Imperial College London

National Heart and Lung Institute

Department of Respiratory Epidemiology,

Occupational Medicine and Public Health

Health Effects of Indoor Nitrogen Dioxide

by

Graziella Favarato

A thesis submitted for the degree of Doctor of Philosophy

May 2015

Declarations

I herewith certify that this dissertation is my original work and that all material included which is not my own work has been properly acknowledged.

Graziella Favarato

Abstract

Rationale: Exposure to indoor NO_2 has been implicated as a cause of respiratory symptoms suggestive of asthma but evidence has been inconsistent.

Objective: To review the existing evidence and examine the effects of indoor NO_2 in adult asthma-related symptoms using data from an adult multi-centre cohort (ECRHS) followed up for 20 years.

Methods: Studies on respiratory health and indoor NO_2 were systematically reviewed and meta-analyses performed. Cross-sectional analyses within a sub-set of ECRHS participants with indoor NO_2 measurements were conducted to assess the associations of asthma severity and wheeze prevalence with NO_2. A regression model was developed to predict indoor NO_2 for a larger ECRHS sample without indoor NO_2 measurements. GEE analyses were conducted to examine the long-term effects of gas cooking and modelled indoor NO_2 on wheeze and asthma score. To investigate the effect of gas-generating NO_2 peaks on asthma exacerbation a panel study was also piloted using a new-to-market portable NO_2 sensor.

Main results: The systematic review identified 50 studies, mainly in children. Results of meta-analyses suggested a significant association between 12-month period prevalence of wheeze and indoor NO_2. Within ECRHS prevalence of wheeze but not asthma severity was associated with measured indoor NO_2. Long-term associations of asthma-related symptoms with predicted indoor NO_2 exposure but not gas cooking were significant. Interpreting this is difficult as the latter analyses (gas cooking) included a larger number of centres and some heterogeneity across centres was observed in the analysis on asthma score. Gas appliances, outdoor NO_2, monthly temperature and country were the main predictors of indoor NO_2. Evaluation of the pilot study recommends better recruitment strategies and independent calibration of NO_2 sensor.

Conclusions: There is some evidence for a link between indoor NO_2 and asthma-related symptoms. Health risks may be small but are applied to a substantial proportion of the population.

.

Table of Contents

Index of Tables

Index of Figures

Acknowledgments

I would like to thank Prof Debbie Jarvis for main supervision of this thesis; John Gulliver for supervision of Chapter 5 on modelling and Roger Newson for statistical guidance; Andre Amaral for commenting on Chapter 2 and Chapter 3; Cosetta Minelli for commenting on Chapter 6; Maria Delgado Saborit for advice on Aeroqual; Ben Barratt for offering the use of ERC King's College facilities and providing access to LAQN data; Asthma UK, Imperial College, South East London Community Air Pollution group, NIHR-CRN for North West London, Gladstone GP practice and Monica Robb for helping with the recruitment of the pilot study; ESCAPE and ECRHS working groups for providing the data; the MRC-PHE Centre for the Environment and Health for funding this PhD; and finally, a particular thank to all women who participated in the study and those who were interested in participating but could not.

Acronyms and abbreviations

$\mu g/m^3$	Micrograms per cubic metre
adj	Adjusted
AER	Air Exchange Rate
ALSPAC	Avon Longitudinal Study of Parents and Children
BHR	Bronchial hyper-responsiveness
BR	Bronchial responsiveness
CH_2O	Formaldehyde
CI	Confidence Intervals
COPD	Chronic Obstructive Pulmonary Disease
CT	Chest Tightness
D+L	derSimonian and Laird – random effect in meta-analysis
ECRHS	European Community Respiratory Health Survey
eNO	Exhaled nitric oxide
ERG	Environmental Research Group, King's College London
ESCAPE	European Study of Cohorts for Air Pollution Effects
ETS	Environmental Tobacco Smoking
FeNO	Fractional Exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
GEE	Generalised Estimating Equation
GINA	Global Initiative for Asthma
GMR	Geometric Mean Ratio
GP	General Practice
GSE	Gas sensitive electrochemical
HDM	House Dust Mite
HEI	Health Effects Institute
het	Heterogeneity
HONO	Nitrous acid
I^2	Index of heterogeneity
ICS	Inhaled Corticosteroids
IgE	Immunoglobulin E
IPW	Inverse Probability Weighting
IQR	Inter-quartile range
IRAS	Integrated Research Application Systems
IRR	Incident Rate Ratio

ISAAC	International Study of Asthma and Allergies in Childhood
ISCO	International Standard Classification of Occupations
I-V	Inverse-Variance – fixed effect in meta-analysis
LRS	Lower Respiratory Symptoms
LRTI	Lower Respiratory Tract Infections
LUR	Land Use Regression
NCICAS	National Cooperative Inner City Asthma Study
NIHR-CRN	National Institute for Health Research Clinical Research Network
NO	Nitric oxide
NO_2	Nitrogen dioxide
NO_x	Nitrogen oxides
O_3	Ozone
OR	Odds ratio
P	P value
PD20	Provocative dose of bronchoconstrictor producing a 20% fall in FEV1
PEF	Peak Expiratory Flow
PEFR	Peak Expiratory Flow Rate
PFC	Perfluorocarbon
PIS	Participant Information Sheet
PM	Particulate Matter
PM_{10}	Particulate Matter with aerodynamic diameter ≤ 10 micrometres
$PM_{2.5}$	Particulate Matter with aerodynamic diameter ≤ 2.5 micrometres
ppb	Parts per billion
ppm	Parts per million
R^2	Coefficient of determination
RH	Relative Humidity
RMSE	Root-mean-square error
SES	Social-Economic Status
SO_2	Sulphur dioxide
SOB	Short of Breath
SPT	Skin Prick Test
TEA	triethanolamine
UFGH	Unflued gas heater
UFP	Ultra Fine Particles
VIF	Variance Inflation Factor
VOC	Volatile Organic Carbon
WHO	World Health Organisation

Conversion

Conversion factor used to convert NO_2 reported in ppb to NO_2 in $\mu g/m^3$ = 1.88

Statistical Conventions

In this thesis an association is defined as statistically significant when the P value is less than 0.05. When discussing the significance of an interaction a statistically significant interaction is when P value is less than 0 .10.

1. Introduction

Over the last decades multiple epidemiological studies have reported on the potential role of inhaled pollutants, such as gases and particles, on respiratory health. Many of these have focused on the role of outdoor air pollutants, although the role of a range of indoor pollutants has also been explored. On average, active adult urban populations in Europe spend 85–90% of their time indoors and indoor exposures may dominate total pollution exposure (Schweizer *et al.*, 2007). Infants, older people and those who suffer from chronic illness or disabilities may spend even more time indoors. With the drive towards improved energy efficiency within homes there is concern that associated decreases in home ventilation patterns may lead to increased exposure to domestic indoor pollutants.

One major source of indoor pollution is the burning of fossil fuels for cooking, heating and hot water. In many European countries natural or bottled gas is the fuel of choice. One of the major indoor pollutants derived from the use of unvented gas appliances is nitrogen dioxide. In this thesis I will examine the role of indoor nitrogen dioxide on respiratory health, particularly asthma and asthma related symptoms in adults in the following steps:

1. A systematic review of epidemiological studies that have reported the association of respiratory health with indoor nitrogen dioxide.

2. Assessment of the association of measured indoor nitrogen dioxide on adult asthma using information from a multi-centre European study conducted in 2002.

3. Development and piloting of a panel study to examine health effects of peak indoor nitrogen dioxide levels on respiratory health in women with asthma.

4. Development of a model that predicts indoor nitrogen dioxide using data from the aforementioned multi-centre European study.

5. Assessment of the relationship of respiratory symptoms in adults with exposure to gas appliances over a twenty-year period within the same cohort study.

1.1 Nitrogen dioxide

Nitrogen dioxide (NO_2) is produced very rapidly from oxidation of nitric oxide (NO), which is a direct product of combustion. In the presence of available oxidants, such as ozone (O_3), oxygen (O_2) or volatile organic compounds (VOCs) nitric oxide is converted into NO_2 as follows:

$$NO + O_3 \rightarrow NO_2 + O_2$$

$$HO_2 + NO \rightarrow NO_2 + OH$$

$$RO_2 \text{ (alkyl peroxide)} + NO \rightarrow RO + NO_2$$

Nitrogen dioxide is a strong oxidant and further reactions take place in presence of ultraviolet light to form ozone (O_3), nitric oxide (NO) and various free radicals. On surfaces such as suspended particles, soil, walls and within aqueous media NO_2 forms inorganic and organic species through multi-phase reactions. Some of these species such as nitrous acid (HONO) and nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are also thought to be associated with adverse health effects (Health Effects Institute, 2010).

Nitrogen dioxide is formed naturally in the combustion generated from wild fires, during electric storms and from soil emission resulting from fertilizers use (Sutton *et al.*, 2011). The main anthropogenic source of NO_2 is combustion of fossil fuel. In the urban environment NO_2 is mainly produced from the combustion of fossil fuel used for transport, power generation, heating and cooking. The main indoor sources of NO_2 are gas stoves and ovens and unvented gas heating appliances.

In UK indoor NO_2 concentrations in homes without gas stoves tend to range between 13μg/m^3 to 40μg/m^3 and in homes with gas stoves between 25μg/m^3 to 70μg/m^3 (Kotzias D et al., 2005). During an episode of gas cooking NO_2 quickly peak to concentrations as high as 1800μg/m^3 (Dennekamp *et al.*, 2001).

1.1.1 NO_2 and mechanism of action on asthma

As a free radical nitrogen dioxide has the potential to induce oxidative stress leading to cell injury and airway inflammation but the mechanisms by which NO_2 may induce respiratory symptoms typical of asthma are still not completely understood. Several mechanisms have been suggested (Gilliland *et al.*, 1999; Kelly, 2003):

1. Oxidative stress and antioxidant depletion.
2. Increased inflammation and airway hyper-responsiveness.
3. Structural changes in the airways leading to asthma.
4. Enhanced response to allergens.
5. Impacts on immunity.

Animals and *in vitro* models have shown that at high concentrations NO_2 produces eosinophilic inflammation, increases epithelial damage, decreases mucin expression and increases baseline smooth muscle tone (Gilmour *et al.*, 1996; Bayram *et al.*, 2001; Persinger *et al.*, 2002; Garn *et al.*, 2003; Ayyagari *et al.*, 2007). However, mathematical airway models of rats, dogs and humans have indicated that there is an interspecies variation in anatomy and respiratory patterns between rodents and humans (Tsujino *et al.*, 2005). In rodents it has been observed that prolonged exposure to NO_2 results in destruction of peripheral airways while studies on mucosal biopsy specimens have suggested that in humans inflammation after exposure to NO_2 is likely to occur in the smaller airways (Blomberg *et al.*, 1997)

Devalia (1994) observed that exposure of human bronchial epithelial cells *in vitro* to NO_2 (760µg/m^3) led to the release of pro-inflammatory cytokines. Antioxidant tissue depletion, such as rapid loss of ascorbic acid, uric acid, lipid peroxidation and depletion of alpha-tocopherol (vitamin E) after exposure to NO_2 has been observed in a large array of *in vitro* models (Halliwell *et al.*, 1992; Kelly and Tetley, 1997) but at extremely high concentrations typically between 2,000µg/m^3 and 20,000µg/m^3. These concentrations do not occur under normal conditions.

1.1.2 Evidence from controlled human exposure studies

Before 1976 there was a consensus that inhalation at concentrations below 2,820µg/m^3 over 2 hours caused little reduction in lung function in both non-asthmatic and asthmatic individuals (Shy *et al.*, 1978). Since then there have been reports of airways changes at concentrations lower than that but findings have been inconsistent and difficult to replicate.

Orehek et al (1976) observed an increase in bronchial-responsiveness in asthmatics at 188µg/m^3 while Koening et al (1987) exposed asthmatic adolescents at 226µg/m^3 and 338µg/m^3 for an hour with intermittent exercises observing no changes in lung function. Bylin et al (1985) exposed asthmatics at 910µg/m^3 for 20 minutes and found that bronchial reactivity increased significantly. Bauer et al (1986) noticed changes in forced expiratory flow rate after exercise in asthmatics at 560µg/m^3 over a 30 minute- period but could not reproduce results in another study.

More consistent evidence has been observed in asthmatics after being challenged with an allergen. Tunnicliffe (1994) observed a decline of FEV_1 in asthmatics when exposed to 760µg/m^3 for one hour followed by a challenge with House Dust Mite (HDM) allergen. Using a similar protocol Strand (1997) observed an increase in allergic response when asthmatics were exposed at the same concentration (760µg/m^3) for 30 minutes.

A meta-analysis (Folinsbee, 1992) of the evidence on acute effects of short-term controlled exposure to NO_2 concluded that increasing concentration of NO_2 was associated with an increase in airways hyper-responsiveness in asthmatics and this was made worse if participants exercised (i.e. increased their minute ventilation). This trend was observed at concentrations as low as 200µg/m^3 in asthmatics but in non-asthmatics was only seen at concentrations above 1,880µg/m^3. The meta-analysis was instrumental in lowering WHO 1-hour exposure guidelines for outdoor NO_2 from 400µg/m^3 to 200µg/m^3 (Graham *et al.*, 1997). Two more recent reviews and meta-analysis (Goodman *et al.*, 2009; Hesterberg *et al.*, 2009) have concluded that experimental data are still inconsistent failing to demonstrate a consistent association of short term exposure to NO_2 with adverse respiratory health at levels below 376µg/m^3.

It has been suggested (Samet and Utell, 1990) that some inconsistencies could be explained by differences in exposure protocols, study design and overreliance on mean group effects making studies difficult to compare. For example, the mode of NO_2 inhalation may vary; it can be carried out via a mouthpiece (i.e. oral exposure) or in a chamber (oral-nasal exposure). Volunteers may be asked to rest or exercise after or during exposure. Duration and frequency of exposure at similar total dose of NO_2 may vary. Statistics such as group mean could be unreliable because of the small study group size. Furthermore, most studies focus on tests of the upper airways but this may be relatively insensitive to any adverse effect as the major site of NO_2 injury is in the smaller airways (Chauhan, 1999).

Controlled human exposure studies are very useful in studying mild and transient responses to an exposure as they are conducted for short duration (typically 1-6 hours) and outcomes are measured shortly after exposure. However, they assess acute responses to one off exposure (often very high) whereas in real life people are exposed to repeated exposure (both low and high) that may lead to acute and chronic health effects.

1.1.3 Evidence from epidemiological studies

Until the 1970s the only reports of human exposure to NO_2 were confined to agricultural and industrial accidents at very high concentrations such as the silo filler's disease (Moskowitz *et al.*,

1964). Early observational studies on the health effects of NO_2 focused on ambient NO_2. One of the initial studies was conducted in Chattanooga (US) and found that ambient NO_2 was associated with respiratory symptoms in school children (Shy *et al.*, 1970). Since then a number of observational and ecological studies examining the health effects of outdoor NO_2 have been published. A recent authoritative report (Health Effects Institute, 2010) on traffic air pollution has concluded that evidence of adverse health effects associated with outdoor NO_2 is suggestive but not sufficient of a causal association. There are difficulties in separating the health effects of NO_2 from the various components present in traffic-related air pollution.

