)	-1.701 ± 1.419	-1.644 ± 1.538	-1.443 ± 1.184	

Table 6.17. Bivariate analysis of the determinants of change score for weight-for-age

Variable	Subgroup (n)	Mean±SD	Statistics
Municipality	Saidpur (89)	0.247±0.481	F=0.154, p=NS (age F=6.885, p<0.05) (baseline WAZ F=11.102, p<0.05)
	Parpatipur (67)	0.215±0.458	
SES	Low (SES1-3) (92)	0.244±0.580	F=0.024, p=NS (age F=6.823, p<0.05) (baseline WAZ F=11.130, p<0.05)
	High (SES4-5) (64)	0.218±0.390	
Average income	Low <3000TK (99)	0.246±0.482	F=0.053, p=NS (age F=6.663, p<0.05) (baseline WAZ F=11.143, p<0.05)
	High >3000TK (57)	0.211±0.452	
Ethnicity	Bangla (90)	0.233±0.473	F=0.351, p=NS (age F=6.674, p<0.05) (baseline WAZ F=11.472, p<0.05)
	Bihari (66)	0.234±0.469	
Maternal education	No (85)	0.277±0.474	F=1.127, p=NS (age F=7.019, p<0.05) (baseline WAZ F=10.404, p<0.05)
	Some (71)	0.181±0.463	
Child present by the stove during cooking	Yes (58)	0.281±0.461	F=0.029, p=NS, (age F=6.007, p<0.05) (baseline WAZ F=19.398, p<0.05)
	No (79)	0.208±0.479	
Cooking location	Indoors (64)	0.255±0.470	F=0.366, p=NS (age F=6.685, p<0.05) (baseline WAZ F=11.410, p<0.05)
	Switchers (91)	0.215±0.474	
Fuel type	Wood (31)	0.159±0.491	F=3.087, p<0.05 (age F=9.842, p<0.05) (baseline WAZ F=1.563, p=NS)
	Golden (21)	0.341±0.524	
	Dung (16)	0.348±0.444	
Cooking frequency	Once (46)	0.0403±0.407	F=5.779, p<0.05 (age F=5.055, p<0.05) (baseline WAZ F=3.133, p=NS)
	More than once (56)	0.211±0.477	
Stove type	Fixed (all)	0.210±0.419	F=0.603, p=NS, (age F=6.703, p<0.05) (baseline WAZ F=11.220, p<0.05)
	Switchers	0.237±0.429	

Table 6.18: Description of socio-demographic factors and cooking practices in terms of their suitability for the use in regression analysis

Variable	Variable type	Subgroup (reference *)	n	%	Statistical power OK	Multicollinearity
Age	Continuous	Youngest	-	-	YES	
		older	-	-		
Presence of a child by the stove during cooking	Dichtomous	Yes *	76	37.6	YES	
		Mixed behaviour	109	54.0		
Cooking frequency	Dichtomous	Once *	62	30.7	YES	Municipality
		More	72	35.6		
Municipality	Dichtomous	Saidpur *	109	54.0	YES	Cooking frequency and ethnicity
		Parbatipur	93	46.0		
Ethnicity	Dichtomous	Bangla *	119	58.9	YES	Municipality
		Urdu	83	41.1		
Maternal ducation	Dichtomous	Uneducated *	108	53.5	YES	
		Some schooling	94	46.5		
Stove type	Dichtomous	Fixed throughout *	55	27.2	YES	
		Mixed behaviour	147	72.8		
Cooking location	Dichtomous	Indoors throughout *	82	40.6	YES	Municipality
		Mixed behaviour and outdoors	120	59.4		
SES	Dichtomous	Low (SES1-3) *	118	58.4	YES	
		High (SES4-5)	84	41.6		
Average income	Dichtomous	Low income <TK3000 *	122	60.4	YES	
		High income >3000	80	39.6		
Total			202	100.0		

Table 6.19: Regression analysis of socio-demographic determinants and cooking practices which may affect the height-for-age z-scores at the baseline and the final surveys

DV	Survey	R	R²	F	p	Model	Reference category	Standardised Beta	t	p	Collinearity Statistics	
Height-for-age	Baseline (167)	0.329	0.109	4.930	0.001	(Constant)			-3.747	0.001	Tolerance	VIF
		Age					-	-0.099	-1.306	NS	0.950	1.053
		Ethnicity					Bangla	-0.270	-3.532	0.001	0.942	1.061
		SES					Low	0.142	1.810	0.072	0.891	1.122
		Maternal education					Uneducated	0.103	1.351	NS	0.946	1.057
	Final (92)	0.342	0.117	2.889	0.05	(Constant)			3.667	0.001		
		Age					-	0.245	2.285	0.05	0.883	1.132
		Maternal education					Uneducated	0.219	2.160	0.05	0.986	1.014
		Child present by the stove during cooking					Present	-0.005	-0.051	NS	0.895	1.118
		Cooking frequency					Once	-0.173	-1.689	0.095	0.970	1,031

Table 6.20: Regression analysis of socio-demographic determinants and cooking practices which may affect weight-for-age z-scores at the baseline and the final surveys

DV	Survey (n)	R	R^2	F	p	Model	Reference category	Standardised Beta	t	p	Collinearity Statistics	
Weight-for-age		0.261	0.068	3.150	0.05	(Constant)			-3.559	0.001	Tolerance	VIF
		Age					-	0.010	0.139	NS	0.953	1.049
		Ethnicity					Bangla	-0.247	-3.279	0.001	0.954	1.049
	Baseline (167)	SES					Low	0.105	1.367	NS	0.926	1.080
		Stove type					Fixed	-0.093	-1.253	NS	0.983	1.017
	Final (92)	No significant predictors could be found										

Table 6.21: Regression analysis of socio-demographic factors and cooking practices which may affect the changes in child growth z-scores between baseline and final surveys

DV	Survey	R	R^2	F	p	Model	Reference category	Standardised Beta	t	p	Collinearity Statistics	
Change score weight-for-age	Baseline to final	0.391	0.153	3.886	0.01	(Constant)			-2.051	0.05	Tolerance	VIF
		Age					-	-0.247	-2.386	0.05	0.917	1.091
		Cooking frequency					Once	-0.212	-2.096	0.05	0.959	10.43
		Child present by stove during cooking					Present	0.090	0.866	NS	0.918	1.090
		Baseline weight-for-age z-score					Baseline	-0.217	-2.134	0.05	0.953	1.049

Table 6.22: Linear regression model of predictors of haemoglobin (baseline and final surveys)

DV	Survey (n)	R	R^2	F	p	Model	Reference category	Standardised Beta	t	p<	Collinearity statistics	
Haemo globin	Baseline (165)	0.558	0.312	18.127	0.001	Constant			17.835	0.001	Tolerance	VIF
		Age					-	-0.204	-3.099	0.01	0.994	1.006
		Ethnicity					Bangla	0.440	6.517	0.01	0.945	1.058
		Stove type					Fixed	0.263	3.961	0.01	0.979	1.022
		Child present by stove during cooking					Yes	-0.007	-0.101	NS	0.939	1.065
	Final (145)	0.397	0.158	6.556	0.001	Constant			17.628	0.001		
		Age					0	0.316	3.928	0.001	0.931	1.074
		Maternal education					Uneducated	0.186	2.396	0.05	0.998	1.002
		Stove type					Fixed	0.089	1.133	NS	0.975	1.020
		Child present by stove during cooking					Yes	0.088	1.083	NS	0.915	1.092

Table 6.23: Regression analysis of socio-demographic factors and cooking practices which may affect the Log-transformed alpha-1-acidglycoprotein status of children

DV	Survey (n)	R	R^2	F	p	Model	Reference category	Standardised Beta	t	p	Collinearity Statistics	
Log-$_{10}$-alpha-1-acid glycoprotein	Baseline (154)	0.290	0.084	3.465	0.001	(Constant)			10.316	0.001	Tolerance	VIF
		Age						-0.111	-1.395	NS	0.949	1.053
		Maternal education					Uneducated	-0.200	-2.558	0.05	0.983	1.017
		Municipality					Saidpur	-0.143	-1.829	0.069	0.983	1.017
		Child present by the stove during cooking					Present	-0.061	-0.768	NS	0.949	1.054
	Final (109)	0.302	0.091	2.612	0.05	(Constant)			-8.879	0.001		
		Age					0	-0.117	-1.221	NS	0.958	1.044
		Stove type					Fixed	0.200	2.076	0.05	0.940	1.064
		Cooking location					Indoors	0.118	1.184	NS	0.873	1.164
		Cooking frequency					Once	-0.048	-0.496	NS	0.951	1.052

Table 6.24: Repeated measures test of the levels of haemoglobin and the selected biomarkers of immunity and the need for medical check

Variable (n)	Medical requested (n)	Baseline mean±SD	Mid-term mean±SD	Final mean±SD	Statistics
Haemoglobin (152)	Yes (72)	103.5±13.6	104.8±12.8	109.3±14.4	F=0.711, p=NS, no age effect seen
	No (80)	107.3±12.6	107.5±13.1	114.0±13.0	
Log_{10}-α-acidglycoprotein (116)	Yes (54)	-0.746±0.157	-0.840±0.200	-0.639±0.171	F=1.467, p=NS, no age effect seen
	No (62)	-0.737±0.187	-0.864±0.171	-0.708±0.140	
(inv.trans.) Albumin (102)	Yes (53)	0.040±0.09	0.049±0.010	0.035±0.008	F=0.146, p=NS, age-effect F=4.262, p<0.05
	No (49)	0.041±0.09	0.052±0.022	0.036±0.011	
C-reactive protein (non-parametric) (CROSS-SECTIONAL)	Yes	Mean: 140.63 (n=170), sum:23907.00	Mean: 124.77 (n=158), sum:19464.00	Mean: 116.30 (n=146), sum:16979.50	Baseline: M-W=308.00, Z=-0.836, p=NS Midterm: M-W=7134.00, Z=-0.077, p=NS Final: M-W=6248.50, Z=-2.825, p<0.01 *M-W=Mann-Whitney
	No	Mean: 132.38 (n=104), sum:13768.00	Mean: 124.04 (n=92), sum:11412.00	Mean; 142.64 (n=108), sum:15405.50	
Height-for-age (167)	Yes (74)	-1.745±1.174	-2.223±1.371	-1.966±1.174	F=1.196, p=NS, age-effect 15.035, p<0.01
	No (93)	-1.916±1.121	-1.949±1.260	-1.997±1.137	
Weight-for-age (177)	Yes (79)	-2.500±1.403	-2.540±1.439	-2.227±1.208	F=0.043, p=NS, age-effect 5.804, p<0.01
	No (98)	-2.615±1.290	-2.581±1.468	-2.316±1.277	
Weight-for-height (131)	Yes (53)	-1.669±1.317	-1.377±1.602	-1.356±1.177	F=1.628, p=NS, no age-effect
	No (78)	-1.675±1.204	-1.737±1.189	-1.365±0.945	

Table 6.25: Socio-demographic determinants and cooking practices within child health groups (healthy vs. other illness)

Variable	Subgroup	Healthy % (n)	Other illness % (n)	Statistical test
Municipality	Saidpur	37.5 (15)	66.6 (62)	X^2=9.296, p<0.01
	Parpatipur	62.5 (25)	34.0 (32)	
Ethnicity	Bangla	67.5 (27)	48.9 (46)	X^2=3.899, p<0.05
	Bihari	32.5 (13)	51.1 (48)	
Maternal education	Uneducated	37.5 (15)	62.8 (59)	X^2=7.244, p<0.01
	Educated	62.5 (25)	37.2 (35)	
Average income	Low <TK3000	55.0 (22)	61.7 (58)	X^2=0.524, p=NS
	High >TK3000	45.0 (18)	38.3 (36)	
SES	Low (SES1-3)	47.5 (18)	57.4 (54)	X^2=1.119, p=NS
	High (SES4-5)	52.5 (21)	42.6 (40)	
Cooking location	Indoors	35.9 (14)	40.4 (38)	X^2=0.237, p=NS
	Other	64.1 (25)	59.6 (56)	
Stove type	Fixed	17.9 (7)	28.7 (27)	X^2=1.628, p=NS
	Other	82.1 (32)	71.3 (67)	

Table 6.26: Description of subgroups of the socio-demographic determinants and cooking an fuel use practice for children with RTI and those with another condition

Predictors	Subgroups (reference marked with *)	Baseline	%	Final	%
Stove type	Fixed *	17	23.3	13	18.3
	Portable or fixed	56	76.7	58	81.7
Municipality	Saidpur*	39	53.4	35	49.3
	Parbatipur	34	46.6	36	50.7
Ethnicity	Bangla *	47	64.4	47	66.2
	Urdu	26	35.6	24	33.8
Maternal education	No Schooling *	36	49.3	33	46.5
	Some schooling	37	50.7	38	53.5
Average Income	Low income <TK3000 *	49	67.1	50	70.4
	High income >3000	24	32.9	21	29.6
SES	Low (SES1-3) *	47	64.4	45	63.4
	High SES (4-5)	26	35.6	26	36.6
Cooking location	Indoors *	30	41.1	31	43.7
	Mixed	43	58.9	40	56.3
Child's presence by the stove during cooking	YES *	42	57.5	38	53.5
	Switchers from Yes to No	31	42.5	33	46.5
Cooking frequency	Once *	34	46.6	37	52.1
	More	39	53.4	34	47.9
Total	Total	73		71	

Table 6.27: Predictors of the risk of RTI: a comparison of healthy (no medical diagnosis) versus RTI

Survey (n)	Binary log. regression	Category	B	Wald	p-value	Odds-ratio	95% CI
Baseline (76)	Child present during cooking	Present *				1.000	
		Absent	-1.360 ± 0.494	7.580	0.01	0.257	$0.097 < X < 0.676$
	Model statistics	Cox & Snell R^2 =0.100	-2 Log likelihood = 95.485	Nagelkerke R^2 =0.134			
Final (61)	No significant predictors could be identified. Very small sample sixe reduced the efficiency of this regression analysis too.						

Chapter 7: Household fuel pollution exposure.

7.1. Introduction

Cooking practices and the presence of a child during cooking were shown to be associated with higher/lower haemoglobin and log10-alpha-1-acidglycoprotein and the prevalence of respiratory infection in this sample. These analyses however employed indirect indicators of household fuel pollution rather than quantitative analysis of air pollutants.

Chapter 7 will describe the quantified particulate matter (size under 2.5µm) and carbon monoxide levels in households selected for indoor air quality testing. This chapter aims to examine which variables are associated with household fuel pollution levels. Child health status and the prevalence of respiratory tract infections will be examined in relation to the household fuel pollutant levels in order to quantify the impact of household fuel pollution on child health and the prevalence of respiratory specific ailments among children in this sample. Lastly this chapter will describe a pilot intervention programme set up in the study sample after the baseline survey, and comment on the uptake of community-based technical and educational intervention to reduce household fuel pollution.

7.2. Household fuel pollution monitoring

A total of 625 household were selected for this field study in northern Bangladesh. Almost ten percent (9.03%) of the total households were randomly selected for air quality monitoring. The sample size was determined as a convenience sample for air quality monitoring in order to maximise data and minimise the cost of air quality analyses. The air quality households represented the overall study sample as well as possible in terms of sample characteristics (household structure and building materials, ventilation methods, fuel use and cooking practices). The sample size for households which were recruited for the air quality monitoring was limited by the high cost of air quality monitoring and the measurement of particulate matter levels in

particular (15 pounds per filter with two filters used per one household for particulate matter measurement).

Real-time (minute-by minute) carbon monoxide and accumulative 12-hour particulate matter (size less than 2.5µm in diameter) were measured in 67 households during the day and night in the kitchen and living room (where applicable) (details of equipment and methodology used provided in Chapter 3). The data was divided into daytime (7am to 7pm) and night-time (7pm to 7am). Personal carbon monoxide exposure of the cook was measured as an accumulative 12-hour value in order to estimate the quantity of carbon monoxide inhaled by the cook. The distribution of particulate matter during the daytime (kitchen and living room) was non-normal but after $\log_{10}$-transformation these displayed normal distribution. Data distribution of <u>night-time</u> particulate matter and carbon monoxide (<u>day and night</u>) data were also non-normal and did not display normal distribution after applying all transformation techniques. The non-normal distribution was due to a high number of zero readings. Consequently, this data was analysed using non-parametric tests. For the data analysis all households selected for air quality monitoring with or without children under the age of five present which could be matched with the background data were included once (n=61). For child health analyses pollution data was available in n=57 of households, with a child or children under the age of five present. Only the youngest child was included in the analyses.

7.2.1. Mean household pollution levels (day and night)

Daytime kitchen carbon monoxide levels ranged from zero to 16 ppm and night-time levels from 0 to 6ppm (Table 7.1, p.217). The range of carbon monoxide levels was similar in both the kitchen and living room during the day (both zero to 16ppm). Night-time carbon monoxide levels in the living room were lower than those observed in the kitchen (0 to 4ppm). Much greater variation was observed in the household particulate matter ($PM_{2.5}$) levels, both during the day and night. During the day the kitchen $PM_{2.5}$ levels ranged from 85 to 3020µg/m^3. The night-time $PM_{2.5}$ levels in both kitchen and living room were less than a third of the measured daytime levels

(Table 7.1, p.217). The World Health Organisation's recommendations for safe particulate matter ($PM_{2.5\mu m}$) mean exposure is $25\mu g/m^3$ over a period of 24-hours (**WHO, 2005**) and mean 8-hour carbon monoxide was set as 9ppm (**US-EPA, 1997, 2006**). Personal exposure to carbon monoxide was measured as an accumulative value over a period of 12 hours during the day. The accumulative personal carbon monoxide levels experienced by the cooks ranged from 10 to 100ppm for a period of 12 hours.

