

HEALTH EFFECTS OF CHRONIC EXPOSURE TO SMOKE FROM BIOMASS FUEL BURNING IN RURAL AREAS

FINAL REPORT
2007

Sponsor
World Health Organization (India)
Sticker No. SE/07/118283

Executor
Chittaranjan National Cancer Institute
37 S. P. Mukherjee Road, Kolkata-700 026
Tel: (033) 2476-5101/5102, Extn. 321; Fax: (033) 2475 7606
E-mail: manasrray@rediffmail.com

Research Team

Dr. Twisha Lahiri, Saswati Chowdhury, Dr. Sanghita Roychoudhury, Shabana Siddique, Madhuchanda Banerjee, Dr. Sayali Mukherjee, Sreeparna Chakraborty, Anindita Dutta, Nandan Kr. Mondal, Anirban Banerjee, Debangshu Das, Manas Datta, Pulin Behari Paul and Dr. Manas Ranjan Ray

CONTENTS

	Page No.
Executive Summary	1-3
CHAPTER – 1: Background and Objective Of The Study	4-22
Background	4-20
Biomass fuel use: global scenario	4-5
Biomass fuel use in Indian subcontinent	5-6
Biomass fuel use in West Bengal	6-7
Leading causes of biomass fuel use in rural India	7-10
Factors influencing emission of pollutants	11-14
Health impact of biomass fuel use	14-20
Scope of the work	21
Objective of the study	22
CHAPTER – 2: Study Protocol	23-33
Study areas	23-24
Organization of health check up camps	24-29
Questionnaire survey for respiratory symptoms	30
Pulmonary function test by spirometry	30-31
Cellular lung reaction to biomass smoke	31-32
Micronucleus (MN) assay	32
Air quality measurements	32
Statistical analysis of data	33
CHAPTER – 3: Results	34-54
Demographic and socio-economic characteristics	34-35
Prevalence of upper respiratory symptoms (URS)	37-38
Prevalence of lower respiratory symptoms (LRS)	38-40
Prevalence of bronchial asthma	40-41
Prevalence of other symptoms	41-42
Prevalence of respiratory symptoms in children	43-44
Effect of biomass smoke exposures on lung function	44-47
Sputum cytology	47-48
Covert pulmonary hemorrhage	49
Greater prevalence of hypertension among biomass users	49
Genotoxic changes in cells exposed to biomass smoke	50
CHAPTER – 4: Discussion and Concluding Remark	55-58
References	59-65

EXECUTIVE SUMMARY

1. Indoor air pollution from burning of traditional biomass fuel such as wood, dung and agricultural wastes for daily household cooking is a major problem in rural India. Although it is known that biomass smoke contains a wide spectrum of potentially toxic compounds, its effect on public health is relatively unexplored. In view of this, the health impact of biomass fuel use in rural India has been evaluated in this study conducted in 2007.
2. A total number of 615 housewives (median age 41 yr) from rural West Bengal who cooked exclusively with biomass fuel (case) and 282 age-matched women from similar neighborhood who used relatively cleaner fuel LPG (control) were enrolled. In addition, 532 and 256 girl children aged 7-10 yr (median age 8 yr) from biomass- and LPG-using families, respectively, were enrolled.
3. The concentration of particulate pollution in indoor air was significantly higher in biomass-using households. For instance, biomass -using kitchen had 3.7-times more PM_{10} and 4-times more $PM_{2.5}$ levels during cooking time when compared with that of LPG-using kitchen. In non-cooking times also, the concentrations of these particulate pollutants were 2-times more in biomass-using kitchen.
4. Cumulative exposures to biomass smoke were associated with greater prevalence of respiratory symptoms, suggesting underlying respiratory illness. Self-declared respiratory symptoms were present in 71% of women who cooked exclusively with biomass fuels in contrast to 28.0% of LPG-users. Most frequent complaint in the former group was chest tightness or chest discomfort (43.3 % vs. 7.3% in control). In addition, biomass users had significantly higher prevalence of sore throat, cough, recurring headache, eye irritation, eye watering, dizziness, muscle pain, tingling and numbness in the extremities.
5. Like the adults, children from biomass using families had 2-times more respiratory symptoms than age- and sex-matched children from LPG using families (70.3 vs. 35.9%, $p < 0.001$). The prevalence of respiratory symptoms both in adults and children was positively correlated with particulate pollutant level in indoor air.

6. Biomass smoke exposures also enhanced the instances of bronchial asthma. Physician-diagnosed asthma was recorded in 6.6% of biomass using women against 4.5% in LPG users. Likewise, medically- diagnosed asthma was present in 6.7% children from biomass using households while 3.9% of children from LPG using families had this problem.
7. Chronic exposures to biomass smoke were associated with impaired lung function. Lung function was reduced in 73.2% of biomass users against 45.4% of controls. Lung function decrement was most prevalent in women who cook predominantly with dung cake and in kitchen adjacent to living areas. Reduction of lung function was positively associated with years of exposure to biomass smoke and low socio-economic status.
8. Like the adults, lung function was significantly reduced in 7 to 10-year old girls from biomass using families: 42.1% of girls from biomass using households had impaired lung function compared with 23% of age-matched girls from LPG-using families.
9. Chronic obstructive pulmonary disease (COPD), a life-threatening breathing problem, was present in 7 % of biomass using women who never smoked in their life. In contrast, only 1.8% of never-smoking LPG users had COPD.
10. Cumulative exposures to biomass smoke were associated with rise in blood pressure. Compared with LPG users, the prevalence of systolic plus diastolic hypertension was 2-times more (14.8 vs. 7.1% in control) in women who cook regularly with wood, dung and crop residues.
11. Women who cook with biomass fuel had several cellular changes in their lungs. Sputum samples of these women contained an excess of alveolar macrophages, neutrophils, eosinophils and lymphocytes, suggesting recurrent bacterial and viral infections and inflammation. Also, they had abundance of iron-laden macrophages in their sputum, suggesting, among others, covert hemorrhage in the lungs.
12. More importantly, women who used to cook with biomass fuels had greater prevalence of metaplasia and dysplasia of airway epithelial cells, which are recognized as early cellular changes towards development of cancer. Moreover, they showed several-fold rise in micronucleus frequency in buccal

and airway epithelial cells that suggest chromosomal damage in these cells. Taken together, these findings indicate greater risk of cancer in the lungs and the airways in rural women who are inhaling smoke from burning biomass during daily household cooking. In agreement with this, population-based cancer registry has illustrated a rapidly increasing trend in the incidence of lung cancer among non-smoking women of West Bengal.

13. In essence, the study has demonstrated that chronic exposures to smoke during daily household cooking with traditional biomass fuel adversely affect lung function, increase the prevalence of respiratory illness including COPD and asthma, and enhances the risk of hypertension and cancer in the lungs and airways.
14. Considering the extensive use of these fuels in the countryside and their potential health hazard, immediate measures should be taken by all concerned to reduce indoor air pollution level by providing better ventilation in the kitchen and through introduction of smokeless chullas. As a long-term policy for public health protection, however, the authority should consider regular supply of cleaner fuel such as LPG to the rural mass at a price the poor people can afford.

BACKGROUND AND OBJECTIVE OF THE STUDY

BACKGROUND

Air pollution is generally perceived as an urban problem associated with automobiles and industries. However, half of the world's population in rural areas of the developing countries is exposed to some of the highest levels of air pollution due to burning of traditional biomass fuels. Biomass is modern jargon for the oldest human energy resource. It means biologically derived material of any kind such as wood, agricultural residues and dung cake that is potentially useful as a source of energy.

Indoor air pollution caused by burning wood and other unprocessed solid biomass can be traced to prehistoric times when man first moved to temperate climates approximately 200,000 years ago. These cold climates necessitated the construction of shelters and the use of fire indoors for cooking, warmth and light. Ironically, fire, which allowed man to enjoy the benefits of living indoors, resulted in exposure to high levels of pollution as evidenced by the soot found in prehistoric caves (Albalak, 1997). Wood was the first fuel that man used and exposure to wood smoke is as old as humanity itself (UNDP, 2004).

BIOMASS FUEL USE: GLOBAL SCENARIO

Over the last 25 years, economic development and modernization has allowed households in wealthier parts of the world to switch to cleaner fuels such as petroleum products (e.g. kerosene, LPG) and electricity (WHO 1997). However, more than 2 billion people of the world, mostly in poor, developing countries of Asia, Africa and Latin America, still rely on solid unprocessed biomass fuels as the primary source of domestic energy (Smith et al., 2004). Of these, 800 million people depend solely on crop residues and dung, although in more than 30 countries wood provides 70% of the energy needs, and in 13 countries it is over 90% (World Energy Council, 1999). It has been observed that people cook with biomass at least once a day in half of the world's households (Smith et al., 2004). Although the proportion of global energy derived from biomass fuel has

fallen from 50% in 1900 to around 13% currently, biomass use is increasing among the poor (WRI, 1999).

About 50% of the gross energy consumption in most developing countries occurs in rural areas. The bulk of this energy is derived from locally available traditional energy resources like wood, dung, agricultural residues and charcoal. Modern energy sources such as electricity and petroleum-based fuels generally provide a small part (2-10%) of the energy consumed by rural people, mainly because of supply and affordability constraints. While the majority of people at risk of exposure to biomass smoke live in rural areas of the world's poorest countries, this is increasingly becoming a problem of poor urban dwellers. Half of the world's wood harvest is now being used as fuel. Poor families expend more than 20% of disposable household income to purchase biomass, or devote more than 25% of total household labor to wood collection (Ramachandra et al., 2003).

BIOMASS FUEL USE IN INDIAN SUBCONTINENT

Wood, agricultural residues and dung cake continue to be one of the major energy sources in India and many other developing countries. The agricultural wastes which have no particular use and lie in the field unutilized and cannot be composted easily end up as fuel. Hay, jute stick, paddy husk, wheat stalks, dried leaves of mango, jack-fruit, coconut, palm and sugarcane, bamboo leaves, branches and roots, cotton roots and stalks, root zone of millets, bajra, wheat husk, lops and tops of fruit trees which are annually pruned are used as fuel in rural areas.

Biomass contribute to one-fourth of the total energy consumed in India. About 33.6 million or 17.5% of all Indian homes use LPG as their primary cooking fuel whereas 78% homes rely on biomass fuels (TEDDY, 1998) and another 3% on coal (NFHS, 1995). Overall, three-quarters of the households of the country still use traditional biomass fuel for cooking and space heating (Holdren et al., 2000; Rehfuss, 2006; WHO, 2006). The number of biomass users in the country is at present 585 million and it is expected to reach 632 million by 2030 (IEA, 2002). Thus, biomass fuels will continue to be an important source of household energy in future. Biomass is extensively used in other south Asian countries also. For instance, 88% of Bangladeshis, 80% of Nepalese, 72% of Pakistanis and 67% of Sri Lankans are dependent on biomass as main household energy source (WHO, 2006, Table 1).

Table 1. Biomass fuel use in India and neighboring countries

Country	Total population in million in 2003	Percentage of population using biomass fuels
India	1065	74
Pakistan	153	72
Bangladesh	146	88
Nepal	25	80
Sri Lanka	19	67

Source: Rehfuess E. *Fuel for life: Household energy and Health*, WHO, 2006

The overall use of biomass in rural domestic sector of India is 1.2-2.1 kg/capita/day (Smith, 1987). A report by the 1992-93 National Council of Applied Economic Research (NCAER) and a survey by World Bank in 1996 in six Indian states stated that about 577 million tons of biomass are used annually in India as a source of domestic energy of which wood constitutes 52%, animal dung 21% and agricultural residues 20% (Table 2).

Table 2. Quantum of biomass fuel use in India

Biomass type	Usage (million tons/year)
Wood	302.1
Dung cake	120.6
Crop residues	115.0
Others	39.3

Source: NCAER and World Bank, 1996

The amount of energy used for cooking depends on many factors such as the type of food cooked, the number of meals cooked, household size, the specific combination of energy source and cooking equipment employed (type of stove, cooking pans), and the way in which cooking devices are used.

BIOMASS FUEL USE IN WEST BENGAL

Like rest of the country, biomass fuel is a steady source of energy for domestic cooking in the majority of rural households of West Bengal, a state in eastern India. Wood, agricultural residues (straw, paddy husk, jute sticks etc.) and dung constitute the major part of biomass fuel in the state. In some cases, these energy sources are used simultaneously with fossil fuels like coal and kerosene. Annual consumption of unprocessed solid biomass fuel in the state has been estimated as 45.6 million tons of which 23.3, 11.9 and 10.3 million tons are contributed by wood, crop wastes and dung cake respectively (Reddy and Venkataraman, 2002; Fig.1). With the growing

deforestation and escalating price of coal and kerosene, more and more rural people of the state are becoming inclined to biomass for domestic cooking.

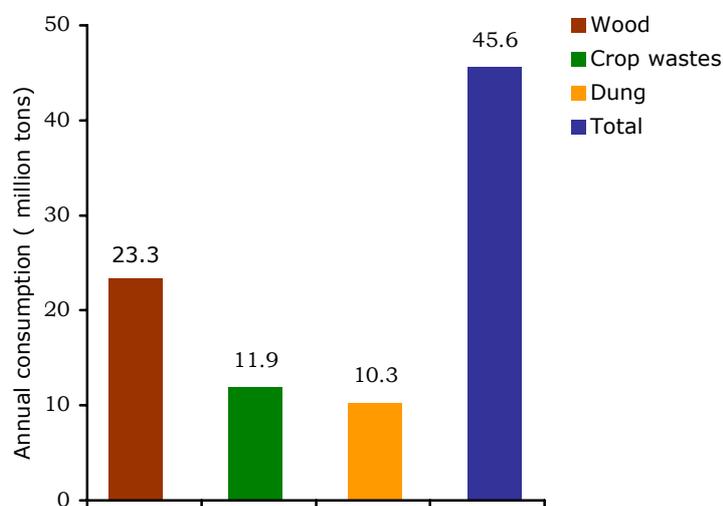


Figure 1. Annual consumption (in million tons) of biomass as fuels for household cooking and room heating in West Bengal

LEADING CAUSES OF BIOMASS FUEL USE IN RURAL INDIA

Owing to population growth and economic development, India's energy consumption is increasing rapidly. Energy and energy technologies have a central role in social and economic development at all scales, from household and community to regional and national. Among its welfare effects, energy is closely linked with public health both positively and negatively, the latter through environmental pollution and degradation. The three main determinants in the transition from traditional to modern energy use are:

- Affordability
- Fuel availability, and
- Cultural preferences

Affordability

The incremental costs of switching over to modern and superior fuels are prohibitive for many rural households. The high operating cost of LPG in India is not favorable to the rural poor who cannot afford to pay for refilling an LPG cylinder every month or

two. It is estimated that households are in a position to switch over to modern fuels when their annual incomes reach Rs.46,000 to 50,000. The affordability of energy-using equipment is just as important as the affordability of fuels. The initial cost of acquiring kerosene and LPG stoves and LPG bottles may discourage some people from switching away from biomass.

Availability

Fuel availability is another important factor. If a modern distribution system is not in place, households cannot obtain access to modern fuels, even if they can afford them. LPG penetration rates are low in many developing countries, partly because distribution infrastructure is lacking. In rural areas, biomass is often perceived as something that is “free” and readily available. This kind of thinking seriously hampers the switch to modern energy. Even when firewood is purchased, it is likely to be cheaper than the cheapest alternative fuel (World Bank, 1995).

Cultural preferences

In some cases, traditions determine the fuel choice regardless of fuel availability and income. For instance, many rich Indian households keep a biomass stove to prepare their traditional *roti* (bread).

Biomass fuel: less efficient and more polluting than petroleum products

In simple devices like the household stoves commonly used in India, biomass fuel does not combust cleanly. Due to poor combustion efficiency, biomass fuel emits a very high quantity of health-damaging particulates during burning (Table 3).

Table 3. Particulate pollution from different types of fuels

Type of fuel	Particles emitted during cooking ($\mu\text{g}/\text{m}^3$)
Wood	1200
LPG and electricity	200-380

Source: Ellegard, 1996

Emissions from biomass burning during domestic cooking

Some of the highest exposure to air pollutants occurs inside homes where biomass fuels are used for daily cooking (Dutt, 1996). Wood consists primarily of two polymers cellulose and lignin. Other biomass fuels also contain these polymers, but their relative proportions differ compared to wood. Besides polymers, small amounts of low molecular weight organic compounds such as resins, waxes and sugars, and inorganic salts are present in biomass. During combustion, pyrolysis occurs and the polymers break apart producing a variety of smaller molecules. Biomass combustion is typically inefficient. As a result, a multitude of partially oxidized health-damaging pollutants are generated.

The list of these pollutants is long (Table 4) and it includes respirable particulate matter with diameter less than 10 (PM_{10}) and 2.5 microns ($PM_{2.5}$) or even less (ultra fine), carbon monoxide (CO), oxides of nitrogen and sulfur. Besides, biomass smoke contains at least five chemical groups recognized by the International Agency for Research on Cancer as known or potential human carcinogens (IARC, 1983). They include polycyclic aromatic compounds such as benzo(a)pyrene and volatile organic compounds such as benzene, toluene and xylene (Sinha et al., 2006). Other toxic compounds are 1,3-butadiene, formaldehyde, and cilia-toxic respiratory irritants such as phenols, cresols and acrolein (Tuthill, 1984; Smith, 1987; Traynor et al., 1987; Koenig et al., 1991; Leonard et al., 2000; Dubick et al., 2002).

The magnitude of air pollution from biomass smoke can be judged from the report that concentration of respirable suspended particulate matter in Indian kitchens is 30 times of the WHO guideline while its outdoor concentration is 2.5 times of the guideline (TERI, 1997; WHO, 1999). Annual concentrations of PM_{10} (particles less than 10 microns in diameter) in ambient air of the Indian cities range from 90–600 $\mu\text{g}/\text{m}^3$. In contrast, a typical 24-hr average concentration of PM_{10} in biomass fuel-using homes ranges from 200 to 5000 $\mu\text{g}/\text{m}^3$ throughout the year, depending on the type of fuel, stove and housing (Smith, 1993; Ezzati and Kammen, 2002). Approximately 5-20% of biomass smoke particulate mass consists of elemental carbon, the composition of the organic fraction varies dramatically with the specific fuel type and with the combustion conditions. The particles are considered as the single best indicator of potential harm. Thus, tens of millions of people in developing countries routinely encounter pollution levels similar to the infamous London killer fog of 1952.

Table 4. Wood smoke emissions

Pollutant	Physical state	Emissions (g/kg wood)
Carbon monoxide	Volatile	80-370
Methane	''	14-25
Volatile organic compounds	''	7-27
Benzene	''	0.6-4.0
Toluene	''	0.15 -1.0
Phenol (and derivatives)	Volatile/Particulate	0.2-0.8
Nitrogen oxides (NO, NO ₂)	Volatile	0.2-0.9
Sulfur dioxide	''	0.16-0.24
Total particle mass	Particulate	7-30
Particulate organic carbon	''	2-20
Particulate elemental carbon	''	0.3 - 5
Oxygenated PAHs	Volatile/Particulate	0.15-1
Benzo(a)anthracene	''	4 x 10 ⁻⁴ - 2 x 10 ⁻³
Benzo(a)pyrene	''	3 x 10 ⁻⁴ - 5 x 10 ⁻³
Dibenzo(a,h) anthracene	''	2 x 10 ⁻⁵ - 2 x 10 ⁻³
Iron	Particulate	3 x 10 ⁻⁶ - 5 x 10 ⁻³

Source: US EPA December 1993

A kilogram of wood when burnt in a typical wood-fired stove can produce significantly harmful levels of gases, particles and other harmful compounds. The concentrations of the emitted pollutants are compared to the respective standard levels in Table 5.