The first observational studies on indoor NO_2 were conducted in UK (Florey *et al.*, 1979) and in US (Keller *et al.*, 1979a; Keller *et al.*, 1979b). Both studies were part of larger studies, The Cleveland Study (Melia *et al.*, 1977) and The Columbus Study, Ohio (Comstock *et al.*, 1981) which examined the respiratory health effects associated with gas cooking. The Columbus Study found no evidence of health effects in those using gas compared to those using an electric stove. Contrarily, The Cleveland Study found that the prevalence of respiratory illness was higher in children from homes where gas was used for cooking than in homes where electricity was used. The authors hypothesised that any observed effect was probably due to NO_2 from the combustion of gas or kerosene (used for heating appliances). Indoor NO_2 levels were much higher in the UK homes than in the US homes. Findings from NO_2 monitoring campaigns in the UK study showed some evidence of an effect in girls and not in boys. In an associated commentary the authors stated: '*Health effects due to indoor nitrogen dioxide levels have become particularly controversial*' (Goldstein and Melia, 1981).

Following these early observations some large studies with more than 1,000 participants were conducted to examine the health effects of indoor NO_2 and gas appliances. A cross-sectional study (Brunekreef *et al.*, 1990; Dijkstra *et al.*, 1990) of over 1,000 Dutch children was conducted to assess the effect of combustion emissions from unvented geysers (a gas appliance for heating water common in Dutch homes until recently). No association of respiratory symptoms/ lung function with indoor NO_2 or unvented geysers was found. As part of the Six City Study, one of the largest multi-city studies on the health effects of ambient air pollution, more than 1,500 children living in US were followed up for a year (Neas *et al.*, 1991). This study was one of the few to control for indoor particles. A composite of several respiratory symptoms was found to be significantly associated with indoor NO_2 as well as having a gas stove. In contrast, a study in more than 1,000 infants living in Albuquerque (US) who were followed up for over a year with NO_2 repeatedly monitored in their bedrooms did not observe any association of duration and/or frequency of respiratory illness with indoor NO_2 or the presence of a gas stove (Samet *et al.*, 1993).

Further studies that examine the health effects of indoor NO_2 have been published since then (see Chapter 2). They are often conducted supplementary to studies on the health effect of gas cooking, the main indoor source of NO_2. Findings have been inconsistent. It has been suggested (Samet and Utell, 1990) that the heterogeneity in results could be explained by issues related to:

- Misclassification of exposure to NO_2; using a proxy to determine the exposure (e.g. use of gas for cooking), assuming that two-week monitored exposure is the same as long-term exposure or failing to take into account exposure to NO_2 peaks may lead to misclassification
- Lack of objective measures of outcome leading to bias when self-reporting symptoms
- Lack of prospective data
- Analysis not taking into account the confounding effect of other combustion by-products (e.g. particles) or other risk factors associated with the outcome
- A possible modifying effect of gender, atopic status, diet and genetic make-up.

The following sections will describe in more details how epidemiological studies measure NO_2 and why this can lead to misclassification, how asthma is assessed and issues related to its assessment and the various risk factors that may also be associated with having asthma.

1.1.4 Measuring NO_2 in epidemiological studies

Chemiluminescence analysers are the 'gold standard' for measuring NO_2 and are used to monitor outdoor NO_2 by environmental departments and local authorities. They provide continuous NO_2 concentration readings in real time but are bulky, expensive, and difficult to transport making them unsuitable for measuring NO_2 levels in people's homes in large scale epidemiological studies.

As an alternative, epidemiological studies measure indoor NO_2 using passive diffusion samplers. These samplers work by passively diffusing NO_2 onto an adsorbent - usually, solution of triethanolamine (TEA). Nitrogen dioxide reacts with a chemically-treated sorbent (extracted post-sampling) and the reaction derivatives are chemically analysed using spectrophotometry[1]. The samplers are placed directly in study participants' homes for one or two weeks providing average concentrations over the exposure period. Epidemiological studies then often use these measurements to assign exposure over a much longer period of time. Passive diffusion samplers

[1] **Spectrophotometry** is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.

do not work for short period of exposure because of the slow sampling rate, determined by the surface of the adsorption media. Several sampler designs have been developed to minimise this problem. While the traditional tube-shaped samplers need to be exposed for at least one week, the radial-shape samplers can be exposed for 72 hours (Yu *et al.*, 2008). Passive diffusion samplers are cheap and simple to use but do not provide adequate information on short-term peaks, which are produced while cooking with gas and may be important when examining health effects of NO_2 (Noy *et al.*, 1990; Franklin *et al.*, 2006). Recently, new devices such as short-term passive samplers and electrochemical gas sensors have appeared on the market. They allow monitoring of NO_2 over much shorter periods and can identify peaks of NO_2 exposure.

For large scale studies even the use of passive diffusion samplers to assess participants' exposure to indoor NO_2 can be expensive and time-consuming and the 'presence of a gas stove' or 'use of gas for cooking' have often been used as proxy indicators of indoor NO_2. The presence of a gas stove has been estimated to be comparable to approximately 30µg/m^3 of long-term exposure to indoor NO_2 (Hasselblad *et al.*, 1992) but in many reports it is not clear whether the term 'gas stove' includes a gas oven as well as a gas hob. Other factors such as the intensity of gas cooking, indoor ventilation and ambient level of NO_2 may also influence the level of indoor NO_2 produced by these appliances.

Epidemiological studies on air pollution from traffic have also traditionally used surrogate measures, such as distances from road, which tend to misclassify exposure because they are not directly estimated from monitoring data. In the last 15 years outdoor air pollution studies have increasingly used exposure models to identify small-area (often at residential level) variations in pollution. These methods use geographic information systems (GIS) to combine geographic information with measurement data from monitoring air pollution stations and/or *ad hoc* monitoring (Jerrett *et al.*, 2005). Several types of models have been developed but dispersion models and land use regression (LUR) are the most common. The ESCAPE[2] study has used land use regression (LUR) to model annual outdoor NO_2 and $PM_{2.5}$ exposures at residential levels in 20 European study areas (Beelen *et al.*, 2009) and is a well-known example of such methods.

To my knowledge so far no large scale epidemiological studies have developed a model to assess indoor NO_2 exposure at household level for assignment to participants in health studies.

[2] **ESCAPE** has provided outdoor NO_2 data for this thesis.

1.2 Asthma

Since it enters the body by inhalation NO_2 has long been suspected of being associated with common respiratory disorders including asthma and asthma related respiratory symptoms. Asthma is a chronic condition that has increased in the last 60 years and affects 300 million people worldwide (Vos *et al.*, 2013). It has been defined as '*the condition of subjects with widespread narrowing of the bronchial airways which changes its severity over short period of time either spontaneously or under treatment.'* (Fletcher *et al.*, 1959) but clinicians and epidemiologists have yet to agree a clear working definition that can be applied in clinical and epidemiological settings.

Asthma is typically associated with intermittent wheezing, shortness of breath (which may come on at rest or after vigorous exercise), chest tightness and cough (often nocturnal). Bronchoconstriction in response to exercise and inhalation of increasing doses (or concentrations) of agents such as histamine or methacholine is a well-recognised feature of this disease. Many people with asthma also develop abnormal immune responses to allergens and will have measureable serum specific Immunoglobulin E (IgE) to one or more aeroallergens, elevated total IgE and other symptoms of allergic disease such as eczema or rhinitis.

A diagnosis of asthma is a clinical one but there is no standardised definition of the type, severity or frequency of symptoms. Diagnosis usually takes into account the patient's clinical history of respiratory symptoms (more than one of wheeze, breathless, chest tightness, cough) and of variable airflow obstruction. More recent descriptions of asthma have also included airway inflammation and bronchial hyper-responsiveness (SIGN, 2014). The most common medications prescribed to people with asthma are inhaled beta-agonists for short term relief of acute symptoms and inhaled cortico-steroids (ICS) for longer term management and reduction of exacerbations.

1.2.1 Assessment of asthma in epidemiological studies

In the absence of a gold standard definition for asthma, interview based epidemiological surveys tend to collect information on the presence of relevant symptoms, treatments, diagnosis and health service utilisation for asthma. In the last 20 years two major international studies, the International Study of Asthma and Allergies in Childhood (ISAAC) (Asher *et al.*, 1995) and the European Community Respiratory Health Survey (ECRHS) (Burney *et al.*, 1996) have developed standardised instruments for the collection of symptom information from children and adults

respectively. As the disease has a variable course, with some people with asthma having prolonged periods when they experience few or no symptoms, most of the questions refer to the presence of symptoms in the previous 12 months.

Asking individuals whether they have asthma will depend on whether they have visited a doctor and been diagnosed with the disease – it is therefore dependent on diagnostic practice. In young adults there may be little variation in this – but in older adults, especially those who smoke, the symptoms of asthma may be labelled as chronic obstructive pulmonary disease. Studies report that there is under treatment of asthma in many countries, and a proportion of adults who report symptoms typical of the disease and who have been diagnosed with asthma, report they only infrequently take medication (Janson *et al.*, 2001).

Some epidemiological studies may include a physical assessment such as lung function tests (peak flow, forced expiratory manoeuvres), airway responsiveness and assessment of atopy (by skin prick tests, serological tests). These objective measures provide additional evidence of the presence of disease.

A person's maximum speed of expiration known as peak flow (PEF) has been measured for many years by many asthma studies to assess the presence and day to day variation of asthma. Peak flow meters are relatively small and cheap but tend to be imprecise. Measurement of daily peak flow is considered to be part of the management program for people with asthma.

The most frequent lung function measurements are Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC) and ratio between FEV_1 and FVC (FEV_1/FVC) arising from a forced expiratory manoeuvre. Participants are asked to take a full inspiration and forcibly exhale the air as hard and fast as they can until they cannot go on. Measurements are made using a spirometer. Although the test is relatively easy participants need to be instructed and may sometimes fail to provide a technically satisfactory manoeuvre. People with asthma may demonstrate evidence of airway obstruction (low FEV_1/FVC ratio) but due to the day to day variability of disease many people with asthma may have results within the normal range. Some studies incorporate a test of post-bronchodilator spirometry where measurements are made after inhalation of a standard dose of beta-agonist inhaler. Lung function measurements are relatively insensitive in detecting early response in small peripheral airway where the major site of NO_2 induced injury has been identified, the transitional zone which is the area between the terminal bronchioles and alveoli (Blomberg, 1997; Chauhan, 1999).

Airway hyper-responsiveness is assessed by asking participants to inhale increasing amounts of an agent (e.g. methacholine) that causes bronchoconstriction. If methacholine is used, a cumulative dose up to 1mg or 2mg is often given and responsiveness measured as rate of change of FEV_1 against the inhaled dose. A fall of 20% or more in FEV_1 after challenge is generally considered indicative of asthma. The challenge test can only be performed in a clinical setting and for this reason it is costly and time-consuming to incorporate into a large scale epidemiological study. People with severe asthma are often excluded from participating because their FEV_1 is already too low to undergo a challenge test.

1.2.2 Assessment of asthma severity

In epidemiological studies various approaches have been used to assess asthma severity, including a composite measure incorporating symptoms and medications as proposed by the Global Initiative for Asthma (GINA), dose-response to methacholine challenge and the amount of endogenous nitric oxide exhaled by an individual (FeNO).

The GINA score is a four-class severity score (intermittent, mild persistent, moderate persistent, severe persistent) based on combination of clinical severity and the daily asthma medication regimen the participant has taken over the last 12 months. Clinical severity is based on the frequency of daytime and night-time symptoms and FEV_1 predicted. Daily medication regimes are classified into four levels according to the daily dose of inhaled corticosteroids (none, low, medium, high dose) (Rabe *et al.*, 2004).

FeNO has increasingly being measured to ascertain the presence or degree of asthma in participants. Nitric oxide is a key signalling molecule involved in a large range of biological functions; in the lungs it is an important mediator of the eosinophilic inflammatory response thus, an elevated FeNO can be suggestive of asthma. However, in patients with non-eosinophilic asthma (up to 50% of total asthma cases) and in smokers exhaled nitric oxide tends to remain low (Douwes *et al.*, 2002).

1.2.3 Risk factors associated with asthma

Epidemiological studies that examine the effect of NO_2 on asthma-related symptoms need to take into account the presence of other risk factors that may also be associated with asthma. Individual characteristics such as gender, age and genotype, occupational exposure, socio-economic status, diet and environmental exposures (e.g. air pollutants, tobacco, airborne

allergens) have been proposed and identified as potential risk factors but the underlying cause or causes of the disease are still poorly understood.

Age is a main risk factor. Children are particularly vulnerable to the effects of air pollution as they breathe 50% more air per kilogram of body weight than adults (Bateson and Schwartz, 2007); 80% of alveoli are formed after birth and development continues through adolescence (Dietert *et al.*, 2000).

Asthma prevalence varies by gender. Boys tend to have more asthma than girls but women more than men. There has been debate on what this reflects: a lower threshold of reporting of symptoms in women; the effect of female sex hormones on asthma; different exposures; increased susceptibility to exposures to other risk factors in women (Leynaert *et al.*, 2012). This has been of particular relevance with regard to exposure to gas cooking (a proxy measure for indoor NO_2) as women have traditionally spent more time cooking than men.

A recent genome wide association study has identified genes associated with asthma in children. In adults the associations were less clear implying that adult asthma is more related to environmental factors than childhood asthma (Bouzigon *et al.*, 2008). With regards to NO_2 there has been a particular focus on genes that regulate anti-oxidant defences produced in the lungs to manage the increased burden of reactive oxygen species (derived from NO_2 inhalation). Variations of these genes, in particular *GSTM1* and *GSTP1* could increase susceptibility to exacerbation of existing asthma and development of new-onset asthma with exposure to NO_2 (Minelli *et al.*, 2011).

Between 9% and 15% of all cases of adult asthma are thought to be implicated with some occupational exposure, mainly to dust and fumes (Venables and Chan-Yeung, 1997). Work exposure to NO_2 is usually associated with people working in ice rinks (Brauer *et al.*, 1993) and agricultural silos. Inside a silo fermentation of forage can lead to extremely high concentrations of NO_2 (over 100,000µg/m^3) which can lead to 'silo-fillers disease' (Moskowitz, 1964).