Table 7.2 (p.218) shows the mean untransformed carbon monoxide and particulate matter levels for kitchens in all houses, and in living rooms in those houses where a separate living room was identified. Some of the study households were one-room (n=28) houses where kitchen and living room were within the same space. In those houses only the kitchen pollution levels were measured. Separate kitchen and living room pollution levels were measured in households with separate kitchen and living room (two room houses n=31) (data was missing on the room number in two households). Table 7.3 (p.219) lists the geometric means calculated for the carbon monoxide and particulate matter pollution levels in the kitchen and living room for day and night. Mean daytime kitchen carbon monoxide was 31.269ppm as compared with 2.161ppm at night. The living room mean values were 6.495ppm and 2.033ppm respectively. The same pattern was observed for the particulate matter levels for day and night.

7.2.2. Patterns of real time carbon monoxide (untransformed) levels and household structure

Figures 7.1 and 7.2 (p.220) illustrate the carbon monoxide levels in one-roomed households. These figures illustrate that the day and night-time carbon monoxide levels varied considerably between households despite having the same structure (one-roomed) and the same cooking frequency. Figures 7.3 to 7.8 (p.220-1) show the carbon monoxide levels between the kitchen and the living room in two-room households.

In two-roomed households carbon monoxide levels were lower in the living room than in the kitchen in some cases (e.g. Figure 7.3 vs. Figure 7.4 (p.220), and Figure 7.5 vs. Figure 7.6 (p.221)). However Figures 7.7 and 7.8 (p.221) show that there were exceptions with some households showing high carbon monoxide levels in the living room than in the kitchen. The factors affecting the distribution of the pollutants will be examined in detail later in this chapter.

7.2.2.1. Comparison of kitchen and living room pollution levels in two-room houses

The levels of household fuel pollutants were examined between the kitchen and living room between day and night in households with two rooms (n=31) in order to examine the spread of the pollution within the households. Log_{10}-transformed particulate matter levels were significantly higher in the kitchens than living rooms during the day (paired t=3.887, p<0.01, n=31) (Table 7.4, p.222). A non-parametric Wilcoxon signed rank test showed that the night-time kitchen particulate matter levels were not significantly different from the living room levels (Z=-1.686, p=NS). The carbon monoxide levels were significantly higher in the kitchen than in the living rooms during the day and night (Z=-2.987, p<0.005, Z=-2.165, p=0.05 respectively).

7.2.2.2. Comparison of kitchen pollution levels in one-room and two-room houses

Comparative analyses were carried out between the kitchen levels in households with one-room only and those households with two rooms. No significant differences in the log-transformed daytime kitchen particulate matter levels could be detected on the basis of the household room number (t=-0.011, p=NS, log_{10}- $PM_{2.5 \text{ (one-room)}}$=2.589 ± 0.381, n=28, log_{10}- $PM_{2.5 \text{ (two room)}}$ =2.590± 0.278, n=31). Non-parametric t-tests showed no differences in the night-time kitchen levels of particulate matter between the one room and two room houses. No difference was detected in the kitchen carbon monoxide levels between one room and two room households during the day or night by using non-parametric tests. From this data it appears that household room number

had no impact on the kitchen particulate matter or carbon monoxide levels in this study sample.

7.2.2.3. Relationship between household carbon monoxide and particulate matter (untransformed) levels

The relationship between the real-time carbon monoxide and accumulative particulate matter levels in the kitchen and living room were examined using non-parametric Spearman's correlation (Table 7.5, p.222) and untransformed data. Daytime pollution levels in the kitchen and living room were highly correlated (r=0.830, p<0.001 and r=0.622, p<0.001 for carbon monoxide and particulate matter respectively). A significant but lower correlation was also found between the kitchen and living-room levels of the two pollutants during the night (r=0.270, p<0.05 and r=0.317, p<0.05 respectively).

7.2.2.4. Personal carbon monoxide exposure and daytime kitchen carbon monoxide exposure

An individual's personal pollution exposure (actual amount of particular pollutant passively inhaled by a person) may be very different from the ambient pollution levels, depending on proximity to the source and time spent near the source of pollution. The 12 hour accumulative personal carbon monoxide exposure ranged from 10 to 100ppm, giving a range of mean one-hour exposure as 0.833 to 8.33ppm (Table 7.1, p.217).

Daytime untransformed carbon monoxide levels measured in the kitchen were correlated with personal accumulative carbon monoxide exposure levels (untransformed) (Spearman correlation r=0.335, p<0.01, n=52). Figure 7.9 (p.223) shows the ranked daytime kitchen carbon monoxide data plotted against ranked personal one-hour carbon monoxide exposure. An examination of untransformed daytime kitchen particulate matter levels showed no significant association with the personal carbon monoxide (accumulative) exposure (R^2=0.205, p=NS, n=52). No association was found between the personal carbon monoxide exposure and mean

number of minutes by which household carbon monoxide levels exceeded the safety threshold of 9ppm.

7.2.3. Length of household fuel pollution exposure

Household carbon monoxide levels were recorded at every minute for a period of 24-hours. The length of carbon monoxide above the safety threshold of 9ppm for 8 hours by the US-EPA (1997, 2006) was tabulated. Most households were exposed to dangerous carbon monoxide (above 9ppm per 8 hours) for some time on a daily basis (Table 7.6, p.224).

7.2.3.1. Carbon monoxide exposure above the safety threshold

No households experienced carbon monoxide levels over 9ppm for longer than a period of 8 hours (Table 7.6, p.224). A fifth of the households were exposed to carbon monoxide above the safety threshold in the kitchen from two to four hours each day. 13.1% of the households experienced 1 to 2 hours of carbon monoxide above the safety threshold daily. Over half of the households experienced dangerous carbon monoxide levels from 15 minutes up to an hour, and an exposure up to 15 minutes accounted for a third of the households. The periods of dangerous carbon monoxide levels in the kitchen at night were much shorter: 20% of the households experienced dangerous pollution levels in the kitchen for longer than 15 min each night. In 7.2% of the households the dangerous pollution levels exceeded one hour at night. Little variation in the dangerous carbon monoxide pollution levels was observed in the living room from day to night. The carbon monoxide levels exceeded the 9ppm safety threshold in the living room in over 70% of the households for up to 15 min each night. However dangerous levels were experienced in the living room for over an hour at night in three cases. This data indicates a more significant health risk from kitchen carbon monoxide exposure than from the living room exposure.

7.2.3.2. Particulate matter pollution exposure exceeding the safety threshold

One hour accumulative particulate matter levels (12-hour accumulative particulate matter levels were divided by 12 hours in order to obtain an hourly accumulative value of particulate matter exposure for each hour of the measurement period) were used to group households into safe and dangerous exposure levels for periods of 12 hours (day and night) and 24-hours using the safety threshold of 25 $\mu g/m^3$ specified by the World Health Organisation as a cut-off for 24-hour exposure (**WHO, 2005**). Almost 65% of the households experienced dangerous particulate matter levels in the kitchen in the daytime for every hour of the day (Table 7.7, p.225). At night almost a fifth of the households were exposed to harmful levels of particulate matter levels in the kitchen. Daytime living room levels of particulate matter exceeded the safety threshold in a quarter of the households whereas at night this dropped down to 12.9%. As the World Health Organisation specifies the 25$\mu g/m^3$ threshold for a period of 24 hours the data was described for this period of time. Almost half (47.8%) of the households were exposed to dangerous particulate levels in their kitchens and over 19.4% of the households had dangerously high pollution levels in the living rooms. Particulate matter levels are likely to have a considerable adverse health impact on children in this community on the basis of the daily 24 hour exposure levels measured in kitchens and living rooms daily. Kitchen particulate matter levels are likely to have a stronger adverse impact on health in these households on the basis of the extensive particulate matter exposure measured in the household kitchens.

7.2.4. House structure and ventilation as the determinant of household fuel pollution levels

Households building materials were described for the whole study sample in Chapter 5 (Table 5.18, p.138). Most households in this subsample selected for air-quality monitoring had roofs made out of corrugated iron (85.9%). Other materials were thatch, bamboo and brick. In this air-quality subsample 80.7% of the households had some cracks and holes to ventilate the households, only one house reported having no ventilation holes. Windows in kitchens were uncommon: only 6 houses had windows,

73.7% did not, and data was missing for 9 cases. 66.7% of households reported the use of a door as the means of ventilation (Chapter 5, Table 5.19,p.138). Walls were built from bamboo in about a half of the households (49.1%) and a third of the houses had brick walls (28.1%). Also thatch (1.8%), corrugated iron sheets (8.8%) and clay (12.3%) were used. The wall type was regrouped into a binary variable (bamboo and thatch versus corrugated iron and brick clay) for the analysis. The levels of carbon monoxide and particulate matter in houses with walls made of different building material were compared.

The log-transformed daytime kitchen particulate matter levels in the kitchen were not significantly associated with the household wall material using the binary variable (t=-1.199, p=NS). A non-parametric t-test showed no significant effect of the wall material on the untransformed particulate matter levels in kitchens at night (Mann-Whitney U=339.0 Z=-1.070, p=NS), neither the untransformed carbon monoxide levels in the kitchen during daytime nor night-time (Mann-Whitney U=293.0 Z=-1.836, p=NS, Mann-Whitney U=388.0, Z=-0.322, p=NS). Daytime living room pollution levels were not affected by the wall material either, neither were: untransformed particulate matter (night): Mann-Whitney U=93.5, Z=-0.502, p=NS, untransformed carbon monoxide (day): Mann-Whitney U=73.0 Z=-1.440, p=NS, untransformed carbon monoxide (night) Mann-Whitney U=77.5, Z=-1.389, p=NS. No association was found between the wall material and the log-transformed kitchen volume either (t=-1.570, p=NS, n=48).

7.3. Sample characteristics, cooking- and fuel-use behaviour and the air-quality monitoring

The background characteristics and the cooking- and fuel-use practices are shown in Table 7.8 (p.226). The parameters highlighted in blue were selected as suitable for statistical analyses because of their sub-group sizes (n>10). The effect of municipal location on the particulate matter and carbon monoxide levels were tested to see whether the nature of a municipality is likely to affect the daily pollution levels experienced by the household members. Ethnicity within Saidpur was shown to

influence the cooking behaviour: the Bihari families were more likely to cook indoors than the Bangla households in Saidpur (Chapter 5). The effect of municipality and ethnicity on the measured pollution levels were tested.

7.3.1. Municipal effect

The municipal effect on the pollution levels was examined using a t-test (parametric and non-parametric). No municipal difference in the $\log_{10}$-transformed particulate matter levels in the kitchens measured during the day was observed (t=1.955, p=NS, n=61). Neither were significant differences observed in the kitchen night-time particulate matter (untransformed) levels (Mann-Whitney: 358.0, Z= -1.270, p=NS, n=61). Non-parametric analysis showed that the daytime carbon monoxide levels in kitchens in Saidpur appeared higher than those in Parpatipur (Mann-Whitney: 312.5, Z= -1.973, p<0.05, n=61). Interestingly significantly higher night-time carbon monoxide (untransformed) levels in the kitchens in Saidpur rather than in Parpatipur were detected (Sum of ranks$_{Saidpur}$: 1389.0, Sum of ranks$_{Parpatipur}$: 502.0, Mann-Whitney: 202.0, Z=-3.958, p<0.001). The municipal effect on the particulate matter and carbon monoxide levels in the living room of the two-room houses (only) could not be tested due to insufficient sample size (two-room houses in Saidpur n=26 and Parpatipur n=4).

7.3.2. Ethnicity

Ethnicity was significantly associated with the daytime kitchen $\log_{10}$-transformed particulate matter levels when tested using the t-test (Bangla mean=2.526±0.335SD, n=33, Bihar mean=2.706±0.323SD, n=28, t= -2.125, p<0.05, n=61) but not with the daytime living room $\log_{10}$-transformed particulate matter levels (two room households only) (t= -1.461, p=NS). Night-time untransformed particulate matter levels (kitchen or living-room) were not significantly associated with ethnicity. It was not possible to test the ethnic effect within Saidpur only, due to insufficient sub-group sizes.

Significantly higher daytime and night-time kitchen carbon monoxide levels were found in Bihari rather than Bangla households (daytime: Sum of ranks $_{Bangla}$:806.0, Sum of ranks$_{Bihari}$:1085.0, Mann-Whitney: 245.0 , Z=-3.192, p<0.001, n=61 and

night-time: Sum of ranks $_{Bangla}$:821.5, Sum of ranks$_{Bihari}$:1069.5 , Mann-Whitney: 260.5 , Z=-3.231, p<0.001). No ethnic effect was seen on the living room levels of carbon monoxide in two room houses during the day or night (daytime: Mann-Whitney:67.0, Z=-1.831, p=NS and night-time: Mann-Whitney: 64.5, Z= -2.118, p=NS).

7.3.3. Household fuel usage and pollution levels

Log_{10}-transformed particulate matter levels and carbon monoxide (untransformed) levels were measured for four cooking fuel types in the kitchen of all houses (Table 7.9, p.227). From the daytime kitchen data the patterns of carbon monoxide and particulate matter levels produced by each fuel type appear quite different. Wood and dung appeared to produce the highest particulate matter pollution levels, whereas the highest carbon monoxide levels were detected in households where wood and golden were burned as fuel. Wood appears to be the source of highest pollution levels for both pollutants (daytime kitchen levels). Very similar particulate matter and carbon monoxide pollution ranking was observed in the kitchens at night. Golden appears to produce the highest ranked carbon monoxide and particulate matter levels at night. Dung remained as the lowest source of the two pollutants at night. The ranked carbon monoxide levels in the kitchen appear much lower during the day than that observed at night. Due to small sub-group sizes of some fuel types, the fuel groups needed to be regrouped for further analyses. When the fuel type was tested as a binary variable: wood (n=30) versus other biomass fuels (n=31) no association between the fuel type and the kitchen pollution levels was found.

7.3.4. Cooking frequency

Cooking frequency (binary grouping once per day versus more than once a day) was not significantly associated with the daytime log_{10}-transformed particulate matter levels measured in the kitchen (t=0.593, p=NS, n=61). Neither were the night-time particulate matter levels in the kitchen associated with the household cooking frequency (mean rank$_{once}$=29.2 and mean rank $_{more than once}$= 32.2, Mann-Whitney: 388.5, Z=-0.608, p=NS). Carbon monoxide (untransformed) levels in the kitchen at

night were significantly higher in the households where cooking was done more than once a day than those households which only cooked once per day (mean rank$_{once}$=23.2 and mean rank $_{more\ than\ once}$= 35.4, Mann-Whitney: 257.0, Z=-2.862, p<0.005). Daytime kitchen carbon monoxide levels were not associated with the household cooking frequency (mean rank$_{once}$=31.1 and mean rank $_{more\ than\ once}$= 30.3, Mann-Whitney: 411.5, Z=-0.297, p=NS). Living room pollution levels could not be tested in the two room households for any effect of cooking frequency, due to small sub-group sizes (once per day n=6, more than once per day n=24).

Stove type (binary), duration of cooking breakfast, lunch or dinner or cooking location (binary) showed no association with the carbon monoxide or particulate matter levels in the households.

From the analyses described above the particulate matter pollution and the carbon monoxide levels in the households appear to be affected very differently by household factors. In order to design an efficient intervention any factors which may influence the two patterns observed for the levels of particulate matter and carbon monoxide need to be carefully considered.

7.3.5. Municipal effect on safe and dangerous pollutant exposures

Very few houses in Parpatipur had two rooms so any differences in the living room pollution levels could not tested for the municipal effect. Households exceeding the safety threshold particulate matter levels measured in kitchens during the day were significantly more prevalent in Saidpur (75.7%) than in Parpatipur (45.8%) (X^2=5.622, p<0.05, n=61) (Table 7.10, p.228). The night-time kitchen exposure levels could not be tested between municipalities due to a small sample size of dangerous particulate matter exposure in Parpatipur. However dangerous particulate matter levels were detected in kitchens in almost a fifth of the households at night in both municipalities, which indicates a continuing health risk from particulate matter exposure at night. Over half of the households in Saidpur and over a third in

Parpatipur were grouped into dangerous exposure level groups by their 24-hour
particulate matter exposure, according to the WHO criteria **(WHO, 2005).**

Households with dangerous levels of particulate matter (using 24-hour mean values)
were not more likely to cook indoors or more frequently, using the chi-square test.
Fuel type was not associated with the particulate matter pollution exposure groups.

Comparing dangerous levels of carbon monoxide in the kitchen during the day for
more than 15 minutes (Table 7.11, p.228): almost half (41.7%) of the households in
Parpatipur experienced dangerous levels of carbon monoxide from 15 minutes to one
hour compared with 16.2% in Saidpur. Approximately the same percentage of
households experienced dangerous levels of carbon monoxide for one to two hours in
Saidpur (13.5%) and in Parpatipur (12.5%). A quarter (24.3%) of households in
Saidpur experienced dangerous levels of carbon monoxide for two to four hours
compared with 12.5% in Parpatipur. More households in Saidpur (10.8%) experienced
dangerous levels for four to eight hours compared with only 4.2% in Parpatipur.
Dangerous kitchen carbon monoxide levels were observed at night in all households
in Parpatipur up to 15 minutes, whereas in Saidpur approximately a third of the
households were exposed to dangerous carbon monoxide levels for up to four hours.

7.3.5.1. Continuous data on duration of carbon monoxide exposure and locality and cooking practices

The number of minutes by which carbon monoxide levels exceeded the threshold of
9ppm set by US-EPA in 1997 was tested for any associations with the household
location, SES status, wall material, and cooking practices. Wall material of the
households was not shown to have a significant effect on the length of time by which
the carbon monoxide levels exceed the safety threshold. Log_{10}-transformed daytime
dangerous carbon monoxide exposure in the kitchen was not significantly higher (t=-
1.639, p=NS) in households with walls made of brick, clay, corrugated iron sheets or
wood (mean: 1.687±0.677SD, n=23) than households with bamboo or thatch walls
(mean: 1.376±0.698SD, n=31). Municipality, SES status, stove type, number of
rooms, cooking frequency (binary), fuel type (binary), and cooking location (binary)

were not significantly associated with the dangerous carbon monoxide exposure in the kitchen during the day.