Table 5. Pollutants generated from burning one kilogram of wood

Pollutant	Typical concentrations*	Standards/guideline set to protect health	Number of times in excess of standard/guidelines
Carbon monoxide (ppm)	129	8.6	15
Particles (µg/m ³)	3300	100	33
Benzene (µg/m ³)	800	2	400
1-3, butadiene (µg/m ³)	150	3	50
Formaldehyde (µg/m ³)	700	100	7

Source: Ezzati et al., 2000; *, From burning 1 kg of wood in a traditional stove in a 40 m³ kitchen with 15 air changes per hour, ppm= parts per million.

The vulnerable group

People of the developing countries are typically exposed to very high levels of indoor air pollution for 3 to 7 hours a day (Engle et al., 1997). Since it is always the women who cook daily household meals, their exposure is much higher than men's (Behera et al., 1988). Young children are often carried on their mother's back while she is cooking, so that from early infancy, children spend many hours breathing smoke (Albalak, 1997).

FACTORS INFLUENCING EMISSION OF POLLUTANTS

1. Fuel type: dung cake is most polluting

Daily average concentrations of PM₁₀ in kitchen and living areas of rural households of Andhra Pradesh were found significantly different for different fuel types (Fig. 2; Balakrishnan et al., 2004). The concentrations were highest in dung-using households, followed by wood, kerosene, and LPG-using households, although the outdoor concentrations were not significantly different across fuel types.

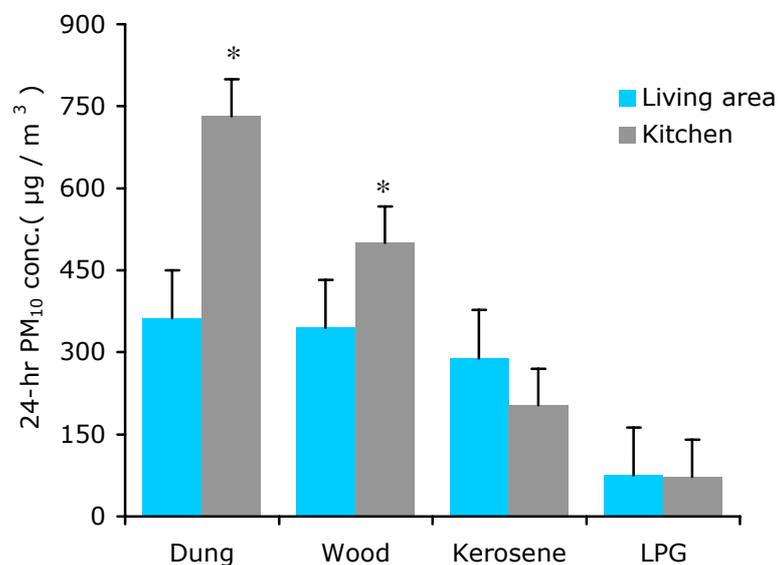


Figure 2. Distribution of 24-h average concentrations of PM₁₀ in kitchen and living areas across fuel types. Bars represent standard deviation of mean * , p<0.05 (Source: Balakrishnan et al., 2004).

2. Kitchen type

Cooking areas in many Indian households are poorly ventilated and half of them do not have separate kitchen (Mishra and Retherford., 1999a). Exposure to indoor air pollution from biomass burning vary with the kitchen type (Balakrishnan et al., 2002). Four common kitchen types are present in rural India:

- a separate enclosed indoor kitchen with partition
- an enclosed indoor kitchen with no partition
- a separate enclosed outdoor kitchen, and
- an open outdoor kitchen (i.e., open air cooking).

Among biomass using households, concentrations of air pollutants were significantly higher in enclosed indoor kitchens as compared to outdoor kitchens but not significantly different between enclosed indoor kitchen types (Fig. 3). Since dispersion is much higher outdoors, outdoor kitchens resulted in lower concentrations close to the stove. Living area concentrations were also significantly higher in indoor-enclosed kitchens as compared to outdoor kitchens.

3. Age and activity of the people

Women who cook with biomass had highest 24-h average exposure concentrations than the non-cooks. Men in the age group 16-50 years experience lowest exposures presumably because they mostly have outdoor jobs (Fig. 4).

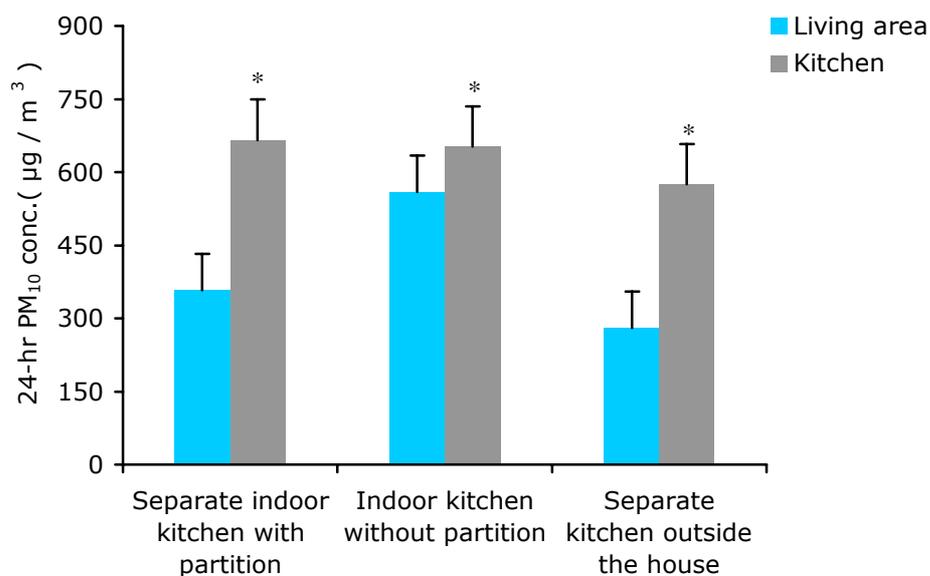


Figure 3. 24-hr kitchen area concentrations of PM₁₀ in different kitchen configurations. Bars represent standard deviation of mean. *, p<0.05 (Source: Balakrishnan et al., 2004)

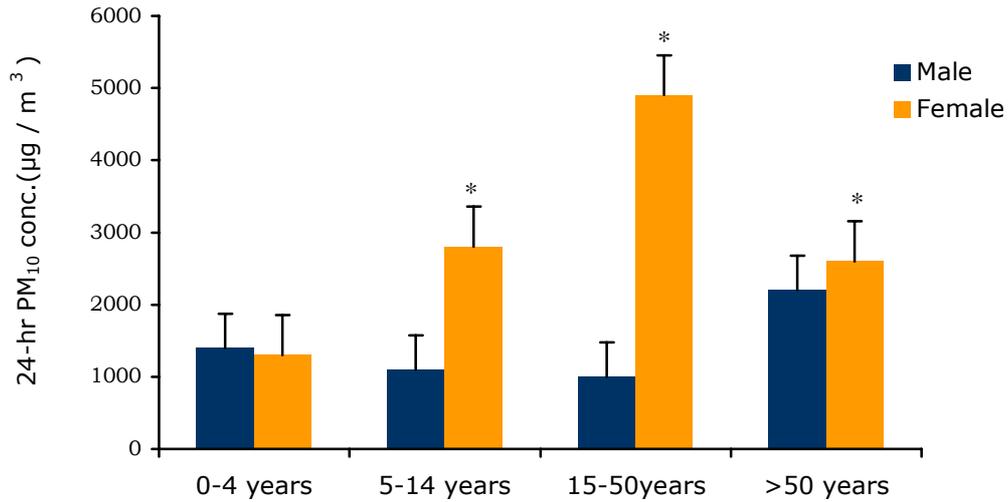


Figure 4. Average daily exposure of PM₁₀ from biomass burning in relation to age and gender. Bars represent standard deviation of mean. *, p<0.05 (Source: Ezzati et al., 2000).

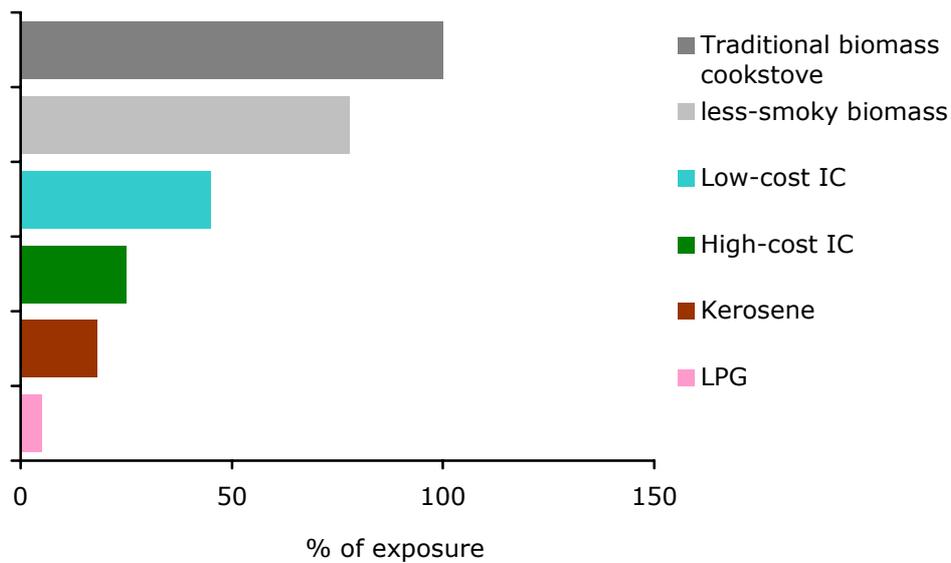


Figure 5. Effectiveness of different exposure interventions compared with traditional cook stoves. IC, improved cook stove (Source : WHO, 2000).

4. Type of cook-stove

It is apparent from Fig. 6 that compared with LPG and kerosene, traditional biomass-using cook stoves release several times more air pollutants in the cooking areas. Use of less smoky biomass such as charcoal significantly reduces the emission and exposure, but still it is higher than LPG and kerosene. Improved cook stove for

biomass burning, on the other hand, further reduce the exposure and the high cost devices appeared to be more efficient in this regard than the low cost ones. In any case, biomass use even in most advanced high cost improved cook stoves generates more air pollution than that of LPG and kerosene using cook stoves (Fig. 5).

HEALTH IMPACT OF BIOMASS FUEL USE

Excess mortality

The health impact of biomass smoke containing high concentrations of particulates and other pollutants can be devastating because for every 20 $\mu\text{g}/\text{m}^3$ rise of PM_{10} in ambient air over the standard, 1% increase in total daily mortality occurs (Samet et al., 2000). Most people are aware that outdoor air pollution can damage their health. But fewer know indoor air pollution often cause greater harm.

Globally, indoor air pollution from biomass fuel use is responsible for 1.6 million deaths due to pneumonia, chronic respiratory disease and lung cancer. Biomass fuels accounts for 2.9 % of all deaths per year worldwide, and 3.7% of the overall disease burden in developing countries. In India, 400,000 to 2 million premature deaths occur per year due to indoor air pollution with a majority of deaths occurring in children under five due to acute respiratory infections (pneumonia; Awasthi et al., 1996; Mishra et al., 1997; Smith, 1999; Bruce et al., 2000). There is also strong evidence of impact on women, up to 34,000 deaths resulting from chronic obstructive disorders (Smith, 2000). In contrast, mortality due to outdoor air pollution is 200,000 to 570,000 representing about 0.4 to 1.1 % of total annual deaths (WHO, 2002). In fact, indoor smoke from biomass burning is the most important health hazard after malnutrition and lack of safe water and sanitation (Fig. 6).

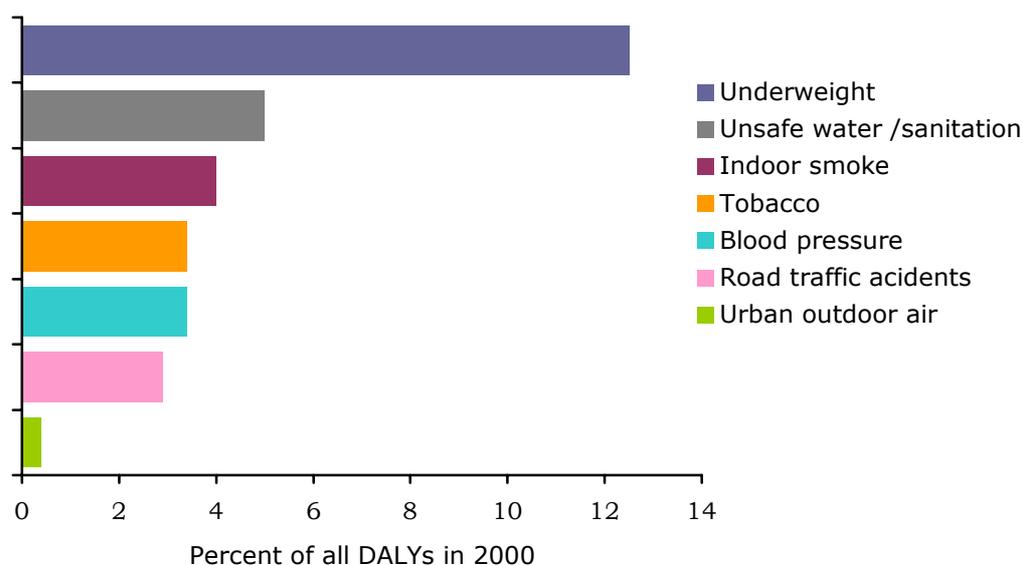


Figure 6. Comparison of health impact of environmental and other conditions in India in terms of Disability-Adjusted Life Years Lost (Source : WHO, 2001,2002).

Excess morbidity

Biomass smoke exposure increases the risk of common and serious diseases of both children and adults (Bruce et al., 2000). It has been causally linked to acute respiratory infections (ARI), chronic obstructive pulmonary diseases (COPD), otitis media, tuberculosis, asthma, low birth weight, cataract and blindness, lung cancer, cancer of nasopharynx, larynx (Smith, 2000; Bruce et al., 2000; Ezzati et al., 2001) and uterine cervix (Velema, 2002).

i. Increase in ARI

Acute respiratory infections (ARI) is the most common cause of illness in children and a major cause of death throughout the world. Among children under five years of age, 3-5 million deaths annually have been attributed to ARI, of which 75% are from pneumonia. ARI accounts for 6.5% of global burden of disease (WHO, 2001). Lower respiratory infections including infection of the lung with pneumonia being the most serious form alone accounts for about 1 million childhood deaths (Smith et al., 2002). Studies in developing countries have shown that young children living in households using biomass fuel have two to three times more risk of serious ARI than unexposed children after adjustment for potential confounders (Smith et al., 2000).

Exposure to indoor air pollution from biomass burning doubles the risk of pneumonia and is responsible for 900,000 deaths annually (WHO, 2005).

Tuberculosis

Tuberculosis is a major health problem in India. Approximately 500,000 persons die from tuberculosis each year in India (WHO, 1997). There have been three published studies to date examining the association between biomass smoke exposure and tuberculosis (two from India, one from Mexico) (Mishra et al., 1999 and Perez-Padilla et al., 2001). An analysis of data from 200,000 Indian adults as part of the Indian National Family Health Survey (1992-93) found that persons living in biomass –using households had more instances of tuberculosis than persons living in households that use using cleaner fuels with an adjusted odds ratio of 2.58 (95% CI: 1.98-3.37) [Mishra et al., 1999]. Biomass smoke exposure can explain up to 59% of rural and 23% of urban cases of tuberculosis in India (Mishra et al., 1999). Increased risk of tuberculosis may result from reduced resistance to infection as exposure to smoke interferes with mucociliary defenses and decreases antibacterial property of lung macrophages.

Reduction in lung function

Biomass fuel users have FVC values less than 75% of predicted (73.42 ± 0.9 ; mean \pm SE) compared to kerosene or LPG users where the respective value is greater than 75% of predicted. The absolute values of FVC, FEV_1/FVC , FEV_1 and PEFr are lowest among biomass and mixed fuel users. FVC is affected most by indoor air pollution more so with biomass fuel. The impairment seems to be more of the restrictive (parenchymal) type (Behera et al., 1994).

Chronic Obstructive Pulmonary Disease (COPD)

Chronic obstructive pulmonary disease (COPD) that includes emphysema and chronic bronchitis (CB) is a lung disease in which the lung is damaged, making it hard to breathe. It is the 4th leading cause of death in the U.S. The symptoms of COPD as described by National Heart, Lung and Blood Institute (NHLBI) include: cough sputum production shortness of breath, especially with exercise, wheezing (a whistling or squeaky sound during breathing), and chest tightness. The Global Strategy for the Diagnosis Management and Prevention of Chronic Obstructive Lung Disease has recognized indoor air pollution as a risk factor for COPD (Global Initiative for Chronic Obstructive Lung Disease, 2001). Indoor air pollution is responsible for

approximately 7,00,000 out of the 2.7 million global deaths due to COPD (WHO, 2005). The prevalence rates of CB in communities exposed to indoor biomass smoke have been reported to be high (Pandey, 1984; Pandey et al., 1985; Behera and Jindal, 1991; Perez-Padilla et al., 1996; Albalak et al., 1999; Golshan et al., 2002).

Bronchial asthma

Asthma is a chronic respiratory disease characterized by sudden attacks of labored breathing, chest tightness, and coughing. Of the limited research that does exist on this subject, some studies have found a positive association between cooking smoke and asthma (Mohammed et al., 1995; Xu et al., 1996; Pistelly, 1997; Thorn, et al., 2001). Data from India's second National Family Health Survey, 1998-99 suggest exposure to cooking smoke is strongly associated with prevalence of asthma among elderly men and women(≥ 60 years of age) [Mishra , 2003].

Cardiovascular risk

Chronic inhalation of smoke in biomass users resulted in significant reduction in hemoglobin level and erythrocyte counts and elevation in total leukocytes, neutrophils and platelet counts (Ray et al. 2003). The absolute number of P-selectin-expressing platelets was 8.3 times higher in biomass fuel users, suggesting excess cardiovascular risk in biomass users (Ray et al., 2007).

Change in immune defense

Particulates emitted from biomass combustion may affect specific and non-specific host defense. Air pollutants commonly found in biomass smoke have been associated with compromised pulmonary immune defense in both animals and humans (Chang et al., 1990; Fujii et al., 2001; Mukae et al., 2001). Biomass smoke particles often contain transitional metals, especially iron, which induce production of reactive oxygen species (ROS) that may catalyze redox reactions in human lung epithelial cells, leading to oxidative stress and increased production of mediators of pulmonary inflammation (Aust et al., 2002).