Ecological studies show that as urbanisation increases prevalence of asthma-related symptoms increases too (Stewart *et al.*, 2001). In high income countries prevalence of asthma differs across different ethnic groups living in the same geographical areas. It has been suggested that such differences could be explained by genetic pre-disposition but this may reflect socio-economic differences. Higher prevalence of asthma has been observed in people of low-socio economic status, who may have restricted access to health service leading to poor control of disease (Rona, 2000) or be exposed to other risk factors such as higher air pollution levels (Fecht *et al.*,

2015), poor housing conditions (e.g. mouldy homes), a diet low in anti-oxidants (Allen *et al.*, 2009) or obesity (Beuther *et al.*, 2006). A recent conference paper has reported that overweight/obese children with asthma living in inner-city communities were more likely to have symptoms when exposed to indoor NO_2 that those who were not overweight/obese (Breysse *et al.*, 2012)

Since asthma is a disease of the respiratory airways, inhalation of harmful substances other than NO_2 has been implicated with respiratory symptoms. Exposure to second-hand tobacco smoke has long been recognised to be associated with respiratory symptoms. Children of parents who smoke are likely to have more respiratory symptoms associated with asthma than children whose parents do not smoke (Cook and Strachan, 1997). In adults evidence of an association of asthma with active smoking is less consistent (Accordini *et al.*, 2012). Tobacco smoking generates some NO_2 but the amount can be negligible and dependent on the intensity of smoking (Leaderer *et al.*, 1986).

Exposure to airborne allergens (i.e. mould spores, pollens, house dust mites, pet allergens) has long been identified as potential risk factors for the development and exacerbation of asthma (Platts-Mills, 1992) and findings from clinical studies have suggested that exposure to an allergen may enhance the effect of exposure to NO_2 in atopic people (Folinsbee, 1992).

Finally, along with NO_2 other air pollutants have been associated with the disease. Particles generally labelled under the term 'PM' (particulate matter) are generated at the same time as NO_2 during combustion of fossil fuel. Similarly to NO_2, they have oxidant properties which can cause oxidative stress in the airways leading to lung injury and inflammation (Kelly, 2003). Studies have reported that during episodes of a high level of outdoor air pollution the number of emergency hospital visits related to asthma morbidity and other respiratory diseases increases (Atkinson *et al.*, 2001; Lin *et al.*, 2003). Whether the effect is due to particles or NO_2 (or perhaps other components in the air pollution mixture) is not so clear and disentangling the possible adverse health effects of the two remains difficult (Katsouyanni *et al.*, 2001; Sarnat *et al.*, 2001).

1.3 Air quality guidelines for NO_2

On the basis of existing epidemiological and clinical evidence WHO annual guidelines have set the limit to indoor and outdoor NO_2 at the value of 40μg/m^3 for annual average exposure and at

value of 200µg/m^3 for 1-hour average daily maximum concentration (Graham, 1997). These values have also been adopted by the EU as a legally binding standard (limit value) (Directive 2008/50/EC). Indoor guidelines have been re-confirmed by a most recent WHO review of indoor air quality guidelines (World Health Organization, 2010).

The current guidelines for annual average exposure were originally based (Graham, 1997) on a meta-analysis published in 1992 (Hasselblad, 1992). The meta-analysis summarised the findings of 11 studies that assessed the respiratory health effects associated with measured indoor NO_2 (4 studies) or the use of gas stove as a proxy measure of indoor NO_2 exposure (7 studies). It concluded that a 30µg/m^3 increase (comparable to the increase resulting from exposure to a gas stove) in indoor NO_2 exposure was associated with a 20% increase in respiratory symptoms in children. On the basis of this conclusion and assuming a background level of 15µg/m^3 (to avoid the most severe exposure) WHO annual guidelines were proposed.

Since the publication of the meta-analysis (1992) several more studies have been published and more systematic reviews have been conducted but there has been no concerted effort to conduct a formal meta-analysis of the epidemiological studies (Basu, 1999; Nitschke, 1999; Brauer, 2002; Fuentes-Leonarte *et al.*, 2009; World Health Organization, 2010)

The meta-analysis conducted by Hasselblad will be discussed in more details in the following chapter (Chapter 2) and an updated meta-analysis that includes most recent findings of studies that measured indoor NO_2 (rather than gas proxy studies) will be presented.

1.4 Summary

Exposure to NO_2 has been implicated as a cause of respiratory symptoms suggestive of asthma but evidence has been inconsistent. Indoors the main source of NO_2 is gas cooking and epidemiological studies have often used the presence of a gas stove as a proxy indicator for indoor NO_2. This can be imprecise and may lead to exposure misclassification error. Improving exposure assessment may help to solve some of these inconsistencies.

Clinical studies suggest that asthmatics could be at higher risk at short-term high exposure to NO_2. As short-term high concentrations of NO_2 are emitted during gas combustion asthmatics

could be at higher risk when cooking with gas but epidemiological evidence of short-term effects of indoor NO_2 in asthmatic adults is scarce.

There is no gold standard definition for asthma and asthma severity; physical assessment of study participants can be costly and is recommended to be used in addition to standardised questionnaires that have been developed for the collection of symptoms. As the disease has a variable course, with some people with asthma having prolonged periods when they experience few or no symptoms most of the questions refer to the presence of symptoms in the previous year but the prospective collection of symptoms repeatedly over the year may be of help in understanding the effects of long-term exposure to indoor NO_2 on asthma-related symptoms. Some risk factors associated with asthma may modify the effect of indoor NO_2 on asthma, particularly in the long-term

WHO recommends that annual average indoor and outdoor exposure to NO_2 should not exceed the annual average level of 40µg/m^3. Nitrogen dioxide is present in indoor air with some evidence that levels exceed these guidelines in homes, particularly those that have unvented gas appliances and poor ventilation. The guidelines were based on the conclusion of a meta-analysis published in 1992 and since then an array of studies have been published but the meta-analysis has not been updated yet.

1.5 Hypothesis

Indoor NO_2 exerts an effect on human respiratory health at indoor levels associated with gas cooking.

1.5.1 Objectives

1. To review the evidence of published literature on the association of respiratory health and long-term exposure to indoor NO_2 - **Chapter 2**

2. To examine whether exposure to indoor NO_2 affects the severity of asthma in a susceptible group, i.e. adults (who generally cook and hence are more exposed to indoor NO_2) with asthma - **Chapter 3**

3. To develop a study that can examine the health effects of indoor NO_2 peaks generated from gas cooking in people with asthma - **Chapter 4**

4. To model exposure to indoor NO_2 by qualifying the sources and factors which determine the level of indoor NO_2 - **Chapter 5**

5. To assess the health effect of long term-exposure to gas cooking and (modelled) indoor NO_2 levels in adults and determine whether observed effects are modified by sex, smoking, atopy and asthmatic status - **Chapter 6** .

2. Systematic review of epidemiological studies on the association of respiratory health and indoor NO_2

2.1 Introduction

2.1.1 Background

It is not generally known that the current WHO outdoor air quality guidelines for annual average exposure to NO_2 are based on a meta-analysis (Hasselblad, 1992) of **indoor** studies that was conducted over 30 years ago. The meta-analysis summarised the evidence of an association of respiratory illness in children and indoor NO_2 in studies published up to 1992. It combined 4 studies that measured indoor NO_2 with 7 studies that assessed the use of a gas stove as a proxy measure of NO_2 exposure. It was assumed that homes with a gas stove had an additional 30µg/m^3 of average NO_2 compared to homes without a gas stove. Respiratory illness included any respiratory symptoms, from 'non-specific' to a composite measure of symptoms of wheeze, colds, coughs going to the chest, shortness of breath and bronchitis. The meta-analysis concluded that a 30µg/m^3 increase in NO_2 exposure was associated with a 20% increase in respiratory symptoms in children (OR 1.19, 95% CI 1.09, 1.30 from a random effect estimate based on DerSimonian and Laird method (DerSimonian and Laird, 1986). The risk of wheeze slightly increased (OR 1.27, 95%CI 1.02 to 1.58) when studies in which gas stove was used as a proxy for average NO_2 exposure were removed from the analysis.

The Hasselblad meta-analysis is quite remarkable as it is one of the first examples of using meta-analysis to synthesise evidence from different studies. Its findings were fundamental to help draw the WHO recommendations for annual outdoor NO_2 in 1997 (Graham, 1997). The guidelines were proposed on the basis of an NO_2 background level of 15µg/m^3 and the fact that significant adverse health effects occur with an additional level of 28µg/m^3 or more. The value of 40µg/m^3 was recommended to avoid the most severe exposures.

Although there have been attempts to incorporate evidence from more recent studies into the setting of this guideline no further attempts at meta-analysis had been conducted by 2011. Updating of the meta-analyses could strengthen the evidence base upon which both indoor and outdoor guideline values are set, particularly as more studies have been conducted and changes

in home ventilation and perhaps gas sources (e.g. from coal gas to natural gas) may have led to differences in health effects.

Synthesis of available evidence should provide further scientific support to the NO_2 air quality guidelines, identifying dose-response relationships and identifying sub-populations and life stages that may be at greater risk of experiencing effects from pollutant exposure. In light of an increasing literature on the health effects of measured indoor levels the original meta-analysis published in 1992 should be updated, gaps in the current epidemiological work identified and further appropriate research identified.

2.1.2 Objectives

1. To review and replicate the original meta-analyses that identified associations of respiratory illness, including asthma with NO_2
2. To conduct a systematic review of epidemiological studies which have assessed the association of respiratory symptoms with directly measured indoor NO_2 levels
3. To carry out meta-analyses of the effect estimates reported by the studies identified from the systematic review where appropriate
4. To identify current gaps in epidemiological studies examining health effects of indoor NO_2.

2.2 Method

2.2.1 Replication of Hasselbald meta-analysis

The meta-analysis carried out by Hasselblad and colleagues (Hasselblad, 1992) was replicated to understand how the combined effect of indoor NO_2 on respiratory illness was estimated and possibly, to replicate the same method using more recent publications. Only those publications included in the meta-analysis and measured indoor NO_2 were considered (Melia, 1980; Melia *et al.*, 1982; Dijkstra, 1990; Neas, 1990).

Three of these papers reported symptoms prevalence in grouped categories of NO_2 exposure but did not report a dose response relationship (Melia, 1980; Melia, 1982; Dijkstra, 1990). A dose-response estimate was calculated using the same method as first described by Hasselblad (1980): '*Since most studies of gas stove exposure ..... show an approximate increase of 30µg/m*^{3}

in NO_2 levels, the slope was multiplied by 30 to get the increase due to gas stove exposure, and then converted to an odds ratio by exponentiation. All of this assumed that the logarithm of the odds ratio was linear in NO_2 exposure (pg 665)'. Grouped NO_2 levels were fitted to a log-normal distribution, the expected mean values for each interval taken and a grouped logistic regression was run to calculate an effect estimate. The meta-analysis was carried out using current standard statistical techniques for meta-analysis (DerSimonian and Laird, 1986).

As in Hasselblad, the combined effect of the meta-analysis was presented per 30µg/m^3 of indoor NO_2, i.e. the assumed long-term indoor NO_2 average concentration associated with a gas stove and separate intercepts for girls and boys were computed using Melia's data (Melia *et al*, 1980, Melia *et al*, 1982).

2.2.2 Literature search

Epidemiological studies reporting an association between measured indoor NO_2 exposure and respiratory health were identified by running a search string in three main bibliographic databases: Medline, Embase and ISI Web of Science.

The search string used Boolean logic 'AND' to link the key words for exposure (NO_2 and NO_2 related species), the outcome (respiratory health) and location (indoor environment). Table 2.1 lists the key words used to identify the studies. Key terms in the same category were linked with the Boolean logic 'OR'. The search string was originated by identifying the key words of some of the most cited papers on indoor NO_2 and respiratory health. As the search aimed to be as inclusive as possible a variety of key terms with similar meaning were used.

Table 2.1 List of key words of the search string used to identify studies on respiratory health and indoor nitrogen dioxide

	Key words
NO_2	NO_2 **OR** HONO **OR** Nitrogen dioxide **OR** Nitrogen oxides **OR** Nitrous acid **OR** NOx
	AND
Respiratory health	Airway **OR** Allerg* **OR** Asthma* **OR** Breath* **OR** Atop* **OR** Breath* **OR** bronch* **OR** COPD OR Cough **OR** FEV1 **OR** FVC **OR** IgE **OR** Infection **OR** Lung function **OR** Peak flow **OR** PEF **OR** Phlegm **OR** Pulmonary **OR** Reactivity OR Respiratory **OR** Responsiveness **OR** Rhinitis **OR** Shortness of breath**OR** SOB **OR** Wheez*
	AND
Indoors	Bedroom **OR** Classroom **OR** Domestic **OR** Home **OR** Indoor **OR** Kitchen **OR** Personal **OR** Room

* by adding the asterisk at the end of word the search will include any word starting with the letters that precede the asterisk

The literature search was run on 17/10/2011 and repeated on 11/12/2013. No publication year or language limits were included in the search. Results from the searches were saved and merged in the reference management database EndNote X5. Duplicates were removed using EndNote facilities ('Find duplicates').

A study was identified as relevant if:

- It reported an effect estimate of the association between any respiratory health parameter (symptoms, diagnosis lung function, airway inflammation or atopy) and measured indoor NO_2 (either domestic and/or classroom-based in children)
- It reported original results (reviews or studies reporting results published elsewhere were excluded)
- It was peer-reviewed
- Analyses were adjusted for at least sex or age.

Studies on biomass fuel combustion, occupational exposure and high indoor exposures in an ice-rink (there are isolated reports of high levels associated with use of de-icing equipment) were excluded.

Relevant studies were initially sifted by title and abstract relevance and then by full text. The bibliographies of existing reviews and relevant studies were checked for studies that had not been identified by the search.

At each step the PRISMA guidelines were followed (Moher *et al.*, 2009) – and when appropriate deviations from the guidelines noted.

2.2.3 Extraction of data from relevant studies

The full text of each publication identified as relevant was read and data were extracted and recorded into two extraction forms:

- Extraction form Level 1, which included details of the study and its characteristics (e.g. location, study population, exposure assessment);
- Extraction form Level 2, which included details of the health effect estimates (e.g. coefficient size and precision, outcome, statistical method) (see Appendix of Chapter 2 for details of the Extraction forms).

An Access database (Microsoft ACCESS version 2002, Microsoft Corporation, Redmond, WA, USA) was created for the purpose to store the extracted information. Details of the effect estimates were then transferred to Stata. When units were reported in ppb they were converted into $\mu g/m^3$ using the conversion factor 1.88. Whenever possible estimates were recorded as change per $10\mu g/m^3$ of indoor NO_2.

2.2.4 Meta-analysis

Effect estimates were combined using the fixed effect and random effect meta-analysis models (DerSimonian and Laird, 1986). The assumption of a fixed-effect model is that the studies effects share the same underlying average effect while the assumption of a random-effect model is the effects are coming from a common underlying distribution of effects.

A meta-analysis was carried out if:

- Effect estimates shared the same outcome and epidemiological measure (e.g. prevalence)
- Effect estimates could be standardised to a change in health effect for a $10\mu g/m^3$ increase in NO_2
- At least four effect estimates in study populations which were independent to each other could be included.