Significantly longer dangerous carbon monoxide exposure was detected in the kitchen at night in households in Saidpur than in Parpatipur (mean rank: 35.50 and 24.06 respectively, Mann-Whitney: 277.5, Z=-2.658, p<0.01, n=61) (Table 7.11, p.228). SES, wall material, number of rooms, kitchen volume, cooking location and - frequency, the duration of cooking for breakfast, stove type or the fuel type were not a significant determinant of the length of dangerous kitchen night-time carbon monoxide exposure. Cooking practices, municipal, SES or housing factors had no impact on the length of living room dangerous carbon monoxide exposure (day or night) or the significance could not be tested due to small subgroup sizes.

7.4. Child growth and immunity and household fuel pollution (in air-quality monitored households)

Results from the previous chapter indicated that those children who were present during cooking (independent of their age) had lower haemoglobin status and poorer growth and health. This section aims to examine the effects of $PM_{2.5}$ and CO, together with cooking and fuel-use behaviour, on child health status. Details of socio-demographic characteristics and cooking and fuel-use practices between households where air quality was monitored and those excluded from the monitoring are provided in Table 7.12 (p.229). The households where air quality was monitored were not significantly different from those households which were not included in the air quality subsample for their socio-demographic characteristics or cooking and fuel-use practices (p=NS). The air quality monitored households different from the non air quality monitored households only in terms of the cooking location. The non-air quality monitored households were more likely to cook indoors whereas households where air quality was monitored cooked outdoors or on the veranda (X^2=9.078, p<0.01). The characteristics of the children in households selected for indoor air pollution measurement (n=57) were compared with the youngest child of the remaining households (non-air quality monitored households) (Table 7.13, p.230).

Only the youngest child was selected from each household for the health analysis, in order to avoid bias. For the analysis of data child health and growth outliers were excluded using the criteria described in Chapters 5 and 6. Data on the presence or absence of the child by the stove during cooking or some of the immune markers was missing for some of the households which reduced the sample size to n=47 or less depending of the variable tested (Table 7.13, p.230). The youngest only children from air-quality monitored households were not significantly different from non air-quality monitored households in terms of the levels of selected biomarkers of immunity (haemoglobin, log10-transformed-alpha-1-acidglycoprotein and inversely transformed albumin) or growth z-scores (height-for-age, weight-for-age and weight-for-height) (p=NS). Constraints of statistical analysis include a higher likelihood of accepting null hypothesis erroneously with small sample size. The small sample may not be a true representative of characteristics truly present in the source sample which the subsample aims to represent. With a larger sample size the true diversity of a population is likely to be correctly demonstrated. The sample size selected for air quality monitoring was expected to introduce constraints in terms of data analysis however the sample size was expected to suffice for some basic analysis in case of an even distribution of characteristics across subgroups needed to be tested. However, in this case subgroup sizes were often less than 10 households which is insufficient for efficient statistical analyses. The observed difference in the cooking location between households where air quality was monitored and non-air-quality monitored households could indicate a strong character difference between the two types of households. Or it could be due to a biased sampling of households which did not cook indoors for air quality monitoring. However, the households were randomly selected for air quality monitoring to avoid such bias.

7.4.1. Child health and immunity in households above and below the WHO safety thresholds

Haemoglobin levels in children were examined from households with safe and dangerous 1-hour accumulative particulate exposure for 12-hours per day. Night-time values or 24-hour mean values could not be tested as the number of households within

the dangerous exposure group was too low for statistical analysis (only n≤9 or less).

No significant differences (Table 7.13, p.230) were detected in haemoglobin levels in

children from households where the daytime one-hour mean particulate levels in the

kitchen exceeded the 25µg/m^3 safety threshold of the World Health Organisation

compared to children from households below the safety threshold (WHO, 2005)

(mean Hb 104.2±15.3g/L n=16 below safety threshold, mean Hb 105.5±13.9g/L n=26

above safety threshold, t= -0.294, p=NS). Log-transformed AGP showed no

association with the mean daytime particulate matter exposure in the kitchen

(mean$_{safe}$: -0.750, n=14 and mean$_{dangerous}$: -0.719, n=26, t=-0.565, p=NS,). No night-

time association could be tested due to a small sample size. Log$_{10}$-transformed

duration of carbon monoxide exposure above the 9ppm safety threshold in the kitchen

or 1hr accumulative mean particulate matter levels during the day were not

significantly correlated with log-transformed AGP levels of the children. Albumin

(inversely transformed) levels were not associated with any other pollution exposure

measures described above. Growth z-scores (height-for-age, weight-for-age, weight-

for-height) were not associated with particulate matter exposure groupings. The

growth z-scores did not correlate significantly with the duration of dangerous carbon

monoxide exposure or particulate matter levels as described above.

7.4.1.1. Child health status (healthy, unwell with other than RTI, and RTI) and household pollution

At baseline 39.1% (n=18) of the children in air-quality households (children n=46)

were reported as healthy by their mother. 41.3% (n=19) of the children were unwell

with a condition other than respiratory infection and 19.6% (n=9) of the children had

moderate or severe respiratory infection according to a medical diagnosis. Chi-square

test between the child health status (healthy, unwell with no RTI, RTI) groups and

safe and dangerous 24-hour particulate matter pollution exposure was not possible due

to the small sample size within the unwell with other than respiratory disease group.

The requirement for medical diagnosis was not significantly associated with the safe

and dangerous particulate matter pollution exposures (X^2= 0.334, p=NS, n=47). One

way ANOVA was carried out to test for differences in household fuel pollution levels

between the children in the three health groups. No significant differences were found

in the log_{10}-transformed daytime kitchen particulate matter levels (F=2.068, p=NS, n=47). Non-parametric ANOVA using untransformed carbon monoxide levels (day and night) and night-time particulate matter levels showed no significant differences between the pollution levels within the child health groups (X^2= 4.322, X^2= 0.677 and X^2= 1.887, p=NS, n=47 respectively). No significant differences were found in the log_{10}-transformed daytime kitchen levels of dangerous carbon monoxide exposure in the households with children in the three health groups (F=0.769, p=NS, n=47).

7.5. Pilot intervention to reduce the household fuel pollution exposure in the study community

7.5.1. Intervention to reduce the household fuel pollution exposure

The longitudinal health intervention work aimed to reduce the personal exposure to household fuel pollutants in the households in communities in Saidpur and Parpatipur in northern Bangladesh. The intervention activities were carried out in the study community by Concern Worldwide, Village Education Resource Centre (VERC) and Winrock International, Bangladesh. The intervention consisted of two parts: a technical part (an improved stove design) and an educational/behavioural component which were both designed and finalised after the baseline field survey in October 2005 using the baseline data on household cooking practices and specific reports by household cooks for their own needs for a stove.

7.5.1.1. Educational/behavioural intervention

The educational/behavioural intervention aimed to educate household members on the health dangers of daily household fuel pollution exposure. Households were also advised on simple ways to reduce daily pollution exposure. Educational activities were specifically targeted at household women and primary cooks and attempted to identify healthy behaviours which could be encouraged in the community as a means for reducing household fuel pollution exposure. During the baseline survey a sideline behavioural assessment (focus groups) was carried out where women were asked questions about their needs for stoves. This aimed to identify the main benefits and disadvantages of the stove women used for daily cooking as well as characterise

women's fuel-use behaviour. On the basis of the information obtained from these focus groups, together with the data from meetings with stakeholders at a local government level (municipality chairmen, ward commissioners, teachers, religious leaders and community health volunteers) messages of behavioural change were developed.

The following health messages were identified:

1. Indoor smoke is a risk factor for pneumonia, bronchitis and asthma

2. Avoid smoke in the house, especially when with your young baby or a child

3. Keep children away from the stove smoke when cooking

4. Ventilate your kitchen properly during and after cooking

5. Cook on a raised platform to improve ventilation

6. Dispose of waste materials appropriately

Once the simple behavioural changes were identified the study communities were targeted with educational activities in order to increase the knowledge of "healthy behaviours" via educational drama plays, bill boards and posters distributed around the community. These activities aimed to illustrate the potential health dangers from household fuel pollution exposure and simple behavioural changes which households could do, which could lead to an improvement in health as a result of lower pollution exposure. Figures 7.10 and 7.11 (p.228) show examples of bill boards and posters used for educational activities in the study communities.

7.5.1.2. Technical intervention

The technical intervention consisted of setting up an improved stove design in households in order to reduce pollution exposure. As part of this intervention a local stove repair and maintenance network was established to support the adoption and the sustainability of the improved stove in the community. The design of the improved stove was based on the traditional mud stove used widely in the community. The new stove was made from local clay and mud and it could be made by the household members themselves. The fuel combustion was improved and the stove had two

cooking holes for pots in order to reduce cooking time as two dishes could be cooked simultaneously. Chimneys were fitted where possible, using cheap metal tubing, which was affordable and easily available in the local market. Once the improved stove was ready for dissemination in the field a training course on stove making was arranged for thirty local community health volunteers (in January 2007). The health volunteers were taught on how to make their own improved stove out of the local clay and to fit a chimney where possible. The trained individuals were then encouraged to teach their new stove making skills to their neighbouring households in order to enhance the passive spread of the technical intervention in the study community.

Dissemination of the improved stove into the study community as well as setting up the support network for stove making and maintenance and arranging a supply of cheap metal chimney tubing in the local market was carried out by Village Education Resource Centre (VERC) on behalf of Winrock International. Winrock International is a non-charitable organisation which specialises in energy efficiency and environmental sustainability in developing countries around the world. The work in the field was carried out in collaboration with Concern worldwide (Bangladesh).

7.5.3. Success of educational and behavioural interventions at the community level

Households were interviewed at the beginning and the end of the study for their knowledge and understanding of health messages to avoid household fuel pollution exposure. At the beginning of the study over half of the mothers (52%) reported having their young child present near the stove during cooking. Less than a third of the mothers reported this behaviour at the end (29%). Knowledge of health dangers of household fuel pollution exposure (as a health risk for respiratory symptoms such as coughs, wheezing, COPD and asthma) increased drastically throughout the field work (baseline: 32%, final: 82%). Almost half of mothers (46%) reported Concern Worldwide as a source of knowledge about the health risks of household fuel pollution from the smoke. The new improved stove was found in 9.6% of the households and 7.9% of the households had built a new separate kitchen for cooking purposes to reduce household fuel pollution exposure. Chimneys were found in 10.1%

of the households. Chimneys were absent from all study households at the start of the work.

The success of the technical intervention in the form of an improved stove design unfortunately could not be tested. This was due to a lack of finances to carry out the pre-planned set of air quality measurements in the field at the end of the study. Hence the efficiency of the improved stove design in improving the air quality in households which had adapted to the use of the improved stove by the end of the study (9.6% households, as described in the previous section) could not be tested. The data from the women's focus groups unfortunately was not made available for all partners in this work. Therefore the effectiveness of the behavioural and educational intervention could not be analysed in this thesis other than the reporting of knowledge change as reported in section 7.5.3.

7.6. Summary

Most households experienced high carbon monoxide and particulate matter (2.5μm) levels according to the safety guidelines by World Health Organisation (**WHO, 2005**) for particulate matter and US-EPA (**US-EPA, 1997**) for carbon monoxide. Significant diurnal variation was seen in the levels of the two pollutants. Night-time pollution levels were significantly lower than daytime levels with a 30% reduction in the carbon monoxide levels and up to a 60% drop in the particulate matter levels at night. The number of rooms in a household was not associated with the measured pollution levels. A positive correlation was seen between the ambient and personal carbon monoxide levels which could make the ambient carbon monoxide levels a good proxy of personal exposure to this pollutant.

More porous household wall material (thatch or bamboo) was linked with shorter duration of carbon monoxide exposure above the 9ppm safety threshold. Night-time carbon monoxide (but not particulate matter) levels were significantly higher in Saidpur than Parpatipur. More common evening cooking is likely to explain the

lingering night-time carbon monoxide levels in Saidpur, together with brick wall housing.

Analysis of child health and growth within air-quality households showed no evidence of an increased prevalence of ill-health due to household fuel pollution exposure. This was mostly due to a very small sample size.

On the basis of reported knowledge on adverse effects of household fuel pollution on health (increase risk of COPD, more frequent and more severe respiratory infections and asthma) a drastic improvement in the general knowledge was seen by the end of the study among mothers in this sample. The improved stove was also seen in approximately ten percent of the households by the end of the field study which may indicate a positive attitude towards the stove among the community members.

Tables and figures

Table 7.1: Ranges of real pollution levels in 67 households selected for air quality monitoring

Measurement taken		Carbon monoxide	Particulate matter	Personal carbon
Location	Period	(ppm)	$(PM_{2.5}\mu m)/(\mu g/m^{3)}$	monoxide(ppm)
Kitchen	Day	0 – 16	85 – 3020	10 – 100 (12 hour accumulative
	Night	0 – 6	25 – 505	
Living room	Day	0 – 16	81 – 3020	
	Night	0 – 4	27 – 536	
Kitchen	24-h average	0 – 8	61 – 1546	0.833 – 8.33 1 hr accumulative mean -
Living room	24-h average	0 – 8	60 – 1546	
Kitchen	24-h accumulative	0 – 16	122 – 3525	
Living room	24-h accumulative	0 – 16	199 – 3556	

Table 7.2: Mean levels of carbon monoxide and particulate pollution in kitchen (all) and living rooms (only 2-roomed houses) during the day and night.

Measurement taken		Non-parametric Carbon monoxide (ppm) (n)	Particulate matter $(PM_{2.5}\mu m)/(\mu g/m^{3)}$ (n)	Particulate matter
Location	Period			Data type
Kitchen	Day	3.443 ± 3.543 (61)	2.609 ± 0.339 (61)	Log_{10} (n+1)
	Night	0.771 ± 1.175 (61)	180.9 ± 128.6 (61)	Non-parametric
Living room	Day	1.871 ± 2.334 (31)	2.372 ± 0.277 (31)	Log_{10} (n+1)
	Night	0.710 ± 1.101 (31)	164.2 ± 96.7 (31)	Non-parametric
Kitchen	24-h average	2.311 ± 1.893 (61)	361.9 ± 251.0 (61)	Non-parametric
Living room	24-h average	1.484 ± 1.480 (31)	228.6 ± 114.3 (31)	Non-parametric

Table 7.3. Geometric means of carbon monoxide and particulate matter pollution levels

Measurement taken		Non-parametric Carbon monoxide	Particulate matter
Location	Period	(ppm)	$(PM_{2.5}\mu m)/(\mu g/m^{3)}$
Kitchen (n=61)	Day	31.269	6.810E+139
	Night	2.161	3.551E+78
Living room (n=31)	Day	6.495	1.768E+92
	Night	2.033	2.101E+71
Kitchen (n=61)	24-h average	10.089	2.986E+136
Living room (n=31)	24-h average	4.410	1.868E+99

Figure 7.1: Carbon monoxide levels in one room house with two cooking periods

Carbon monoxide monitoring (household P-711) Same kitchen and living room - ONE-ROOM hh - two cooking periods
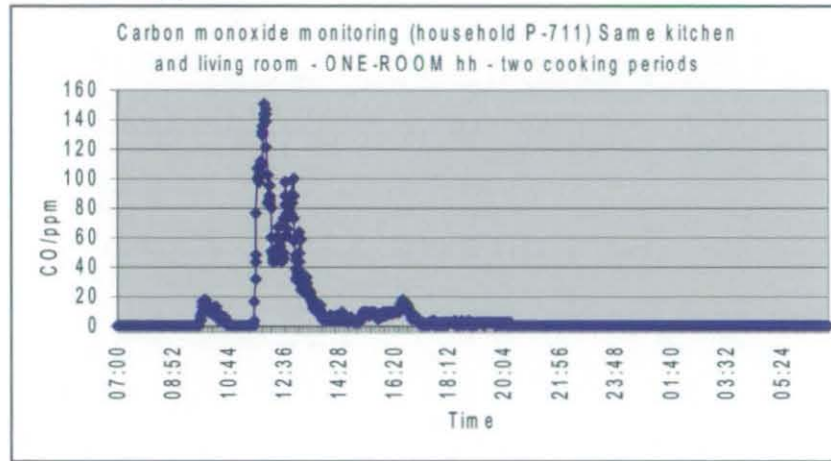
Figure 7.3: Carbon monoxide levels in kitchen (two room house) with two cooking periods

Carbon monoxide monitoring (household P-810) Kitchen, TWO-ROOM hh -two cooking periods
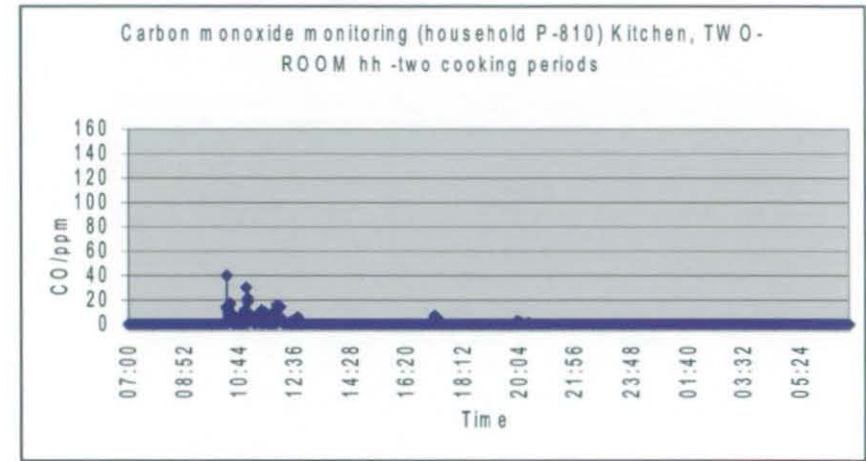
Figure 7.2: Carbon monoxide levels in one room house with three cooking periods