Hormonal changes

Biomass smoke contains steroid disruptors and the causative agents were identified as polycyclic aromatic hydrocarbons and their derivatives, substituted phenolic compounds, aromatic carbonyl compounds and higher molecular weight alcohol and ketones (Wu et al., 2002).

Eye irritation and cataract

Eye irritation from smoke is widely reported (Ellegard, 1997). There is also preliminary evidence that a biomass smoke exposure is associated with blindness. An adjusted odds ratio of 1.3 for blindness has been reported in women who cooked with biomass (Mishra et al., 1999b). A hospital-based case-control study in Delhi comparing LPG with biomass fuel use found adjusted odds ratio of 0.62 (95% CI: 0.4-0.98) for cataracts i.e. LPG users had lower risk of the disease (Mohan et al., 1989). Conversely, an adjusted odds ratio of 2.4 for biomass use for blindness in biomass users was found in a case control study in Nagpur (Zodpey and Ughade, 1999). Animal studies have demonstrated that biomass smoke damages the lens in rats causing discoloration and opacities. The mechanism is thought to involve absorption and accumulation of toxins, which then lead to oxidative stress (Rao et al., 1995).

Otitis media

Evidence from developing countries suggests a close relationship between biomass smoke exposure and middle ear infection (otitis media) - a condition that causes a considerable amount of morbidity. A strong association was found between exposure to wood smoke in the living environment and the occurrence of otitis media among a population of Nigerian children (Amusa et al., 2005).

Low birth weight and perinatal mortality

Cooking with biomass doubles the risk of stillbirth (Mavalankar et al., 1991; Ardayfio et al., 1993). Low birth weight (LBW; birth weight < 2,500g), an important risk factor for infant mortality and morbidity, is common among biomass users (Boy et al., 2002). Conditions that interfere with transplacental delivery of nutrients and oxygen usually cause varying degrees and types of intra-uterine growth retardation (IUGR) and consequent low birth weight. Carbon monoxide (CO) emitted from combustion of wood when inhaled combines with hemoglobin to form

carboxyhemoglobin (COHb), a much more stable compound that does not readily give up oxygen to peripheral tissues and organs, including fetus. Studies have shown that exposure to biomass smoke is associated with COHb levels of 2.5-13% against a critical level of 2.5% COHb according to WHO guidelines is less than 2.5% (Dary et al., 1981; Behera et al., 1988). COHb levels from biomass smoke exposure is comparable to environmental tobacco smoke and active smoking (WHO, 1999).

Genotoxic effects

Cooking with biomass is a major contributor of mutagens in breathing air (Alfheim et al., 1983). Wood combustion is responsible for 75% of the exposure to particle-associated organics and 20% of cancer risk (Lewtas et al., 1992). Smoke emitted from burning biomass increases the frequency of cytogenetic alterations in blood lymphocytes of exposed populations, possibly because of exposure to mutagens present present in biomass fuels. A study in India has shown greater frequency of micronucleus (MN) formation and other chromosomal abnormalities in lymphocytes of biomass users compared with users of LPG (Musthapa et al., 2004). The relative MN frequency in relation to fuel type was in the order of cowdung > wood> kerosene >= LPG.

Increased risk of cancer

Biomass smoke contain many potentially carcinogenic compounds including polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene volatile organic compounds (VOCs) such as benzene, 1,3-butadiene, styrene, xylene and aldehydes. It has been estimated that biomass use increases cancer risk by 30-80 times (Zhang and Smith, 1996; Table 6).

Table 6. Lifetime cancer risks from different cooking fuels

	LPG	Kerosene	Wood
Exposure ($\mu\text{g}/\text{m}^3$) *			
Benzene	1.7-4.5	5.9-16	58-150
1,3-Butadiene	0.04-0.11	0.02-0.05	11-30
Cancer risk ($\times 10^6$)			
Benzene	14-37	48-130	470-1200
1,3-Butadiene	11-34	5.3-14	3200-8400

*Lifetime averaged exposure level when ventilation rate is 15 per hour. Cancer risk = lifetime exposure x cancer potency. The published cancer potencies are : benzene, $8 \times 10^{-6} (\mu\text{g}/\text{m}^3)^{-1}$ (WHO, 1987; Wallace, 1991); 1,3-butadiene, $2.8 \times 10^{-6} (\mu\text{g}/\text{m}^3)^{-1}$ (EPA, 1995). [Zhang and Smith, 1996]

i. Lung Cancer

Biomass smoke exposure is recognized as an important risk factor in the causation of lung cancer among women in addition to tobacco smoke (Behera et al., 2005). Lung cancer is the 5th most prevalent cancer type in West Bengal among women who never smoke in their life. An overwhelming majority of these women used to cook regularly with biomass fuels. Therefore, it is likely that cumulative exposure to biomass smoke has contributed significantly to the genesis of lung cancer in these women.

ii. Cancer of the nasopharynx and larynx

Biomass smoke has been implicated as a cause of nasopharyngeal carcinoma (Clifford, 1972) although this is not a consistent finding (Yu et al., 1985). A recent study, from South America, reported an adjusted odds ratio of 2.7 (95% CI: 2.2-3.3), and estimated that exposure to wood smoke accounted for around one third of such cancers in the region (Pintos et al., 1998).

iii. Cervical cancer

There is strong evidence that exposure to biomass smoke increases the risk of invasive cancer of uterine cervix. This association was investigated in women with cervical neoplasia in Honduras (Velema, et al. 2002). It has been reported that biomass smoke activates Human Papilloma Virus (HPV), Type 16 and 18 that are instrumental for the development of cervical cancer in women. On the other hand biomass smoke contains benzene (Smith et al., 2000) that may cause leukemia and other human cancers, aplastic anemia and other bone marrow disorders, which are potentially fatal if left untreated (IARC, 1982; Rinsky et al., 1987).

Poverty and health: a vicious cycle

Poverty is an important, probably the most important determinant of health. This is clearly demonstrated by a close interrelationship between household energy, poverty and health. Reliance on simple biomass fuels holds back development because it impairs health and restricts opportunities for education and income generation, while poverty prevents households breaking out of this reliance because poor families cannot afford the higher cost of cleaner fuels and the appliances required. The Energy and Resources Institute (TERI) has estimated that chronic exposure to PM₁₀

from biomass fuel use in India is responsible for illness and deaths that may cost the nation several billion rupees (Saxena and Dayal, 1998).

Lacunae in the present understanding

According to the 2004 assessment of the International Energy Agency, the number of people relying on biomass fuels for cooking and heating will continue to rise. The reliance on biomass fuels appears to be growing as a result of population growth along with unavailability or non-affordability of cleaner fuel. Wood is the most commonly used biomass fuel in India but scarcity of wood forces these poor people to shift to inferior fuels like dung and agricultural residues. Despite the magnitude of this problem, health impacts of indoor air pollution from biomass fuel use in India or elsewhere have not become a central focus of research, development and policy-making. In view of this, assessment of health impact of biomass smoke exposure especially in women who cook regularly with these fuels in poorly ventilated kitchen in the rural areas of the country seems extremely important.

SCOPE OF THE WORK

The respiratory tract is the major entry point of airborne pollutants and lung is the ultimate target organ for their adverse effects. Sustained exposures to smoke can lead to acute lung injury and even death (Laffon et al., 1999). The injury may not be restricted to the lungs because biomass smoke contains fine and ultra fine particles (Tesfaigzi et al., 2002) that readily cross the alveolar-capillary barrier and reach vital organs of the body through circulation (Nemmar et al., 2002). It is conceivable therefore that cumulative biomass smoke exposure could lead to pulmonary and systemic health impairment. Unfortunately, very little attention has been focused so far in India on this important aspect of public health. In view of this, we have investigated the pulmonary and systemic effects of chronic biomass smoke exposure in a group of rural women who cook solely with biomass fuels and have compared the results obtained from a group of control women who use relatively cleaner fuel LPG for daily household cooking. In addition, the effect of biomass smoke on respiratory health of the children has been investigated.

OBJECTIVE OF THE STUDY

- To evaluate the pulmonary and systemic effects of chronic exposure to biomass smoke, and
- To prepare a database on respiratory and general health status of rural populace in relation to biomass fuel use.

STUDY PROTOCOL

STUDY AREAS

The study was conducted in 2007 in West Bengal, a state in eastern part of India. About 72% of people of the state live in the villages where houses are made up of either mud and thatch or brick. The roof was made up of terracotta tiles, tin or hay over a bamboo structure. Small windows, usually 1 to 2 per room, provide ventilation of the rooms. In kitchen, however, ventilation arrangements such as chimney and/or exhaust fans and windows are usually absent. About 32% of people of the state live below the poverty line. Administration at the grass root level is supervised by democratically elected village councils called *Panchayats*. The main livelihood of the people in these rural areas is agriculture, handloom weaving and pisciculture. The study areas were selected in far away places distant from industrial and vehicular pollution sources in order to focus specifically on indoor air pollution. The number of motor vehicles in the sampling areas was negligible as bicycles and cycle rickshaws were the main form of transport. Moreover, there was no air polluting industries within a radius of 5 kilometers. Therefore, vehicular emissions in these areas were minimum and industrial pollution was almost absent.

LIVING CONDITION

Male-dominated society confers multiple domestic responsibilities to the womenfolk in rural India. Women are not encouraged to take up any outdoor job. Instead, they spend most of their time indoor attending to daily domestic cooking, housekeeping, childbearing, child rearing, needle work, making dung cakes, steaming of paddy to make parboiled rice, and attending to household chores. Their staple diet is rice with fish curry along with locally available vegetables. Rural families traditionally use biomass such as wood, dung, and agricultural residues as cooking fuel in poorly ventilated earthen ovens. LPG distribution network in India is mainly concentrated in urban and suburban areas, leaving vast areas of the country dependent on traditional biomass fuels. But after liberalization of economy by the Government of India in early 90's, LPG distribution system has slowly started operating in rural areas. As a result, some villages, especially those closer to the district or sub-

divisional headquarters, are getting regular supply of LPG. However, there remains the problem of poverty and consequent restricted affordability. Even in some families that have opted for LPG, biomass is still being used to cut fuel cost. Therefore, mixed fuel use is a reality in rural India. However, in this study we did not include mixed fuel users and concentrated only on exclusive biomass and LPG users.

Organization of health check-up camps

Health check-up camps were organized with active help and co-operation from village Panchayats, local clubs and voluntary organizations in rural areas of Nadia, Burdwan, North and South 24-Parganas and Medinipur (East and West) districts of West Bengal, a state in eastern India, in 2007. The objective and plan of the study were explained to the local people and organizations. These organizations informed and invited local people to attend makeshift health camps held usually in Panchayat office or local club room or at open roadside places (Plate 1, 2 & 3) from early morning till evening.

Participants

Adults

Six hundred fifteen women (age 41 ± 1.3 yr) from Nadia, Burdwan, North and South 24-Parganas and Medinipur (East and West) districts of West Bengal who used to cook exclusively with biomass fuels (case) and 282 age- matched (age 39 ± 1.9 yr) women from similar neighborhood who used LPG as cooking fuel (control) were enrolled in this study (Table 7).

Children

In addition, 532 school-going girls, aged 7-10 years (median age 8 yr) from biomass using households and 256 children (girls) from LPG-using households (age 7-10 yr, median 8 yr) were enrolled (Table 7).



a



b

Plate 1: Cow dung cakes are being dried in mud wall (a), and a woman and her daughter returning home collecting crop wastes from the field (b) in a village in West Bengal.



Plate 2: A village woman cooking with biomass (a), and a health camp in progress in a village in North 24-parganas district of West Bengal (b)

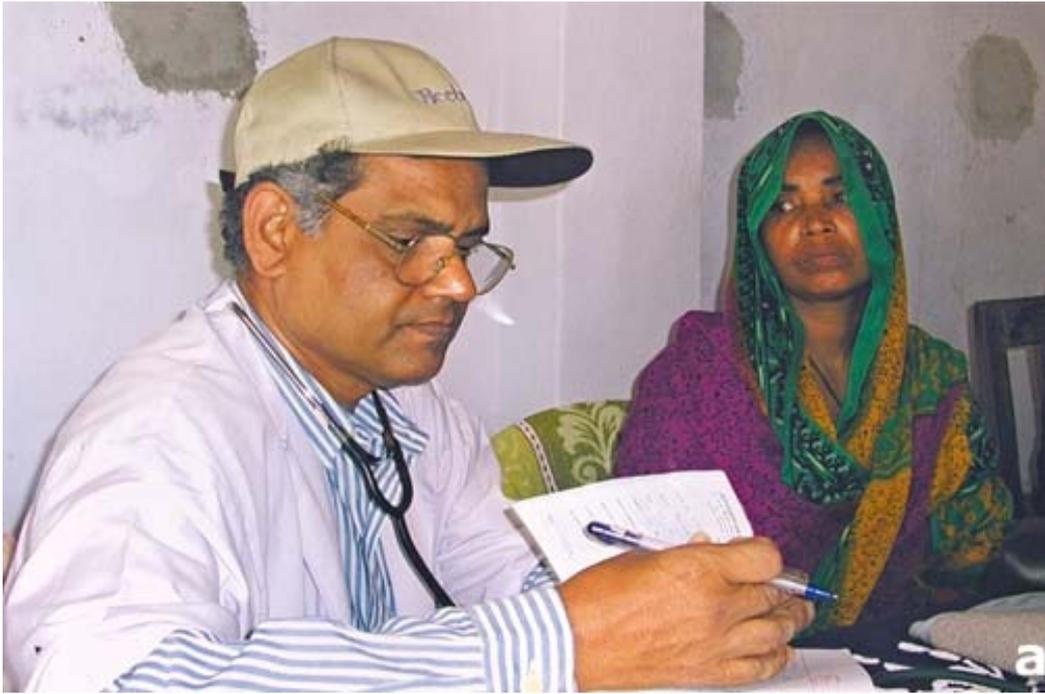


Plate 3: A road-side health camp is in progress at Sabang, West Medinipore district of West Bengal

Table 7. List of the villages where health camps were organized and the number of participants

Village	District	Participants			
		LPG user		Biomass user	
		Adult	Children	Adult	Children
Taki	24-Parganas (North)	55	47	82	62
Mohar	Medinipur (West)	-	-	52	36
Sabang	Medinipur (West)	12	18	39	45
Rautara	Medinipur (East)	-	-	62	42
Rajapur	24-Parganas (North)	18	22	84	79
Falta	24-Parganas (South)	45	41	62	67
Gayeshpur	Nadia	64	83	87	78
Sagar	24-Parganas (South)	-	-	54	46
Jamalpur	Burdwan	88	45	93	77
Total		282	256	615	532

Inclusion and exclusion criteria

The inclusion criteria were apparently healthy women (and their children) from villages who used to cook at least 5 days a week for the past 5 years or more exclusively with either biomass or LPG. In order to minimize the role of pollutants from industrial and vehicular sources, those villages were selected that had no factory within a radius of 5 km and cycle/bicycle was the principal mode of local transport. Women who were currently pregnant or breastfeeding or taking oral contraceptives, had recent or past history of ailments such as tuberculosis and malignancy were currently under medication, or had extreme body mass index (BMI; weight in kg/height in meter² less than 15.0 and greater than 40.0) were excluded. For calculation of BMI, we measured height and weight of the participants with shoe removed at the site of interview.

Personal interview

To negate any possible bias, the interviews were held in the *Panchayat* office or in a community hall and not in the residence of participants. The interviewers first described the study protocol to the prospective women participants. Thereafter each participant was interviewed separately in complete privacy twice by two different interviewers. A third female investigator randomly interviewed

one out of every five participant, filling out parallel forms, noting her observation and finally cross-checking them with those of previous two investigators to prevent any bias. No appreciable difference in the quality of data was obtained between the interviewers. Other members of the research team carried out the job of coding, recording, entering, and analyzing data in the laboratory in SPSS 10.0 statistical package (Chicago, IL, USA). Bias and human error was prevented at this step by parallel running of the data by two independent groups (Plates 2, 3).

Background information

Each subject was asked to answer a structured questionnaire for information about age, body mass index (BMI), diet, religion, marital status, general and reproductive health, occupation, socio-economic status, fuel type, kitchen location, cooking duration, lifestyle etc. As many women in control and biomass user groups were poorly educated, the investigators explained the questions to all the participants in local dialect (Bengali) during personal interview and recorded their answers in the questionnaires on their behalf. Collected data were categorized as follows: age of participant was stratified into 18-25, 26-33, 34-41, 42-45 years; BMI was categorized as 15.0-18.4, 18.5-24.9, 25.0-30.0, 30.1-40.0 kg/m²; dietary habits as totally vegetarian /mixed food habit; religion as Hindu/Muslim/Christian; occupation as housewife with- domestic chores only / domestic plus agricultural work / self-employed with remuneration or without remuneration. Question was specifically asked whether they were involved in mixing and/or spraying of agricultural pesticides (yes/no). Occupation of spouse was categorized into agriculture based job/ handloom weaving/ other.

Establishment of socio-economic status

Socio-economic status (SES) was ascertained following the procedure of Srivastava (1978) and Tiwari et al., (2005) by scoring 0 to 10 of seven indicators: house, material possession, education, occupation, monthly income, land/house cost, social participation and understanding. Scores of seven profiles were added and classified into 3 categories of SES-low, medium and high.

A. QUESTIONNAIRE SURVEY FOR RESPIRATORY SYMPTOMS

Questionnaire survey

Information about the prevalence of respiratory symptoms experienced in the past 1 year and last 3 months, frequency of the signs and symptoms, history of medication were collected. Respiratory symptoms were broadly grouped into two: upper respiratory symptoms (URS) like sinusitis, running or stuffy nose, sore throat, common cold and fever, and lower respiratory symptoms (LRS) like dry cough, cough with phlegm, wheezing and chest discomfort (Pope and Dockery, 1999). In addition, prevalence of headache, eye irritation, tingling etc. has also been evaluated.

B. PULMONARY FUNCTION TEST BY SPIROMETRY

Lung function tests by spirometry were performed with informed consent of the participant. The tests were performed according to the methods suggested by the American Thoracic Society (ATS, 1995) using a portable, electronic spirometer (Spirovit SP-1, Switzerland). Before performing the pulmonary function test, the height and weight of the subject was measured with shoes removed. Each subject performed at least three forced expiratory maneuvers while sitting with free mobility and nose closed with a nose clip to prevent passage of air through the nose to ensure reproducibility of results. Using a computer assisted quantitative assessment the best maneuver for acceptance was determined. The data were compared with predictive values based on age, sex, height and ethnic group. The following spirometric parameters were recorded for analysis:

1. Forced Vital Capacity (FVC), i.e. the volume of air in liters that can be maximally forcefully exhaled
2. Forced Expiratory Volume at 1 second (FEV_1), i.e. volume of air (in liter) that is forcefully exhaled in one second.
3. Ratio of FEV1 to FVC (FEV_1/FVC), expressed as percentage
4. Forced Expiratory Flow at 25-75% ($FEF_{25-75\%}$) or Maximal Mid-expiratory Flow Rate (MMFR), which is the average expiration flow rate during the mid 25-50% portion of the FVC
5. Peak Expiratory Flow Rate (PEFR) – the peak flow rate during expiration

The abnormalities that could be detected by spirometry tests are obstruction, restriction and combined lung defects. In *obstructive lung diseases* such as emphysema or chronic bronchitis, the FEV₁ is reduced disproportionately more than the FVC resulting in an FEV₁/FVC ratio less than 70%. In restrictive lung disease, the FVC is reduced less than 80% of predicted. In combined lung disease both FVC and FEV₁/FVC ratio are decreased. FVC less than 80% and FEV₁/FVC less than 70% are considered to be combined lung function.