Only one estimate per study population was included. If monitoring was carried out in more than one room (e.g. kitchen, bedroom living room) and effect estimates were reported for each room only one estimate was selected; in this case, the estimate associated with measurement in the living room was chosen (this was a pragmatic decision as this was the room where most studies

conducted NO_2 monitoring). If a publication presented only stratified analysis by a particular factor then the estimate of each stratum was selected. For example, if the stratification was by gender, then both estimates for males and females were selected. If results were stratified by season because repeated measurements were taken through the year, a combined estimate was computed by taking the weighted average of the log of the odds ratio and then exponentiating.

Results of the meta-analyses were presented using forest plots. Each line of the forest plot represents an effect estimate and its 95% confidence intervals. The effect estimates were standardised (per 10μg/m^3) to describe the increase in risk associated with a 10μg/m^3 of increase indoor NO_2. Results were presented in subgroups determined by the population under study:

- Infants from the general population
- Children from the general population
- Adults from the general population
- Schools (children attended same schools and indoor NO_2 measurements were taken inside the schools)
- High-risk groups (i.e. having a family member with asthma)
- Asthmatics.

The summary effect (I-V Overall and D+L Overall) was reported for each sub-group. 'I-V Overall' stands for Inverse-Variance and is often named as the 'fixed effect'; 'D+L Overall' stands for DerSimonian and Laird, who first introduced the standard techniques for meta-analysis, and is commonly named as the 'random effect'. Heterogeneity between studies was assessed by calculating the I^2 of Higgins and Thompson (Higgins *et al.*, 2003), which describes the percentage of total variation across studies that is due to heterogeneity rather than chance. A value of 0 indicates no observed heterogeneity; the larger the I^2 the greater the heterogeneity. Since in absence of heterogeneity the random effect does not differ from the fixed effect, I will focus on the random effect results.

Studies included in the meta-analysis were assessed for publication bias through the visual inspection of funnel plots and formal statistical tests (Begg and Mazumdar, 1994; Egger *et al.*, 1997). The Begg's test is an adjusted rank correlation test based on Kendall's tau for the association between the effect estimate and their variance. The Egger's test plots the regression line between precision of the studies (independent variable) and the standardised effect (dependent variable). It assumes that when there is no publication bias the intercept (the bias coefficient) is equal to zero; as bias increases the bias coefficient is expected to increase.

Egger's test has more statistical power than Begg's test but the power of both tests is limited particularly for moderate amounts of bias or when meta-analysis are based on a small number of small studies (n<10). In the absence of linear trend between precision and standardised effect Begg's test will perform better than Egger's test as the former assesses bias by examining the correlations by rank (Sterne *et al.*, 2000).

Sensitivity analyses were carried out to investigate the influence of study characteristics on the meta-analysis results. A quality score weighting was initially considered and eventually discarded as inappropriate for the scope of this meta-analysis

Statistical analyses were carried out in Stata 12.1 (release 12.1; StataCorp. College Station, TX, USA). The *meta* commands were used to run the meta-analysis, forest plots and funnel plots.

2.2.5 Summary of studies not included in the meta-analysis

Since the meta-analyses did not include all studies identified by the systematic review, the final part of the Results section summarises the significant findings reported by all studies and focuses on reviewing those studies and those topics that it was felt were not adequately covered by the meta-analyses, i.e. studies in adults, studies in people with asthma, studies in people with COPD, studies on lung function, studies on FeNO, studies that adjusted for other air pollutants, studies that reported stratified analysis by effect modifier.

2.3 Results

2.3.1 Replication of Hasselblad meta-analysis

Among the 11 studies included in the Hasselblad meta-analysis four studies incorporated measured indoor NO_2 (Melia, 1980; Melia, 1982; Dijkstra, 1990; Neas, 1990). All four studies were in children aged between 5 and 11.

Two studies were UK based (Melia 1980; Melia 1982). They were carried out in different populations two years a part living in the same area (Middlesbrough) and included between 100 and 200 children. Indoor NO_2 was measured with Palmes diffusion tubes for one week in the children's bedroom and living room and the prevalence of any 'respiratory condition', a composite

of one or more respiratory conditions (i.e. usually cough in morning, day or night, colds going to the chest, ever wheezing or whistling attack of asthma or bronchitis in the last 12 months) was assessed with the use of questionnaire to be filled in by the child's parents.

One study (Dijkstra, 1990) was conducted in The Netherlands. It was a large prospective study of 775 children, whose symptoms were assessed twice over a 2-years period and weekly average NO_2 measured in children's kitchen, living room and bedroom. The outcome considered in the Hasselblad meta-analysis was a composite of any wheeze (i.e. wheezy or whistling sounds in the chest in the last year), cough (i.e. cough on most day, for at least 3 months consecutively, in the autumn-winter season) or asthma (i.e. attacks of shortness of breath with wheezing in the last year).

One study was conducted in the US (Neas, 1990). It examined 1286 children, who were part of a larger cohort (Six US Cities Study). Indoor NO_2 was measured in each child's home for two weeks during the cold season and repeated in the warm season. Parents completed a questionnaire that reported symptoms during the previous year. Effect estimates for several symptoms were reported and none reached statistical significance. The estimate for lower respiratory symptoms, a composite of the occurrence during the prior year of one of five symptoms (shortness of breath with wheezing, persistent wheeze, chronic cough, chronic phlegm and bronchitis) was selected as in Hasselblad.

Neas (1990) study is the only one to report an effect estimate on a continuous NO_2 scale; the estimate was also adjusted for sex, age, parental history of respiratory conditions, socio-economic status and indoor particles. Dijkstra *et al* (1990) and Melia *et al* (1980) presented estimates on a categorical scale and as in Hasselblad, they were combined together to estimate an effect per 30μg/m^3 indoor NO_2 exposure by fitting a lognormal distribution to the exposure categorical data.

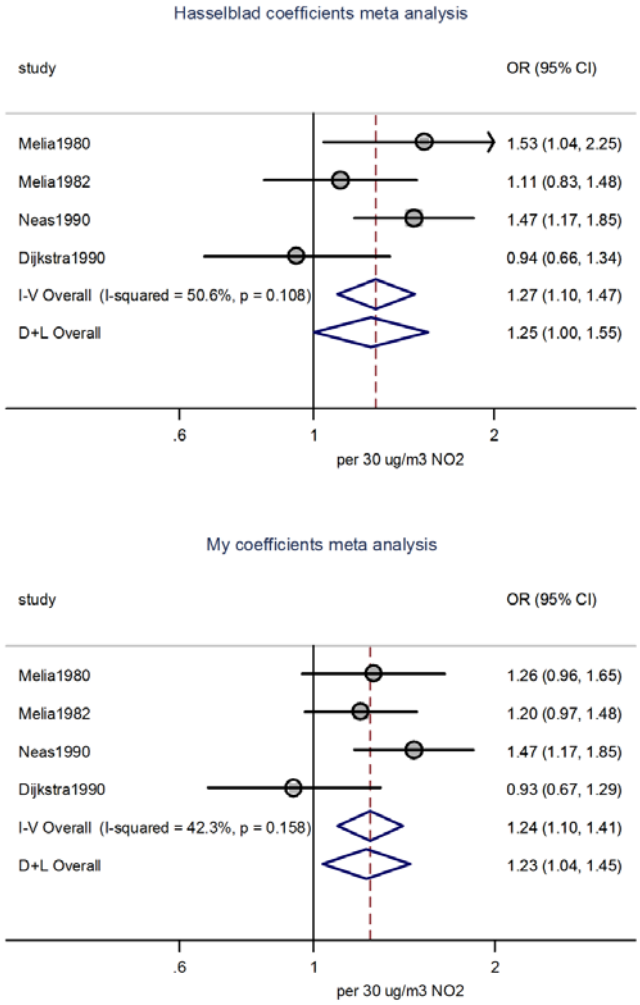


Figure 2.1 Meta-analysis of studies included in Hasselblad (1992) reporting an association between respiratory symptoms and measured indoor NO_2 – published results (top) and replication of analysis (bottom)

Figure 2.1 shows the results of the replication of the original analysis and those from the Hasselblad analysis. The replicated coefficient beta (β=0.077) of Melia study (1980) was different from the coefficient estimated by Hasselblad (β=0.015). I contacted the author for clarification but he did not have any of the old documentation that could help me (personal communication 24/01/2012). The fixed effect (OR 1.24; 95% CI 1.10, 1.41 per 30μg/m^3 NO_2) and the random effect (OR 1.23; 95% CI 1.04, 1.45 per 30μg/m^3 NO_2) of the replicated meta-analysis were very close to the effects estimated by Hasselblad.

Please, note that the common metric currently reported in the literature of epidemiological studies on outdoor and indoor NO_2 is 10µg/m^3 increase (rather than 30µg/m^3) of NO_2. This is the risk metric that I will use in this thesis. For future reference, the combined random effect reported by Hasselblad is OR 1.08 (95%CI 1.01, 1.16) per 10µg/m^3 increase of indoor NO_2.

2.3.2 Literature search

The search in the bibliographic databases yielded 1624 results of which 596 were duplicates and removed. After assessing the abstract for relevance further 963 studies were removed. The inspection of all the reviews and the bibliographies of the 48 relevant studies identified two additional studies. The full text of the remaining 67 studies was read and 17 irrelevant studies were excluded for the following reasons:

- Not original studies (Melia *et al.*, 1985; Gent *et al.*, 2012; Belanger *et al.*, 2013)
- Not adjusted (Erdei *et al.*, 2003)
- Examined risk factors for personal exposure only (Ponsonby *et al.*, 2001)
- On urinary nitrate as a biomarker of exposure to nitrogen dioxide and its association with asthma prevalence (Ciuk *et al.*, 2001)
- No effect estimate reported because NO_2 levels were too low (Martins *et al.*, 2012), or effect estimate not significant:(Smedje *et al.*, 1997; Diette *et al.*, 2007)
- No effect estimate reported and/or could not be interpolated from graphs and data provided (Ng *et al.*, 2001; Pilotto *et al.*, 2004; Howden-Chapman *et al.*, 2008; Marks *et al.*, 2010; Gul *et al.*, 2011; Hulin *et al.*, 2011; Yeatts *et al.*, 2012).

The Hasselblad's meta-analysis included two studies which were not retrieved by the search: Melia (1980) and Neas (1990). Melia (1980) was published in *Clinical Respiratory Physiology* (the precursor of *European Respiratory Journal*), a journal not indexed by the three bibliographic databases. Results of the same study were previously published in another article (Florey, 1979), which was retrieved by the literature search. Neas (1990) is a conference proceeding, which was not included the systematic review because of not being not peer reviewed. Results of this conference proceeding were published the following year in a peer-reviewed journal (Neas, 1991) and the article was identified by the literature search.

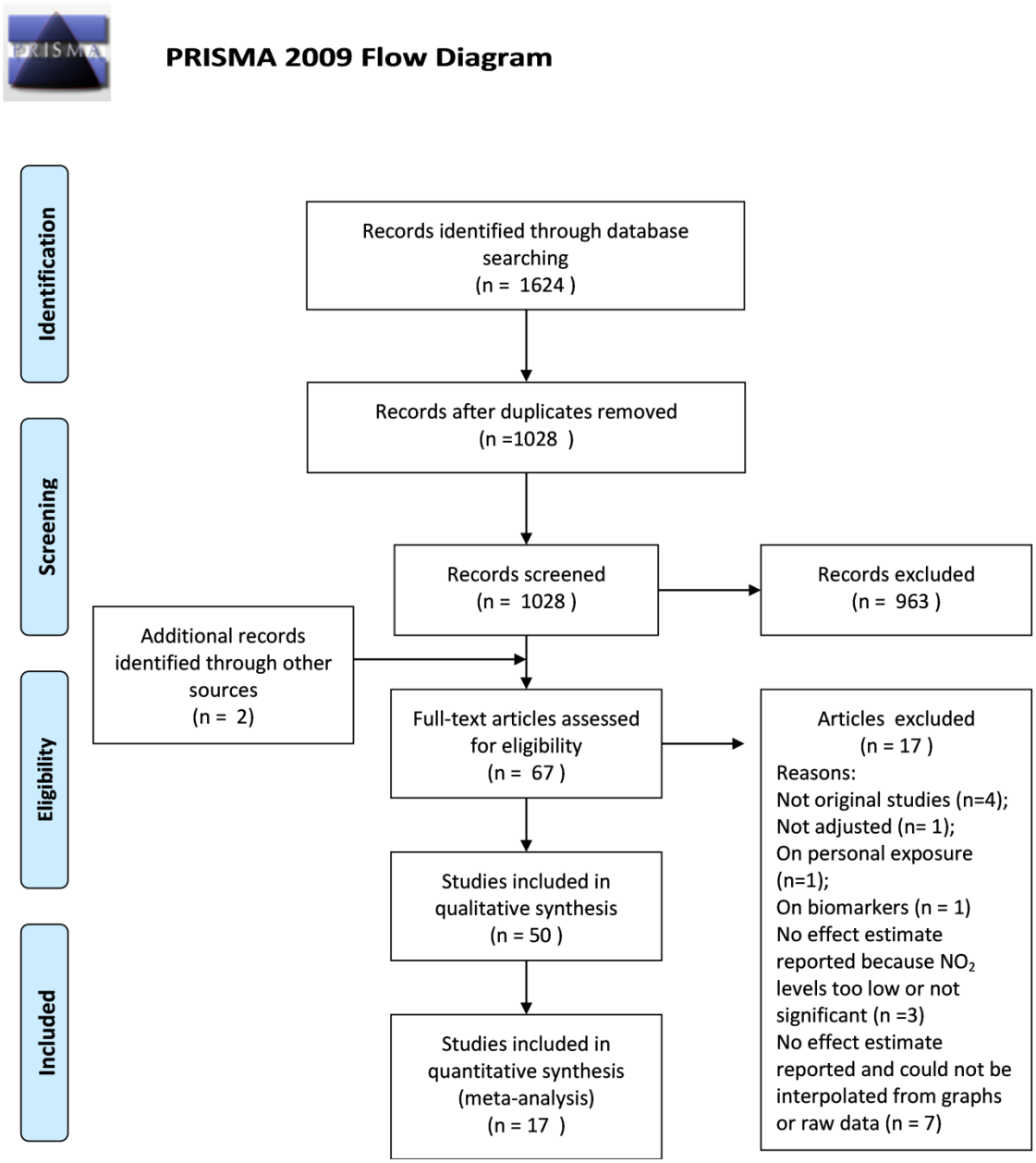


Figure 2.2 PRISMA flow diagram of literature search up to 11/12/2013 for studies on the association of respiratory health and indoor NO_2

2.3.3 Summary of the reviewed studies

The literature search identified 50 studies reporting an association between measured indoor NO_2 and respiratory health. A brief summary of each study is given in Table 2.2. Studies have

been listed in alphabetical order by first author in order to facilitate browsing the table. Each study has been summarised by the following characteristics:

- Design and Participants - it includes details of study design, population and location where it was carried out
- Exposure assessment - it includes details of NO_2 monitoring (e.g. type of monitor, frequency and duration of monitoring, room where monitoring took place) and reported indoor NO_2 levels
- Outcome assessment - it includes details of the health outcomes and the epidemiological measure (e.g. prevalence, incidence)
- Statistical analysis - it includes details of statistical analysis, the type of effect estimate (e.g. odds ratio, hazard ratio) and details of any stratified analysis
- Adjusted for - it lists the confounders the study adjusted the analyses for
- Significant associations - it lists the outcomes whose association with indoor NO_2 was significant ($P<0.05$).