Carbon monoxide monitoring (household S-142) Same kitchen and living room - ONE-ROOM hh - three cooking periods
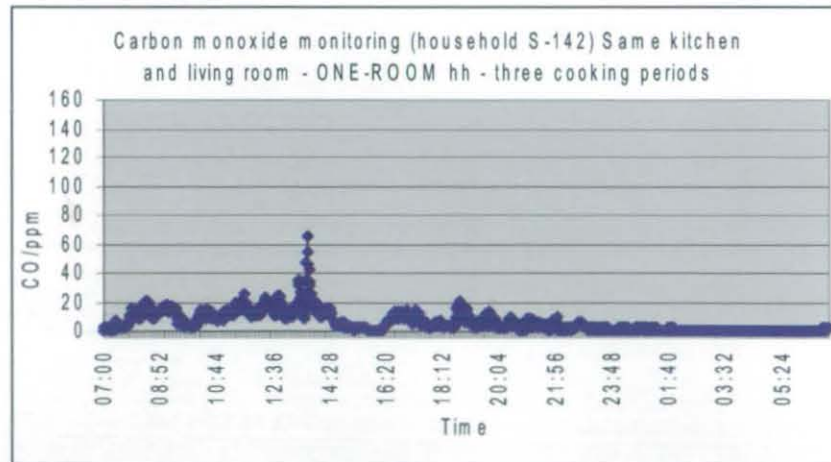
Figure 7.4: Carbon monoxide levels in living room (two room house) with two cooking periods

Carbon monoxide monitoring (household P-810) Living room, TWO-ROOM hh - two cooking periods
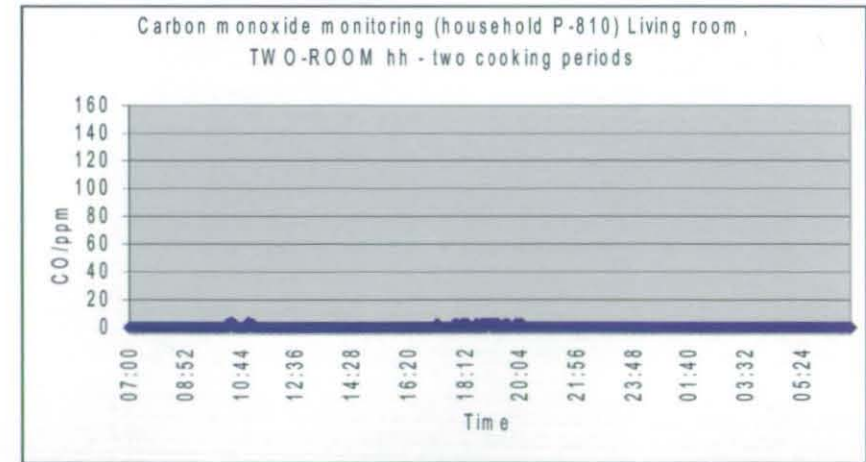
Figure 7.5: Carbon monoxide levels in kitchen with three cooking periods (household S-143)

Carbon monoxide monitoring (household S-143) Kitchen, TWO-ROOM hh - three cooking periods
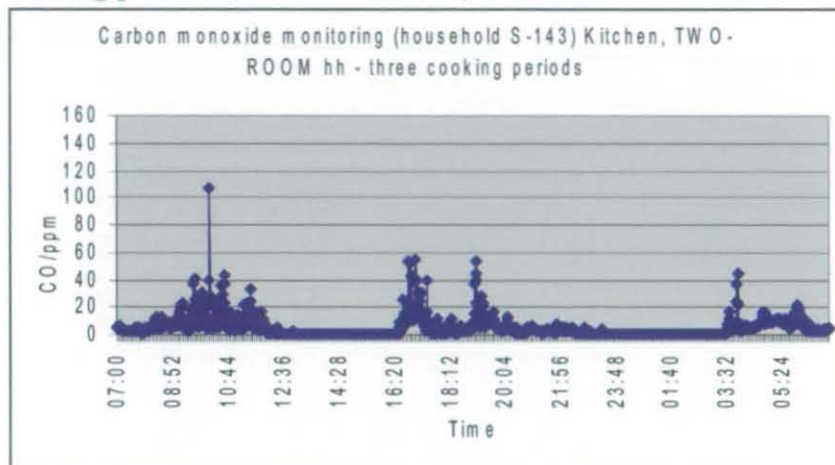
Figure 7.7: Carbon monoxide levels in kitchen with three cooking periods (household S-311)

Carbon monoxide monitoring (household S-311) Kitchen, TWO-ROOM hh - three cooking gperiods
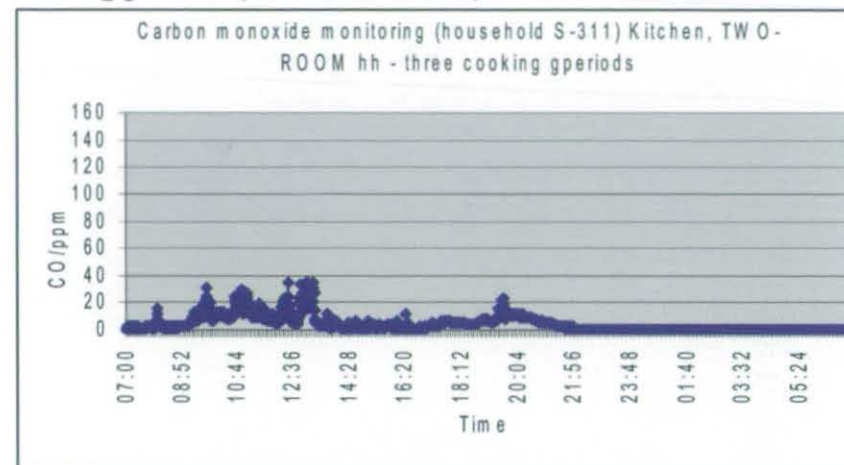
Figure 7.6: Carbon monoxide levels in living rooms with three cooking periods (household S-143)

Carbon monoxide monitoring (household S-143) Living room, TWO-ROOM hh - three cooking periods
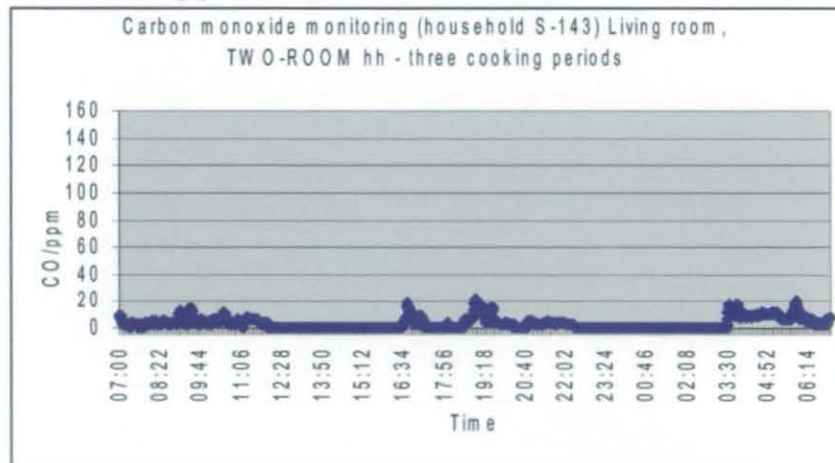
Figure7.8: Carbon monoxide levels in living room with three cooking periods (household S-311)

Carbon monoxide monitoring (household S-311) Living room, TWO-ROOM hh- three cooking periods
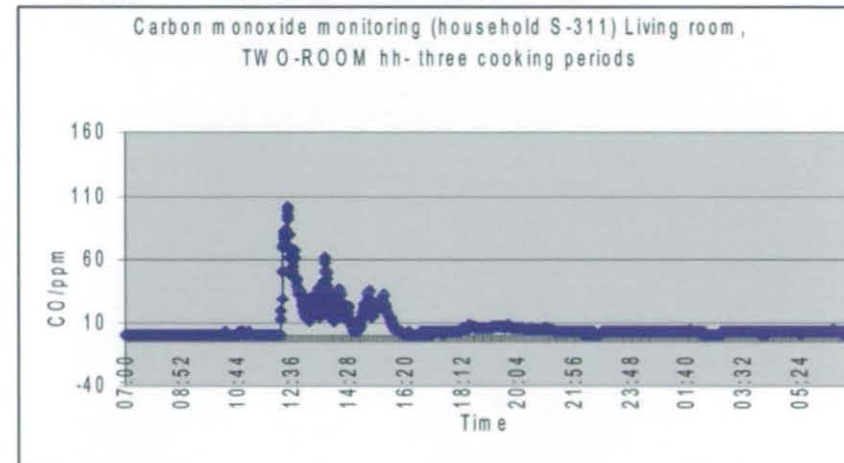
Table 7.4: Comparison of kitchen and living room pollutant levels in households with two rooms.

	Two room houses	Pollutant	Log-transformed particulate matter		Untransformed particulate matter		Untransformed carbon monoxide	
			Paired t-test		Non-parametric Wilcoxon signed rank test		Non-parametric Wilcoxon signed rank test	
Area	kitchen	day	2.590 ± 0.278 (n=31)	t=3.887 p<0.01	-		Mean −ve rank: 10.5 (n=16) Mean +ve rank: 7.17 (n=3), ties=12	Z=-2.987 p<0.003
	living room		2.372 ± 0.277 (n=31)			-		
	kitchen	night	-		Mean −ve rank: 17.31 (n=16), Mean +ve rank: 10.75 (n=12), ties=3	Z=-1.686 p=NS	Mean −ve rank: 4.71 (n=7) Mean +ve rank: 3.0 (n=1), ties=23	Z=-2.165, p=0.03
	living room		-	-				

Table 7.5: Correlation between the pollutant levels (daytime and night time) in kitchen and living room

Non-parametric Spearman's correlation						
Pollutant measured			Carbon monoxide (untransformed levels)			
			Kitchen		Living room	
	Location	Period	Day	Night	Day	Night
Particulate matter (untransformed levels)	Kitchen	Day	r=0.830, p<0.001			
		Night		r=0.270, p<0.05		
	Living room	Day			r=0.622, p<0.001	
		Night				r=0.317, p=NS

Figure 7.9: Spearman's correlation showing the relationship between real-time ambient carbon monoxide and personal carbon monoxide exposure
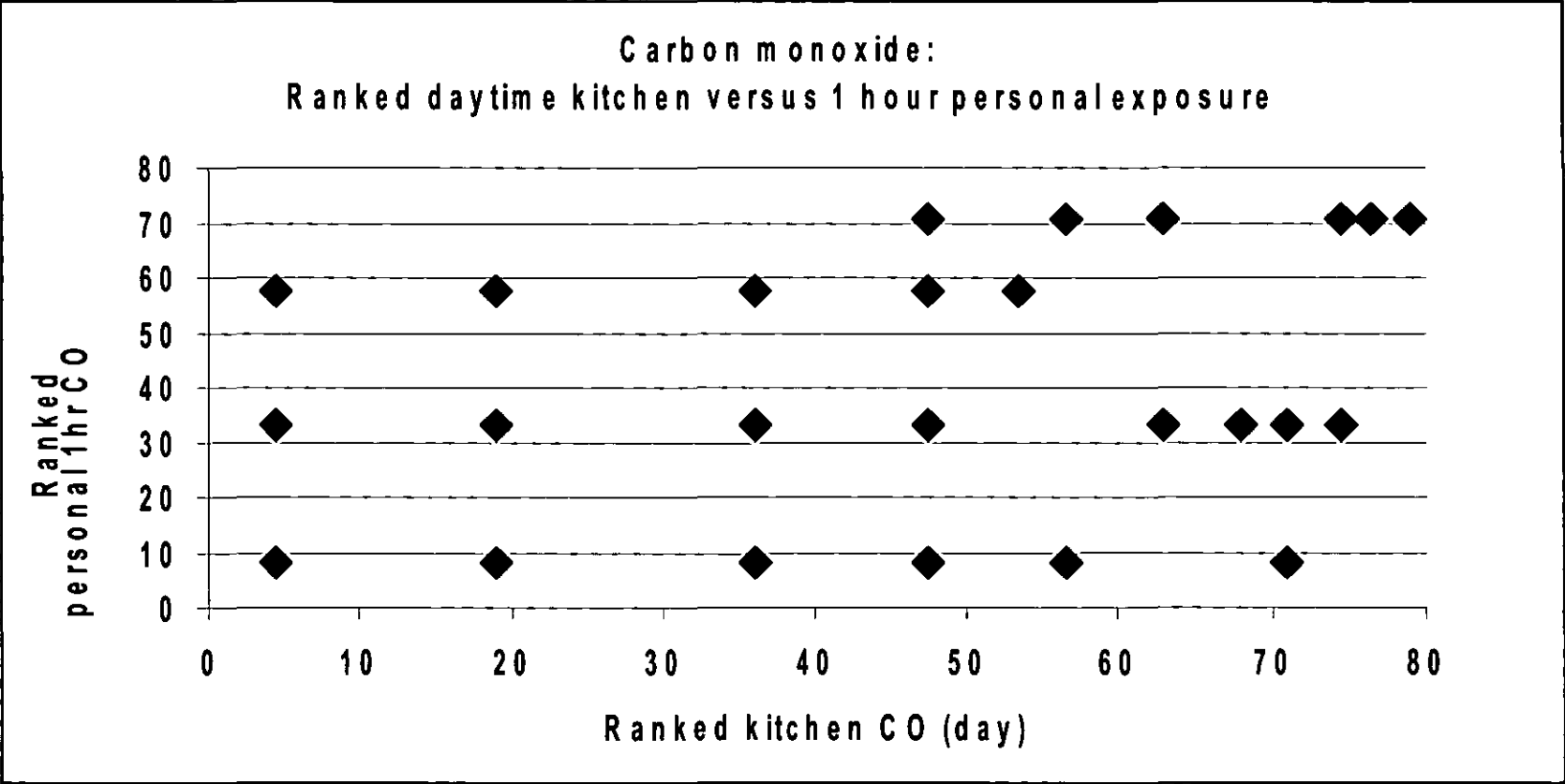
Table 7.6: Length of dangerous carbon monoxide exposure in households

Time groups (min)	Percentage (and number) of households			
	Kitchen Day % (n)	Kitchen Night % (n)	Living room Day% (n)	Living room Night% (n)
0-15	32.8 (20)	78.7 (48)	71.0 (22)	77.4 (24)
16-60	26.2 (16)	13.1 (8)	9.7 (3)	12.9 (4)
61-120	13.1 (8)	4.9 (3)	6.5 (2)	3.2 (1)
121-240	19.7 (12)	3.3 (2)	12.9 (4)	6.5 (2)
241-480	8.2 (5)	0 (0)	0 (0)	0 (0)
Over 480	0 (0)	0 (0)	0 (0)	0 (0)
Total	61	61	31	31

Table 7.7: Prevalence of safe and dangerous 1hr-accumulative mean particulate matter levels in households

Particulate matter per 1 HR average	Percentage (and number) of households					
	Kitchen Day % (n)	Kitchen Night % (n)	Kitchen 24hr % (n)	Living room Day % (n)	Living room Night % (n)	Living room 24hr % (n)
Safe < 25ug/m3	36.1 (22)	82.0 (50)	52.2 (32)	74.2 (23)	87.1(27)	80.6 (25)
Dangerous > 25ug/m3	63.9 (39)	18.0 (11)	47.5 (29)	25.8 (8)	12.9 (4)	19.4 (6)
Total	61	61	61	31	31	31

Table 7.8: Background variables within air quality households (n=61)

Background variables	Sub-groups	n	%
Municipality	Saidpur	37	60.7
	Parpatipur	24	39.3
Ethnicity	Bangla	34	55.7
	Bihari	27	44.3
Education of household head	No	34	55.7
	Yes	27	44.3
Maternal education	No	31	50.8
	Yes	30	49.2
Cooking location	Indoors	30	49.2
	Separate kitchen building	5	8.2
	Open air	10	16.4
	Veranda -2walls	16	26.2
Cooking location	Indoors	35	57.4
	Open air (including veranda)	26	42.6
Stove type	Biomass portable chimneyless	18	29.5
	Biomass fixed chimneyless	43	70.5
Cooking frequency	One	22	36.1
	Two	24	39.3
	Three	15	24.6
Duration of cooking (breakfast)	None	26	42.6
	<1hr	22	36.1
	Over 1hr	13	21.3
Duration of cooking (breakfast)	None	26	42.6
	<1hr and over	35	57.4
Duration of cooking (lunch)	None and <1hr	7	11.5
	1-2hrs	44	72.1
	>3hrs	10	16.4
Duration of cooking (dinner)	None	38	62.3
	<1hr up to 3hrs	23	37.7
Child present	Yes	51	83.6
	No	5	8.2
Fuel type 4 groups	Wood	32	52.5
	Golden	11	18.0
	plant- derived	11	18.0
	Dung	7	11.5
Fuel type	Wood and Golden	43	52.5
	Others	18	47.5

Table 7.9 Day and night-time carbon monoxide and particulate matter pollution levels in kitchen and living rooms produced by different fuel types

Pollution levels	Time	Data type	Location	Pollution levels per fuel type			
				Wood % (n)	Golden % (n)	Plant-derived % (n)	Dung % (n)
Carbon monoxide (ppm)	Day	untransformed	Kitchen	32.2 (32)	33.1 (11)	30.5 (11)	23.1(7)
		untransformed	Living-room	13.0 (14)	19.1 (10)	18.2 (6)	14.0(1)
	Night	untransformed	Kitchen	27.3 (30)	44.8 (11)	35.0 (11)	20.3 (7)
		untransformed	Living-room	12.3 (14)	21.1 (10)	17.3 (6)	9.5 (1)
Particulate matter (μg/m^3)	Day	Log-transformed	Kitchen (log)	2.65 ± 0.36 (32)	2.59 ± 0.37 (11)	2.58 ± 0.27 (11)	2.51± 0.35 (7)
		Log-transformed	Living-room	2.32 ± 0.32 (14)	2.40 ± 0.21 (10)	2.47 ± 0.34 (6)	2.24 (1)
	Night	untransformed	Kitchen	28.7 (32)	44.9 (11)	35.6 (11)	13.0 (37)
		untransformed	Living-room	12.1 (14)	21.9 (10)	16.9 (6)	6.6 (1)

Table 7.10: Prevalence of safe and dangerous 1hr accumulative particulate matter exposure in households in the two municipalities