C. DIAGNOSIS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

COPD was initially diagnosed on the basis of symptoms of chronic bronchitis (presence of cough and expectorations on most of the days for at least three months in a year for two consecutive years or more). Confirmation of diagnosis and further classification of COPD were based on spirometric measurements following the criteria of Global Initiative for Chronic Obstructive Lung Diseases [GOLD] as shown in Table 8 (Pauwels et al., 2001).

Table 8. Classification of COPD following the criteria of GOLD

Stage of COPD	Severity	Spirometric value	Symptom
I	Mild	FEV ₁ /FVC <70% FEV ₁ 70-79% of predicted	With or without chronic symptoms like cough, sputum expectoration, dyspnea
II a	Moderate	FEV ₁ /FVC <70% FEV ₁ 51-69% of predicted	''
II b	Severe	FEV ₁ /FVC <70% FEV ₁ 30-50% of predicted	''
III	Very Severe	FEV ₁ /FVC <70% FEV ₁ <30% of predicted	Chronic respiratory failure

(Ref: Pauwels et al., 2001)

D. CELLULAR LUNG REACTION TO BIOMASS SMOKE

Sputum cytology

The participants were instructed to wash their mouth with saline water and to cough vigorously to expectorate sputum. The samples were collected in a sterile plastic container. Smears were made on clean glass slides from the non-transparent high

viscosity part of each sample. The slides were semi-dried in air, and fixed in appropriate fixatives immediately at the site of collection and brought to the laboratory at Kolkata for staining. The smears were fixed for 30 minutes in ethyl alcohol for Papanicolaou staining, 20 minutes in buffered formalin (40% formaldehyde in 0.1M phosphate buffer, pH 7.4, 3:1, v/v) for non-specific esterase, 10 minutes in 10% formalin for Perl's Prussian blue reaction. Papanicolaou (Pap) staining for cytology was done following the method of Hughes and Dodds (1968). Staining for non-specific esterase (NSE), a marker enzyme for macrophages was done by Fast Blue B method (Oliver et al., 1991). Perl's Prussian blue reaction was done to identify deposition of ferric iron (hemosiderin) in alveolar macrophages, by the method of Pearse (1985).

E. MICRONUCLEUS (MN) ASSAY

Exfoliated epithelial cells from buccal mucosa and sputum were smeared on slide, dried in air and fixed with cold solution of 1% glutaraldehyde in 1/15M phosphate buffer (pH 7.5) for 20 min. Then the slides were stained by Feulgen reaction essentially by the modified procedure of Belien et al. (1995). At least 2000 cells for each person were analyzed under light microscope (Leitz, Germany). The slides were illuminated with a halogen light source and the fields of vision were filtered with a monochromatic green filter ($\lambda = 550 \text{ nm}$) for which Feulgen stain shows maximum absorption. An object was considered as MN if it fulfils the established criteria (Belien et al., 1995).

F. AIR QUALITY MEASUREMENTS

The concentrations of PM_{10} in cooking areas during cooking as well as non-cooking hours were measured by portable, real-time aerosol monitor (DustTrak™, model 8520, TSI Inc., MN, USA). The instrument contains 10-mm nylon Dor-Oliver cyclone, operates at a flow rate of 1.7 liters per minute and measures particles load in the concentration range of $1\mu\text{g}-100\text{mg}/\text{m}^3$. Since biomass-using women cook in a sitting position 2-3 ft away from the open chullah (make-shift oven), the monitor was placed in the breathing zone of the cook 2.5 ft above the floor level on a wooden stool 3 ft away from the chullah. LPG users, on the other hand, generally cook in a standing position, and the monitor was placed accordingly at a height of 4 ft and 3 ft away from the oven.

G. STATISTICAL ANALYSIS OF DATA

The collected data were processed and analyzed in SPSS 10.0 (Statistical Package for Social Sciences, Chicago, IL, USA) software. The results were statistically analyzed by Student's *t* test and Fisher's exact χ^2 -test, and $p < 0.05$ was considered significant.

RESULTS

DEMOGRAPHIC AND SOCIO-ECONOMIC CHARACTERISTICS

Biomass users and control women were comparable with respect to age, smoking habit, marital status, BMI, food habit, religion and cooking hours. However, biomass users were more exposed to environmental tobacco smoke due to their spouses' smoking habit and had lower education and family income than LPG-using controls (Table 9).

Table 9. Demographic characteristics of women participants

Characteristics	LPG user (n = 282)	Biomass user (n = 615)	p value*
Age in years, mean \pm standard error	39 \pm 1.9	41 \pm 1.3	NS
Age (in yr) distribution (%)			
20-30	15.6	16.3	NS
31-40	37.3	36.6	NS
41-50	30.1	29.3	NS
51+	17.0	17.9	NS
Body mass index (kg/m ²), mean \pm SE	23.9 \pm 0.7	21.9 \pm 2.3	NS
BMI (kg/m ²) distribution (%)			
<18.5	2.2	2.6	NS
18.5-24.9	74.6	75.8	NS
25.0-30.0	19.8	18.9	NS
>30.0	3.4	2.6	NS
Smoking habit (%)			NS
Current smoker	0	0	
Never smoker	100	100	
Smoker in family	28.0	69.0	0.001
Food habit (%)			
Vegetarian	3.5	3.1	NS
Mixed	96.5	96.9	NS
Years of schooling (%)			
0	1.4	2.8	0.001
Up to 5	27.6	62.8	0.001
5+	70.9	34.4	0.001
Marital status (%)			NS
Married	100	100	
Family size (%)			
Small (up to 4 members)	50.7	33.0	0.05
Medium/large (4+members)	49.3	67.0	0.05

Cooking hours/day, mean \pm SE	2.8 \pm 0.8	3.5 \pm 0.2	NS
Kitchen location (%)			
Separate	71.6	34.5	0.001
Religion (%)			
Hindu	89.7	87.3	NS
Muslim	10.3	12.7	NS
Family income per month in rupees, mean \pm SE	3650 \pm 121.3	1750 \pm 59.6	0.001

n, number of premenopausal rural women; *, *p* values from χ^2 -test (Fisher's exact test) and student's *t*-test, whichever applicable, NS, statistically not significant

Two-third (65.5%) of biomass fuel-using households did not have separate kitchen in contrast to only 28.4% of LPG-using families without separate kitchen. In biomass using households, wood was the most commonly used fuel. It was the major fuel in 480 (78%) households. In 92 (15%) households, agricultural residues such as dried leaves, jute stick and paddy husk were the principal fuel type. In remaining 43 families (7%) dung cake was the principal biomass fuel for domestic cooking. However, in majority of the households a mixture of wood, dung and agricultural wastes were used. The number of members in the family of biomass users varied from 2-12 against 2-11 in LPG-using families. The family size was categorized into 2 groups: (i). small (up to 4 members) and (ii) Medium and large (more than 4 members). Two-third of biomass users (412 out of 615) had medium and large families compared with half (139/282) of the control subjects.

Elevated level of indoor air pollution in biomass-using households

Concentrations of particulate pollutants during cooking and non-cooking hours are presented in Fig. 8. During cooking hours the concentration of PM₁₀ in biomass using kitchen was 3.7- times more than that of LPG-using kitchen (625 vs. 169 $\mu\text{g}/\text{m}^3$, $p < 0.001$). Even in non-cooking hours, PM₁₀ level was more than double when compared with that of LPG-using kitchen (204 vs. 93 $\mu\text{g}/\text{m}^3$, $p < 0.001$). Similarly, the concentration of PM_{2.5} in biomass using kitchen was 4-times higher during cooking (312 vs. 77 $\mu\text{g}/\text{m}^3$, $p < 0.001$) and 2.4-times higher during non-cooking hours (108 vs. 45 $\mu\text{g}/\text{m}^3$, $p < 0.001$, Fig. 7).

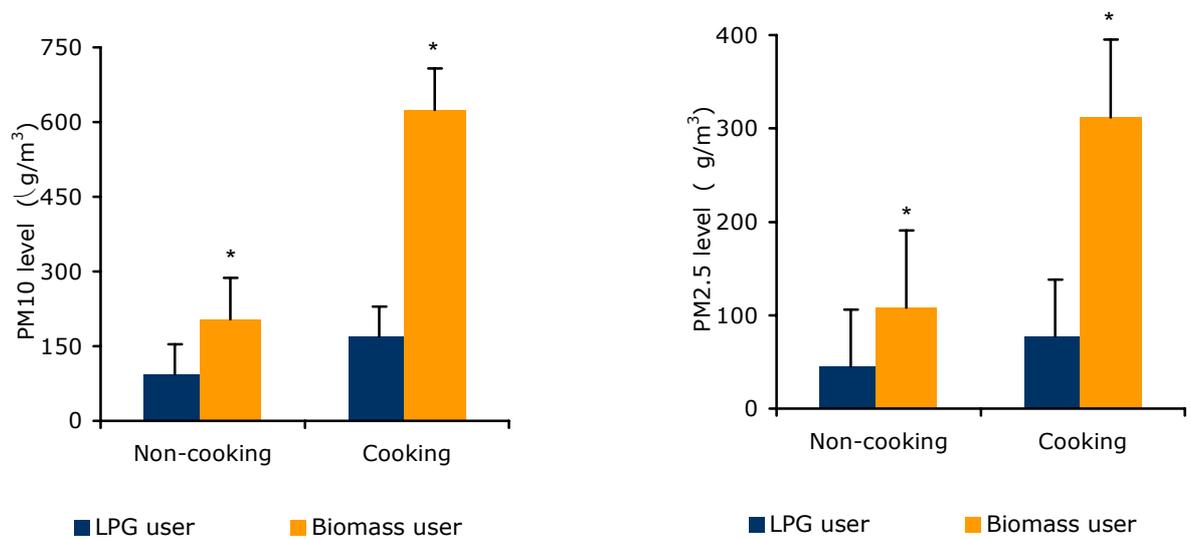


Figure 7. Particulate pollution in kitchen of biomass and LPG users of rural West Bengal during cooking and non-cooking hours

Greater prevalence of respiratory symptoms among biomass users

One or more respiratory symptoms were present in 585 out of 615 (95.1%) women in past 12 months who cooked with biomass fuels in contrast to 162 out of 282 (57.4%) of those who cooked exclusively with LPG (Fig. 8). The difference between these two groups with respect to prevalence of respiratory symptoms was highly significant ($p < 0.001$).

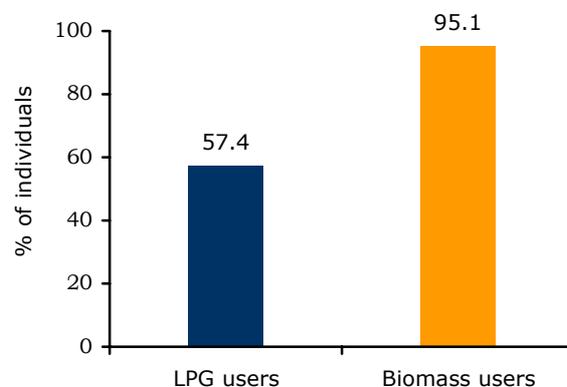


Figure 8. Prevalence of respiratory symptoms in past 12 months in women who cooked regularly with LPG and biomass fuels in rural West Bengal

PREVALENCE OF UPPER RESPIRATORY SYMPTOMS (URS)

Upper respiratory symptoms (URS) represented by sinusitis, runny or stuffy nose, sore throat and common cold with fever were present in 90.1% of biomass users (554/615) in contrast to 52.1% of LPG-using controls (Table 10). URS was more prevalent in wood and dung users, women from larger family, and in the age group of 31-40 years. After controlling potential confounders such as age, tobacco chewing, ETS and SES by multivariate logistic regression analysis, use of biomass fuel was found to be positively associated with the prevalence of URS (Odds ratio [OR] = 2.6, 95% confidence interval [95%CI] 1.3-5.6), but in conditional logistic regression analysis there was no significant correlation between URS and exposure years (OR= 0.6, 95% CI, 0.3-3.1).

PREVALENCE OF INDIVIDUAL SYMPTOMS UNDER URS

Sinusitis

Sinusitis was present in 36.7% of biomass using women against 24.8% of LPG users ($p < 0.05$). There was a close correlation between sinusitis and lifetime exposures (exposure years) to biomass smoke (OR=1.53, 95%CI 1.3-6.7).

Runny or stuffy nose

Runny or stuffy nose and sneezing are symptoms associated with rhinitis. In nearly 90% cases, rhinitis is caused by hypersensitivity i.e. allergic reactions to a host of environmental allergens including pollen and fungal spores. The symptoms were present in 61.9% of biomass users participated in this study against 24.2 % of controls ($p < 0.001$). A close correlation was found between runny nose and lifetime exposures to biomass smoke (OR =1.7, 95% CI, 1.3-9.5).

Sore throat

Sore throat was present in 59.2% of biomass users participated in this study. In contrast, only 28.4% of LPG-users from similar neighborhood had this symptom. A positive correlation was observed between the prevalence of sore throat and lifetime exposure to biomass smoke (OR=1.5, 95% CI, 1.8-2.9).

Common cold and fever

Common cold with fever was reported by 70.7% of biomass users compared with 39.0% of LPG users. The prevalence of this symptom was positively associated with PM₁₀ level and the extent of exposure (OR= 1.6, 95% CI, 1.1-14.6) after controlling potential confounders such as age, spouse's smoking, tobacco chewing habit and SES.

Table 10. Prevalence (%) of upper respiratory symptoms in past 12 months in women who cook regularly with traditional biomass fuels

Symptom	LPG-using control (n=282)	Biomass user (n=615)
Sinusitis	24.8	36.7*
Runny or stuffy nose	24.2	61.9*
Sore throat	28.4	59.2*
Common cold with fever	39.0	70.7*
Overall URS	52.1	90.1*

n, number of women participants; many participants had more than one symptom; *, $p < 0.05$

PREVALENCE OF LOWER RESPIRATORY SYMPTOMS (LRS)

Lower respiratory symptoms (LRS) include dry cough, cough with phlegm (sputum production), wheezing breath, chest discomfort and difficulty in sleep due to breathing problem. The prevalence of one or more symptoms under LRS in the past 12 months was 88.1% (542/615) in biomass users of this study compared with 49.3% (139/282) in LPG users ($p < 0.001$; Fig. 9, Table 11).

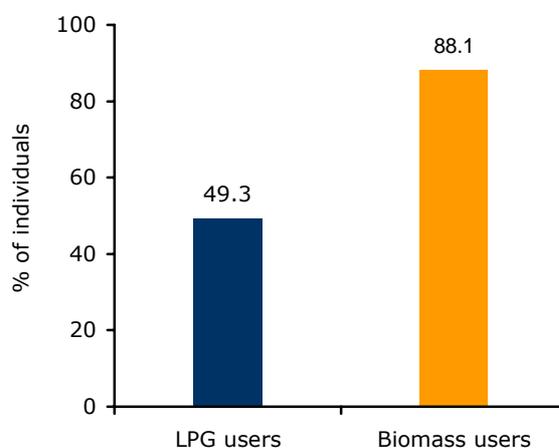


Figure 9. Prevalence (%) of lower respiratory symptoms in biomass and LPG using women

There was appreciable difference in LRS prevalence among biomass users with or without separate kitchen. In women with separate kitchen, 85% had LRS. In contrast, 92% of women without separate kitchen had LRS. The risk factors of LRS were tobacco chewing habit, environmental tobacco smoke (ETS, i.e. passive smoking) wood and dung use, larger family, older age group and low socio-economic conditions. In univariate logistic regression analysis, exposures to biomass smoke were strongly associated with LRS (OR= 8.4, 95% CI, 6.2-11.3). After controlling potential confounders such as age, tobacco-chewing habit, ETS and SES in multivariate analysis, biomass smoke exposure was still significantly positively associated with LRS (OR= 1.5, 95% CI, 1.5-15.5).

PREVALENCE OF INDIVIDUAL SYMPTOMS UNDER LRS

Dry cough

The prevalence of chronic cough, both dry and sputum-producing (wet), was significantly higher in biomass users compared with LPG-using controls. Dry cough was present in 46.0% of biomass users compared with 26.2% of LPG users. The difference between biomass and LPG users in this regard was highly significant ($p < 0.001$). The prevalence of the symptom was positively associated with years of exposures to biomass smoke. The risk factors of dry cough were ETS and dung use. After controlling potential confounders, exposure to biomass smoke was found positively associated with chronic dry cough (OR= 1.6, 95% CI, 1.3-6.7).

Cough with phlegm (wet cough or sputum-producing cough)

The prevalence of wet cough was 59.3% in biomass users compared with 28.0% in controls ($p < 0.001$). The risk factors of sputum-producing cough were ETS and dung use. After controlling potential confounders in multivariate logistic regression analysis, exposure to biomass smoke was found positively associated with cough with phlegm (OR= 1.5, 95% CI, 1.1-2.4).

Chest discomfort or chest pain

The prevalence of chest tightness or chest pain was present in more than half (52.8%) of biomass users enrolled in this study. In contrast, only 23.0% of LPG users had this symptom ($p < 0.001$). The risk factors of chest discomfort were ETS,

predominantly dung use, absence of separate kitchen and low SES. After controlling potential confounders in multivariate logistic regression analysis, exposure to biomass smoke was found positively associated with chest discomfort (OR= 2.6, 95% CI, 1.5-11.5).

Shortness of breath

The prevalence of shortness of breath was present in 69.1% of the biomass users compared with 30.5% of controls ($p < 0.001$). The risk factors were ETS, predominantly dung use, absence of separate kitchen and low SES. After controlling potential confounders in multivariate logistic regression analysis, exposure to biomass smoke was found positively associated with shortness of breath (OR= 1.5, 95% CI, 1.2-8.0).

Wheezing breath

Wheeze or whistling sound during breathing was present in 18.2% of biomass users compared with 5.3% of LPG-using controls. The risk factors of wheeze were ETS, predominantly dung use and low SES. After controlling potential confounders in multivariate logistic regression analysis, cumulative exposure to biomass smoke was found positively associated with wheezing breath (OR=2.6, 95% CI, 1.6-11.1).

Table 11. Prevalence (%) of lower respiratory symptoms in past 12 months in women who cook regularly with traditional biomass fuels

Symptom	LPG-using control (n=282)	Biomass user (n=615)
Dry cough	26.2	46.0*
Cough with phlegm	28.0	59.3*
Chest discomfort	23.0	52.8*
Shortness of breath	30.5	69.1*
Wheeze	5.3	18.2*
Overall LRS	49.3	88.1*

n, number of women participants; many participants had more than one symptom; *, $p < 0.001$

PREVALENCE OF BRONCHIAL ASTHMA

In rural areas of West Bengal, physician-diagnosed asthma was found more prevalent in women who cook regularly with biomass fuels compared with age-

matched LPG-using women from similar neighborhood (6.2% vs. 3.9%, $p < 0.05$; Fig. 10).