.

Table 2.2 Summary of studies' characteristics

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Annesi-Maesano 2012	cross-sectional study (French Six Cities Study) 6590 children from 108 primary schools (401 randomly chosen classrooms) age: 9-10 years -Bordeaux, Clermont-Ferrand, Cre´teil, Marseille, Strasbourg, Reims (France)	Radiello passive diffusion samplers placed in classrooms for 5 days; range (33th-66th percentiles) 23.7 µg/m^3 - 31.6 µg/m^3 no gas sources mentioned -also indoor $PM_{2.5}$, indoor CH_2O	Asthma prevalence in the last year (defined using ISAAC questionnaire) rhinoconjunctivitis prevalence skin prick testing (SPT) for 10 common allergens exercise-induced asthma (EIA) prevalence	Marginal model using GEE approach with independent working correlation structure using the city as stratum stratified by atopic status OR reported by tertiles of NO_2 exposure on graphs	age, sex, passive smoking, paternal or maternal history of asthma, allergic diseases, dampness, gas appliance, ethnicity, SES	Past year asthma (stronger for allergic asthma)
Belanger 2003	-prospective (1 year) high-risk birth cohort , Yale Childhood Asthma Study (YCAS) 849 new-borns with an asthmatic sibling (see Belanger 2006 for siblings' analysis) recruited between 1996-98 Connecticut and Massachusetts, US *-same cohort as in van Strien 2004*	Palmes tube placed in living area for 10-14 days. 459 homes with $NO_2 \leq$ 10ppb (=19 µg/m^3); 390 homes with NO_2 >10ppb 34 % homes with gas stove	wheeze, frequency of persistent cough reported monthly for one year and analysed as none, <30days, ≥30days over a year. symptoms recorded by parent in a daily diary	Ordered logistic regression Stratified analysis by mother's asthmatic status (yes/no) OR on a continuous scale (18.8 µg/m^3);	maternal education, mould, sex, dust mites, cockroaches, cats, dogs, smoking at home	Persistent cough in children whose mother did not have asthma

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Belanger 2006	retrospective (1 year) study (YCAS study) 728 children with active asthma (siblings of YCAS cohort, see Belanger 2003); age: 66.5% younger than 6 years, 33.5% between 6 and 12 years Connecticut and Massachusetts, US	Palmes tubes placed in main living area for 10 to 14 days single-family homes: *median?* 14.3 (IQR 14.3) µg/m^3 ; 23.5% of gas stoves multi-family housing *median* 35.5 (IQR 36.8) µg/m^3 ; 23.5% of gas stoves	wheeze, persistent cough, SOB, chest tightness assessed for the month before sampling NO_2 number of days with respiratory symptoms in the year previous to NO_2	-logistic regression and Poisson regression (for days of symptoms); Stratified analysis by multi-family and single-family housing OR on a continuous scale (37.6 ug/m3)	age, season of sampling, ethnicity, mould/mildew, water leaks, maintenance medication use -smoking not included because of being a source of NO_2	Wheeze, SOB, and chest tightness in multi-family housing
Belanger 2013	Prospective study (1 year) (Study of Traffic, Air quality and Respiratory Health) 1342 children with active asthma; age:5-10; Connecticut and Massachusetts, US, period: 2006-2009	Palmes tubes placed in dayroom and bedroom exposed for one month and repeated every 3 months throughout the year (4 in total) overall mean 19.6 µg/m^3 , IQR (8.5-23.5 µg/m^3) no details of gas usage	asthma severity score according to GINA (0= no symptoms, 1=mild transient, 2=mild persistent, 3=moderate persistent, 4=severe persistent), frequency of wheeze, frequency of night symptoms, frequency of rescue medication use	Hierarchical ordered logistic regression using a Bayesian approach OR reported by quartile and as continuous using a threshold model in log; increment is reported by 5-fold increase frequency of symptoms reported as zero days, 1-3 days, 4-19 and more than 19 days per month	-SES, age, sex, general atopy, season, indoor allergens, ethnicity, mother's education, smoking in the home (for asthma severity score analysis) mother's education, smoking, ethnicity were replaced with 'maintenance medication use' in the analysis of symptoms because of collinearity with the 'use of rescue medication'	Asthma severity, wheeze, night symptoms and rescue medication use

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Berwick 1989	- Panel study (Yale Health and Heating Study) lasting 12 weeks during which participants' parents were phone-interviewed 6 times - 113 children Age: <13 yrs -New Haven, Connecticut, US	- Palmes tubes placed in kitchen, living room and bedroom for 2 weeks - in the kitchen: (kerosene heater + gas stove 168.4 $\mu g/m^3$), gas stove (76.9 $\mu g/m^3$); in the bedroom: (kerosene heater + gas stove 196.9 $\mu g/m^3$, gas stove 53.7 $\mu g/m^3$) -49% housing with kerosene, 51% with no kerosene	Presence of lower respiratory symptoms (fever, chest pain, productive cough, wheeze, bronchitis, pneumonia or asthma) over a 12-week period	-Logistic regression with results stratified by age (<7yrs and ≥7yrs); -OR on a continuous scale (per 30 $\mu g/m^3$)	age, sex, history of respiratory illnesses, Hollingshead scale (an SES measure)	LRS in children aged above 7
Braun-Fahrlander C. 1992	- Panel study lasting for 6 weeks during which NO_2 and symptoms are daily recorded -625 children Age:0-5 yrs -Zurich, Basel	-Palmes tube placed in the room where child stays more frequently and changed weekly for 6 weeks -31.31 $\mu g/m^3$ average in Basel; 27.31 $\mu g/m^3$ average in Zurich - 35.5% of homes with gas cooking	cough during day, cough at night, breathing difficulty sore throat, runny nose, fever, earache combined as URI	-Poisson regression (for the daily rate of symptoms incidence) - linear regression for the mean duration of symptoms -Relative risk and duration rate per 20 $\mu g/m^3$ NO_2	sex, SES, history of asthma, history of bronchitis, history of frequent colds, passive smoking	N.S.

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Brunekreef 1990	- Panel study lasting for 2 years during which lung function is measured 4 times (same study as in Dijkstra 1990) -876 children; Age: 6-12 years -The Netherlands	-Palmes tubes placed in kitchen, living room and child's bedroom - mean (IQR): 23.6 (12-24) µg/m^3 in homes without kitchen geyser, 40.3 (24-50) µg/m^3 in homes with vented kitchen geyser, 71.7 (46-73) µg/m^3 in homes with unvented kitchen geyser	FVC, FEV_1, PEF, MMEF	-Linear regression for % differences in lung function by the year of the clinical test -beta coefficient reported on categorical scale (0-20 µg/m^3 , 21-40 µg/m^3 , 41-60 µg/m^3 , >60 µg/m^3).	Height, Weight Gender parental education smoking at home	N.S.
Carlesten 2011	-Intervention study (to reduce suspected indoor allergens) of birth cohort (Childhood Asthma Primary Prevention Study) followed during first year of life (at birth and at 4, 8, 12 months) and then at age 7 - 380 high-risk (i.e. with at least one first-degree relative with asthma or two first degree relative with immunoglobulin E-mediated allergic disease) children; Age: 7 at last follow-up -Vancouver and Winnipeg, Canada	-Palmes diffusion tubes in child's bedroom for 2 week measured during first year of life. - median (range): 18.8 µg/m^3 (4.5-69.2 µg/m^3) -37 (9.7%) homes with gas stove over a total 380	Diagnosis of asthma, bronchial hyperactivity (BHR) at age 7, atopy at age 1, atopy at age7 urinary cotinine.	-Logistic regression -OR for binary exposure using median as the cut-off point -Main focus of analysis is on the effect of a combined exposure to dog and indoor pollution (NO_2 , ETS).	group allocation (intervention), race, sex, history of asthma, maternal education, city of residence, season -confounders selected using a stepwise approach	Asthma and BHR when NO2 interacting with Can-F1 positive

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Dijkstra 1990	- Prospective study; symptoms assessed two times, lung function measured four times over a 2- year period (same study as in Brunkreef 1990) -832 children; Age: 6-12 yrs -The Netherlands	-Palmes tubes placed in kitchen, living room and child's bedroom in most of the homes of the study population - mean (IQR): in homes without kitchen geyser: 23.6 (12-24) µg/m^3 , in homes with vented kitchen geyser: 40.3 (24-50) µg/m^3 , in homes with unvented kitchen geyser: 71.7 (46-73) µg/m^3 -15.3% had a vented kitchen geyser in the home, and 16.5% had an unvented kitchen geyser in the home.	cough, wheeze, asthma, FVC, FEV_1, PEF, MMEF FVC growth FEV_1 growth PEF growth MMEF growth	-Linear regression for % differences in lung function -logistic regression for respiratory symptoms. Estimates on a categorical scale (0-20 µg/m^3 , 21-40 µg/m^3 , 41-60ug/m3, >60 µg/m^3). [*OR estimated on a continuous scale per combined symptoms estimated using Hasselblad's method]*	age, sex, parental education	N.S.
Emenius 2003	-nested case-control within BAMSE birth cohort study -cases= children with recurrent wheezing matched with controls by date of birth. - 540 children Age: 0-2 -Stockholm, Sweden	- Palmes tubes placed in living room for 4 weeks - 22.6ug/m3 in homes with gas stove; 16.4 µg/m^3 in homes without gas stove - 8.52% of homes with gas stove -also outdoor NO_2	recurrent wheezing (i.e.3 or more episodes of wheezing not associated with common colds after 3 months of age, combined with symptoms of bronchial hyperactivity, i.e. cough during sleep, play and laughter or inhaled steroid treatment)	-Conditional logistic regression. -Stratified by ETS -OR for period prevalence (1 year) at age 1 and age 2 and lifetime prevalence up to age 2 per quartiles of exposure. [NO_2 *reported as a continuous variable (per 10 ug/m3* NO_2 *) in the text.]*	parental atopy, sex, maternal smoking, maternal age, breastfeeding (<6 months, >=6 months)	Recurrent wheezing when indoor NO_2 interacting with ETS

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Esplugues 2011	- retrospective study of a birth cohort sub-sample (INMA), -352 children (50% of total cohort) Age: 1 -a study on outdoor and indoor NO2 -Valencia, Spain	-Passive samplers for 2 weeks (sampler and location where sampler was placed unspecified) after birth -median:18.1ug/m3 (IQR 14.5 µg/m^3); mean:19.7 µg/m^3 - 41.7% of homes with electric cooker; 58% with gas cooker (natural 34%, butane 22%, propane 2%) (from Esplugues 2010) -also outdoor NO_2	Cumulative 12 months incidence of lower respiratory tract infection (i.e. any episode of bronchitis, bronchiolitis or pneumonia during 1st year of life) cumulative incidence of wheeze, cumulative incidence of persistent cough (lasting for longer than 3 weeks) -health data collected retrospectively	logistic regression OR per 10ug/m3 increment [30 outcomes reported but no Bonferroni correction]	sex, daycare, smoking at week 12 of pregnancy, season of birth, season of measurement, number of persons who live together, zone of residence	Persistent cough
Farrow 1997	-prospective study of birth cohort (ALSPAC); Follow-up: 1year -921 new-borns Age: 3-12 months age -Avon, UK	- Palmes tube placed in infants' bedroom for 10 days -median: 12.8 µg/m^3 , range (1.1 – 161.7 µg/m^3) -no details of gas usage	20 outcomes assessed during the 2-week monitoring period including cough, wheezing, earache, high temperature, breathlessness	logistic regression ORs reported on the basis of doubling the level of NO_2 . *[To get an OR on a continuous scale for meta analysis the median has been taken as a numerical value to compare the odds ratios.]*	maternal education, mould, age, smoking at home, parity, maternal age, preterm birth	Diarrhoea

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Fischer 1985	-cross-sectional (period: 1982) and longitudinal analysis (start date: 1965) in a cohort sub-sample - 97 adult non-smoking women living in rural area - Some of the participants already in Fischer 1989 - Vlagtwedde, The Netherlands	-Passive diffusion tubes placed in kitchen, living room and bedroom for one week in the winter - average: 10-391 µg/m^3 in kitchens, 8-198 µg/m^3 in living rooms, 8-53 µg/m^3 in bedrooms (period: 1982-83)	- IVC and FEV_1 every three years -FVC, PEF, MEFV (period: 1982)	-longitudinal analysis for IVC and FEV_1 decline from 1965 to 1982 (age group 40-60 year only, N=81) - Cross-sectional analysis (all ages) in 1982 using multiple linear regression -Estimates as beta with standard error *(longitudinal analysis did not take into account within individual variation)*	Age, Height, passive smoking, educational level	FEV1 and MEFV and living room indoor NO2 (negative association)
Fischer 1989	-cross-sectional analysis of a longitudinal study (1965-1982, same as in Fischer 1985) -612 adult women - Vlagtwedde (rural), Vlaardingen (industrial), The Netherlands	-Palmes diffusion tubes for 1 week in kitchen, living room, bedroom, measured in 1982/83 - range from 17 µg/m^3 bedroom in rural area to 96 µg/m^3 in kitchen (urban area); unvented geyser (kitchen 120(sd 55) µg/m^3 ; vented geiser (kitchen 71(sd 53) µg/m^3); no geiser (kitchen 49(sd30) µg/m^3)	FEV_1, PEF, MMEF, IVC	linear regression for difference in lung function -stratified by smoking status and residential area (rural, urban)	age, passive smoking, height, educational level	Lung function (non-smoking rural participants-negative association)

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Flamant-Hulin 2010	-sub-sample of cross-sectional study (French Six Cities Study, see Annesi-Maesano 2012) -104 children ((34 asthmatic and 70 non-asthmatics) in 33 classrooms of 14 schools, Age: 10.3 years average -Bordeaux, Clermont-Ferrand, Cre´teil, Marseille, Strasbourg, Reims (France)	Radiello passive diffusion samplers placed in classrooms for 5 days; - Exposure range:15-33 $\mu g/m^3$ -PM2.5, CH_2O also measured indoors -no details of gas usage	-FeNO measured during the week of air quality monitoring	linear regression analyses using marginal models with GEE approach stratified on asthmatic status -stratified by atopic status; *[effects presented graphically but no numerical information given]*	atopy, parental education, geographical origin of mother, family history of allergy, passive smoking during childhood.	Increase in FeNO in atopic children
Florey 1979	- cross-sectional (The Cleveland Study) - 428 children from 10 schools; Age: 6-7 years Middlesbrough, UK	-passive diffusion tubes in kitchens and 25% random sample also in children's bedrooms for one week - range: 11.3-353.4 $\mu g/m^3$ and 9.4-596.0 $\mu g/m^3$ in homes with electric cooker (n=87) and gas cooker (n=428) respectively; -outdoor NO_2 also measured	$FEV_{0.75}$ PEFR MMF Any respiratory illness (usually cough in morning, day or night, colds going to the chest, ever wheezing or whistling, attack of asthma or bronchitis in the last 12 months)	- Linear model by exposure category	Age Height weight	N.S.