PM$_{2.5}$ 1 HR mean	Saidpur			Parpatipur		
	Kitchen day % (n)	Kitchen night % (n)	Kitchen 24hr % (n)	Kitchen day % (n)	Kitchen night % (n)	Kitchen 24hr % (n)
Safe < 25ug/m3	24.3 (9)	81.1 (30)	43.2 (16)	54.2 (13)	83.3 (20)	66.7 (16)
Dangerous > 25ug/m3	75.7 (28)	18.9 (7)	56.8 (21)	45.8 (11)	16.7 (4)	33.3 (8)
Total	100 (37)	100 (37)	100 (37)	100 (24)	100 (24)	100 (24)

Table 7.11: Length off dangerous carbon monoxide exposure in households in the two municipalities

Time groups (min)	Saidpur		Parpatipur	
	Kitchen day % (n)	Kitchen night % (n)	Kitchen day % (n)	Kitchen night % (n)
0-15	35.1 (13)	64.9 (24)	29.2 (7)	100 (24)
16-60	16.2 (6)	21.6 (8)	41.7 (10)	0
61-120	13.5 (5)	8.1 (3)	12.5 (3)	0
121-240	24.3 (9)	5.4 (2)	12.5 (3)	0
241-480	10.8 (4)	0	4.2 (1)	0
Total	37	37	24	24

Table 7.12: Comparison of socio-demographic characteristics and cooking and fuel-use practices of air-quality and non-air quality monitored households

Variable	Subgroup	No % (n)	Yes % (n)	Statistic
Municipality	Saidpur	54.4 (141)	63.3 (31)	X^2=1.301, p=NS
	Parpatipur	45.6 (118)	36.7 (18)	
Ethnicity	Bangla	59.5 (154)	51.0 (25)	X^2=1.206, p=NS
	Bihari	40.5 (105)	49.0 (24)	
Education (household head	No	53.3 (138)	61.2 (30)	X^2=1.048, p=NS
	Some	46.7 (121)	38.8 (19)	
Maternal education	No	52.9 (137)	49.0 (24)	X^2=0.253, p=NS
	Some	47.1 (122)	51.0 (25)	
Literacy	Illiterate	25.5 (55)	51.1 (24)	X^2= 12.038, p<0.01
	Literate	74.5 (161)	48.9 (23)	
Average income	Low <TK3000)	57.9 (150)	69.4 (34)	X^2=2.255, p=NS
	High >TK3000)	42.1 (109)	30.6 (15)	
SES	Low (SES1-3)	57.9 (150)	63.3 (31)	X^2=0.487, p=NS
	High (SES4-5)	42.1 (109)	36.7 (18)	
Child present by stove during cooking	Yes	47.5 (106)	54.5 (24)	X^2=0.723, p=NS
	Switchers	52.5 (117)	45.5 (20)	
Cooking location	Indoors	36.2 (93)	59.2 (29)	X^2=9.078, p<0.01
	Other	63.8 (164)	40.8 (20)	
Fuel type	Wood	51.9 (54)	60.0 (15)	Not possible to test
	Golden	26.0 (27)	32.0 (8)	
	Dung	22.1 (23)	8.0(2)	
Cooking frequency	Once	45.8 (76)	44.1 (15)	X^2=0.032, p=NS
	More than once	54.2 (90)	55.9 (19)	
Stove type	Fixed biomass	26.5 (68)	34.8 (17)	X^2=1.139, p=NS
	Other	73.5 (189)	65.3 (32)	

Note: Differences in the fuel choice households where air-quality was monitored and those where no monitoring was done could not be tested due to small subgroup sizes of dung n=2 in air quality monitored households. The subgroups size for golden (n=8) was also borderline (n=5 as a threshold for Chi-square group sizes).

Table 7.13: Comparison of child health and immunity variables of air-quality and non air-quality monitored households (controlling for age as a covariate)

Variable	No mean (n)	Yes mean (n)	Unvariate test
Haemoglobin	105.1±13.4 (241)	103.9±14..2 (44)	F=0.101, p=NS
Log_{10}- alpha-1-acidglycoprotein	-0.750±0.174 (232)	-0.746±0.174 (43)	F=0.016, p=NS
Albumin (1/x)	0.042±0.010 (236)	0.041±0.009 (43)	F=0.350, p=NS
C-reactive protein	Mean rank: 139.8 (231)	Mean rank:131.9 (45)	X^2=0.365, p=NS
Height-for-age	-1.866±1.206 (257)	-1.898±1.075 (47)	F=0.018, p=NS
Weight-for-age	-2.500±1.382 (255)	-2.635±1.134 (47)	F=0.393, p=NS
Weight-for-height	-1.631±1.227 (247)	- 1.710±1.115 (45)	F=0.164, p=NS

Figure 7.10: Educational poster illustrating simple behavioural change to avoid household fuel pollution exposure
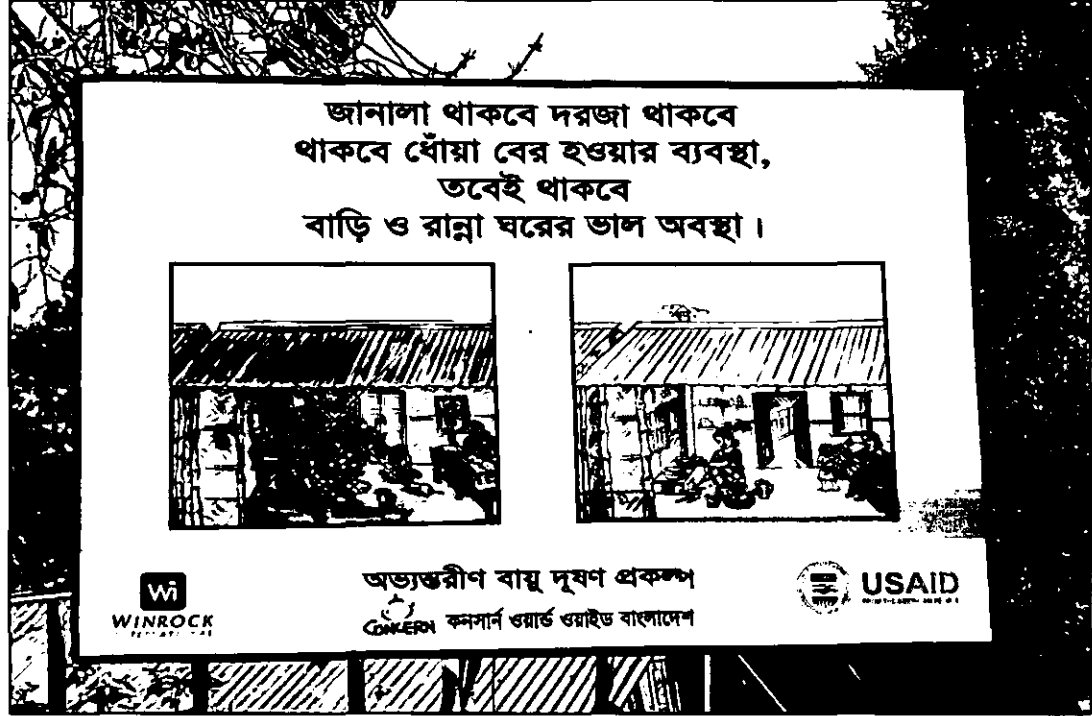
Figure 7.11: Educational poster illustrating simple behavioural change to avoid household fuel pollution exposure

Chapter 8 – Discussion and Conclusions

8.1. Introduction

This PhD thesis aimed to study the effect of household fuel pollution from cooking practices on the prevalence of respiratory tract infections (or RTI) and poor immunity among children under the age of five in semi-urban and rural communities in Bangladesh, using measured pollution levels (carbon monoxide and particulate matter) and data on child health (maternally reported, medical and immune status). This study also included a two-part intervention (technical and behavioural components) which aimed to reduce the household fuel pollution exposure through sustainable behavioural changes in daily lives in the study community and the use of an improved stove design to lower the harmful pollution exposure.

This PhD work aimed to find answers to the following research questions posed:

1. To assess the relative contribution of cooking practices, fuel-use and ventilation methods as well as housing structure to household pollution levels measured by particulate matter and carbon monoxide.

2. To examine whether household fuel pollution is a risk factor for respiratory infections in young children after controlling for household socio-demographic background and the cooking and fuel-use practices.

3. To examine risk factors for poor immune status and under-nutrition in young children, since these will also increase susceptibility to respiratory tract infections.

8.2. An assessment of the relative contribution of cooking practices, fuel-use and ventilation methods as well as housing structure to household pollution levels measured by particulate matter and carbon monoxide.

Socio-demographic and behavioural settings, in which the child health, growth and air-quality were monitored, were studied. The ethnic diversity observed in the study communities (Bangla and Bihari) allowed an interesting review of the effect of ethnicity on household fuel pollution exposure, cooking and fuel use practices and the related child health and growth. The examination of the socio-demographic background showed that long-term immigrants, the Bihari people, were the poorest in terms of average income and commonly belonged to the low SES. Similarly high SES households were more educated, had a higher average income and were of Bangla origins. Cooking was commonly done indoors, in a separate kitchen building, outdoors or on a veranda. Most households used a basic chimneyless mud stove which was very inefficient in fuel combustion and heat transfer, as reported previously (**Berruta, Edwards, Masera, 2008**). The cooking times were long and commonly no ventilation was in place in most households to reduce the health hazard from smoke exposure, as suggested by previous works (**WHO, 2002: Dasgupta et al, 2006**).

In rural Parpatipur, households more commonly cooked outside using a portable stove as the nature of the settlement allowed the use of space around the house for cooking. Households used wood and dung as cooking fuel. According to the fuel cleanliness classification by Smith dung is on the lowest rung of the ladder in terms of energy efficiency (**Smith *et al*, 1994**). Dung was abundant in the area due to the presence of livestock and for this reason the common choice for fuel. Cooking on dung may be less efficient in terms of time and fuel consumption, as well as producing more smoke. Fuel type may also explain the observed lower cooking frequency in Parpatipur. It is possible that more efficiently burning fuels such as golden and wood were not commonly available in the local market in the rural Parpatipur. Semi-urbanised Saidpur was a high density settlement (households per kilometre^2) where households cooked two or three times a day using wood or golden as fuel. The fuel choice most likely reflected the lack of collectable fuels,

such as dung, in the area. Common practice of indoor cooking may reflect the highly dense nature of the settlement.

This thesis showed no reflection of average income on cooking location, whereas lower SES households were more likely to cook outdoors in both municipalities. Interestingly, despite their lower SES status indoor cooking was very common among the Bihari ethnicity. Indoor cooking may contribute significantly to health danger from daily household fuel pollution exposure. The lack of ventilation reported by a majority of households (data missing for 45% of the households) may heighten the pollution exposure in the study communities. Cooking indoors may indicate a potential for an increased health danger among the Bihari in particular. Interestingly the household fuel choice was also ethnically driven (tested within Saidpur only). Golden which appeared as the choice of fuel for the Bihari families in Saidpur is a form of compressed rice husk which is commonly used as an alternative to wood or other fuels. Its cost may be more attractive to poorer households, such as the Bihari (low SES). The analysis however showed no link between the average income and the choice of fuel. The assessment of income in this study included all cash income that the household members received per month. It did not take into account any other form of income which may blur the results and explain the strange relationship between the SES and income in this study sample. In Bangladesh in-kind income is a common practice for paying for services and jobs in Bangladesh and may contribute to a large portion of overall family income in many families.

Chapter 7 described the measured carbon monoxide (CO) and particulate matter (PM<2.5μm) pollution levels in the air-quality monitored households. Air-quality monitoring revealed high carbon monoxide and particulate matter (size less than 2.5μm) exposure (CO= 0-16ppm and PM=25 - 3020μg/m^3) which greatly exceeded the safety thresholds set by the World Health Organisation and the US-EPA (PM$_{2.5\mu m}$ >25μg/m^3 and CO>9ppm) **(US-EPA, 1997: WHO, 2005).** The measured pollution levels clearly indicated a danger of adverse health effects from household fuel pollution exposure. The measured pollutant levels in this Bangladeshi sample are comparable with other research publications **(Albalak, Frisancho, Keeler,**

1999: Ezzati and Kammen 2001A,B: Röllin *et al*, 2001: Dasgupta, 2006: Rinne, 2007).

A great variation in the pollutant levels between day and night was observed. Night-time pollution levels were significantly lower than the daytime levels which indicates a lower health risk from household fuel pollution at night, as predicted (carbon monoxide levels= 60%, particulate matter =30%). The measured household particulate matter ($PM_{2.5\mu m}$) and carbon monoxide levels showed a municipal effect: the more densely populated Saidpur had carbon monoxide levels lingering in the households for longer and at night in particular. In Saidpur higher night-time carbon monoxide levels could be due to a higher cooking frequency. Also dangerous particulate matter exposure ($PM_{2.5\mu m} > 25\mu g/m^{3)}$ was more prevalent in household kitchens during the day (75.7%) (18.9% at night) and in Parpatipur daytime dangerous particulate matter levels were found in 45.8% of households (16.7% at night), which indicates a significant health risk. This could explain the greater prevalence of poor health and immunity observed among young children in Saidpur, despite the better socio-economic status and wealth in this municipality, which are normally associated with better health.

The difference in the rate of pollutant clearance may suggest that ventilation methods or household arrangements may be more efficient at clearing one type of pollution than another **(Freeman and Tejada, 2002: Brauer and Saxena, 2002)**. An examination of the effect of household room numbers on the measured pollution levels in the kitchen did not show an association, which contradicts what has been suggested by other authors **(Dasgupta *et al*, 2006).** In larger households pollutants could spread around more evenly leaving the kitchen levels lower. However the household size could possibly explain the lack of effect of room numbers on the pollution levels: in this study household room division was not always a clear-cut case: even solid walls separating two rooms often had gaps and cracks which allowed air exchange. Some discrepancy in the classification of the households into one-room and two-room households could have happened which could explain this unusual result. More thorough analysis of household volume, room arrangement and information about alternative ventilation methods are needed to identify their effect on the household fuel pollution exposure and the risk of respiratory infections in

this community. The effectiveness of ventilation could not be studied in this work as ventilation was rare in the study sample. Ventilation is likely to have a highly beneficial effect on reducing the health risk from household fuel pollution exposure as suggested in other studies **(Brauer and Saxena 2002: Ezzati and Kammen, 2001A,B, 2002A,B: Dasgupta *et al.* (2004a, 2004b))**.

Significant municipal differences in the recorded night-time carbon monoxide levels could not be explained by the household wall material using this data., which contradicts previous research which suggests an effect of household building material on the rate of pollutant clearance **(World Bank, 2002: Brauer and Saxena, 2002)**. This was surprising as other studies have suggested lower pollution levels in households with more porous walls (low air tightness - e.g. bamboo and thatch) **(Dasgupta *et al*, 2006)**. The authors suggested that Bangladeshi houses with mud walls have much higher particulate matter levels ($253\mu g/m^3$ higher) than houses with thatched walls due to a higher sealing effect of the mud walls (controlling for fuel type) **(Dasgupta *et al*, 2006)**.

The use of wood as a fuel was very common in Saidpur and among the Bihari ethnicity in particular. The use of dung and plant-derived fuels which were identified as low-health risk fuels was particularly widely practiced in Parpatipur which again highlights the influence of the municipal nature of human behaviour. The thesis data could not clearly show a link between cooking location or fuel-use and the measured pollution levels due to small sub-group sizes. This thesis did not find a link between the fuel type and the measured pollution levels as has been suggested by other research publications (e.g. **Röllin *et al*, 2001: Balakrishnan *et al*, 2002: Rinne *et al*, 2007: Kilabuto, Matsuki, Nakai, 2008**). Often, comparisons are made between biomass fuels against cleaner fuels such as kerosene, biogas and even electricity. Perhaps in this study community factors other than the fuel type are greater determinants of the household fuel pollution levels. Over 98% of the households in this sample used biomass fuel and often two or three types of fuels were burned at once, which is likely to blur the relationship between a fuel type and pollution levels.

This PhD work found a moderate relationship between personal maternal exposure to carbon monoxide and ambient household carbon monoxide levels (R^2=0.335, p<0.01, n=52). A much stronger association was found in a Guatemalan study using 'plancha' and open fires (R^2=0.850, p<0.001, n=55) **(Naeher *et al*, 2001)**. This study states a moderate correlation between ambient particulate matter levels and personal carbon monoxide levels (R^2=0.650, p<0.001, n=41). This association was not found in this PhD. The discrepancy could be due to the different behavioural factors of the extent of pollution exposure experienced by mothers in Bangladesh and Guatemala. Personal household fuel pollution needs to be studied using a larger sample size and real-time monitoring of pollutants.

In this thesis it was not possible to provide a clear cause-effect relationship between the measured household fuel pollution and the prevalence of medically diagnosed respiratory diseases, due to a low sample size of air quality households and young children within them. However the evidence on the surprisingly high prevalence of respiratory infections among children suggests an association between the measured high pollution levels and an increased risk of moderate or severe respiratory infections in children. The study showed that due to the extent of household fuel pollution experienced by households in this community there is a great health danger from household fuel pollution on children in this sample.

8.3. An examination of household fuel pollution as a potential risk factor for respiratory infections in young children after controlling for household socio-demographic background and the cooking and fuel-use practices.

This thesis examined any associations between the respiratory health of children, child growth, and socio-demographic determinants, household structure and cooking- and fuel use behaviour. Acute respiratory infections (or ARI) have been reported as the leading cause of mortality among children under the age of five globally **(Tomaskovic, Boschi-Pinto, Campbell, 2004: Wardlaw, Salama, Johansson, 2006)**. This thesis identified respiratory tract infections (or RTI) as the most significant disease load in the study community. Medically diagnosed

respiratory diseases were highly prevalent among these Bangladeshi children (around 20% throughout the fieldwork) which is consistent with the data on the prevalence of RTI previously reported by the Bangladeshi Demographic Health Survey 2004 (22.2%) **(BDHS, 2004)**. The proportion of children identified with RTI did not significantly change throughout the fieldwork. A temporary dip of the prevalence of RTI by 5.9% during the midterm survey most likely reflected an increase in the prevalence of other illnesses (diarrhoeal diseases, skin infections, ascariasis) due to the timing of the survey in the early parts of the annual monsoon season (midterm survey: June-July 2007). An increased prevalence of fungal and diarrhoeal diseases due to an increased humidity and moisture of the monsoon has been previously reported **(Hashizume *et al*, 2007)**. Health complaints other than moderate or severe RTI reported by doctors consisted of upper airway diseases (URTI – 85%). Only 15% of all other illnesses reported by doctors included skin infections, diarrhoeal diseases and parasitic infections.