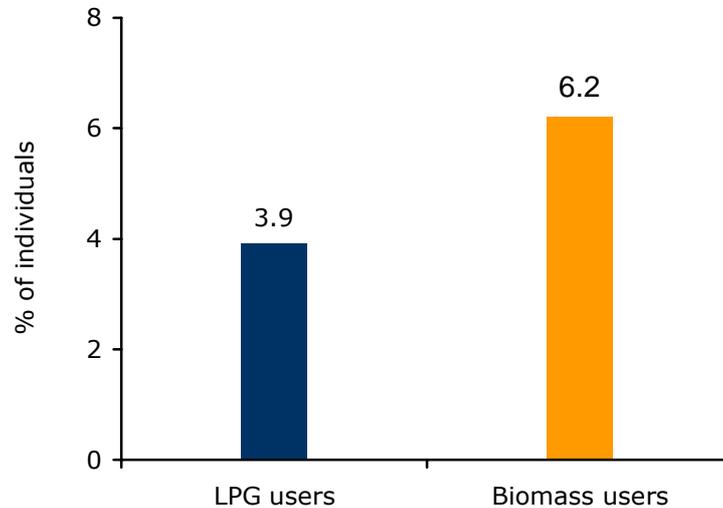


Figure 10. Prevalence (%) of physician-diagnosed asthma

Asthma prevalence correlated positively with age and exposure years. The prevalence was highest in 40-60 year age group. On the other hand, obese women and those with large family (>10 members) had highest asthma prevalence.

PREVALENCE OF OTHER SYMPTOMS

Recurrent headache

Headache was a frequent problem in women who cook regularly with LPG or biomass. However, biomass users had greater prevalence of this problem as recurrent headache in past 12 months was present in 84.4% women compared with 64.5% of LPG users, and the difference was statistically significant ($p < 0.001$). Important confounding factors were ETS and low SES. After controlling potential confounders such as age, ETS and SES in multivariate logistic regression analysis, exposure to biomass smoke was positively associated with headache (OR= 3.2 , 95% CI, 2.6-16.8).

Eye irritation and eye watering

Eye irritation including burning, dryness and itching in the eyes in past 12 months was reported by 69.3% of biomass users compared with 34.7% of LPG users. Similarly, eye watering was also significantly more prevalent among biomass users (57.1% vs. 8.5%). Eye irritation was more prevalent in women cooking with agricultural residues followed by dung and wood users. After controlling potential confounders in multivariate logistic regression analysis, exposure to biomass smoke was found positively associated with eye irritation (OR= 1.8% CI, 1.2-2.6).

Dizziness

Frequent dizziness was present in 41.6% of biomass users as compared with 11.3% of LPG users, and the difference was highly significant ($p < 0.001$). The risk factors were ETS, adjacent kitchen, predominantly dung use and tobacco chewing. After controlling potential confounders in multivariate logistic regression analysis, exposure to biomass smoke was found positively associated with headache (OR= 6.7, 95% CI, 3.3-13.6).

Muscle pain

Frequent pain in the muscles was present in 76.7 % of women who cook exclusively with biomass fuels against 44.0% of age-matched LPG users from same neighborhood. Muscle pain correlated positively with age and exposure years. The prevalence was highest in dung cake users.

Tingling and numbness

Tingling and numbness in past 12 months was reported in 62.3% of biomass users as compared to 31.2% of LPG users. The risk factors were ETS, wood and dung use and low SES. After controlling potential confounders in multivariate logistic regression analysis, exposure to biomass smoke was found positively associated with tingling and numbness (OR= 3.0, 95% CI, 1.8-5.2). The problem was positively associated with age and exposure years. The prevalence was highest in predominantly dung cake and wood users compared with agricultural waste users.

PREVALENCE OF RESPIRATORY SYMPTOMS IN CHILDREN

One or more respiratory symptoms were present in the past one year in 87.4% girl children from biomass using households in contrast to 51.5% of age-matched (7-10 yr) girls from LPG-using families ($p < 0.001$). On the other hand, 70.3% children aged 7-10 years from biomass-using families had respiratory symptoms in past 3 months compared with 35.9% children from LPG-using families.

Table 12. Prevalence (%) of respiratory symptoms in 7-10 year-old children in past three months

Parameter	Children from LPG using families (n=256)	From biomass using families (n=532)
URS	26.9	61.6*
LRS	23.0	50.0*
Overall	35.9	70.3*

*Many children had more than one symptom; *, $p < 0.001$ in Chi-square test*

In past 3 months, URS was present in 61.6% in biomass users' children in contrast to 26.9% in control group. Similarly, half (50%) of the biomass users' children had LRS compared with 23% of children from LPG-using households (Table 12, Fig.11). Thus, children from biomass users' families had significantly higher prevalence of respiratory symptoms, implying greater chances of underlying respiratory diseases.

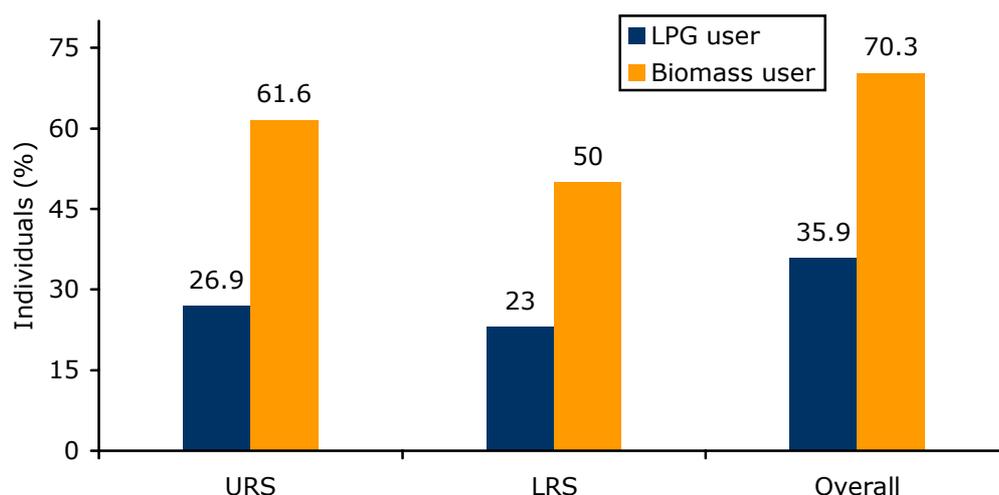


Figure 11. Prevalence (%) of respiratory symptoms in children from biomass fuel and LPG-using families

Children from biomass using households had greater prevalence of all the respiratory symptoms under URS (sinusitis, runny or stuffy nose, sore throat and common cold with fever) and LRS (dry or wet cough, chest discomfort, shortness of breath and wheeze) [Table 13].

Table 13. Prevalence (%) of respiratory symptoms in 7-10 year-old children in past three months

Symptom	LPG (n=256)	Biomass (n=532)
Overall URS	26.9	61.6*
Sinusitis	4.2	12.4*
Runny or stuffy nose	17.2	45.5*
Sore throat	11.7	47.1*
Common cold with fever	17.2	53.9*
Overall LRS	23.0	50.0*
Dry cough	9.8	18.8*
Cough with phlegm	13.7	28.2*
Chest discomfort	8.6	21.4*
Shortness of breath	4.7	20.7*
Wheeze	5.8	10.9*
Asthma	3.9	6.7*

**, p<0.05 compared with children from LPG using households*

Asthma

Medically diagnosed bronchial asthma was present in 3.9% of children from LPG user families while 6.7% children from biomass using households had asthma (P<0.05, Table 13)

EFFECT OF BIOMASS SMOKE EXPOSURES ON LUNG FUNCTION

In women

Lung function was significantly reduced in two-third (75.4%) of biomass users as compared with 36.2% of controls (p<0.001). Restrictive type of lung function deficit (FVC less than 80% predicted) was predominant in both the groups, but the prevalence was about 2-times more in biomass users (50.7 vs. 28.0, p<0.001). Similarly, obstructive type of lung deficit (FEV₁/ FVC less than 70% predicted) was more than doubled in women who cook with biomass (16.9 vs. 7.1% in control). A combination of both types of deficits was several-fold increased in biomass users (Table 14; Fig 12).

Table 14. Assessment of pulmonary function test by spirometry

Lung function	LPG user (n=282)	Biomass user (n=615)
Normal	63.8	24.6*
Reduced	36.2	75.4*
<i>Restrictive</i>	28.0	50.7*
<i>Obstructive</i>	7.1	16.9*
<i>Combined</i>	1.1	7.8*

Results are expressed in % of individuals; *, $p < 0.05$ compared with control in Chi-square test

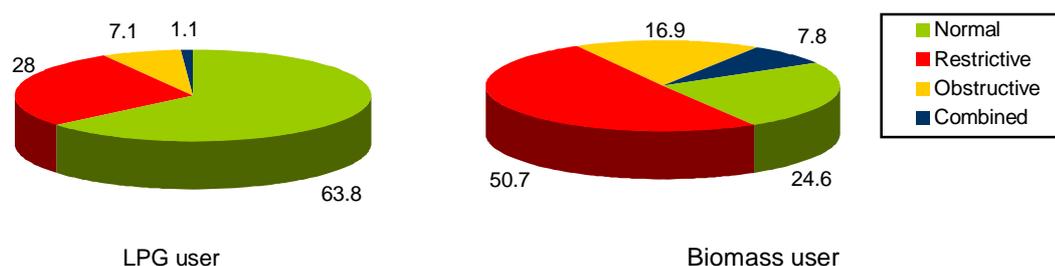


Figure 12. Prevalence (%) of lung function impairment among biofuel users and control subjects

In children

Lung function was significantly reduced in 42.1% of girls from biomass using households. In contrast, 23% of age-matched girls from LPG-using families had reduced lung function ($p < 0.001$, Table 15). Restrictive type of lung function deficit was predominant in both the groups, but the prevalence was 1.8-times more in children of biomass users. Similarly, obstructive and combined types of lung deficits were significantly higher in children from biomass using households.

Table 15. Lung function of children from LPG and biomass-using households

Lung function	LPG (n=256)	Biomass (n=532)
Reduced	23.0	42.1*
<i>Restrictive type</i>	19.1	34.9*
<i>Obstructive type</i>	2.3	4.5*
<i>Combined type</i>	1.5	2.6*

Results are expressed in % of individuals; *, $p < 0.05$ compared with control in Chi-square test

Greater prevalence of COPD in biomass using women

COPD is characterized by symptoms of chronic bronchitis (presence of cough and expectorations on most of the days for at least three months in a year for two consecutive years or more) and reduced lung function. COPD was present in 7.0% of biomass users while only 1.8% of LPG-users had COPD (Fig. 13). Thus the poor, rural women of West Bengal who cook regularly with dung, wood and agricultural wastes suffer 3.9-times more from life-threatening COPD than their neighbors who can afford cleaner fuel LPG, and the difference between these two groups with respect to the prevalence of the disease was highly significant ($p < 0.001$).

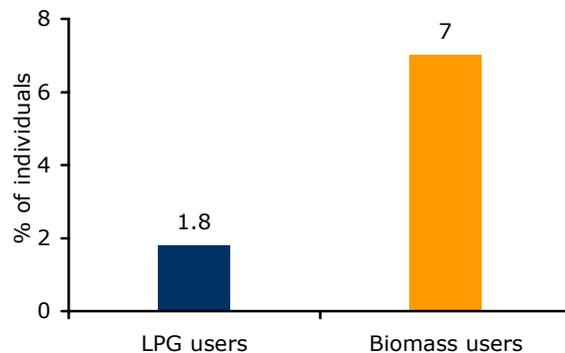


Figure 13. Prevalence of chronic obstructive pulmonary disease (COPD) among biomass and LPG users

Lung function decrement was more in women cooking with biomass fuel in adjacent kitchen than those cooking in separate kitchen. Women cooking regularly in adjacent kitchen had reduced FVC, FEV₁, FEV₁/FVC, and PEF values compared with those having separate kitchen, but the changes were not statistically significant ($p < 0.05$).

Poorest lung function in dung users

Women cooking with dung had greatest prevalence of lung function decrement as 85.0% women of this group had reduced lung function compared with 69.0% and 65.7% of crop residue and wood users respectively. Reduction in mean FVC was more in women who used predominantly dung cakes followed by agricultural residues and wood (Fig. 14).

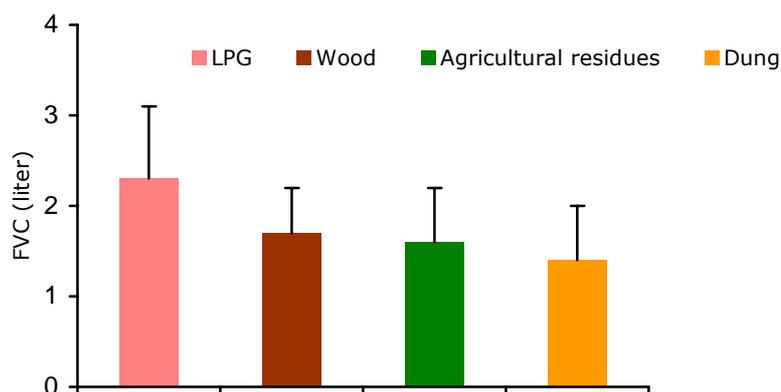


Figure 14. Reduction of FVC in relation to cooking with different types of biomass. Bars represent standard deviation of mean

SPUTUM CYTOLOGY

Airway inflammation in biomass users

Rural women who used biomass for domestic cooking showed significant rise ($p < 0.05$) in the number of alveolar macrophages (AM) and inflammatory cells in sputum. Neutrophils, eosinophils and lymphocytes in the sputum of biomass users were elevated by 1.6-, 2.4- and 1.9-fold respectively relative to control suggesting infection, inflammation and allergic reactions in the lungs (Table 16; Plate 4).

Table 16. Differential counts of cells (mean \pm SE) present in sputum of biomass and LPG-using women

Cell type	Cells per high power field (hpf) of microscope	
	LPG user (n=282)	Biomass user (n=615)
Eosinophil	2.7 \pm 0.3	6.6 \pm 0.7*
Lymphocyte	2.6 \pm 0.2	4.8 \pm 0.4*
Neutrophil	40.9 \pm 0.8	65.5 \pm 6.5*
Macrophage	3.7 \pm 0.8	8.1 \pm 1.8*
Epithelial cell	6.5 \pm 0.7	7.6 \pm 0.7

* $p < 0.05$ compared with respective control values; hpf, high power field (40X objective, 10X eye piece)

Alveolar macrophage (AM) count: remarkably increased in biomass users

Biomass users showed remarkable increase in AM number in sputum. NSE-positive AM per high power field (hpf) of light microscope (10x eye piece and 40x objective) was 11.4 \pm 0.3 in biomass users in contrast to 3.2 \pm 0.5 in LPG users ($p < 0.05$; Plate

4). Spearman's rank correlation analysis (r_s value) revealed a positive correlation of lifetime exposure of biomass smoke and AM/hpf ($r_s = 0.362$, $p < 0.01$, Table 28, Plate 5). Moreover, the AMs of women cooking with biomass fuel were highly keratinized, heavily loaded with phagocytosed particles and were larger in size (Plate 5).

Cytological changes in airway epithelial cells

Two important cytopathological findings in sputum of biomass users were the presence of metaplasia and dysplasia of bronchial epithelial cells. Metaplasia was present in 5.3% of LPG users. In contrast, 22.4% sputum samples of the biomass users had metaplasia of airway cells ($p < 0.001$). Dysplasia of epithelial cells was found in 4.1% of biomass users compared with 1.1% of LPG users (Fig. 15). After controlling potential confounding factors, a positive correlation was observed between biomass smoke exposure and occurrence of metaplasia (OR=1.35, 95% CI, 1.14-3.32; Fig. 15, Plate 6).

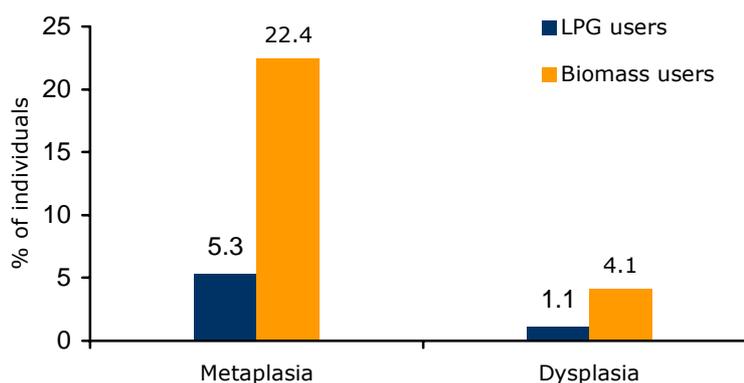


Figure 15. Prevalence of abnormal sputum cytology among biomass and LPG users

Other cytological changes in sputum in biomass users were goblet cell hyperplasia suggesting excessive pollution load, aggregates of ciliated columnar epithelial cell, indicating airway damage, Ciliocytophthoria, suggesting viral infections, and multinucleated columnar cell, suggesting genotoxicity (Table 17)

Table 17. Percentage of individuals with altered sputum cytology

Finding	LPG users	Biomass user
Squamous metaplasia	5.3	22.4*
Dysplasia of airway cells	1.1	4.1
Goblet cell hyperplasia	3.9	7.3
Ciliocytophthoria	0.5	3.0*
Aggregates of columnar epithelial cells	3.5	11.1*
Multinucleated columnar cell	0	0.7*

*, $p < 0.05$ compared with control

COVERT PULMONARY HEMORRHAGE

In order to investigate whether chronic exposure to biomass smoke was associated with microscopic hemorrhage inside the lung, Perl's Prussian blue reaction was carried out in sputum for detection of hemosiderin iron in AM, because pulmonary hemorrhage is generally associated with abundance of iron-laden macrophages (siderophages) in sputum. Results showed that 38% of AM of the biomass users had iron deposits compared with 8% of iron-laden macrophages in sputum of LPG users. Thus, the biomass users had 5-fold rise in the percentage of siderophages. The increase in the total number of siderophages in sputum was even greater. Women using biomass fuel had a mean of 12.1 siderophage per hpf compared with 0.6 siderophage/hpf in LPG users, thereby demonstrating 20-fold increment over the LPG users ($p < 0.001$; Table 18; Plate 7).

Table 18. Iron deposition in alveolar macrophages

Siderophage in sputum	LPG users	Biomass user
Number/hpf	0.6 ± 0.2	12.1 ± 6.0*

*Results are mean ± SD; *, $p < 0.001$ compared with control*

GREATER PREVALENCE OF HYPERTENSION AMONG BIOMASS USERS

Biomass fuel users had significantly higher prevalence of systolic as well as diastolic hypertension compared with LPG users (Table 19). About 15% of biomass-using women had systolic plus diastolic hypertension compared with 7.1% of LPG-users.