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Garrett 1998	-Panel study lasting one year during which NO2 was monitored 5 times and data on respiratory symptoms experienced during the monitoring period were collected -148 children (53 are asthmatic and 61% have at least one +ve skin prick test) Age: 7-14 yrs. -a study on NO_2 and gas stoves. -Victoria, Australia	Passive diffusion tubes placed in child's bedroom, living room and kitchen repeated for 4 days x 5 over one year in 80 households *(more than one child per household)* -median:11.6 $\mu g/m^3$; range (10th-90th percentile): 5.01-27. $\mu g/m^3$	-frequency of lower respiratory symptoms (cough, SOB, waking SOB, wheeze, asthma attack, chest tightness, cough in the morning, chest tightness in the morning) -PEFR measured for 2 weeks in winter and 2 weeks in late spring -Results for symptoms score and PEFR only partially reported	-logistic regression -also stratified by atopic and non-atopic. -analysis repeated substituting NO_2 with gas stove OR per 10 $\mu g/m^3$ NO_2 *[Analyses do not take into account household clustering]*	sex, parental asthma, parental allergy	N.S. (significant for cough and chest tightness when considering gas stove) N.S. difference between atopic and non-atopic
Gillespie 2011	- secondary analysis of a clustered, randomised intervention trial (Housing, Heating and Health Study, see Howden-Chapman 2008) lasting for 112 days during which symptoms are recorded daily in a diary - intervention: homes having their unflued gas heater been replaced with a less polluting heating (replaced with heat pump, wood pellet burner, flued gas)	-Passive diffusion tubes placed in living rooms for 4-week period x 4 - Geometric mean: 11.4 $\mu g/m^3$ -also outdoor NO_2 measured	evening FEV_1, morning FEV_1, evening PEFR, morning PEFR; asthma symptoms severity score (scale 0-3) for: cough at night, cough during the day, cough on waking, wheeze at night, wheeze during the day, wheeze on waking preventer use, preventer use, reliever puffs per day, lower respiratory symptoms and upper respiratory symptoms	-Linear mixed-effect model with random effects for repeated measures within individual and fixed effect of NO_2 on health outcome - 'change in mean symptom rate' and change in lung function effect per logged unit increase in NO_2 level	age, sex, parental asthma, smoking, outcome at baseline, region, ethnicity, effect of intervention, low income N.B. groups were randomised	-change in mean symptoms score (for all symptoms but preventer use) - change in evening and morning FEV_1 (negative association)

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
	-349 children with asthma Age: 6-13 yrs -New Zealand					
Hansel 2008	-prospective study (Baltimore Indoor Environment Study of Asthma in Kids) – participants visited home 3 times over a 12-months period and asked about symptoms in the previous two week -150 children with asthma Age: 2-6 yrs; -Baltimore, US	-Ogawa badges placed in child's sleeping room for 72 hours x 3 (baseline, 3 months, 6 months) -Mean: 56.4 $\mu g/m^3$; range:5.5-740.7 $\mu g/m^3$ -83% homes with gas stove (12% reporting using gas stove/oven for heating) -also indoor $PM_{2.5}$	Number of days over 2 weeks with the following symptoms: -Daytime wheezing, coughing or chest tightness, -slowing activity due to asthma, wheeze, chest tightness or cough -limited speech due to wheeze -wheeze, cough, or chest tightness while running -coughing without a cold -nocturnal awakenings due to cough, wheeze, shortness of breath or chest tightness	Negative binomial with generalised estimating equations -logistic regression models -stratified by atopic status IRRs (incidence rate ratios) and ORs on a continuous scale	caregiver education level, age, sex, season of sampling, PM2.5, Second hand Smoke, distance from kerb, type of street in front of home, race	Limited speech due to wheeze and nocturnal awakening due to cough, wheeze, SOB or chest tightness (nocturnal symptoms stronger in atopic children)
Hansel 2013	-prospective study; -84 adult former smokers with moderate or severe COPD and a mean FEV1 of 48.6% predicted; Age: mean 68.9 (sd 7.4) yr -Baltimore, US	-Ogawa passive diffusion badges; monitored over a 1-week period in the participants' bedroom and main living area at baseline, 3 months, and 6 months. -Living area: mean 22.9 (SD 22.8) $\mu g/m^3$; bedroom :mean 20.3 (SD19.9) $\mu g/m^3$ -no details of gas appliances	FEV_1% predicted, dyspnoea, wheeze, nocturnal symptoms, usual cough, usual phlegm, frequency of inhaler use, SGRQ (St George Respiratory Questionnaire), any exacerbations, sever exacerbations. All outcomes were assessed at each home visit, which took place at baseline, 3 months and 6 months. Exacerbations were	generalised estimating equations model for repeated measurements	Age Sex Education Season of sampling FEV_1 % predicted Air nicotine Hair nicotine	dyspnoea and inhaler use (living area NO2); nocturnal symptoms and severe COPD (bedroom NO_2); increase in FEV_1 % predicted (living area NO_2)

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
		-also PM2.5 measured	assessed by questionnaires at each clinic visits and monthly by phone			
Hoek 1984	-case-control study with cases being children from the data of the city School Health Service reporting suffering from asthma, bronchitis , frequent cough or colds and allergy -128 cases and 103 controls ; Age: 6 yrs	Palmes diffusion tubes in kitchen, living room and bedroom for 1 week -range; kitchen 110-789 µg/m^3 , living room 17-277 µg/m^3 , bedroom 10-146 µg/m^3 -no gas details given	Asthma Bronchitis Cough Wheeze Breathlessness combined symptoms *[prevalence period non found in text]*	-logistic regression - OR (90% CI) to an increase of the 10 log NO2 concentrations with once unit NO_2 time weighted [*standardisation to 10 ug/m^3carried out using estimates reported in Li 2013]*	age, sex, parental education, bedroom heating, mother smoking, home humidity, parental respiratory symptoms	N.S.
Jarvis 2005	-Sub-sample of a longitudinal study (ECRHS) started in 1990 -276 adults; mean age: 43 -a study mainly focusing on indoor nitrous acid. -Ipswich and Norwich, UK	passive diffusion tubes for NO2 measured in kitchen away from window for 14 days - NO_2 median 24.66 µg/m^3 (IQR 14.55, 42.00 µg/m^3); HONO median 3.10 ppb (IQR 2.05, 5.09) -57.7% of homes with HONO measurements had mostly used gas for cooking -HONO also measured	change in FEV_1% predicted, FEV_1/FVC,	linear regression for change in lung function effect estimate on continuous scale	age, sex, dampness, city, season of lung testing, season of measurement, smoking status, pack years of smoking, occupational group	N.S. (but significant association with HONO)

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Kattan 2007	-Panel study lasting for 1 year with assessment at baseline and 3, 6, 9 and 12 months) – National Cooperative Inner-City Asthma Study (NCICAS) -1528 children with current asthma with NO2 measurement in 663 homes; Age: 4-9 yr (NCICAS); - Eight inner cities, US (Bronx NY, East Harlem NY, St Louis MO, Washington DC, Baltimore MI, Chicago Ill, Cleveland Ohio, Detroit Mitch	-Palmes tube in child's sleeping area for 7 days -median: 56 µg/m^3 , 24% of children exposed to NO_2 >75.2 µg/m^3 -gas stove in 87.8% of homes	Asthma morbidity (4 or more days with wheeze during a two-week period, any unscheduled medical visit for asthma in the past 3 months and PEF <80% predicted, health service used in the previous 3 months)	-longitudinal binomial regression model. results presented high (>75 percentile) vs low; - stratified by atopy and season (<15.6 C vs >=15.6 C) -Effect estimates on binary scale (cut-off point 99.6 µg/m^3 = 75^{th} centile)	smoking at home, family history of asthma, use of steroid medication, family income, study site, psychosocial status	asthma symptoms; decrease in PEF in non-atopic children (only when levels of indoor NO2 are high)
Kim 2011	-cross-sectional study on home, school and outdoor environment -1028 children from 34 classroom; mean age 10yr; -*Three cities*, Korea	-passive diffusion samplers (IVL) placed on a wall in each classroom for 7 days during November or December -19 µg/m^3mean, range 7-38 µg/m^3 -no details of gas usage - also measured outdoor NO_2 and indoor/outdoor , O3, formaldehyde, UFP	wheeze, doctor diagnosed asthma, current asthma (medication or/and asthma attacks during the last 12 months)	-generalised linear and latent mixed model with three-levels (individual-school-city) and random intercept -OR on a continuous scale (per 10 µg/m^3 NO_2)	age, sex, ETS, furry pets, pollen allergy, home environment	NS

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Magnus 1998	nested case (*see definition in outcome*)-control within the Oslo Birth Cohort -153 one-to-one matched pairs of children -age: 2 years	Passive diffusion tubes exposed for 2 weeks in kitchen, child's bedroom, main living room and in day-care site if child attending it -child's sleeping room mean (range) 13.2 (5-33) µg/m^3 -no details of gas given -also measured outdoor NO_2	cases must have developed at least 2 episodes of bronchial obstruction during first 2 years of life or one episode lasting for more than 4 weeks	conditional logistic regression OR on a continuous scale [in text]	gender, parental asthma, maternal education, ETS, birth weight, breastfeeding	N.S
Matsui 2013	-prospective study, participants followed for 1 year and clinically assessed at baseline, 3, 6, 9 and 12 months at same period as NO2 monitoring -146 children with persistent asthma Age:5-17 years -Baltimore, US	- Ogawa passive diffusion badges placed in child's bedroom for 5-7 days x 4 within two weeks of clinic visit -median 39.1 (IQR: 26.3-58.3) µg/m^3 -gas details: NR - Also indoor $PM_{2.5}$	FEV_1/FVC % predicted, FeNO, Acute visit to emergency department, Oral corticosteroid burst, Reversibility, Beta-agonist use, Maximum symptoms days, Wheeze, Cough, Chest tightness, Exercise-related symptoms	Logistic regression (exposure of interest is endotoxin) Estimates on binary exposure for NO_2 (high NO_2 ≥37.6 µg/m^3 vs low NO_2 <37.6 µg/m^3)	age, sex, lot of endotoxin assay, airborne mouse allergen, total IgE, controller medication air nicotine *the following were considered but not included in the final model because of no confounding effect: SES, season, PM*	wheeze, cough, chest tightness, exercise-related symptoms when high NO_2 levels interacting with endotoxin (negative association)
Melia 1982	-cross-sectional study (The Cleveland study) -191 children in 183 homes living in homes using gas for cooking Age: 5-6 years -Middlesbrough UK	Palmes diffusion tubes for one week in the child's bedroom and living room -bedroom range: 8.8 to 302.3 µg/m^3 ; living room range : 16.9 to 549.3 µg/m^3 .	one or more respiratory conditions (usually cough in morning, day or night, colds going to the chest, ever wheezing or whistling, attack of asthma or bronchitis in the last 12 months)	-Generalised linear modelling -estimates on a tertile scale (0-37.6 µg/m^3 , 37.6-75.0 µg/m^3 , >=75.2 µg/m^3)	age, sex, smoking at home, social class	N.S.

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
		- 100% of participants' homes use gas for cooking				
Mi 2006	-cross-sectional study looking at the influence of building ventilation, NO_2 , O_3 and formaldehyde in classrooms -1414 children from 30 classes in 10 different schools; Age: 13-14 yrs. -Shanghai, China	Badge diffusion sampler (IVL) for seven days in each classroom (n=30) -min-max valued in classrooms: 33-86 µg/m^3 -outdoor NO_2 measured (47-83 µg/m^3) -no heating system in the building; indoor NO_2 probably from outside sources	current wheeze, daytime breathlessness, nocturnal breathlessness, asthma attacks, asthma medication, current asthma in previous 12 months	logistic regression OR per 10 µg/m^3 increment	age, sex, dampness, smoking	Current asthma and asthma medication
Mukala 2000	-Panel study lasting for 13 weeks with daily recording of symptoms -162 children in 12 days care centers (8 in more polluted central areas and 4 in cleaner suburban areas) Age: 3-6 -Helsinki, Finland	- Palmes tubes placed inside day-care centers for 13 week - only tubes that had collected for 168(+-24 hr) were accepted for analysis; -also indoor and outdoor NO_2 exposure measured with Palmes tubes placed on children 's garnments - max median : 47 (range 22-83) µg/m^3 in spring/central area; min median : 23 (range 14-33) µg/m^3 sping/suburban area -no details of gas usage	cough episodes recorded daily	Poisson regression using GEE option with independent correlation structure assuming that correlation of responses followed the first-order autoregressive process. - stratified by season (winter, spring). -Estimates on a categorical scale and continuous scale *(in text)*	parental education, ETS, allergies, oven (gas/electric), day care centre	NS

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Neas 1991	-a sub-sample of a large cohort study (The Harvard Six Cities) assessed every year for 3 consecutive years -1567 children selected with a stratified strategy to obtain 70% (achieved 58%) smoking households and 70% (achieved 48%) with major NO_2 sources (gas cooking stove or kerosene heater) Age:7-11	-Palmes tubes in 2 consecutive 1-week sampling periods in winter and summer; in kitchen, activity room and child's bedroom -household without a major indoor NO_2 source: mean 16.2 µg/m^3 ; households with a major indoor NO_2 source (48%): 44.2 µg/m^3 -. Gas stove 623/751; kerosene heater 156/751 -PM also measured	Previous year prevalence of: LRS (any SOB or wheeze or cough or phlegm or bronchitis combined), SOB, chronic wheeze, chronic cough, bronchitis, hay fever, earache Also FVC, FEV_1 mL, FEV_1/FVC, $FEV_{0.75}$, FEF_{25-75}, FEV_{25-75}/FVC,	logistic regression models -generalized estimating equations for incidence -stratified by gender and smoking at home OR per 28.2 µg/m^3	age, sex, city, parental history of bronchitis, parental history of asthma, parental college education, single parent family status, respirable particulates at home -analysis stratified by gender and smoking homes *-no adjustment for indoor smoking*	LRS (when stratified significant only ic girls and in smoking homes)
Nitschke 2006	- panel daily lasting for 12 weeks with symptoms recorded daily in a diary and NO_2 exposure measured in schools and home; schools had taken part in intervention trial (see Pilotto 2004) -174 children with asthma from 18 schools Age: 5-13 yr -Adelaide, Australia	-passive diffusion badges in schools on 3 consecutive days over three times and at home for 3 days over one time at home (home exposure recorded from time children arrive back from school until bedtime) during winter season -Measurements are based on max exposure values as a proxy for peaks exposure: -kitchen: range 5.6-795.2 µg/m^3 ; median 41.4 µg/m^3 Classroom: range 16.9-577.2 µg/m^3 , median 73.3 µg/m^3	chest tightness, cough wheeze, breathing difficulty, breathlessness on exertion, daytime asthma attacks, night asthma attacks, outcomes measured as symptoms counts, FEV_1% taken at beginning and end of study	negative binomial model for symptoms rate and symptom counts exposure: maximum NO_2 level at home (and school) -sub-group analysis in children sensitised to HDM for modifying effect of HDM with NO_2 -relative risk per 18.8 µg/m^3increase in NO_2 *[32 outcomes and not*	age, sex, smoking at home, ethnicity, hayfever, parental education, clustering by school, serious respiratory illness before age 2	Difficult breathing during the day (school exposure) and at night (kitchen exposure), asthma attacks at night and night-time wheeze (kitchen exposure), decrease in FEV_1 % predicted (kitchen exposure), (In sensitised children: if Der p low then wheeze at night increases, if Der p is high then wheeze at night decreases)