The high prevalence of respiratory related ill-health in the study community is likely to be due to the daily chronic exposure to high levels of household fuel pollutant experienced by the study households. Earlier studies have suggested lowered immune defences as a result of chronic daily exposure to indoor air pollution from domestic fuel use **(Smith, 1993: Pandey *et al*, 1991: Ekici, 2005)**.

The effect of the measured household fuel pollution on child health and the prevalence of RTI could not be thoroughly examined in this thesis work due to a small sample size of children in households where air quality was monitored. Neither could the effect of cooking and fuel-use practices on the health risk from household fuel pollution exposure be examined using real measured pollution levels (the small subgroup sizes in the analyses hindered the work). Health danger from the measured high levels of carbon monoxide and particulate matter is evident in this Bangladeshi community. Health damage from household fuel pollution has been suggested as independent of the intensity of the exposure **(Ezzati and Kammen, 2001B)**. Previous research suggests harmful health effects as a result of an exposure to particulate matter just slightly above the background levels **(WHO, 2005)**.

The three sets of data on child health status were consistent with each other. Maternally reported ill-health (discussed to a lesser degree in this thesis) was consistent with the medical reports as suggested by Rousham et al (**Rousham et al, 1998**). The use of the three way method in this thesis provided a very thorough assessment of the child health at all levels of severity of community-wide ill-health.

8.4. An examination of risk factors for poor immune status and under-nutrition in young children, since these will also increase susceptibility to respiratory tract infections

The analysis of subclinical health status using the selected biomarkers of immunity and haemoglobin levels indicated an alarming state of poor health and immunity among young children in this Bangladeshi sample. A large portion of children were anaemic (using 110g/L as a cut-off as by **WHO, 1992b**) and the albumin status (reflecting nutritional status as well as being a negative acute phase protein) was very low for a majority of the children. The infection markers c-reactive protein and alpha-1-acidglycoprotein were elevated in a significant proportion of the children indicating a high prevalence of active infection among children in this community. The high household pollution levels, the high disease burden from respiratory tract diseases and the poor state of immunity supports the theory that a continuous mucosal irritation from chronic household fuel pollution exposure (**Kulkarni and Grigg, 2008**) is likely to reduce general immune defences in exposed individuals (**Brauer, 1998, WHO, 2004**).

A study in Malawi suggested very low levels of protein synthesis and breakdown in children hospitalised for an acute respiratory infection and malnutrition in Malawi (**Manary et al, 1997**). In this thesis the albumin levels or the levels of other selected biomarkers of immunity did not reflect the health status groupings of the children (healthy, RTI). A study from Nepal has shown a link between albumin and SES in Nepal. Children from a lower socio-economic class had significantly lower albumin levels than those from better off families ($p < 0.0001$) (**Panter-Brick et al, 2001**). The findings in thesis were again inconsistent with the described study. Neither SES nor average monthly income were reflected in the child health and growth measures

examined in this study. This could indicate a highly prevalent community-wide state of poor health and immunity among the children in this study sample irrespective of the SES or wealth status of the households. Perhaps both groups of children (healthy and RTI children) in this Bangladeshi study were malnourished and had poor albumin status and immunity. It could be that a long-term chronic effect of household fuel pollution exposure may affect the growth status through a chronic immune-stimulation. Previous research has suggested chronic immune-stimulation which is a highly energy demanding process as an underlying reason for growth retardation **(Lunn *et al*, 2001)** or reduced immune defences **(Wouter *et al*, 2005: Kulkarni and Grigg, 2008).** The use of haemoglobin and the selected biomarkers of immunity revealed the great extent of poor health and immunity among the children in the study community. However, the immune markers were less informative in distinguishing between the healthy and medically diagnosed RTI cases in the sample. This could be due to the extensive prevalence of poor health and immunity in the community and highly immune-challenged and malnourished young children **(Wieranga *et al*, 2005).**

The poor nutritional status of children was reflected as high prevalence of stunting, underweight and wasting observed throughout the fieldwork in this study. A 1.7 times higher prevalence of RTI in malnourished Bangladeshi children with low weight-for age scores has been reported in an earlier study **(Rahman *et al*, 1990).** This thesis work did not show any pattern in the growth z-scores between healthy and RTI groups of children. Despite the lack of SES or income effect on child growth detected in this thesis work the reported high prevalence of growth retardation and RTI in this community is in agreement with the previously reported link between poor growth and an increased risk of acute respiratory infections **(Rahman *et al*, 1990: Nandy *et al*, 2005).** Interestingly the ethnicity was reflected in the degree of growth faltering in this study. The Bihari children were significantly more stunted and underweight and more anaemic in this study and more likely belonged to the lower SES status (as previously discussed). Therefore the SES effect could be indirectly reflected via the ethnic difference in this study, as a part of the more complex setting.

This thesis found no association between the cooking and fuel use practices and child growth using longitudinal GLM analysis. The observed patterning in the height-for-age and weight-for age z-scores among these Bangladeshi children could only be explained by age. The change score for weight-for-age z-score was significantly associated with cooking frequency; a higher cooking frequency indicated lower improvement in the weight-for-age z-score in children. This is interesting as higher SES status was suggested to be linked with more frequent cooking. This would indicate a more severe adverse effect of cooking fuel pollution on the child growth in households which are expected to be better off in terms of higher SES status, better income and standard of life. Interestingly, household stove choice was a significant predictor of child immune status using $\log_{10}$-alpha-1-acidglycoprotein and haemoglobin. $\log_{10}$-alpha-1-acidglycoprotein levels were also predicted by ethnicity whereas the patterning of the haemoglobin was municipally-driven. Inversely transformed albumin levels showed only a significant age-effect. This study showed a poorer health in terms of elevated $\log_{10}$-alpha-1-acidglycoprotein levels (as positive acute phase protein) in children from Bihari households using a fixed stove. Lower haemoglobin levels were associated with Saidpur and especially among children from households cooking on a fixed stove design. Cooking practices in this community were significantly associated with poorer health status. The poorer health (in terms of the selected immune markers) showed a greater health danger from household fuel pollution exposure in high SES households which were more likely to use a fixed stove design.

Linear and logistic regression analyses clearly indicated a strong effect of ethnicity, maternal education, presence of a child by the stove, stove type and cooking location and –frequency on child health and growth. The study showed a more significant blueprint of cooking behaviour (such as stove type, cooking location and the presence of a child by the stove during cooking) on the levels of the selected levels of immune markers but not on the growth z-scores. The regression analyses showed that Bihari children whose mothers were uneducated and cooked on a fixed stove were the worst off in terms of an anaemia ($R=0.558$, $R^2=0.312$, $F=18.127$, $p<0.001$). This pattern was reflected by AGP levels too. Growth data was more closely linked with the SES status and the maternal education in this study. Children from low SES Bihari households with an uneducated mother and where a child

stayed by the stove during cooking were the worst off in terms of the severity of stunting (R=0.329, R^2=0.109, F=4.930, p<0.001) and low weight-for-age (R=0.261, R^2=0.068, F=3.150, p<0.05). This is in agreement with the recent study from Bangladesh which highlights the importance of SES and maternal education as the predictors of child growth status **(Moshena, Mascie-Taylor, Goto, 2010)**. The use of CDC2000 growth reference instead of the World Health Organisation growth references to estimate the growth status of children may lead to some inaccuracies. Perhaps a link between the cooking practices and child growth could be established with the use of WHO 2006 standards.

The use of a fixed stove model instead of a portable model indoors potentially provides a source of a continuous health hazard on a daily basis which exposes all household members to the harmful effects of household fuel pollution. In this study indoor cooking using a fixed stove model was commonly practiced by the Bihari households. Currently no research has examined the effect of stove type in the form of a simple fixed or a portable mud stove on the pollution exposure levels or related child health outcome. Other works have compared the effect of more efficient stoves such as a 'plancha' with a chimney, PATSARI stove, LPG and electric stoves against the inefficient chimneyless mud stoves with pollution levels **(Schei *et al*, 2004: Bruce *et al*, 2004: Romieu *et al*, 2009)**. This thesis work suggests that different cooking practises were strong determinants of child health and the prevalence of moderate/severe respiratory tract infections in this study sample. The evident lack of effect of the SES status or average monthly income on child health among these Bangladeshi children may indicate a widely prevalent health hazard, irrespective of socio-economic status.

The findings of this thesis also provide suggestive evidence of the role of maternal pollution exposure as a very strong determinant of the child's survival of an infantile infectious disease through low birth weight **(Green, Mead, Turner, 1974: Chan *et al*, 1989: Maisonet et al, 2001: Mishra, Retherford, Smith, 2005)** as well as intra-uterine conditions and maternal inflammatory response to particulate matter pollution **(Dexter et al, 2000)**. The birth weights of children in this thesis work were unfortunately not recorded, hence the intra-uterine pollution exposure could not be examined. The thesis work would also benefit from an inclusion of thorough

assessments of maternal lung function and immune analysis in order to estimate the long-term effect of maternal exposure to pollutions using chronic obstructive pulmonary disease as a simple indicator. This study could not examine the link between the fuel choice and the health outcome in children using regression, as the use of longitudinal groupings of fuel type reduced the sample size below to n=43, which is too low for any regression analysis (as according to **Harris, 1975**).

8.5. Methodological considerations

This section will review methods applied in this study and their contribution to the work.

8.5.1. Filter paper blood sampling technique

This PhD work examined the suitability of a filter paper method as a technique for blood sampling in field conditions with no appropriate laboratory conditions or facilities available. Its non-invasive nature and lack of requirement for specific storage systems for the samples made it very attractive for the use in field studies such as described in this thesis (which include child participants in particular), as recommended by previous studies (e.g. **McDade, 2001, Panter-Brick** *et al*, **2001**). This work showed that the filter paper method was very well accepted by the study participants due to its minimally invasive nature.

Quantitative immune assays applied (ELISA and immune precipitation techniques) in this thesis suggested a less informative and discriminating nature of alpha-1-acidglycoprotein (or AGP) as a marker of infection. The poor ability of AGP to predict the immune status could also be due to the blood sampling method applied in this study. High environmental temperatures to which the blood spot samples were exposed may have caused some molecular instability and damaged the proteins of interest and may have reduced the concentration of this protein. The levels of c-reactive protein from filter paper samples were very low throughout the study. Similarly low c-reactive protein levels were obtained from filter paper samples collected in laboratory conditions for a test purpose. This would suggest that the fibrous structure of the filter paper could affect the c-reactive protein concentration as suggested by McDade (**McDade, 2001**). However in this thesis

work c-reactive protein data maintained its good discriminating nature despite the blood sampling technique and provided very useful information about the child immune health. Perhaps AGP levels were more affected by the storing conditions, high ambient temperatures and humidity to which the samples were exposed prior to the storing. It could also be that the set of immune markers examined in this study were not discriminate enough to be used as an indicator of health in a community with an extreme community-wide disease load such as RTI as well as highly prevalent malnutrition (**Wieranga *et al*, 2005**).

The validation study examined the accuracy of the filter paper technique as an alternative blood collection method. A comparison was made between the concentrations of CRP and albumin from venous liquid whole blood and finger-prick blood samples collected on filter paper. The study showed a large reduction in the protein concentration when the filter paper was used as a method of collection for finger-prick blood samples instead of the use of venous liquid whole blood. The filter paper concentration of CRP was less than a quarter of the measured levels in the liquid whole blood. Only half of the albumin concentration of the liquid whole blood was recovered from the filter paper samples. The study therefore showed a significant effect of the blood collection method on the protein concentration levels, which is consistent with earlier research findings (**McDade, 2001**). It may be protein concentrations are reduced due to a binding of the blood proteins onto the fibrous filter paper. Some proteins may bind to the fibres of the filter paper tighter than others, which may have a great influence on their concentrations. Then only a percentage of their real concentration can be efficiently eluded by the use of washing buffer (see Chapter 4 for details of the sample treatment). However the loss observed in the CRP concentrations in this thesis work was much higher than previously reported by McDade (**McDade, 2001**).The reduction of the blood protein levels (CRP and albumin) could also be affected by the use of the finger-prick blood sample technique itself. Blood protein concentrations may be diluted by tissue fluids when collected from extremities such as fingers or heels, especially if the finger is milked or squeezed to increase the blood flow. This thesis highlights the need to carry out a detailed study on the effect of filter paper on blood sampling on the concentrations of proteins of interest. The use of hematocrit analysis with blood

spot analysis as a control for any environmental effect on protein levels in blood spots has been suggested **(Mei *et al*, 2001).**

The validation study also included a small-scale study of the effect of environmental conditions such as high heat and humidity (such as observed in Bangladesh) on blood protein concentration on filter paper. A significant reduction of the c-reactive concentration was observed as a result of short (few days) or long (two weeks) exposure of the samples to high heat and humidity. This study showed no significant effect of high heat except on the c-reactive protein concentrations. It could be that the heat only may only cause a slow denaturation of the blood protein and affects its binding to the filter paper. Additionally the humidity may lead to a slow chromatographic effect of the denatured blood protein reducing the concentration on the filter paper even further. Previous research has suggested a chromatographic effect on blood samples as a potential downside of the filter paper method for blood sampling ((**McDade and Shell-Duncan, 2002).** It cannot be assumed that all proteins are affected by the filter paper matrix or environmental conditions in a universal way. The discrepancies in the levels of the selected biomarkers of immunity observed in this study could be due to a different sensitivity of the immune markers to the sample collection methods or environmental conditions. The data from the validation study highlighted the importance of examining the effect of the sample collection method on each protein of interest. It is essential to work out a conversion factor (filter paper X = whole liquid blood, where X is conversion factor) individually for each blood protein under investigation. The use of a universal conversion rate to calculate the whole liquid blood concentrations from the filter paper blood samples could lead to huge inaccuracies.

8.5.2. An assessment of the intervention

An improved stove design was introduced into this Bangladeshi community very close to the end of the fieldwork and its spread relied on passive transfer. The final survey saw an increase in the prevalence of stoves with chimneys from zero percent (baseline survey) to over 10% which is positive. However due to the shortness of the effect of the technical intervention in the communities it is not possible to

conclude on its effectiveness in reducing household fuel pollution exposure in the study households (and the related adverse health effects). The final survey data indicated that the improved stoves were well accepted by households. The educational and behavioural intervention involved educating households on health dangers from household fuel pollution exposure and the risk of respiratory infections in children and it was well targeted: it was mainly aimed at household mothers. Bill boards and posters were visibly displayed in the community, households were aware of the campaign and it was well accepted by the household mothers. The health messages and the improved stove were designed on the basis of the women's needs identified in the focus groups which makes the intervention programme more sustainable. A good understanding of socio-cultural settings, which are tightly linked with the household cooking behaviour (Ezzati *et al*, 2004) will improve the uptake and the sustainability of an intervention. Both interventions were of very low cost or no cost to the households and their simplicity and accessibility to all will strengthen their uptake in this community. Passive transfer is beneficial as it empowers the households to spread the intervention. However without any further educational support work to ensure the knowledge transfer this intervention may just be ignored in the study community in the long-term.

8.5.3. An ideal interventional study design

This PhD study would have benefited from a period (2 months of weekly assessments) of more intensive health assessment of the very young children (under the age of 12 months) in order to obtain a clearer picture of the respiratory health damage from households fuel pollution in this community. An inclusion of a thorough assessment of maternal respiratory health would have helped to provide information about the long-term effect of household fuel pollution exposure on the pulmonary health. Young children (due to their shorter exposure in terms of age) are unlikely to exhibit clear signs of health impact of fuel fume exposure.

The use of selected biomarkers of immunity is a very effective way to measure children's general health. However, the use of a more direct assessment of tissue damage of the pulmonary tissue may have been more informative. However, at the time of the study a lack of pulmonary-specific biomarkers of immunity (more

informative biomarkers of pulmonary damage as a result of household fuel pollution exposure) led to the choice of biomarkers used in this thesis. The biomarkers selected for the study were well-known and widely used in clinical studies for a long-time which strengthened their value as indicators of immune health in this study.

This PhD work saw an improvement in the child health and growth. The effect of the technical intervention on the prevalence of RTI could not be examined due to the late dissemination of the work (due to delays and complications in the field - see Chapter7for details). Behavioural intervention aimed to reduce household pollution exposure through small simple changes in the daily lives of household members such as keeping children away from cooking. This method has been suggested as a potentially effective behavioural intervention in earlier publications (**Ezzati, Saleh, Kammen, 2000: Dasgupta** *et al*, **2006**). Indeed, this thesis work has shown the presence of a child by the stove as a significant determinant of health status and the risk of RTI. The observed improvement in the immune status of children could therefore indicate the beneficial influence of the behavioural intervention. Chapter 7 highlights a considerable reduction in the number of households reporting young children being present by the stove during cooking by the final survey (in other words an increase in the healthier behaviours).

It must however be understood that even a simple behavioural change may not be as simple as it looks. Perhaps household do not have resources to keep children separated from their mother during the long task of cooking: there might not be other family members to look after very young children. Households may not have a safe play area outside the house, where children could be left safely. In some cases the weather conditions are not suitable for keeping children outside during cooking, for example during the heavy monsoon rains or cold climates. The nature of the settlement is likely to influence the effectiveness of a technical intervention. A reduction in the household pollution exposure in the densely populated Saidpur is likely to have a more significant effect on child health than in Parpatipur due to availability of open space and cooking areas outside, but the health risk from domestic pollution exposure may be harder to determine. These points need to be

carefully considered when designing a successful intervention to reduce household fuel pollution exposure. Perhaps in such situations a technical intervention in the form of a more efficient and cleaner burning stove with a chimney may be a more suitable solution in reducing the adverse health impact from household fuel pollution exposure. Behavioural change however can help to save lives in communities where household fuel pollution is a significant health problem, but where benefits from economical improvements such as a nationwide installation of biogas stoves or even electricity cannot reach them in the near future **(Budds, Biran, Rouse, 2001)**.