Table 19. Prevalence (%) of hypertension

	LPG user (n=282)	Biomass user (n=615)
Systolic hypertension		
Stage I	11.3	16.6*
Stage II	3.5	10.6*
Diastolic hypertension		
Stage I	8.8	15.9*
Stage II	5.9	8.5*
Systolic + Diastolic	7.1	14.8*

** $p < 0.05$ compared with LPG user*

GENOTOXIC CHANGES IN CELLS EXPOSED TO BIOMASS SMOKE

The biomass users showed significant increment ($p < 0.05$) in the frequency of micronucleated (MN) cells in buccal and airway epithelium. The rise was 2.2-fold in case of buccal epithelial cells, and 3.5-fold in airway epithelial cells (Table 20). MNs are formed as a result of chromosomal breakage. Therefore, the finding suggests increase in chromosomal damage in cells that directly at the route of exposure to biomass smoke.

Table 20. Micronuclei count in exfoliated buccal epithelium (mean \pm SD)

Micronucleus /1000 cells	LPG user	Biomass user
Buccal epithelial cells	1.9 \pm 0.6	4.2 \pm 1.9 *
Airway epithelial cells	1.8 \pm 1.1	6.3 \pm 1.5*

* $p < 0.05$ compared with LPG user

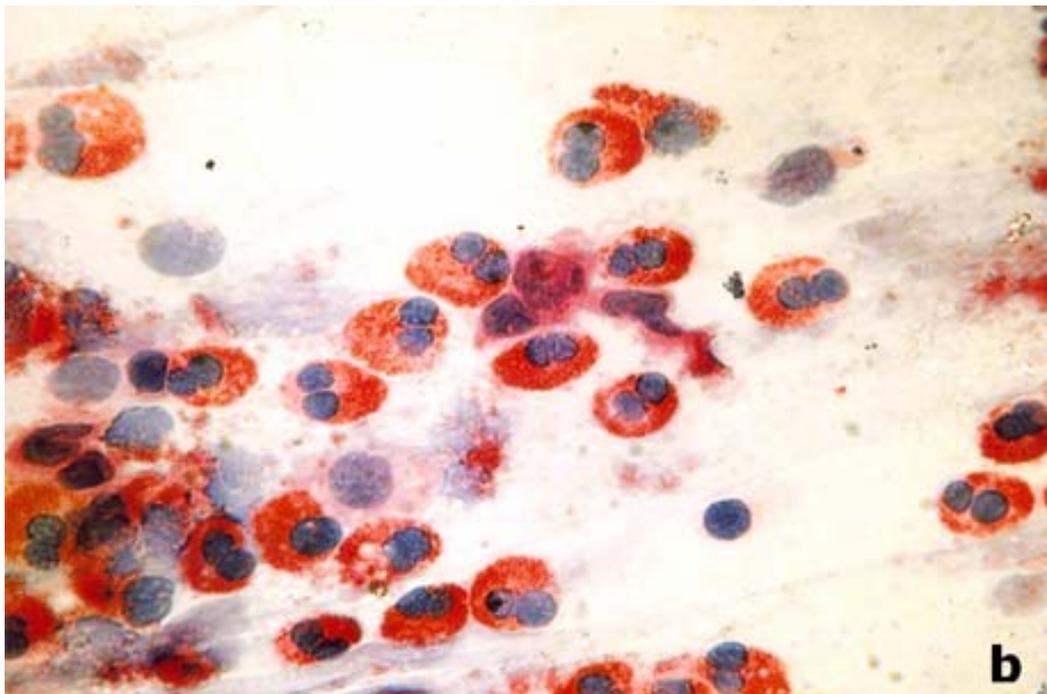
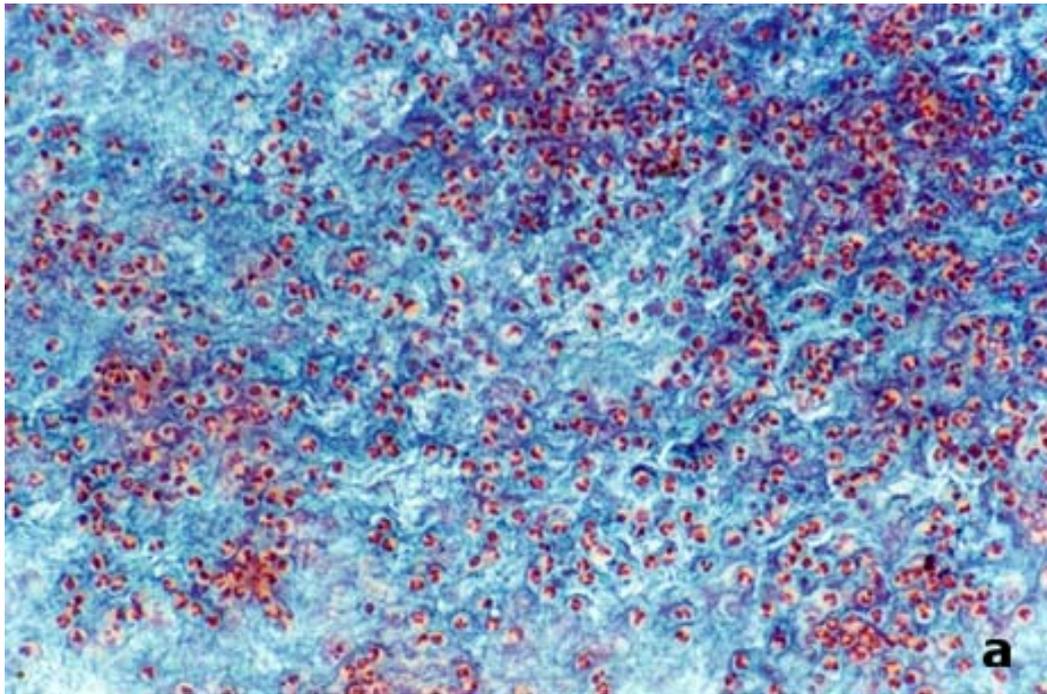


Plate 4: Photomicrographs of sputum samples of biomass using women showing massive increase in the number of neutrophils (a) and eosinophils (b), suggesting bacterial infection and airway allergy, respectively. Papanicolaou-stained, x 200 (a), x1000 (b)

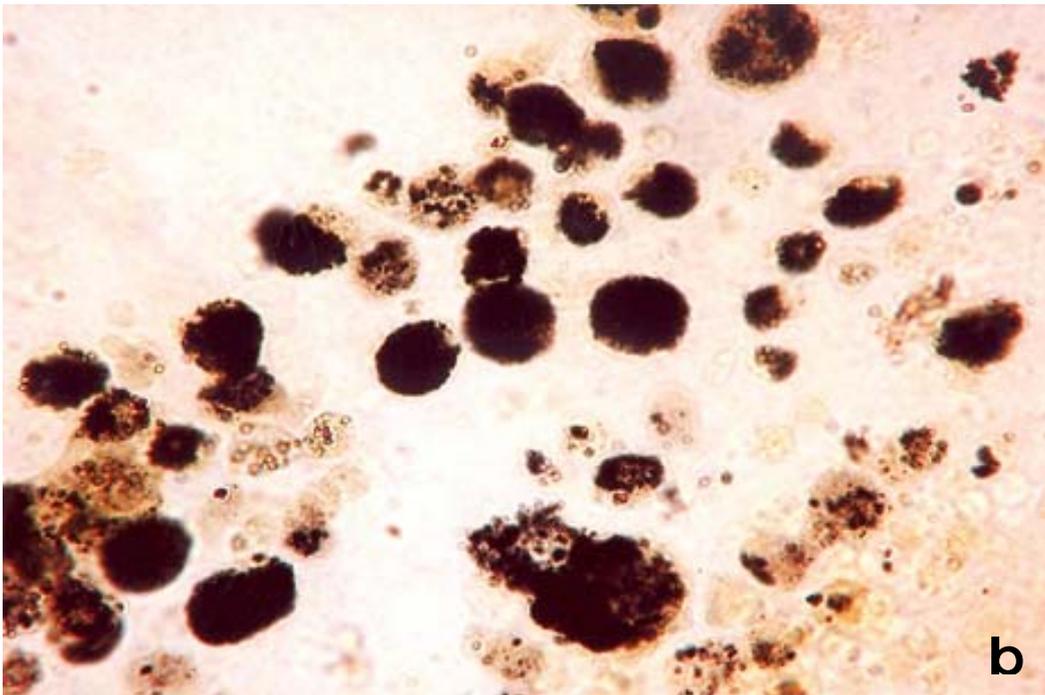
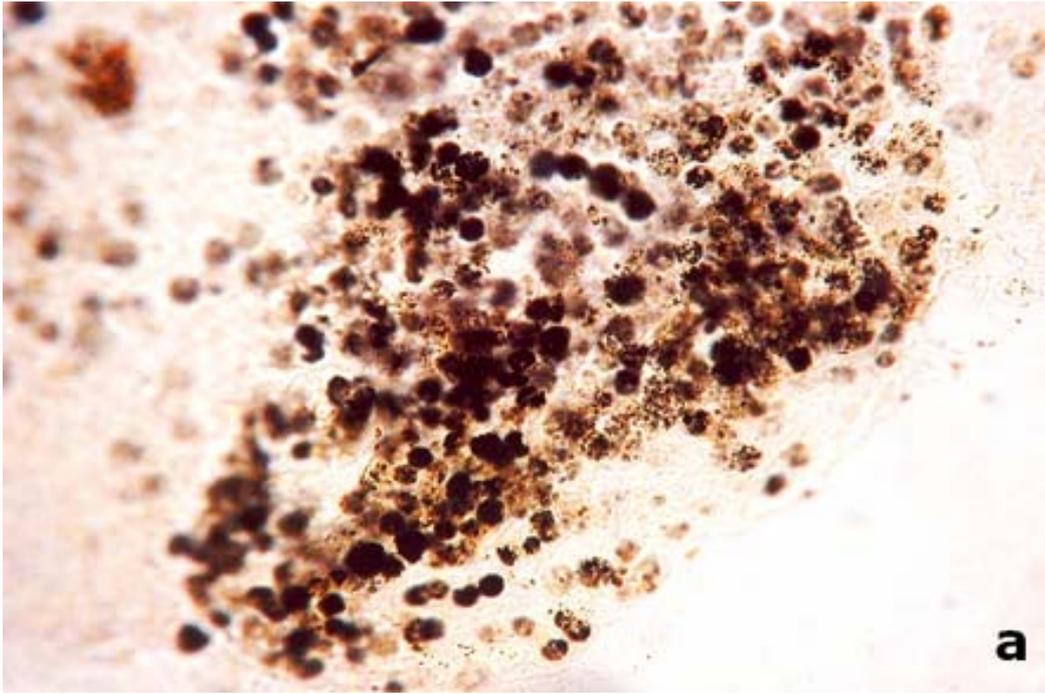


Plate 5: Photomicrographs of sputum of biomass-using women showing heavy carbonaceous particle deposition in alveolar macrophages suggesting high particulate pollution load. Papanicolaou-stained, x400 (a), x1000 (b).

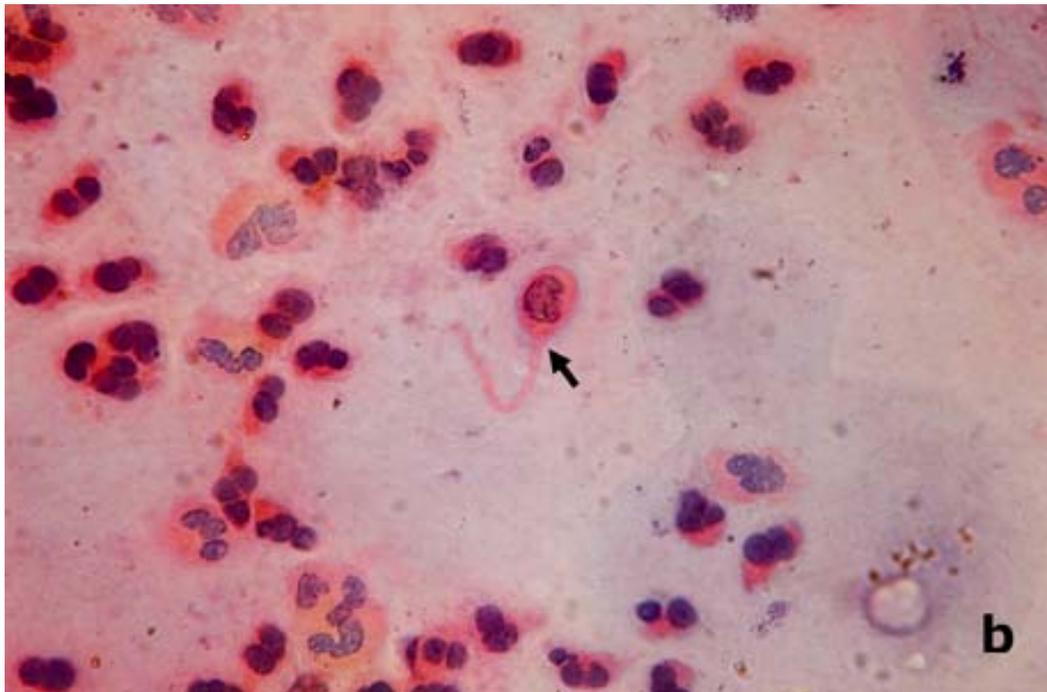
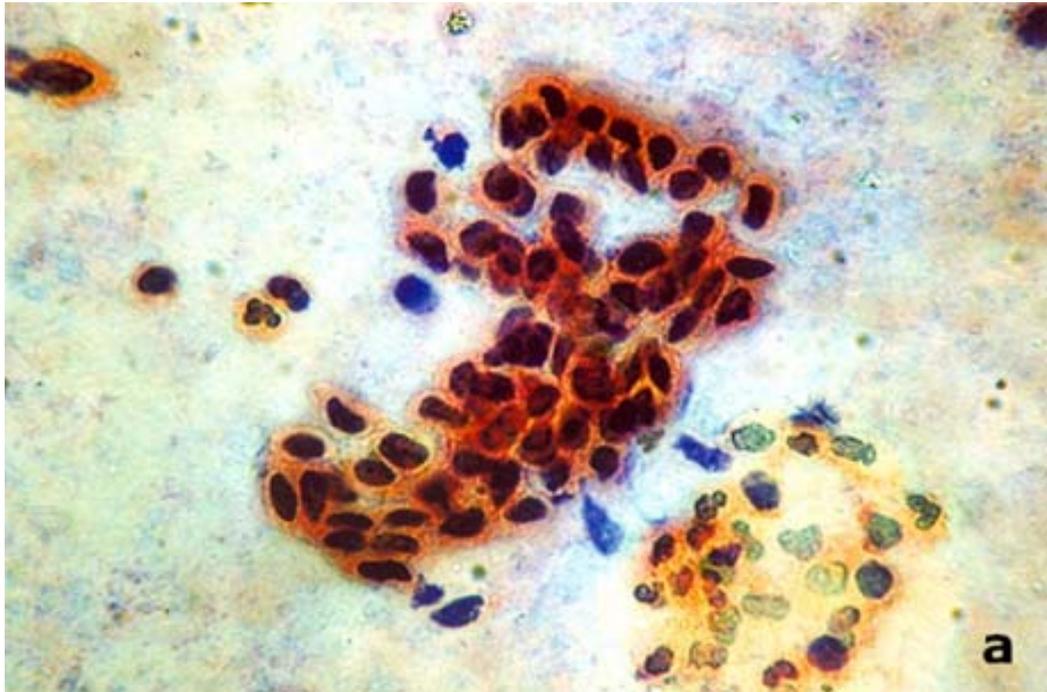


Plate 6: Cellular changes in airway cells of biomass users: airway epithelial cell metaplasia (a) and dysplasia (arrow, b). Papanicolaou-stained, x1000 (a & b).

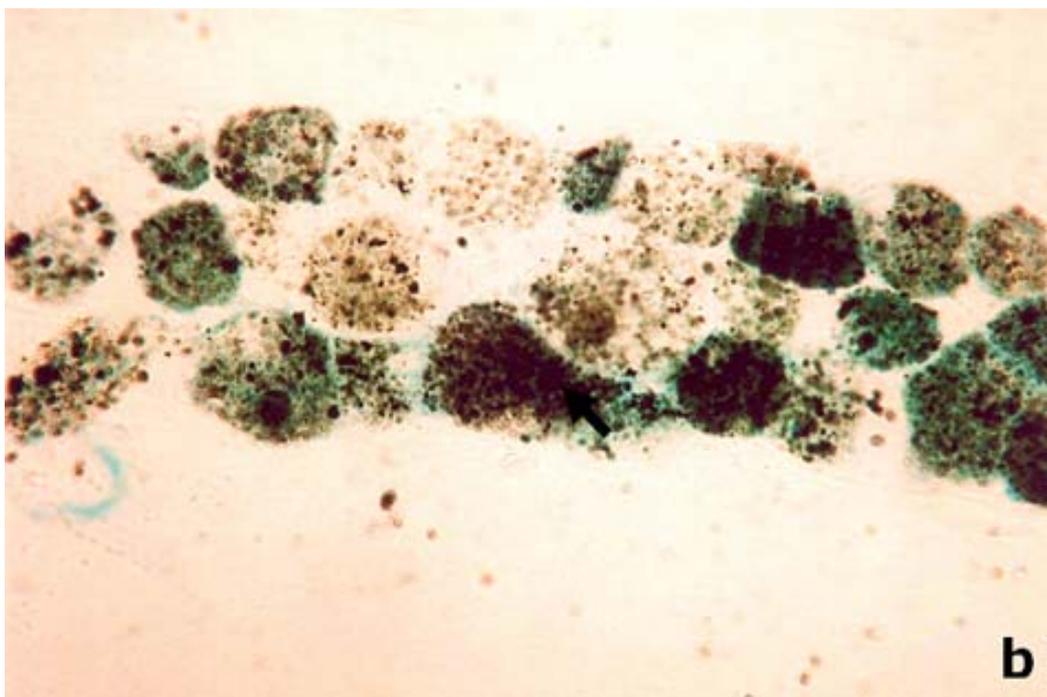
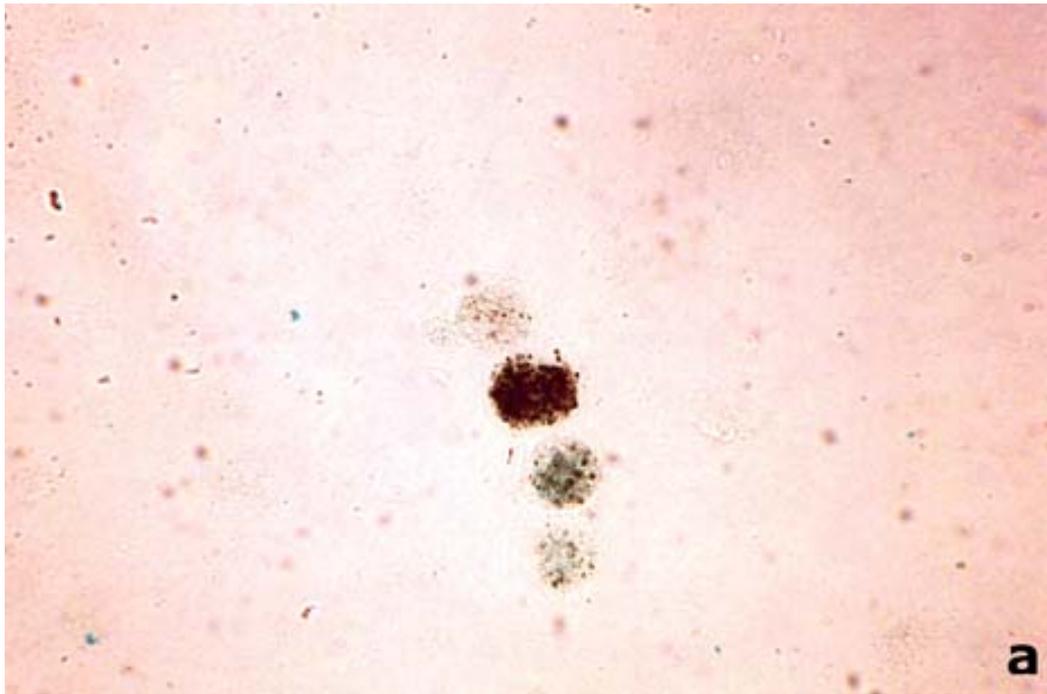


Plate 7: Photomicrographs of sputum showing abundance of iron-laden alveolar macrophage in biomass users (b) while only two mildly positive cells are present in sputum of LPG user (a). Perl's Prussian blue reaction, x1000.