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
		-use of gas cooking or UFG at home		*adjusted for Bonferroni]*		
Osman 2007	cross-sectional study on indoor air quality, HEART (Home Environment and Respiratory Health) - 148 adults with severe COPD - Age: mean 69 years (SD 8.2) -Aberdeen UK	-Palmes tubes in living room and bedroom left for 1 week in the living room and bedroom -median (IQR): living room 14.7 (8.8-23.3) µg/m^3 , bedroom 13.3 (7.3-19.6) µg/m^3 -no gas details reported -indoor $PM_{2.5}$ and endotoxins also measured	COPD health status measured using the St George's Respiratory Questionnaire	-Linear regression -estimate reported as beta with SE -stratified by smokers and non-smokers	age, lung's function	N.S.
Pilotto 1997	-panel study on indoor classroom (n=41) and homes (n=121) with unflued gas appliances exposure over a winter period (March-Sep) during which children kept a daily symptoms diary -388 children from 41 classrooms in 4 schools with unflued gas heating and 4 schools with electric heating -Sidney, Australia	- passive diffusion badges to open when gas appliance on and close when appliance off for two weeks -Classroom monitored daily over 9 alternated weeks for 6-hous school day daily -Classroom: electric (mean range 7-48.9 µg/m^3), unflued gas (7.5-248.2 µg/m^3) -Homes: 100% unflued gas appliances and non-smoking 83% of homes with NO_2 between 75.2 and 150.4	hoarse voice, sore throat, cough with phlegm, dry cough, sneeze, stopped up nose, runny nose, wheeze, cold, absent from school	-generalised linear mixed model (GLMM) with binomial error distribution and logit link specification that takes into account classroom correlation -mean symptoms rate -OR (proportion of children with at least one symptoms per day) for binary exposure (<75. µg/m^3 vs >75.2 µg/m^3) *-analysis do not take*	age, sex, area, history of asthma, early severe chest illness in non-smoking homes only	Mean difference for daily rate of symptoms (cough with phlegm, cold, absent from school) and absence for school

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
		$\mu g/m^3$.		*into account individual correlation*		
Quackenboss 1991	-panel study study on the exposure assessment approaches to estimate personal exposure to indoor and outdoor NO_2 and PM - 400 children (30 asthmatics) age 6-15 yrs	-Palmes diffusion tubes in the kitchen, main living area and child's bedroom for two weeks. -median (IQR): kitchen (electric) 13.6(9.2-19.4) $\mu g/m^3$, kitchen (gas) 36.8 (26.0-58.5) $\mu g/m^3$; bedroom (electric) 11.5 (7.5-17.7) $\mu g/m^3$; bedroom (gas) 26.4 (19.0-34.3) $\mu g/m^3$ -Also outdoor NO_2 and PM (indoor and outdoor) measurements. - 100% gas ranges	PEFR, acute respiratory illness, allergic symptoms	-random effects longitudinal model for PEFR; -regressive logistic model for occurrence of daily symptoms - results reported only for children with asthma (n=30) and children with no lifetime history of asthmatic symptoms OR per 10 $\mu g/m^3$ *[see text]*	not clear	Morning PEFR and high bedroom NO_2 in asthmatic children (negative association)
Raascho-Nielssen 2009	-prospective birth cohort study (Copenhagen Prospective Study on Asthma in Childhood, COPSAC) -411 high-risk infants (with asthmatic mother) Age:18 months -Copenhagen, Denmark	Palmes diffusion tubes in children's bedrooms three times over 18 months of life for 10 weeks in each occasion - Median (bedroom): NO_2 7.5 $\mu g/m^3$ (range 5^{th} -95^{th} 3.3-17.0 $\mu g/m^3$) -gas details not reported -NOx, $PM_{2.5}$, BS, CH_2O also measured indoors	Daily wheezing symptoms recorded in diary over 18 months from birth. Wheezing (any symptoms severely affecting the child's breathing such as noisy breathing (wheeze or whistling sounds), breathlessness SOB or persistent, troublesome cough)	-logistic regression (for symptoms) - linear regression (for number of symptoms/days) Estimates per quintile of exposure	sex, area, lung function ($FEV_{0.5}$) at 1 month of age, mother's education	N.S.

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Samet 1993	-prospective study (from birth to 18 months) during which mother reported daily occurrence of symptoms every 2 weeks -1205 healthy infants from homes without smokers -Albuquerque, New Mexico, US	-Palmes diffusion tubes measured placed for two weeks in child's bedroom year around - homes with gas stove measurements repeated every two weeks. -22% of homes measures greater than 37.6 $\mu g/m^3$ -79% of homes with gas cooking, 21% electric cooking	Wet cough wheeze Upper illness (runny or stuffy nose occurring at least in two consecutive days) All lower illness (at least one day of cough or wheeze)	Generalised estimating equations (lagged and unlagged estimates) ratio and duration of illness on a categorical scale -incidence rate (i.e. ratio number of events to number of days at risk) -duration of illness	maternal education, age, sex, ethnicity breastfeeding in infancy, parental history of asthma, parental history of allergic diseases, season, household income, birth order -only non-smoking homes were selected	NS
Sarnat 2012	Panel study lasting 16 weeks during which eNO is measured every week - 58 current asthmatic children from two schools in Mexico and two schools in US across the border ; Age: 6-12 -border across Mexico and US	Ogawa passive diffusion samplers measuring exposure in computer room, library and classroom for 96 hours -Indoor NO_2 levels were 40.0, 6.6 and 14.9 $\mu g/m^3$ in 3 schools but in the other one were 157.7 $\mu g/m^3$ (which is extremely high, but the school is located adjacent a bus station) - no details of gas appliances in schools; indoor NO_2 source is NO_2 from traffic Also measured indoor/outdoor PM_{10}, PM_{10-}	Exhaled NO, respiratory symptoms	Generalised estimating equations (for percentage change in eNO per IQR increase (= 35.7 $\mu g/m^3$)	school, indoor NO (as it may interfere with exhale NO measurements), ambient temperature, relative humidity	eNO (stronger effect with outdoor NO_2)

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
		$_{2.5}$, PM_{25}., BC and outdoor NO_2				
Shima 2000	-prospective study looking at the effect of outdoor and indoor NO2 and assessing children with a questionnaire every year for 3 years -842 children living in urban, sub-urban and rural districts; Age: 9-10 yrs at baseline -Chiba prefecture, Japan	Passive diffusion badge-type sampler (Toyo Roshi) measuring 24-h average in living room twice (winter 1993 and summer 1993) -vented: mean ($\mu g/m^3$) annual 34.6, winter 45.1, summer 28.6 unvented: mean($\mu g/m^3$) annual 60.9, winter 141.2, summer n.a. -61.1 % homes with unvented heaters -also outdoor NO_2	Asthma prevalence and incidence (3 yr) of (i.e. 2 episodes of wheezing with dyspnoea that had ever been given the dx of asthma), Wheeze prevalence and incidence bronchitis prevalence and incidence	-logistic regression -stratified by gender - OR per 18.8 $\mu g/m^3$ -No cluster analysis by area	sex, history of respiratory illnesses, breastfeeding in infancy, parental history of allergic diseases, respiratory diseases under 2 years of age, parental smoking, use of unvented heater in winter, outdoor NO_2, history of allergic diseases	N.S.

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Simoni 2002	- cross-sectional study of sub-sample in Simoni 2004 -383 adults age 15-72 years - Delta Po, Italy	Palmes diffusion tubes for 2 weeks (1 week in winter, 1 week in summer) installed in kitchen, living room and in 1 or 2 bedrooms. median: 37.6 µg/m^3 (winter), 26.3 µg/m^3 (summer); kitchen (winter) 62.0 µg/m^3 ; kitchen (summer) 37.0µg/m^3 -$PM_{2.5}$ also measured - no gas details available	PEF(mean, AMP/MEAN, MAX/MIN) as binary variable ARI (acute respiratory illnesses with fever) WFRI (chronic bronchitis and/or asthmatic symptoms without fever and without ARI) IRR (irritation without fever)	Logistic regression for daily indices of exposure (weekly mean x daily time of exposure) -sub-group analysis for winter/ summer and chronic bronchitis, OR on binary scale (median as cut off point)	age, sex, smoking, asthma chronic, bronchitis chronic, rhinitis	Bronchitis/asthmatic symptoms in winter and PEF in people with chronic respiratory disease (asthma or bronchitis)
Simoni 2004	-cross-sectional study (sub-sample in Simoni 2002) -421 adults living in one urban and one rural areas Age: mean 40 years -Delta Po, Pisa (Italy)	Palmes diffusion tubes for 2 weeks (1 week in winter, 1 week in summer) installed in kitchen, living room and in 1 or 2 bedrooms. mean (kitchen-Pisa): 28.2 µg/m^3 (winter) 24.4 µg/m^3 (summer); mean (kitchen-Po Delta): 62.0 µg/m^3 (winter), 28.2 µg/m^3 (summer) gas furnace : Pisa 85%, PoDelta 97%	ARI (acute resp illnesses i.e. runny nose, sore throat, sputum from the chest, chest cold, SOB) WFRI (bronchitis and/or asthmatic sx (sputum from the chest, SOB, attack of SOB, wheeze) without fever and without ARI) IRR (irritant symptoms) GENER (non-specific symptoms) Max amplitude PEF	Logistic regression. Estimates per high vs low exposure Results reported only for the winter period	age, sex, area, smoking	ARI
Smith 2000	-panel study lasting 6 weeks -129 participants with current asthma living in	Passive diffusion lapel badge monitor to be attached to participant when arriving home from daily activities; badge cover removed when cooking	chest tightness, cough, wheeze, breathlessness, breathlessness on	GEE with imputation (for missing data). - Lag 0 and lag 1 considered in analysis	age, sex, area, smoking, SO_2,	chest tightness (lag 0 and lag 1), daytime/night time asthma attacks (lag 0), breathlessness on

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
	104 households Age: any (adults and children). -Port Adelaide, Australia	and heating and time recorded; cover replaced at bed time and time recorded; one week cycle x 6. - median range: 7.0-275.7 µg/m^3; IQR within subject 1.1-288.6µg/m^3 -64% households with gas for cooking and heating	exertion, daytime asthma attacks, night asthma attacks recorded in diary. *Asthma attack definition*: any asthmatic episode involving breathlessness and/or wheezing and/or chest tightness and/or coughing that interrupts ongoing activities or requires some procedures, e.g. resting or using nebulizer to resume normal and comfortable breathing	- stratified by age group - daily asthma symptoms and daily NO2 exposure -OR on a continuous scale -*household not treated as cluster in the analysis* *70 outcomes but not corrected for Bonferroni*	O_3, wind, relative humidity, highest level of education at home, minimum temperature, total fungal spore counts, *Cladosporium*, *Alternaria*	exertion (lag 1) in participants of age ≤ 14 years; cough (lag 1) in participants of age 35-49
Sunyer 2004	Cross-sectional analysis of a birth cohort study (Asthma Multicentre Infants Cohort Study AMICS) 1611 infants followed up for a year Ashford (UK), Barcelona and Menorca (Spain)	Palmes diffusion tubes for 2-week period installed in the living room median : UK 10.9µg/m^3, Barcelona 86.3µg/m^3, Menorca 22.3µg/m^3 gas stove use: UK 60%, Barcelona 74%, Menorca 72%. Gas heater use: UK 83%, Barcelona 34%, Menorca 25%	- cumulative incidence of LRTI (defined as a positive answer to "has a doctor ever told you that your son/daughter has had a chest infection?") and use of antibiotics during first year of life; - frequency of LRTI symptoms (recorded retrospectively by mothers)	logistic regression Estimates by category (<=9.4, 9.4-18.8, 18.8-56.4, >56.4 µg/m^3)	parental atopy, sex, maternal smoking, family size, breastfeeding in infancy, social class, parental asthma, season	N.S.