8.6. Study constraints

Statistical analyses applied in this study were often hindered by the small subgroup sizes available. No power calculations were undertaken to define the effective sample size prior to the fieldwork. The low sample size affected the analysis of the effect of household fuel pollution data and the effect of the measured pollutant levels of the observed child health, growth and immunity. These interesting points could not be analysed efficiently which was very unfortunate. With a larger sample size the strong study design of this thesis could have enhanced the understanding of the mechanism and the causal pathway of the significance of household fuel pollution exposure in child health and growth outcomes in developing countries. This study has highlighted the importance of power calculations as a method of maximising the effect and the chance of detecting informative variation within the dataset and a success of worthwhile statistical analyses.

The stove intervention took a very long time to design and implement in the study community and its benefits could not be assessed within the time frame of the work. The households benefited from the improved stove only for two months before the final field survey (March 2007) which is too short to detect any benefits related to the intervention. The assessment of the health benefit would require a time lapse of at least one to two years before any improvement in the health status could be linked with the intervention, as suggested by **Smith *et al*, 2007**. The spread of the intervention relied on passive information transfer (word-of mouth by the

community members), which may be slow. Also the knowledge transfer is highly dependent on the interest and efforts of the community in receipt of the intervention.

Air-quality was monitored at the baseline only due to the high cost of the measurements. No measurements of household fuel pollution levels were taken during the final survey when the improved stoves were in place in some households. Pollution monitoring at the end of the study would have allowed for the comparison of the pre and post intervention pollution levels providing very useful information about the real efficiency of the stove intervention in reducing pollution levels in the communities. A second pollution monitoring was included in the initial plan for this PhD work, however due to the high cost of monitoring and data processing afterwards this had to be dropped. The sample size of the households partaking in the air-quality monitoring was too small for an effective use of the measured pollution data. The small sample size can reduce the lack of variation observed within the sample which may lead to inaccurate conclusions about the sample. The sample diversity can be greatly affected by sampling errors which may cause a heavily biased data set, if the sample size is small. For this reason the low sample size can easily be non-normal which itself reduces the efficiency of any statistical analysis. Now a greater diversity of pollution monitors suitable for field conditions, with no access to electricity, are available. Portable, battery-powered monitors which measure real-time carbon monoxide and particulate matter data are likely to be a more reliable and accurate method of examining pollution levels at much lower cost than the previously used method, which is a great advantage.

8.7. Strengths of the PhD study

This study efficiently monitored the actual real-time carbon monoxide and accumulative particulate matter (2.5μm) levels in air-quality households during the day and night. This thesis work has provided solid data on the extent of household fuel pollution exposure experienced by households in the study sample. Also the health status of children under the age of five years was thoroughly monitored using maternal reporting, medical diagnosis and the analysis of selected biomarkers of immunity to cover all cases and severities of health problems among the children. This study attempted to link direct, measured pollution data with measured health

data from effective health diagnosis, instead of relying on a self-reported health history and reported pollution levels. This is a great strength of this study design. The use of the three way assessment of health provides very accurate data on the health status of children in this study.

The use of longitudinal data on child growth, health and immunity and the analysis of the predictors of this data using longitudinal sets of socio-demographic determinants and details on cooking and fuel-use practices has made the results accurate and reliable. The method applied has taken into account any changes in the cooking and fuel-use behaviour which may lead to strange patterning in the child health and growth observed. These changes are a natural part of people's lives and should be carefully considered when designing a sustainable intervention to reduce pollution exposures.

This study was a community based study which used real health and pollution data from communities. This study was able to provide community-wide information about ill-health among children in this community, which makes the data more realistic and powerful. Many previous household fuel pollution studies have used patients referred to a hospital for treatment due to respiratory illness. This kind of study design allows monitoring of the health impacts and the severity of respiratory infections as a result of household fuel pollution only in a minority of people. In most cases of respiratory infections individuals do not end up in hospitals for treatment. Hospital-based studies exclude this segment of the society, which weakens this type of study design. The study design used in the thesis work enabled an examination of child health and household fuel pollution levels at the community levels, reaching all members of the society in their natural surroundings. This type of study design is likely to provide very accurate and unbiased data on the health impacts of household fuel pollution.

The behavioural and technical interventions applied in this study benefitted from their design: they could be carried out at low cost, or even no cost to the participating household and they were designed to suit all segments of the society. This type of intervention could be successfully applied to a large-scale intervention to reduce household fuel pollution exposure. The nature of this intervention makes

it applicable for use in other countries which are burdened with health dangers from household fuel pollution exposure. Whilst developing countries are waiting for a national input into tackling the fuel pollution problem this type of intervention might be effective and reach everyone in all segments of a society. The behavioural intervention (with a design which is locally suitable) in particular is a very useful method of reducing household fuel pollution exposure at a large-scale effectively and at minimum cost and invasiveness to the lives of the communities.

8.8. Future directions:

The literature review in Chapter 2 showed the lack of power of studies which use verbally reported health and pollution exposure measures to draw conclusions about the adverse health outcomes of household fuel pollution. Recently better methods have been designed to effectively assess the pulmonary health of women and children in communities. The Guatemalan RESPIRE study is a good example of a health intervention with an effective assessment of pulmonary function using the ratio of forced expiratory volume to forced vital capacity (FEV: FVC) and spirometry as measures. Personal exposure to fuel pollution was via carbon monoxide in the exhaled breath as a measure of carboxyhaemoglobin (to measure blood carbon monoxide concentration) (**Diaz *et al*, 2007**). It would be useful to apply similar methods to further studies to examine pulmonary health as a result of household fuel pollution exposure.

At the time of the start of this PhD, no method of measuring real-time particulate matter levels was available, or at least not at an affordable cost. Now technology is available to study household particulate matter and carbon monoxide using methods including light portable monitors which can even be worn by the household cook. This enables a measurement of real-time personal PM and CO exposure in the households, which could be very useful in determining a real relationship with ambient and personal pollution exposures accurately, as well as when examining the effect of ventilation or fuel type on household fuel pollution levels. Minute-by-minute assessment of particulate matter pollution levels would help to determine any changes in the particulate matter pollution levels throughout the day, rather than relying on accumulative measurements (as used in this thesis). Mean daily averages

of pollution levels have been suggested as poor indicators of daily pollution exposure by household members **(Ezzati and Kammen 2001A,B)**. At the time of this thesis the only way personal carbon monoxide exposure could be measured was by accumulative carbon monoxide measures using a Dräeger tube worn in clothes. For carbon monoxide an accumulative measurement as an estimate of personal exposure is particularly poor, due to the mechanism in which carbon monoxide exposure can be dangerous to the health. Sudden very high levels of carbon monoxide can be deadly.

Authors Kulkarni and Grigg have developed an effective method of assessing the particulate matter load in the alveolar macrophages as a result of fuel pollution exposure **(Kulkarni and Grigg, 2005)**. The particulate matter load of alveolar macrophages can be measured from sputum samples (sputum induction is carried out by an ultrasonic nebuliser) and cell smear from the sputum is examined under a light microscope. This method provides a direct method of assessing the pulmonary particulate matter content as a response to fuel pollution exposure, which would be very advantageous in examining the direct particulate matter load in the lungs as a result of household fuel pollution exposure.

8.9. Conclusion

This PhD work has contributed to the knowledge of health outcomes of household fuel pollution exposure using measured data on child health and growth, immune status as well as air quality monitoring. So far no study has examined the effect of a stove type in terms of a fixed or portable chimneyless biomass stove on quantified child health, growth and immune status. Previous studies have compared the health effect of biomass fuels versus clean fuels, **(Röllin *et al*, 2001; Balakrishnan *et al*, 2002; Dasgupta *et al*, 2006)** but no studies have examined the effect of different types of chimneyless biomass stoves and the impact of the stove design and its related use on child health. This PhD study has shown a significant influence of stove type on child health in this Bangladeshi community. This study also clearly indicated that the relationship between child growth and household fuel pollution is not straightforward. The quantified child growth measures showed no association with common cooking practices of this study community. However, the poorer relative

change of growth status was associated with higher cooking frequency indicating higher exposure to household fuel pollutants by the household members in this community.

No study so far has quantified the presence of medically examined respiratory tract infection and examined an association between the effect of the presence of a child by the stove during cooking and RTI. This study has shown the presence of a child by the stove as a significant risk factor for RTI. The author of this study was not aware of any studies examining the actual effect of the presence of a child by the stove during cooking on the health outcomes. Most studies have attempted to link household fuel pollution with health outcomes (**Perez-Padilla** *et al*, **1999; Naeher** *et al*, **1999; Ezzati and Kammen, 2001A,B, 2002A,B; Balakrishnan** *et al*, **2002;**), but have not examined the behavioural patterns of the household members in detail. The effect of household number and air tightness of a household on the pollution levels have also been reported (**World Bank 2002; Brauer and Saxena, 2002**). A previous study has shown a fast reduction of pollution levels when moving away from the stove within a household (**Ezzati and Kammen 2001A, B**). This type of information is of very high significance as it is important to identify unhealthy behaviours observed and their relationship with the adverse health outcome in order to find a suitable and effective behavioural change to solve or to reduce (at least) household fuel pollution exposure. This study has highlighted a new direction for research into adverse health outcomes of household fuel pollution. This is essential to examine daily behaviours of household members including children in order to efficiently target efforts to reduce the adverse health outcome due to chronic household fuel pollution exposure.

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Appendix 1: Child health questionnaire and medical examination record.

A1.1. Household identification data:

	General Information	
1	District	Nilphamari 1 Dinajpur 2
2	Municipality	Saidpur 1 Parbatipur 2
3	Ward	☐☐
4	Cluster	☐☐
5	Household ID Number	☐☐☐
6	Date of interview	Day ☐☐ Month ☐☐ 2005
7	Name and code of enumerator	Name:_____ ☐☐
8	Status of interview	Completed 1 Incomplete 2
9	Name of Household Head	Name:_____
10	ID no of Respondent	☐☐
11	Primary or secondary cook	Primary cook 1 Secondary cook 2
12	Selected for IAP monitoring	Yes 1 No 2

A1.2. Child health questionnaire:

Interviwerr: If 2-59 months aged child is available in the household, ask Q. E16-E20 to mother or caretaker or else skip to QF1.				
	Youngest child		**Next to youngest child**	
Write the name and ID of child:	Name:_____ ID □□		Name:_____ ID □□	
Date of birth of child:	Day Month Year □□ □□ □□□ Don't know 99999997		Day Month Year □□ □□ □□□ Don't know 99999997	
Has the _____ in your (Name) household been unwell in the last 2 weeks?	Yes 1 No 2		Yes 1 No 2	
In the last 2 weeks, has the youngest child in your household experienced any of the following? (Read out)				
Illness	**Yes**	**No**	**Yes**	**No**
Blocked or runny nose	1	2	1	2
Sore throat	1	2	1	2
Ear discharge	1	2	1	2
Fever	1	2	1	2
Cough	1	2	1	2
Asthma	1	2	1	2
Wheezing or whistling breath	1	2	1	2
Breathing faster than usual with short fast breaths	1	2	1	2
Unable to breastfeed, or feed	1	2	1	2
Lower chest indrawing	1	2	1	2
Diarrhea	1	2	1	2
Interviewer: Check Q.E18a to E18k and circle in appropriate code.	**One or more than one code 1 is circled 1 None of code 1 is circled 2 → E20**		**One or more than one code 1 is circled 1 None of code 1 is circled 2 → E20**	
Interviewer: If children with any of the symptoms numbered from E18d-E18k arrange for medical examination.				
Did you seek treatment for this child for any of these conditions?	Yes.............................1 No.................................2 ⌐ E20 ◄———┘		Yes.............................1 No.................................2 ⌐ E20◄——┘	

| Please specify for which conditions you seek treatment?

(Multiple answer) | Blocked or runny nose.. 01
Sore throat 02
Ear discharge 03
Fever.............................. 04
Cough 05
Asthma........................... 06
Wheezing or whistling
   Breath 07
Breathing faster than usual
   with short fast breaths.. 08
Unable to breastfeed or
   Feed 09
Lower chest indrawing ... 10
Diarrhea .... 11 | Blocked or runny nose... 01
Sore throat........................02
Ear discharge 03
Fever 04
Cough..............................05
Asthma............................06
Wheezing or whistling
   Breath............................07
Breathing faster than usual
   with short fast breaths.08
Unable to breastfeed or
   Feed............................. 09
Lower chest indrawing....10
Diarrhea ....   11 |

What foods and/or liquids did the youngest child take yesterday from morning until night?

Type of food	Yes No Times	Yes No Times
1. Breast milk	1 → 2	1 → 2
2. Water	1 → 2	1 → 2
3. Porridge	1 → 2	1 → 2
4. Semi solids	1 → 2	1 → 2
5. Animal milk	1 → 2	1 → 2
6. Solid food	1 → 2	1 → 2

Interviewer: Measure the weight and height of the child and record the values in the box.

Using Uni scale, accurately take and record weight:	KG Gram Mother &child both ☐☐ ☐☐ Only mother ☐☐ ☐☐	KG Gram Mother &child both ☐☐ ☐☐ Only mother ☐☐ ☐☐
Using Height board, accurately asses and record height: (Interviewer: For <24 month by lying and for 24 and >24 month by standing)	CM ☐☐☐.☐ (e.g. 99.5cm or 121.7cm)	CM ☐☐☐.☐ (e.g. 99.5cm or 121.7cm)
Blood prick sample taken?	Yes 1 No 2 E26 Not possible 3	Yes 1 No 2 E26 Not possible 3
Using hemocue, record hemoglobin:	Hemoglobin ☐☐☐ (e.g 102g/L or 95g?l) Tube/ ☐☐☐ Blood spot paper no	Hemoglobin ☐☐☐ (e.g 102g/L) Tube/ ☐☐☐ Blood spot paper no
	Go to E16 for next child if none then go to F12	
	Youngest child	**Next to youngest child**

	Youngest child	**Next to youngest child**

Interviewer:Check E18d to E18k and circle in appropriate code.	One or more than one
	  code 1 is circled 1
	Give the questionnaire
	for medical examination
	None of code 1 is circled 2
		One or more than one
		  code 1 is circled **1**
		Give the questionnaire
		for medical examination
		None of code 1 is circled 2

Interviewer: Please check the filled in questionnaire and then give thanks to resonpodent.

| Finishing time: | Hour ☐☐   Minute ☐☐ | |

A1.3. Medical examination record:

Only for children who have reported one or more symptoms on question E18d-E18k.		
	Youngest child	**Next to youngest child**
Write the name and ID of child:	Name:_____ ID # ☐☐	Name:_____ ID #☐☐
Check and record Child's Temperature:	In centigrade:	in centigrade:
Breathing rate: Please note method of counting for breathing rate	Breaths/min: Method:	Breaths/min: Method:
Activity of child while counting breathing rate?	Sleeping 1 Lying 2 Breastfeeding/sitting 3 Crying 4	Sleeping 1 Lying 2 Breastfeeding/sitting 3 Crying 4
Presence of chest indrawing?	Yes 1 No 2	Yes 1 No 2

Severity of chest indrawing
 	
(circle all that apply)
	Below rib cage and
	   intercostal muscles 1
	Above the sternum 2
	Around collar bones and
	   around back of thorax 3
		Below rib cage and
		   intercostal muscles 1
		Above the sternum 2
		Around collar bones and
		   around back of thorax 3

Chest auscultation notes	**Severity of respiratory infection:** Normal chest sounds 0 Mild 1 Moderate 2 Severe 3	**Severity of respiratory infection:** Normal chest sounds 0 Mild 1 Moderate 2 Severe 3
Is the child on any current medication?	Yes 1 No 2 **Details:** **Respiratory illness** **Other**	Yes 1 No 2 **Details:** **Respiratory illness** **Other**
Details of any medication prescribed on date of examination	**Respiratory illness** **Other**	**Respiratory illness** **Other**

Appendix 2:   Ethical approval for the study methods

Ref No: R05/P22

LOUGHBOROUGH UNIVERSITY
ETHICAL ADVISORY SUB-COMMITTEE

RESEARCH PROPOSAL
INVOLVING HUMAN PARTICIPANTS

Title: Risk factors for indoor air pollution and respiratory infections among
mothers and children in Bangladesh

Applicants: Dr E Rousham, P Nasanen, Dr S Saha

Department: Human Sciences

Date of clearance: 22 March 2005

Comments of the Sub-Committee:

The Sub-Committee was satisfied with the qualifications and experience of the
investigators, and with the details provided on the nature of the research, and the procedures
to be followed. The Sub-Committee agreed to issue clearance to proceed subject to the
following conditions:
- That the investigators provided confirmation of approval from Concern's ethical
committee when available.
- That the investigators clarified with the University's Finance Office that their
insurance cover was adequate for all aspects of the proposed work.

Appendix 3: Preparation of washing and coating buffers for Enzyme-Linked-Immuno-Assay (or ELISA)

A3.1. Introduction:

ELISA assay was used to detect the concentration of c-reactive protein, and alpha-1-acidglycoprotein levels from finger prick blood samples collected on filter paper. The detailed description below shows how washing buffer and coating buffers were made freshly from reagents for the immune assays (following DAKO (UK) method).

A3.2. Reagent preparations:

The following quantities of reagents were measured for the preparation of coating buffer and washing buffer using digital weighing scales with an accuracy of 0.001g.