DISCUSSION AND CONCLUDING REMARK

This study has demonstrated a multitude of health problems in women who were chronically exposed to biomass smoke during daily household cooking and their children who spend a lot of time with their mothers assisting them in the kitchen. They had higher prevalence of respiratory symptoms, reduced lung function, bronchial hypersensitivity and airway inflammation when compared with LPG using women from similar neighborhood. Respiratory and cardiovascular effects of air pollution are primarily mediated by fine and ultrafine particles respectively, and the concentrations of PM₁₀ and PM_{2.5} in indoor air during cooking was 3 to 4-times higher in biomass-using homes than LPG-using households. Biomass such as wood emits more particles than LPG or electricity during cooking and fine and ultrafine particles constitute a bulk of the biomass smoke (Morawska and Zhang, 2002). The inhaled ultrafine particles readily cross alveolar-endothelial barrier and circulates in the body and reaches the important organs such as liver within minutes (Nemmar et al, 2002). Therefore, particulate pollutants present in biomass smoke could have played a key role in mediating inflammatory changes and hemorrhage in the lungs.

Smoke inhalation is recognized as an important cause of acute lung injury and associated high mortality rate (Laffon et al., 1999). In the present study biomass users had significantly lower FVC, FEV₁, PEF and FEF_{25-75%} values than LPG users, suggesting significant reduction of lung function. Restrictive type of impairment was predominant. Like the present finding, several investigators have reported reduction in lung function in women chronically exposed to biomass smoke (Pandey, 1984; Behera et al., 1994; Dutt et al., 1996; Perez-Padilla et al., 1996; Amoli, 1998; Albalak et al., 1999; Laffon et al., 1999; Pauwels et al., 2001; Golshan et al., 2002; Arslan et al., 2004; Regalado et al., 2006).

Lung defense against inhaled particles and gaseous pollutants include innate mechanism such as aerodynamic filtration, mucociliary clearance, particle transport and detoxification by alveolar macrophages, as well as local and systemic innate and acquired antiviral immunity. Exposure to particulate pollution has been shown to increase the number of alveolar macrophage (AM) in sputum (Nobutomo, 1978; Brain, 1986; Mylius and Gullvag, 1986; Talbot et al., 1987; Hornby and Kellington,

1990; Mauderly, 1994; Kyi et al., 2000; Lahiri et al., 2000 a, b). Since AM are recognized as the first line of cellular defense in the lung, marked rise in the number of AM in sputum of biomass users of this study may indicate upregulation of lung defense in the face of greater challenge from airborne pollutants.

Biomass users suffered from airway inflammation, as evident from sputum neutrophilia and eosinophilia. In an earlier study increase in the number of neutrophils has been reported in response to wood smoke exposure (Tesfaigzi et al., 2002), and influx of neutrophils from circulation to the airways has been found following exposure to PM₁₀ (Salvi et al., 1999; Ghio et al., 2000). Accumulation of more active neutrophils in the lungs could be helpful for better antimicrobial defense (Klut et al., 2000). Sputum eosinophilia, on the other hand, could partially explain cough because sputum eosinophil count > 3% is usually associated with eosinophilic bronchitis, wheeze and chronic cough (Ayik et al., 2003). Sputum eosinophilia, as found in non-smoking biomass-using women of this study, is a common finding in smokers with bronchial allergy (Maestrelli et al., 1996; Komori et al., 2001).

Several cytological changes were found in sputum of the biomass users. Exposures of airways to environmental toxins (ozone, endotoxin, cigarette smoke) or allergens induce proliferation of epithelial cells (Tesfaigzi, 2002). Sheets of ciliated and non-ciliated columnar epithelial cells were often found in sputum of women cooking regularly with biomass fuel. These cells line the respiratory surface of the upper airways. The appearance of these cells in large sheets or clusters suggests damage to the respiratory epithelium following chronic smoke exposure. The change could be detrimental to respiratory physiology because it will affect the efficacy of the mucociliary escalator for pollutant disposal. Moreover it will facilitate entry of the microorganisms into the lung tissues thereby enhancing pulmonary infection.

Multinucleated columnar epithelial cells, metaplasia and dysplasia of airway epithelial cells, seen in a large number of biomass users of this study, are present more often in subjects with high risk of cancer development. Metaplasia is the initial morphological change in the journey towards neoplasia (Grubb, 1994). Squamous metaplasia usually develops as an adaptive response to toxic insults and the cells behave differently from that of normal airway epithelium. Metaplasia often leads to dysplasia when cell turnover becomes more rapid (Snead et al., 2003). Although it is rarely seen in sputum samples of non-smokers, metaplasia with atypia, a hallmark of cytological change in the lower respiratory tract after carcinogen exposure (Kamei et al., 1993), and a risk factor for lung cancer (Vine et al., 1990), has been

frequently recorded in rural women using biomass of this study. Metaplasia of squamous epithelial cells is an indicator of predisposition to chronic obstructive pulmonary disease (Madison et al., 1984) and lung cancer (Djuricic and Plamenac, 1999). Increased prevalence of squamous metaplasia in biomass-using women of rural West Bengal therefore signifies a greater risk of lung diseases including cancer.

Women of rural West Bengal who cook with biomass had abundance of siderophages in sputum. It may suggest covert pulmonary hemorrhage. Our study also revealed that women chronically exposed to biomass smoke had greater prevalence of micronucleus formation in buccal and airway epithelial cells suggesting genetic damage. Micronuclei (MN) are formed in cells as a result of breakage in chromosome. The assessment of MN in exfoliated cells is a useful tool to study the degree of cytogenetic damage in target tissues by human carcinogens (Belien et al., 1995). Elevated frequency of MN does indicate increased probability of carcinogenesis. Biomass smoke contain many potentially toxic compounds including polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene volatile organic compounds (VOCs) such as benzene, 1,3-butadiene, styrene, xylene and aldehydes. Biomass use increases cancer risk by 30-80 times (Zhang and Smith, 1996).

In essence, the study has demonstrated a multitude of health problems in women of rural West Bengal who were chronically exposed to biomass smoke during daily household cooking. They had higher prevalence of respiratory symptoms, reduced lung function, bronchial hypersensitivity, airway inflammation, and diminished antioxidant defense and platelet hyperactivity with consequent high risk of cardiovascular diseases relative to LPG-using women from similar neighborhood. The changes were obvious even in women who never smoked in their life, hence the findings cannot be attributed to tobacco smoking, although exposures to environmental tobacco smoke (passive smoking) because of smoking habit of male member(s) of the family could have played a role. Even after controlling this potential confounder in multivariate logistic regression analysis, exposure to indoor air pollution from biomass burning was found to be positively associated with these changes. The precise mechanism by which biomass smoke has mediated the toxic effects is currently unknown, but high level of particulate pollutants present in smoke could have played a major role. It is important to mention in this context that respiratory and cardiovascular effects of air pollution are primarily mediated by fine and ultrafine particles respectively, and the concentrations of PM₁₀ and PM_{2.5} in indoor air during cooking was 3 to 4-times higher in biomass-using homes than LPG-using households.

CONCLUDING REMARK

Millions of poor people of the country who cannot afford cleaner fuel have no other alternative but to use traditional biomass for cooking and room heating. In the process, their health becomes adversely affected, as demonstrated in this study. The victims are generally women who cook with these fuels and their children who spend a long time with their mothers. The administrators, policy makers as well as the victims are largely unaware about the harm biomass fuels are causing on their health. In fact, air pollution and related health hazards are considered as a problem of urban life while the villages are treated as abode of peace, tranquility and freshness. No wonder, there is no standard for indoor air quality in the country. Therefore, there is no question of maintaining the indoor emission level within standard. Moreover, since the victims are mainly women and children and that too from poor rural areas, they suffer in silence while everybody seems busy with so many 'important' issues.

Against this backdrop, the findings can serve as an eye-opener. The need of the hour is regular monitoring of health of the biomass users, extensive research on the mechanism of biomass-smoke toxicity and susceptibility, and medical intervention for those diagnosed with respiratory or systemic health problems. As long-term measures the authority should consider introduction of smokeless chullas (oven with chimneys) and proper kitchen ventilation in all biomass using households and supply of cleaner fuel such as LPG at an affordable price to the rural people. If necessary subsidy should be given to the needy as it will save money on account of treatment of diseases associated with biomass use.

REFERENCES

- Albalak R. Cultural Practices and Exposure to Particulate Pollution from Indoor Biomass Cooking: Effects on Respiratory Health and Nutritional Status among the Aymara Indians of the Bolivian Highlands, Doctoral Dissertation, University of Michigan, 1997.
- Albalak R, Frisancho AR, Keeler GJ. Domestic biomass fuel combustion and chronic bronchitis in two rural Bolivian villages. *Thorax*, 54, 1004-1008, 1999.
- Alfheim I, Lofroth G, Moller M. Bioassay of extracts of ambient particulate matter. *Environ Health Perspect*, 47, 227, 1983.
- American Thoracic Society (ATS). Standardization of Spirometry. *Am J Respir Crit Care Med* 152, 1107-1136, 1995
- Amoli K. Bronchopulmonary disease in Iranian housewives chronically exposed to indoor smoke. *Eur Respir J* 11, 659-663, 1998.
- Amusa YB, Ijadunola IK, Onayade OO. Epidemiology of otitis media in a local tropical African population. *West Afr J Med.*, 24, 227-230, 2005.
- Anderson HR. Air pollution and trends in asthma. *Ciba Found Symp*, 206, 190-207, 1997.
- Ardayfio-Schandorf E. Women's health status in Africa: Environmental perspectives from rural communities. *Health Care for Women International*, 14, 375-386, 1993.
- Arslan M, Akkurt I, Egilmez H, Atalar M, Salk I. Biomass exposure and the high resolution computed tomographic and spirometric findings. *Eur J Radiol* 52, 192-199, 2004
- Aust AE, Ball JC, Hu AA, Lighty JS, Smith KR, Straccia AM, Veranth J, Young WC. Particle characteristics responsible for effects on lung epithelial cells. *Res Rep Health Eff Inst.*, 110, 67-76, 2002.
- Awasthi, S, Glick H, Fletcher R. Effect of Cooking Fuels on Respiratory Diseases in Preschool Children in Lucknow, India. *American Journal of Tropical Medicine and Hygiene*, 55, 48-51, 1996.
- Ayik SO, Basoglu OK, Erdinc M, Bor S, Veral A, Bilgen C. Eosinophilic bronchitis as a cause of chronic cough. *Respir Med.*, 97, 695-701, 2003.
- Balakrishnan K, Sankar S, Parikh J, Padmavati R, Srividya K, Venugopal V, Prasad S, Pandey VL. Daily average exposures to respirable particulate matter from combustion of biomass fuels in rural households of southern India. *Environ Health Perspect* 110, 1069-1075, 2002.
- Balakrishnan K, Sambandam S, Padmavathi R, Mehta S, Smith KR. Exposure assessment for respirable particulates associated with household fuel use in rural districts of Andhra Pradesh, India. *Journal of Exposure Analysis and Environmental Epidemiology*, 14, S14-S25, 2004.
- Behera D, Dash S, Malik S. Blood carboxyhaemoglobin levels following acute exposure to smoke of biomass fuel. *Indian J Med Res*, 522-42, 1988.
- Behera D, Jindal SK. Respiratory symptoms in Indian women using domestic cooking fuels. *Chest*, 100, 385-388, 1991.
- Behera D, Jindal SK, Malhotra HS. Ventilatory function in nonsmoking rural Indian women using different cooking fuels. *Respiration* 61, 89-92, 1994
- Belien JAM, Copper MP, Braakhuis BJM, Snow GB, Baak JPA. Standardization of counting micronuclei: definition of a protocol to measure genotoxic damage in human exfoliated cells. *Carcinogenesis* 16, 2395-2400, 1995
- Birring SS, Brightling CE, Bradding P, Entwisle JJ, Vara DD, Grigg J, Wardlaw AJ, Pavord ID. Clinical, radiologic, and induced sputum features of chronic obstructive pulmonary disease in nonsmokers: a descriptive study. *Am J Respir Crit Care Med*. 166, 1078-83, 2002.
- Boy, E., Bruce, N.G. and Delgado, H. Birth weight and exposure to kitchen wood smoke during pregnancy in rural Guatemala, *Environ. Health Perspect*, 110, 109-114, 2002.
- Brain JD. Toxicological aspects of alteration of pulmonary macrophage function. *Ann Rev Pharmacol Toxicol* 26, 547-565, 1986
- Bruce N, Perez-Padilla R, Albalak R. Indoor air pollution in developing countries: a major environmental and public health challenge for the new millennium, *Bull. World Health Org.*, 78, 1078-1092, 2000.

- Chang JC, Distler SG, Kaplan AM. Tobacco smoke suppresses T cells but not antigen-presenting cells in the lung associated lymph nodes. *Toxicol Appl Pharmacol*, 102, 514-523, 1990.
- Clifford P. Carcinogens in the nose and throat: nasopharyngeal carcinoma in Kenya. *Proceedings of the Royal Society of Medicine*, 65, 682-686, 1972.
- Daigler GE, Markello SJ, Cummings KM. The effect of indoor air pollutants on otitis media and asthma in children. *Laryngoscope*, 101, 293-296, 1991.
- Dary O, Pineda O, Belizan JM. Carbon monoxide in dwellings in poor rural areas of Guatemala. *Bull Environ Contam Toxicol*, 26, 24-30, 1981.
- Djuricic S, Plamenac P. The effect of sex factors on cytologic changes in the sputum of young adults exposed to urban air pollution. *Srp Arh Celok Lek* 127, 16-20, 1999.
- Dubick MA, Carden SC, Jordan BS, Langlinais PC, Mozingo DW. Indices of antioxidant status in rats subjected to woodsmoke inhalation and/or thermal injury. *Toxicology*, 176, 145-157, 2002.
- Dutt D, Srinivasa DK, Rotti SB, Sahai A, Konar D. Effect of indoor air pollution on the respiratory system of women using different fuels for cooking in an urban slum of Pondicherry. *Natl Med J India* 9, 113-117, 1996
- Ellegard, A. Cooking smoke and respiratory symptoms among women in low-income areas of Maputo. *Environ. Health Perspect* 104, 980-985, 1996.
- Engle PL, Hurtado E, Ruel M. Smoke exposure of women and young children in highland Guatemala: predictions and recall accuracy. *Human Organisation*, 56, 408-417, 1997.
- Erkicic S, Coskun Ozsarac MD, Kullu S. Sputum cytology for the diagnosis of lung cancer. *Acta Cytol* 47, 1023-1027, 2003
- Ezzati M, Kammen D. The health impacts of exposure to indoor air pollution from solid fuels in developing countries: Knowledge, gaps and data needs. *Environ. Health Perspect*, 110, 1057-1068, 2002.
- Fujii T, Hayashi S, Hogg JC, Vincent R, van Eeden SF. Particulate matter induces cytokine expression in human bronchial epithelial cells. *Am J Respir Cell Mol Biol*, 25, 265-271, 2001.
- Ghio AJ, Kim C, Devlin RB. Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. *Am J Respir Crit Care Med*, 162, 981-988, 2000.
- Giovagnoli MR, Alderisio M, Cenci M, Nofroni I, Vecchione A. Carbon and hemosiderin-laden macrophages in sputum of traffic policeman exposed to air pollution. *Arch Environ Health*, 54, 284-290, 1999.
- Global Initiative for Chronic Obstructive Lung Disease. National Institutes of Health, National Heart, Lung and Blood Institute. Publication 01-2701, October 2001.
- Gold JA, Jagirdar J, Hay JG, Addrizzo-Harris DJ, Naidich DP, Rom WN. Hut lung. A domestically acquired particulate lung disease. *Medicine (Baltimore)*, 79, 310-317, 2000.
- Golshan M, Faghihi M, Roushan-Zamir T, Marandi MM, Esteki B, Dadvand P, Farahmand-Far M, Rahmati S, Islami F. Early effects of burning rice farm residues on respiratory symptoms of villagers in suburbs of Isfahan, Iran. *Int J Environ Res*, 12, 125-131, 2002.
- Grobbelaar JP, Bateman ED. Hut lung: a domestically acquired pneumoconiosis of mixed aetiology in rural women. *Thorax*, 46, 334-340, 1991.
- Grubb C. *Diagnostic Cytopathology, A text and colour atlas*. Churchill Livingstone, London. pp. 65-112, 1994.
- Hughes HE, Dodds TC. *Handbook of Diagnostic Cytology*, E&S Livingstone, Edinburgh, pp. 215-217, 1968.
- IARC. Some industrial chemical and dyestuffs. *IARC Monogr Eval Carcinog Risks Hum*, 29, 95-148, 1982.
- IARC. Polynuclear aromatic compounds, part 1: chemical, environmental and experimental data. In: *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans IARC Monographs Vol. 32*, pp. 1-453, 1983.
- IEA. *World Energy Outlook 2002*. Energy and Poverty (Chapter 13), posted on <http://www.worldenergyoutlook.org/weo/pubs/weo2002/energypoverity.pdf>, 2002.
- Jeffery PK. Structural and inflammatory changes in COPD : a comparison with asthma. *Thorax*, 53, 129-36, 1998.

- Kamei T, Kohno T, Ohwada H, Takeuchi Y, Hayashi Y, Fukuma S. Experimental study of the therapeutic effects of folate, vitamin A and vitamin B₁₂ on squamous metaplasia of the bronchial epithelium. *Cancer*, 71, 2477-2483, 1993.
- Keatings VM, Collins PD, Scott DM. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med.*, 153, 530-534, 1996.
- Klut ME, Whalen BA, Hogg JC. Flow cytometric analysis of defensins in blood and marrow neutrophils. *Eur J Haematol*, 64, 114-120, 2000.
- Koenig JQ, Pierson WE. Air pollutants and the respiratory system: Toxicity and pharmacologic interventions. *Clin. Toxicol.*, 29, 401-411, 1991.
- Komori M, Inoue H, Matsumoto K, Koto H, Fukuyama S, Aizawa H, Hara N. PAF mediates cigarette smoke-induced goblet cell metaplasia in guinea pig airways. *Lung Cellular Mol Physiol*, 280, L436-L441, 2001.
- Kyi M, Miyazaki Y, Inoue T, Miyake S, Matsukawa A, Yoshizawa Y. Acute effects of smoke exposure on the cellular and cytokine profile in isolated perfused lungs. *Resp Physiol*, 123, 143-151, 2000.
- Laffon M, Pittet JF, Modelska K, Matthay MA., Young DM. Interleukin-8 mediates injury from smoke inhalation to both the lung endothelial and the alveolar epithelial barriers in rabbits. *Respir Crit Care Med*, 160, 1443-1448, 1999.
- Lahiri T, Ray MR, Mukherjee S, Basu C, Lahiri P. Marked increase in sputum alveolar macrophages in residents of Calcutta: Possible exposure effect of severe air pollution. *Current Science*, 78, 399-404, 2000a.
- Lahiri T, Roy S, Basu C, Ganguly S, Ray MR, Lahiri P. Air pollution in Calcutta elicits adverse pulmonary reaction in children. *Ind J Med Res*, 112, 21-26, 2000b.
- Leonard SS, Wang S, Shi X, Jordan BS, Castranova V, Dubick MA. Woodsmoke particles generate free radicals and cause lipid peroxidation, DNA damage, NFkappaB activation and TNF-alpha release in macrophages. *Toxicology*, 150, 147-157, 2000.
- Lewtas J, Lewis C, Zweidinger R, Stevens R, Cupitt L. Sources of genotoxicity and cancer risk in ambient air. *Pharmacogenetics*, 2, 288, 1992.
- Lojda Z, Gossrau R, Stoward PJ. Histochemical methods for esterases. In: *Histochemistry Theoretical and Applied*. Stoward PJ, Pearse, A.G.E. (eds). Vol III, Churchill Livingstone, London. pp. 639-652, 1991.
- Madison R, Afifi AA, Mittman C. Respiratory impairment in coke oven workers: Relationship to work exposure and bronchial inflammation detected by sputum cytology. *J Chron Dis*, 37, 167-176, 1984.
- Maestrelli P, Calcagni PG, Saetta M, Bertin T, Mapp CE, Sanna A, Veriter C, Fabbri LM, Stanescu D. Integrin upregulation on sputum neutrophils in smokers with chronic airway obstruction. *Am J Respir Crit Care Med*, 154, 1296-1300, 1996.
- Mauderly JI. Toxicological and epidemiological evidence for health risks from inhaled engine emissions. *Environ Health Perspect*, 102, 165-171, 1994.
- Mavalankar DV, Trivedi CR, Grah RH. Levels and risk factors for perinatal mortality in Ahmedabad, India. *Bull. WHO*, 69, 435-442, 1991.
- Mehta S. Characterizing Exposures to Indoor Air Pollution From Household Solid Fuel Use, in *Environmental Health Sciences*, University of California, Berkeley, 2003.
- Mishra V, Retherford R. Cooking Smoke Increases the Risk of Acute Respiratory Infections in Children. *National Family Health Survey Bulletin*, No. 8, IIPS Mumbai and East-WestCenter, Honolulu, 1997.
- Mishra VK, Retherford RD, Smith KR. Biomass cooking fuels and prevalence of tuberculosis in India. *International J. Infect. Dis.*, 3, 119-129, 1999a.
- Mishra VK, Retherford RD, Smith KR. Cooking with biomass fuels increases the risk of tuberculosis. *Natl Fam Health Surv Bull.* , 13, 1-4, 1999b.
- Mishra V. Effect of indoor air pollution from biomass combustion on prevalence of asthma in the elderly. *Environmental Health Perspective*, 111, 71-77, 2003.
- Mohammed N, Ng'ang'a L, Odhiambo J, Nyamwaya J, Menzies R. Home environment and asthma in Kenyan school children: a case-control study. *Thorax*, 50, 74-78, 1995.

- Mohan M, Sperduto RD, Angra SK, Milton RC, Mathur RL, Underwood BA, Jaffery N, Pandya CB, CHHabra VK, Vajpayee RB. India-US case-control study of age related cataracts. *Arch.Ophthalmol*, 107, 670-676, 1989.
- Morawska L, Zhang JJ. Combustion sources of particles. 1. Health relevance and source signatures. *Chemosphere*, 49, 1045-1058, 2002.
- Morrow PE. Possible mechanism to explain dust overloading of the lungs. *Fundam App Toxicol*, 10, 369-384, 1988.
- Mukae H, Vincent R, Quinlan K, English D, Hards J, Hogg JC, van Eeden SF. The effect of repeated exposure to particulate air pollution (PM₁₀) on the bone marrow. *Am J Respir Crit Care Med*, 163, 201-209, 2001.
- Musthapa MS, Lohani M, Tiwari S, Mathur N, Prasad R, Rahman Q. Cytogenetic biomonitoring of Indian women cooking with biofuels: micronucleus and chromosomal aberration tests in peripheral blood lymphocytes. *Environ Mol Mutagen*, 43, 243-249, 2004.
- Mylius EA, Gullvag B. Alveolar macrophage counts as an indicator of lung reaction to industrial air pollution. *Acta Cytol*, 30, 157-162, 1986.
- National Family Health Survey (MCH and family Planning): India 1992-93. International Institute for population Sciences, Mumbai, 1995.
- Nemmar A, Hoet PH, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, Vanbilloen H, Mortelmans L, Nemery B. Passage of inhaled particles into the blood circulation in humans. *Circulation*, 105, 411-414, 2002.
- Nobutomo K. Air Pollution and cytological changes in sputum. *Lancet*, 11, 523-526, 1978.
- Office of Technology Assessment, *Fueling Development: Energy Technologies for Developing Countries*, Congress of the United States: Washing, D.C. p. 324, 1992.
- Oliver C, Lewis PR, Stoward PJ. Histochemical methods for esterases. In: *Histochemistry Theoretical and Applied*. Stoward PJ, Pearse, A.G.E. (eds). Vol III, Churchill Livingstone, London. pp. 607-618, 1991.
- Pandey MR. Domestic smoke pollution and chronic bronchitis in a rural community of the Hill Region of Nepal. *Thorax*, 39, 337-339, 1984.
- Pandey MR, Regmi HN, Neupane RP, Gautam A, Bhandari DP. Domestic smoke pollution and respiratory function in rural Nepal. *Tokai J Exp Clin Med.*, 10, 471-481, 1985.
- Pauwels RA, Buist AS, Calverely PMA, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*, 163, 1256-1276, 2001.
- Pearse AGE. Pigments and pigment precursor. In: *Histochemistry, Theoretical and Applied*. Stoward PJ and Pearse AGE (eds). 4th edition, Vol 2, Churchill Livingstone, Edinburgh, pp. 874-928, 1985.
- Perez-Padilla R, Regalado J, Vedal S, Pare P, Chapela R, Sansores R, Selman M. Exposure to biomass smoke and chronic airway disease in Mexican women. A case-control study. *Am J Respir Crit Care Med.*, 154, 701-706, 1996.
- Perez-Padilla, R. Perez-Guzman, C. Baez-Saldana, R. and Torres-Cruz, A. Cooking with biomass stoves and tuberculosis: a case-control study. *Int. J. Tuberculosis Lung Dis*, 5, 1-7, 2001.
- Pintos J, Franco EL, Kowalski LP, Oliveira BV, Curado MP. Use of wood stoves and risk of cancers of the upper aero-digestive tract: a case-control study. *Int J Epidemiol.*, 27, 936-940, 1998.
- Pistelly R. Woodsmoke and asthma: a controversial issue. *Am J Respir Crit Care Med*, 155, A941, 1997.
- Platts-Mills TAE, Woodfolk J. Rise in asthma cases. *Science*, 278, 1001, 1997.
- Pope CA III, Dockery DW. Epidemiology of particle effects. In: *Air pollution and health*. Holgate ST, Samet JM, Koren SH, Maynard RL. (eds), *Air Pollution and Health*. Academic Press, San Diego. pp. 673-705, 1999a.
- Ramachandra TV, Kamakshi G, Shruti BV. Bioresource status in Karnataka. doi:10.1016/j.rser.2003.09.001. Copyright © 2003 Published by Elsevier Science Ltd., 2003.
- Rao C, Qin C, Robinson W, Zigler J. Effect of smoke condensate on the physiological integrity and morphology of organ cultured rat lenses. *Curr. Eye Res.*, 14, 295-301, 1995.

- Ray MR, Basu C, Lahiri T. Haematological changes and up-regulation of P-selectin expression in circulating platelets of Indian women chronically exposed to emissions from biomass fuels. *Comp Clin Path*, 580, 491-494, 2003.
- Ray MR, Roychoudhury S, Mukherjee S, Lahiri T. Occupational benzene exposure from vehicular sources in India and its effects on hematology, lymphocyte subsets and platelet p-selectin expression. *Toxicology and Industrial Health*, 23, 167-175, 2007.
- Reddy AKN, Reddy BS. Substitution of energy carriers for cooking in Bangalore. *Energy*, 19, 561-571, 1994.
- Reddy MS, Venkataraman C. Inventory of Aerosol and Sulphur Dioxide Emissions from India: II – Biomass Combustion. *Atmospheric Environment*, 36, 699-712, 2002.
- Regalado J, Perez- Padilla R, Sansores R, Paramo Ramirez JI, Brauer M, Pare P, Vedal S. The effect of biomass burning on respiratory symptoms and lung function in rural Mexican women. *Am J Respir Crit Care Med*, 174, 901-905, 2006.
- Rehfuess E, Corvalan C, Neira M. Indoor air pollution: 4000 deaths a day must no longer be ignored. *Bull WHO*, 84(7), 508, 2006.
- Rinsky RA, Smith AB, Homung R, Fillioon TG, Young RJ, Okun A, et al. Benzene and leukemia: an epidemiological risk assessment. *N Eng J Med*, 316, 1044-1050, 1987.
- Sagel SD, Sontag MK, Wagener JS, Kapsner RK, Osberg I, Accurso FJ. Induced sputum Inflammatory measures correlate with lung function in children with cystic fibrosis. *J Pediatr*, 141, 811-817, 2002.
- Salvi S, Blomberg A, Rudell B, Kelly F, Sandstrom T, Holgate ST, Frew A. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exposure in healthy human volunteers. *Am J Respir Crit Care Med*, 159, 702-709, 1999.
- Samet JM, Domonici F, Frank C, Curriero, Coursac I, Zeger SL. Fine particulate air pollution and mortality in 20 U.S. cities, 1987-1994. *The N Engl J of Med*, 343, 1742-1749, 2000.
- Saxena S, Dayal V. Valuing air pollution in India, *Pacific and Asian journal of Energy*, Tata Energy Research Institute, 8, 208, 1998.
- Seaton A, Soutar A, Crawford V, Elton R, MacNerlan S, Cherri J. Particulate air pollution and the blood. *Thorax*, 54, 1027-1032, 1999.
- Sinha SN, Kulkarni PK, Shah SH, Desai NM, Patel GM, Mansuri MM, Saiyed HN. Environmental monitoring of benzene and toluene produced in indoor air due to combustion of solid biomass fuels. *Science of the Total Environment*, 357, 280-287, 2006.
- Smith KR, Apte MG, Yoying M, Wongsekiarttirat W, Kulkarni A. Air Pollution and the Energy Ladder in Asian Cities. *Energy*, 19, 587-600, 1994.
- Smith KR, Liu, Y. Indoor air pollution in developing countries. In: *Epidemiology of Lung Cancer* (Samet JM, ed) New York : Marcel Dekker, 151-183, 1994.
- Smith KR. The national burden of disease from indoor air pollution in India. In: Raw G, Aizlewood C, Warren P, eds. *Indoor Air 99, the 8th International Conference on Indoor Air Quality and Climate*, August 1999, Edinburgh, London, Construction Research Ltd., 13-18, 1999.
- Smith KR. Indoor air pollution implicated in alarming health problems. In: *Indoor Air Pollution Energy and Health for the Poor*. Newsletter published by World Bank, p. 1, 2000.
- Smith KR. National Burden of Disease in India from Indoor Air Pollution. *Proceedings of the National Academy of Sciences* 97:13286-13293, 2000.
- Smith KR, Samet JM, Romieu I, Bruce N. Indoor air pollution in developing countries and acute respiratory infections in children. *Thorax*, 55, 518-532, 2000.
- Smith KR, Uma R, Kishore WN, Lata K, Joshi V, Zhang J, Rasmussen RA, Khalil MAK. Greenhouse gases from small-scale combustion devices in developing countries, Phase IIa: Household stoves in India. EPA-600/R-00-052 US Environmental Protection Agency, Office of Research and Development, Washington DC, 2000a.
- Smith KR, Uma R, Kishore VVN, Zhang J, Joshi V, Khalil MAK. Greenhouse implications of household fuels: An analysis for India. *Annual Review of Energy and Environment*, 25, 741-763, 2000b.
- Smith KR, Mehta S, Feuz M. Indoor air pollution from household use of solid fuels. In : Ezzati M, Rodgers A, Lopez A, and Murray C (eds) *Comparative quantification of health risks : global and regional burden of disease due to selected major risk factors*, Geneva, WHO, 2002.

- Smith KR, Mehta S, Maeusezahl-Feuz M. "Indoor smoke from household solid fuels", in Ezzati M, Rodgers AD, Lopez AD, Murray CJL. (eds.), *Comparative Quantification of Health Risks: Global and Regional Burden of Disease due to Selected Major Risk Factors*, Geneva, World Health Organization, Vol. 2, pp. 1437-1495, in press, 2004.
- Snead DRJ, Perunovic B, Cullen N, Needham M, Dhillion DP, Satoh H, Kamma H. hnRNP B1 expression in benign and malignant lung disease. *J Pathol*, 200, 88-94, 2003.
- Srestha IL, Srestha SL. Indoor air pollution from biomass fuels and respiratory health of the exposed population in Nepalese households. *Int J Occup Environ Health*, 11, 150-160, 2005.
- Srivastava GP. Socio-economic status scale (Urban), Agra: National Psychological Corporation, 1978.
- Talbot RJ, Morgan A, Moores SR, Matulionis DH. Preliminary studies of the interaction between ²³⁹PuO₂ and cigarette smoke in the mouse lung. *Int J Radiat Biol*, 51, 1101-1110, 1987.
- TEDDY : Tata Energy Data Directory Yearbook. New Delhi, India: TERI, 1998-1999.
- TERI. Biomass Energy Systems, Venkata Ramana, P., Srinivas, S., N., TERI, New Delhi, India, 1997.
- TERI : Tata Energy Data Directory Yearbook. New Delhi, India: TERI, 2001-2002.
- Tesfaigzi Y, Singh SP, Foster JE, Kubatko J, Barr EB, Fine PM, McDonald JD, Hahn FF, Mauderly JL. Health effects of subchronic exposure to low levels of wood smoke in the rats. *Toxicol Sci*, 65, 115-125, 2002.
- Thorn J, Brisman J, Toren K. Adult-onset asthma is associated with self-reported mold or environmental tobacco smoke exposures in the home. *Allergy*, 56, 287-292, 2001.
- Tiwari SC, Kumar A, Kumar A. Development & standardization of a scale to measure socio-economic status in urban & rural communities in India. *Indian J Med Res*, 122, 309-314, 2005.
- Traynor GW, Apte MG, Carruthers AR, Dillworth JF, Grimsrud DT, Gundel LA. Indoor air pollution due to emissions from woodburning stoves. *Environ Sci Technol*, 21, 691-697, 1987.
- Tuthill R. Woodstoves, formaldehyde and respiratory disease. *American Journal of Epidemiology*, 120, 952-955, 1984.
- UNDP, *World Energy Assessment*. United Nations Development Programme: New York City, 2004.
- Velema JP, Ferrera A, Figueroa M, Bulnes R, Toro LA, de Barahona O, Claros JM, Melchers WJ. Burning wood in the kitchen increases the risk of cervical neoplasia in HPV-infected women in Honduras. *Int J Cancer*, 97, 536-541, 2002.
- Vine MF, Schoenbach VJ, Barbara SH, Hulka BS, Koch GG, Samsa G. Atypical metaplasia and incidence of bronchogenic carcinoma. *Am J Epidemiol*, 131, 781-793, 1990.
- Wallace LA. Comparison of risks from outdoor and indoor exposure to toxic chemicals. *Environmental Health Perspect*, 95, 7-13, 1991.
- World Health Organization. *Health and Environment in sustainable development. Five years after the earth summit.*, Report, Geneva, Switzerland, Chapter 3, 1997.
- World Energy Council, FAO *The challenge of rural energy poverty in developing countries*, Report, London, World Energy Council, Annex 1, page 118, 1999.
- World Health Organization. Guidelines for Air Quality. WHO Publications, Geneva, Switzerland, 1999.
- World Health Organization. Guidelines for Air Quality. WHO, Geneva, Switzerland, 2000.
- World Health Organisation: World Health Report, Geneva, WHO, 2001.
- World Health Organization. The World Health Report 2002: Reducing Risks, Promoting Healthy Life, Chapter 4, Quantifying Selected Major Risks to Health, No.8, Environmental Risks, WHO, Geneva, 2002.
- World Health Organization. World Health Organization Health Guidelines for Vegetation Fire Events. World Health Organization, Geneva, Switzerland, 1999. World Health Organisation. Indoor air pollution and health. Fact sheet N°292, 2005.
- World Health Organisation. *Fuels for life : Household Energy and Health*, Rehfues E, WHO Library Cataloguing-in-Publication Data, 2006.
- World Resources Institute, UNEP, UNDP, World Bank. 1998-99 *World Resources: a guide to the global environment*. Chapter in Report, Oxford University Press, Chapter 2, pages 65-67, 1998.

WRI World Resources Institute, UNEP, UNDP, World Bank. 1998-99 World Resources: a guide to the global environment. Oxford University Press, 1999.

Wu WZ, Wang JX, Zhao GF, You L. The emission soot of biomass fuels combustion as a source of endocrine disruptors. *J Environ Sci Health Part A Tox Hazard Subst Environ Eng*, 37, 579, 2002.

Xu X, Niu T, Christiani DC, Weiss ST, Chen C, Zhou Y, Fang Z, Jiang Z, Liang W, Zhang F. Occupational and environmental risk factors for asthma in rural communities in China. *Int J Occup Environ Health*, 2, 172-176, 1996.

Yu MC, Ho JH, Henderson BE, Armstrong RW. Epidemiology of nasopharyngeal carcinoma in Malaysia and Hong Kong. *National Cancer Institute Monograph* 69, 203-207, 1985.

Zhang J, Smith KR. Hydrocarbon emissions and health risks from cookstoves in developing countries. *Journal of Exposure Analysis and Environmental Epidemiology*, 6, 147-161, 1996.

Zodpey SP, Ughade SN. Exposure to cheaper cooking fuels and risk of age-related cataract in women. *Indian J Occup Environ Med*, 3, 159-161, 1999.