A3.2.1 Preparation of coating buffer:

Chemical formula: 0.01M Phosphate buffer (PBS buffer), 0.15M NaCl, pH 7.2

Table 3.1: Reagents required for the preparation of coating buffer

Reagent	Supplier	Product code	1000mL (90 plates)	500mL (45 plates)
NaH_2PO_4-H_2O	Fisher	S9638	0.35g	0.18g
Na_2HPO_4	Fisher	S0876	1.07g	0.54g
NaCl	Fisher	42429-5000	8.97g	4.24g
H_2O			1000mL	500mL

Each reagent was measured before hand. Reagents were added into the deionised distilled water in small quantities one at a time. The volume of the distilled water into which the reagents were added was initially 80% of the total volume prepared in order to account for any changes in the total volume of the coating buffer due to volume replacement by solid reagents and resulting water from chemical reactions

during pH adjustment. A stirrer bar was used to mix the solution during the process
and no reagent was added until the previous quantity had dissolved completely.
Once the reagents had been added the pH of the solution was measured using a pH
meter. The final solution was expected to have a pH of 7.2 ($\pm$0.2). Any deviations
from the aimed pH was corrected by adding drops of hydrochloric acid (to reduce
pH) or sodium hydroxide (to increase pH). Once the correct pH had been obtained
the solution as topped up with deionised distilled water to make the desired quantity
of coating buffer. The coating buffer was stored in a glass bottle with a sealable top
for up to two months in a fridge at +4^{0}C.

A3.2.2. Preparation of washing buffer

Chemical formula: 0.01M Phosphate buffer (PBS buffer), 0.50M NaCl, Tween 20,
pH 7.2)

Table 3.2: Reagents required for the preparation for washing buffer

Reagent	Supplier	Product code	Amount for 9 washes (3 ELISA)
NaH_2PO_4-H_2O	Fisher	S9638	0.35g
Na_2HPO_4	Fisher	S0876	1.07g
NaCl	Fisher	42429-5000	29.22g
Tween 20	Fisher	S196630	1mL
H_2O			1000mL

The reagents of the washing buffer were dissolved into deionised and distilled water
in the same way as described in the previous section (see A2.2.1: Preparation of the
coating buffer). However before making up the total volume of solution 1mL of
Tween$_{20}$ was added using a dropper pipette. Once Tween had been added the total
volume of washing buffer was made up to the aimed one litre. Washing buffer was
stored up to two months, and checked for any fungal formation.

Appendix 3a: Detailed protocol for c-reactive protein ELISA assay

A3a.1. Introduction:

This protocol provides a detailed description of steps involved in the assessment of c-reactive protein levels using Enzyme-Linked-Immuno-Assay (or ELISA) (following DAKO (UK) method).

A3a.2. Preparations DAY BEFORE ELISA:

A.3a.2.1. Sample preparation:

Blood spots were diluted by a factor 1:100 by adding blood spot discs containing 12.5µL of blood into 1.25mL of washing buffer. Samples were left in a fridge overnight so that maximum amount of blood could be washed off the filter paper.

A.3a.2.2. Coating of a MaxiSoap plate with coating antibody for ELISA analysis:

A stock coating antibody was diluted so that the final volume was 10mg/L. (If a stock concentration was 11g/L = 11 000mg/L, a 1:1100 dilution was performed by adding 10µL of antibody stock into 11mL coating buffer). The coating buffer was prepared using the standard DAKO protocol (see appendix 2). The solution was mixed well. The MaxiSoap plate was coated with coating antibody by adding 100µL of coating antibody into each well. Then the plate was wrapped in cling film and kept in a fridge at 4°C.

A.3a.3. Preparations on the MORNING OF THE ELISA RUN:

A.3a.3.1. Plate preparation:

The plate which was coated with coating buffer the night before was washed with washing buffer three times before the samples and the standards were distributed.

The coating buffer was flicked out of the wells and each well was filled with washing buffer, after which the washing buffer was flicked out. This was repeated three times. This was done once the standards had been prepared.

A.3a.3.2. Final standard concentrations:

Final standard concentrations (10μg/L, 8μg/L, 6μg/L, 5μg/L, 4μg/L, 2μg/L and 1μg/L) were prepared as shown in Table below:

Table 3a.1: Preparation of c-reactive protein standards from stock

Standard	Stock	Washing buffer	Dilution
Stock (conc: 163mg/L)	1μL into	99μL	Final concentration: 1630μg/L (1:100 dilution)
10μg/L	12.3μL	1987.7μL	(10μg/L /1630μg/L) x 2000μL = 12.3μL
8μg/L	800μL of 10μg/L	200μL	(8μg/L/10μg/L) x 1000μL = 800μL
6μg/L,	150μL of 10μg/L standard	100μL	(6μg/L/10μg/L) x 250μL = 150μL
5μg/L,	250μL of 10μg/L standard	250μL	(5μg/L/10μg/L) x 500μL = 250μL
4μg/L	250μL of 8μg/L standard	250μL	(4μg/L/8μg/L) x 500μL = 250μL
2μg/L	250μL of 4μg/L standard	250μL	(2μg/L/4μg/L) x 500μL = 250μL
1μg/L	250μL of 2μg/L standard	250μL	(1μg/L /2μg/L) x 500μL = 250μL

A.3a.3.3. Sample distribution:

100μL of the 1:100 dilution of each blood sample and the standards (as described) were distributed into each well in duplicate. Once distributed the plate was incubated in an incubator for two hours at room temperature. The plate was shaken for 10 seconds every 30 min. After the incubation the plate was washed three times with washing buffer (as described previously).

A.3a.3.4. HRP antibody labelling:

The samples were labelled with HRP antibody by adding 100μL of diluted c-reactive protein-HRP antibody into each well using a multichannel pipette. Before

distribution the CRP-HRP was diluted by adding 3μL of CRP-HRP antibody stock into 12mL of wash buffer. This was mixed well using the pipette. The plate was wrapped in cling film and incubated at room temperature for two hours. After the incubation the plate was washed three times with washing buffer (as described previously).

A.3a.3.5. Colour-labelling of the samples antibody-antigen binding using OPD tablets:

OPD tablets were taken out of the fridge about 40 min before the labelling so that they would be of room temperature. A glass tube containing 12mL of deionised and distilled water was covered with foil to prevent light exposure to the OPD tablets (OPD tablets are sensitive to light). 10 minutes before the procedure 4 OPD tablets were added to the tube and the lid was replaced immediately. Once dissolved 4.3μL of 35 Mol hydrogen peroxide was added into the OPD solution and mixed well.

100 μL of OPD solution was then added into each well on the plate. The plate was incubated in dark for exactly 30 min. Colour change from clear to yellow/orange was observed (as an indicator of a successful colour labelling) at the end of the incubation. 100 μL of sulphuric acid (0.5M H2SO4) was added into each well to stop the colour development. Drastic colour change to dark orange was observed.

A.3a.3.6. Reading protein concentrations:

The c-reactive protein concentration in the blood samples was read at wave length: 490nm using the plate reader. Standard curve was set as 4-parameter logistic and no blank subtraction was used.

A3a.3.7. List of equipment and reagents required to perform this ELISA assay

Table 3a.2: A list of reagents and equipment required to perform the c-reactive protein ELISA using DAKO method

Reagents	Company	Cat no.	Storage
Human Serum Protein Calibrator	DAKO	X0923	4°C
Polyclonal antibody to c-reactive protein	DAKO	Q0329	4°C
Polyclonal antibody to c-reactive protein - HRP	DAKO	P0227	4°C
OPD tablet for ELISA	DAKO	S2045	4°C
Hydrogen peroxide (H_2O_2)	Sigma	H1009	4°C
Sodium Phosphate monobasic monohydrate (NaH_2PO_4-H_2O)	Fisher	S9638	
Sodium phosphate dibasic anhydrous (Na_2HPO_4)	Fisher	S0876	
NaCl	Fisher	42429-5000	
Tween 20	Fisher	S196630	
95-97% H_2SO_4	Fisher		
Equipment			
Nunc-Immuno F96 Maxisorp plates with certificates	Fisher	439454A	
Multi-pipette			
Plate reader			
Dry incubator or oven			

Appendix 3b: Detailed protocol for alpha-1-acidglycoprotein ELISA assay (following DAKO (UK) method)

A3b.1. Introduction:

This protocol provides a detailed description of steps involved in the assessment of alpha-1-acidglycoprotein levels using Enzyme-Linked-Immuno-Assay (or ELISA).

A.3b.2. Preparations DAY BEFORE ELISA:

A.3b.2.1. Sample preparation:

Blood spots were diluted by a factor 1:100 by adding blood spot discs containing 12.5µL of blood into 1.25mL of washing buffer. Samples were left in a fridge overnight so that maximum amount of blood could be washed off the filter paper.

A.3b.2.2. Coating of a MaxiSoap plate with coating antibody for ELISA analysis:

Stock coating antibody was diluted so that the final volume was 10mg/L. (If a stock concentration was 11g/L = 11 000mg/L, a 1:1000 dilution was performed by adding 12µL of antibody stock into 12mL coating buffer (The coating buffer was prepared using DAKO protocol, as described in appendix 2). The solution was mixed well. The MaxiSoap plate was coated with coating antibody by adding 100µL of the diluted coating antibody into each well. Then the plate was wrapped in cling film and kept in a fridge at 4°C.

A.3b.3. Preparations on the MORNING OF THE ELISA RUN:

A.3b.3.1. Plate preparation:

The plate which was coated with coating buffer the night before was washed with washing buffer three times before the sample and standard distribution. The coating buffer was flicked out of the wells and each well was filled with washing buffer, after which the washing buffer was flicked out. This was repeated three times. This was done once the standards had been prepared.

A.3b.3.2. Standard concentrations:

Alpha-1-acid glycoprotein stock (0.73g/L) was first diluted 1:100 by adding 10µL of the stock into 990µL washing buffer to obtain a diluted stock concentration of 7300µg/L. The standards (140µg/L, 110µg/L, 90µg/L, 70µg/L, 50µg/L, 30µg/L, 10µg/L and 5µg/L) were prepared as shown in Table below:

Table 3b.1: Preparation of alpha-acidglycoprotein standards from diluted stock

Standard	Stock (conc: 7300µg/L)	Washing buffer	Dilution $(C_2 x C_1)/V_2 = V_1$
140µg/L	28.8µL of stock	1471.2µL	(140µg/L /7300µg/L) x 1500µL = 28.8µL
110µg/L	22.8µL of stock	1477.2µL	(110µg/L/7300µg/L) x 1500µL = 22.8µL
90µg/L,	18.5µL of stock	1481.5µL	(90µg/L/7300µg/L) x 1500µL= 18.5µL
70µg/L,	400µL of 140µg/L standard	400µL	(70µg/L/140µg/L) x 800µL = 400µL
50µg/L	363.6µL of 110µg/L standard	436.4µL	(50µg/L/110µg/L) x 800µL = 363.6µL
30µg/L	266.7µL of 90µg/L standard	533.3µL	(30µg/L/90µg/L) x 800µL = 266.7µL
10µg/L	88.9µL of 90µg/L standard	711.1µL	(10µg/L /90µg/L) x 800µL = 88.9µL
5µg/L	300µL of 10µg/L standard	300µL	(5µg/L /10µg/L) x 600µL = 300µL

A.3b.3.3. Sample distribution:

100μL of the 1:100 dilution of each blood sample and the standards (as described) were distributed into each well in duplicate. Once distributed the plate was incubated in an incubator for two hours at room temperature. The plate was shaken for 10 seconds every 30 min. After the incubation the plate was washed three times with washing buffer (as described previously).

A.3b.3.4. HRP antibody (peroxide-conjugated antibody) labelling:

The samples were labelled with HRP antibody by adding 100μL of diluted alpha-1-acidglycoprotein-HRP (or AGP-HRP) antibody into each well using multichannel pipette. Before distribution the AGP-HRP was diluted by adding 22μL of AGP-HRP antibody stock into 11mL of wash buffer. This was mixed well using the pipette. The plate was wrapped in cling film and incubated at room temperature for two hours. After the incubation the plate was washed three times with washing buffer (as described previously).

A.3b.3.5. Colour-labelling of the samples antibody-antigen binding using OPD tablets:

OPD tablets were taken out of the fridge about 40 min before the labelling so that they would be of room temperature. A glass tube containing 12mL of deionised and distilled water was covered with foil to prevent light exposure to the OPD tablets (OPD tablets are sensitive to light). 10 minutes before the procedure 4 OPD tablets were added to the tube and the lid was replaced immediately. Once dissolved 4.3μL of 35 Mol hydrogen peroxide was added into the OPD solution and mixed well.

100 μL of OPD solution was then added into each well on the plate. The plate was incubated in dark for exactly 30 min. Colour change from clear to yellow/orange was observed (as an indicator of a successful colour labelling) at the end of the incubation. 100 μL of sulphuric acid (0.5M H2SO4) was added into each well to stop the colour development. Drastic colour change to dark orange was observed.

A.3b.3.6. Reading protein concentrations:

The alpha-1-acidglycoprotein concentration in the blood samples was read at wave length of 490nm using the plate reader. Standard curve was set as linear curve and no blank subtraction was used.

A.3b.3.7. List of equipment and reagents required to perform this ELISA assay

Table 3b.2: A list of reagents and equipment required to perform the alpha-1-acidglycoprotein ELISA using DAKO method

Reagents	Company	Cat no.	Storage
Human Serum Protein Calibrator	DAKO	X0908	4°C
Polyclonal antibody to alpha-1-acid-glycoprotein	Acris	BP518	-20C°
Polyclonal antibody to alpha-1-acid glycoprotein - HRP	Acris	BP518HRP	-20C°
OPD tablet for ELISA	DAKO	S2045	4°C
Hydrogen peroxide (H_2O_2)	Sigma	H1009	4°C
Sodium Phosphate monobasic monohydrate (NaH_2PO_4-H_2O)	Fisher	S9638	
Sodium phosphate dibasic anhydrous (Na_2HPO_4)	Fisher	S0876	
NaCl	Fisher	42429-5000	
Tween 20	Fisher	S196630	
95-97% H_2SO_4	Fisher		
Equipment			
Nunc-Immuno F96 Maxisorp plates with certificates	Fisher	439454A	
Multi-pipette			
Plate reader			
Dry incubator or oven			

Appendix 3c: Detailed protocol for immune precipitation technique for the measurement albumin levels

A.3c.1. Introduction:

This protocol provides a detailed description of the steps involved in the assessment of blood albumin levels using a standard immuno-precipitation assay.

A.3c.2. Sample preparation:

Blood spots were diluted by a factor 1:100 by adding blood spot discs containing 12.5µL of blood into 1.25mL of washing buffer. Samples were left in a fridge overnight so that maximum amount of blood could be washed off the filter paper.

A.3c.3. Preparation of standards:

Standard for the standard curve were prepared using stock and dilution buffer as shown in Table 1. Human serum standard was first diluted 1:80 using dilution buffer by adding 50µL of Human Serum Standard into 3950µL of Dilution buffer.

A.3c.4. Distribution of samples and standards:

10µL of diluted blood samples (1:100 dilution) were added to the wells in duplicate and mixed well. 225µL of Reaction buffer was added to each well using a multichannel pipette. The plate was then immediately placed in the plate reader and antibody-antigen reaction speed was recorded.

A.3c.5. Analysis programme

The programme used to read the reaction speed was as follows:

1. Incubation 1: incubation time 5mins/Temperature 37C'
2. Shake 1: Total time 10sec/ON time 10sec/Speed 900rpm
3. Measurement 1: Stepping/Single/Filter 340nm
4. Pause 1: Run plate out ON/Alarm ON/Waiting time 10mins – put 75µL diluted Albumin antibody by multi-channel pipette, then 'continue'

5. <u>Shake 2:</u> Total 10sec/ON time 10sec/Speed 900rpm

6. <u>Measurement 2:</u> Stepping/Kinetic/Interval 30sec/Measure count 10 (total 5mins)/Filter 340nm

7. <u>Measurement 3:</u> Stepping/Single/Filter 340nm

A.3c.6. Label antibody dilution:

0.63mL of Rabbit-antihuman Albumin was added to 7.875mL of dilution buffer to obtain a suitable concentration (1:12.5 dilution) of the label antibody for the reaction. 75µL of the diluted label antibody was added to each well of the NUNC Microwell Plate during the pause (section 4 in the programme – see section A5.5.).

Table 3c.1: Preparation of albumin standards from stock

Standard	Stock	Method	Dilution Buffer
Stock solution	55.75	50µL Human Serum Standard	3950µL (1:80 dilution of human serum standard - 44.6g/L)
1	44.6	400µL Stock (55.7g/L)	100µL
2	41.8125	300µL Stock (55.7g/L)	100µL
3	33.45	375µL Stock (55.7g/L)	250µL
4	27.875	100µL Stock (55.7g/L)	100µL
5	23.894	300µL Stock (55.7g/L)	400µL
6	13.9375	100µL Stock (55.7g/L)	300µL
7	6.98	100 µL of standard 6 (13.9375g/L)	100 µL
8	0		Dilution buffer only

Table 3c.2: Reagents and equipment used to perform albumin immne-precipitation

Reagents	Company	Catalogue number	Storage
Human Serum Standard	DAKO	X0908	4°C
Rabbit Anti-human Albumin	DAKO	Q0328	4°C
Reaction buffer 1	DAKO	S2007	4°C
Dilution buffer	DAKO	S2005	4°C
Equipment			
NUNC Microwell Plates F96 without lid	Fisher	269620	
Multi-pipette			
Plate reader			

Appendix 4: Blood collection into microtainer tubes for the validation tests

Blood was collected on filter paper as well as into heparinised microtainer tubes from a sub-sample of children in the field (N=17) in order to compare the levels of the selected biomarkers of immunity on the filter paper with the actual level in the liquid blood. This was done to validate the use of filter paper as a method for collecting blood in conditions where it was not possible to carry out the appropriate methods of processing blood samples.

The child's finger was pricked using a safety lancet as described previously. Blood drops were scooped up into a microtainer tube using a FloTop scoop which could be attached to the tube. In order to improve the blood flow the child was asked to stand where possible and the child's hand and arm were also gently rubbed to increase the blood flow. Approximately 400-600μL of blood was collected. Tubes were labelled using the same blood ID number as used for the filter paper samples. The tube lid was replaced immediately and the tube was shaken by inverting the tube at least 10 times before it was placed into an ice-cool box for transfer. The liquid blood samples were spun within 1.5 hours of collection using a centrifuge for minimum of 3min at the speed of 15000rpm to separate blood cells from the plasma before storing it at -20°C in a freezer.