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Tavernier 2005	Case-control study (Indoor Pollution Endotoxin Allergen Damp and Asthma in Manchester, IPEADAM) matched for age, sex, sib-ship size, -90 matched children-pairs age 4-17 -Manchester, UK	-passive diffusion tubes kept for one week in the living and in the child's bedroom -no details of NO_2 measurements or gas usage -$PM_{2.5}$, VOC, formaldehyde, endotoxin also measured	cases: asthma diagnosis	Conditional logistic regression OR (no details of NO_2increment or comparisons between categories)	controls matched for age, sex and sib-ship size	N.S
Trishe 2005	-prospective study – women keep record of daily symptoms over one year period - 888 adult women non-smoking -Connecticut and Virginia, US	- Palmes diffusion tubes placed in the main living area for 2 weeks in homes with electric stove and 3 to 6 weeks in homes with kerosene heaters and gas stove in winter. -median: gas space heater (103 $\mu g/m^3$), no gas space heater (23.5$\mu g/m^3$) -34% of women using gas stove - Also SO_2 measured	chest tightness, cough, wheeze, sore throat, runny or stuffy nose, laryngitis.	log-linear Poisson using generalised estimating equations for rate of days with symptoms over frequency use of secondary heating source estimates for number of days with symptoms on categorical scale (≤150.4 $\mu g/m^3$ vs >150.4 $\mu g/m^3$)	race, history of allergies, number of children, dwelling type, residence state, education (only non-smokers)	Wheeze when considering high NO_2 exposure (> 150.4 $\mu g/m^3$ =top quartile) vs lowest quartiles (≤150.4 $\mu g/m^3$)

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
van Strien 2004	-prospective study (YCAS – same cohort as Belanger 2003) during which parents record daily symptoms over a year -652 high risk infants (must have an asthmatic sibling of age younger than 11) Age:0-1 -- Connecticut and Massachusetts, US	-Palmes tubes placed in main living room for a 2-wk period over the year - median 18.6µg/m^3 (IQR 9.6-32.7µg/m^3) -also HONO measured	days of wheeze, days of SOB, days of persistent cough over one year prevalence recorded by parents in a daily calendar	Poisson regression -Stratified by cold and warm seasons. -Estimate as rate ratios on categorical scale (<80 µg/m^3 vs >80 µg/m^3)	maternal education, sex, smoking at home, HONO (for NO_2 analysis) and NO_2 (for HONO analysis), siblings, season of sampling, daycare, living in flat, ethnicity, parental asthma	Cough, SOB when considering highest quartile vs lowest quartile (but not for HONO). Stronger effect in winter
Venn 2003	case-control study; cases= 193children with persistent wheezing and 223 controls; age: 9-11 years; location: Nottingham, UK	-passive diffusion tubes in kitchen over a four week period - Mean levels of NO_2 were higher in subjects from homes where gas was used for cooking (50.4µg/m^3) than where gas was not used (21.2µg/m^3) -CH_20 also measured	morning and evening PEF night-time and daytime symptoms score on a scale 0 to 5 over the 4 week period when NO_2 was measured.	regression model - stratified by atopic status OR reported by quartile	sex, age SES.	N.S.
Vieira 2012	cross-sectional study; 64 children aged 6-10 yr, Sao Paulo, Brasil	- passive diffusion tubes placed in the participant's living room measured for 30 days (2x 15 days) -mean 17.6 (SD 3.9) µg/m^3	asthma medication in the last 12 months, asthma medical diagnosis, wheezing at some time, pneumonia	logistic regression -exposure comparison not clear	age, sex, maternal schooling, smoking in the home, presence of siblings	Doctor diagnosis asthma, wheeze at some time

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Zhang G 2004	-cross-sectional study -88 children Age: 4-6 -Perth, Australia	-Palmes diffusion tubes placed in living room and child's bedroom for 24 hours X 2 -geometric mean (min-max) living room-winter - in non-snoring children homes: 38 (9-314) μg/m^3; in snoring children homes: 48 (6-345) μg/m^3 -in non-snoring homes 57.5% gas cooking; in snoring homes 53.0% gas cooking; in infrequent snoring homes 63.0% gas cooking	snoring	Logistic regression OR on binary scale (low, medium high)	age, sex, pets, smoking at home	Snoring
Zhao 2008	cross-sectional study, survey carried out 1 week before class measurement; -1193 children in 46 classrooms Age: 11-15 -Taijuan, China (see Zhang X 2011)	-IVL Passive diffusion samplers placed in classroom for 7 days (no school had mechanical ventilation) -39.4μg/m^3 indoor classroom average, range 16-62μg/m^3 -no details of gas usage -also outdoor NO_2, ind/out SO_2, O_3, CH_2O measured	cumulative asthma, wheeze or whistling in the chest (12 months prevalence) daytime attacks of breathlessness (12 months prevalence) nocturnal attacks of breathlessness (12 months prevalence) furry pet or pollen allergy (12 months prevalence) respiratory infection (3 months prevalence)	logistic regression with hierarchical model (school-class-student) OR on a continuous scale per 10 μg/m^3	age, sex, parental asthma, parental allergy, ETS, new painting, new floor material, new furniture	Nocturnal attacks of breathlessness (but NS when adjusted for other pollutants)

Author	Design and Participants	Exposure assessment	Outcome assessment	Statistical analysis	Adjusted for	Significant associations
Zhang X 2011	- cross-sectional analysis of Zhao 2008 study, two years after -1143 children in 34 classrooms Age: 11-15 years Taijuan, China (see Zhao 2008)	-IVL Passive diffusion sampler exposed for 7 days in 34 classrooms 39.4µg/m^3 average -no gas heater -also measured SO_2	Any symptoms Improved if away from school Any mucus Any skin problem	Logistic regression with hierarchical model (school, class-student) -HR for new onset or remission OR cross-sectional per 10 µg/m^3 increment	age, sex, parental allergy or asthma	Any symptoms, improved away from school and any mucus

IVL Swedish Environmental Research Institute : IVL brochure. Diffusive samplers for air monitoring, available at: http://www3.ivl.se/affar/miljo_kartl/proj/passive_sampl/PassivaProvtagare.pdf. Swedish, Environmental Research Institute, Ltd..;
Ogawa Ogawa protocol. NO, NO_2, NO_X and SO_2 sampling protocol using the Ogawa sampler, Edition 6.06, June, 2006, available at: http://www.ogawausa.com/protocol.html. Ogawa & Company, USA, Inc.
Palmes Palmes E.D., Gunnison A.F., Dimattio J., and Tomczyk C. Personal sampler for nitrogen dioxide. Am Ind Hyg Assoc J 1976: 37: 570–577.
Radiello Radiello manual. English Edition v. 01-2006, available at: http://www.radiello. com/english/Radiello's manual 01-06.pdf. Fondazione Salvatore Maugeri –

Design and participants

Sixteen studies were published before 2000, the earliest being published in 1979 (Florey, 1979) and the remaining 34 studies from 2000 onwards. Twenty-three studies were carried out in Europe (6 in UK), 15 in US and Canada, 6 in Australia and New Zealand, 5 in China, Korea and Japan and 1 in Brazil. Twenty-nine studies were in children, 11 in infants (age 2 or younger), 9 in adults and 1 (Smith *et al.*, 2000) in participants of any age (from 2 to 84 years). Thirty-four studies were in the general population, 10 in asthmatics, 4 in high-risk groups (defined by having a family member with asthma) and 2 studies in people with a diagnosis of COPD. Seven studies were carried out in school and one study in a nursery.

Seven pairs of studies reported analyses using the same study population (or sub-groups belonging to the same population): (1) (Brunekreef, 1990; Dijkstra, 1990); (2) *(Hansel* et al.*, 2008; Matsui* et al.*, 2013); (3) (Fischer* et al.*, 1985; Fischer* et al.*, 1989); (4) (Annesi-Maesano* et al.*, 2012; Matsui, 2013); (5) (Simoni* et al.*, 2002; Simoni* et al.*, 2004)***; (6) (Zhao *et al.*, 2008; Zhang *et al.*, 2011); (7) (Belanger *et al.*, 2003a; van Strien *et al.*, 2004). The study population of the latter pair of studies was also related (siblings) to the study population in Belanger (2006).

The study sample size varied across the studies ranging from over 1,000 participants (Neas, 1991; Braun-Fahrlander *et al.*, 1992; Samet, 1993; Sunyer *et al.*, 2004; Mi *et al.*, 2006; Zhao, 2008; Hulin, 2011; Kim *et al.*, 2011; Zhang, 2011) to less than 150 (Fischer, 1985; Berwick *et al.*, 1989; Quackenboss *et al.*, 1991; Garrett *et al.*, 1998; Smith, 2000; Ng, 2001; Zhang *et al.*, 2004; Hansel, 2008).

Fifteen studies reported cross-sectional analysis of data collected at a single follow-up within cohort studies. Thirteen studies were cross-sectional and reported cross-sectional analysis; eleven studies were panel studies and 7 studies were case-control (Hoek *et al.*, 1984; Venn *et al.*, 2003; Tavernier *et al.*, 2005), or case-control nested in a cohort (Magnus *et al.*, 1998; Emenius *et al.*, 2003). One cohort study reported longitudinal analysis for lung function decline over a 18-year period (Fischer, 1985) and a Japanese child cohort examined the association of wheeze and asthma incidence and indoor NO_2 over a 3-year period (Shima and Adachi, 2000).Two studies (Pilotto *et al.*, 1997; Nitschke *et al.*, 2006; Gillespie-Bennett *et al.*, 2011) reported secondary analyses of intervention studies that looked at the health effects of replacing unflued gas heaters with flued gas or electric heaters in schools or at home

Indoor NO_2 monitoring

The most common rooms where indoor NO_2 monitoring was carried out were the living room, followed by bedroom and kitchen. Seven studies monitored NO_2 in children's classrooms (Smedje, 1997; Mukala *et al.*, 2000; Mi, 2006; Zhao, 2008; Flamant-Hulin *et al.*, 2010; Hulin, 2011; Kim, 2011; Sarnat *et al.*, 2011; Zhang, 2011; Annesi-Maesano, 2012) or day-care centre (Mukala, 2000). Two studies measured indoor NO_2 exposure both at school and home (Pilotto, 1997; Nitschke, 2006).

All studies measured NO_2 with passive diffusion samplers. Although the design of a passive diffusion sampler can vary, the working principle is similar and can only provide concentration averages based upon the time the sampler is kept open. The majority of studies used a tube-design sampler (Palmes, 1981), eight studies a badge design sampler (Ogawa, Toyo Roshi), four studies used a sampler produced by the Swedish Environmental Research Institute IVL and two studies a sampler with a radial design (Radiello, 2012).

One-week or two-week average concentrations were the most common measurements as most measurement devices are not recommended for less than one week exposure periods. Three studies used samplers that can be used for shorter periods: one study (Shima and Adachi, 2000). limited monitoring to one day in summer and one day in winter and the other two studies (Nitschke, 2006, Hansel, 2008) limited the monitoring to 72 hours repeated three times over the year. All three studies used badges for sampling.

Pilotto (1997) and Smith (2000) attempted to measure short-term exposure to NO_2 during periods of gas appliances use by keeping the passive diffusion badges open only during the time the appliance was on. Simoni (2002; 2004) attempted to interpolate a daily index of personal exposure by measuring weekly average of NO_2 with Palmes diffusion tubes and multiplying the measured concentration by the time participant spent home.

A few studies repeated the exposure measurements throughout the year (Braun-Fahrlander, 1992; Samet, 1993; Garrett, 1998; Raaschou-Nielsen *et al.*, 2010) or at different seasons (Neas, 1991, Shima 2000). Five studies measured indoor NO_2 during the cold season only (Fischer, 1985; Magnus, 1998; Triche *et al.*, 2005; Nitschke, 2006; Kim, 2011).

Indoor NO_2 levels

The reported average levels of indoor NO_2 varied within and between studies. For example, the median of 10-day average measured in UK infants' bedroom (Farrow *et al.*, 1997) was 13µg/m^3 but individual household average concentrations ranged from 1µg/m^3 to 162µg/m^3. In a study conducted in Sweden (Magnus, 1998) the bedroom mean weekly average was 13µg/m^3 but in a study conducted in US inner cities, the National Cooperative Inner-City Asthma Study (Kattan *et al.*, 2007) the reported median weekly average was 56µg/m^3.

In more than 30 studies the 75th centile of measured average NO_2 was above 40µg/m^3. Households or classrooms with unvented gas heating appliances tended to have the highest levels of indoor NO_2. For example, in a Dutch study conducted in the late 1980s (Djjkstra, 1990) the median weekly indoor NO_2 average in homes with an unvented kitchen geyser was 72µg/m^3 while the median in homes with a vented geyser was 40µg/m^3. Households that cooked on a gas stove had higher indoor NO_2 levels than those who cooked on an electric stove. For example, British homes that cooked with gas had a weekly average mean of around 50µg/m^3 while homes that did not use gas for cooking had a mean of 21µg/m^3 (Venn, *20*03).

Studies published before 2000 tended to report higher indoor NO_2 levels than studies published after 2000. Eight of the 10 studies published before 2000 reported mean or median weekly or bi-weekly averages concentrations above 40µg/m^3. A Dutch study (Hoek, 1984) reported extremely high upper range values reaching levels above 700µg/m^3. Studies with extremely high measurements tended to report the presence of unvented geyser heaters (Hoek, 1984; Fischer, 1985; 1989), kerosene appliances (Berwick, 1989) or unflued gas heaters (Florey, 1979; Melia 1982).

Two Australian studies measured indoor NO_2 only when gas appliances were on (Pilotto, 1997; Smith, 2000) and these measurements tended to be higher than weekly averages. In Pilotto (1997) measurements ranged from 75µg/m^3 to 150.4µg/m^3 in homes with unflued gas appliances; in Smith the median ranged from 7µg/m^3 to 276ug/m3.

Ten studies (Magnus, 1998; Mukala, 2000; Mi, 2006; Zhao, 2008; Raaschou-Nielsen, 2010; Flamant-Hulin, 2010; Annesi-Maesano, 2012; Kim, 2011; Zhang X, 2011; Sarnat, 2012) did not report the presence of any gas appliances and indoor NO_2 levels tended to be low. For example, the weekly median indoor NO_2 in Danish children's bedroom was 7.5µg/m^3 (Raaschou-Nielsen, 2010), likely to be influenced by the absence of indoor NO_2 sources. However, when outdoor

NO_2 was high (above 80µg/m^3) indoor NO_2 levels tended to be above 40µg/m^3 (Mukala, 2000; Mi, 2006; Sarnat, 2012).

Health outcomes

Respiratory symptoms were assessed using questionnaires to be completed by a field worker at interview or health diaries to be self-completed or completed by parents (if participant was a child) at home. Questionnaires asked retrospectively whether a symptom or diagnosis had been present usually in the 12 months prior to interview and NO_2 monitoring. Diaries were used to keep a daily record of symptoms over a period that stretched from a couple of weeks (Hansel, 2008) to 18 months (Raaschou-Nielsen, 2010) during which NO_2 would be monitored periodically.

Studies tended to assess symptoms more frequently than lung function. Wheeze was the most common symptom to be assessed (23 studies), followed by cough (14), shortness of breath (10), upper respiratory symptoms (7), chest tightness (6); FEV_1 was the most common lung function measurement to be reported (8 studies).

Statistical analyses

The epidemiological measure of the outcome influenced the choice of statistical analysis. Studies that looked at a prevalence of a symptom or disease (i.e. asthma) over a period of time used logistic regression to estimate the odds ratio of having the symptom. Changes in lung function were estimated using linear regression.

When the symptom was recorded daily the study estimated the frequency using a multi-level mixed model (Pilotto, 1997; Gillispie, 2011; Belanger, 2013) or a generalised estimating equation approach (Samet, 1993; Mukala, 2000; Smith 2000; Triche, 2005; Hansel, 2008; Flamant-Hulin, 2010; Annesi-Maesano 2012; Sarnat, 2012; Hansel, 2013).

Two studies (Samet, 1993; Smith, 2000) considered the lag effects (0 day and 1 day) of indoor NO_2 exposure.

Health effect estimates were reported as a continuous association per increment of NO_2 (µg/m^3 or ppb) or by comparing the lowest exposure vs higher exposures, divided into percentiles.

Adjustment for confounders

As required by inclusion criteria all studies adjusted the analysis for at least one confounder: sex, age, some indicators of socio-economic status (mainly parental education if participants was a child or educational level if participant was an adult), exposure to active or passive smoking, parental history of asthma or atopy, history of respiratory disease, season when indoor NO_2 monitoring was carried out, indoor allergen level (pets, cockroaches, fungal spores) and presence of dampness or mould at home.

Fifteen studies did not adjust exposure to environmental tobacco smoke (ETS) or stratify for active smoking. The authors of a study conducted in children living in Boston argued that '*smoking (environmental tobacco smoke) is a source of NO_2 exposure*' (Belanger, 2006, pg 298) and therefore not included as a confounder. A Canadian study excluded ETS exposure after applying a stepwise regression approach to determine the confounders to include in the analysis (Carlsten *et al.*, 2011). Two studies (Pilotto, 1997; Samet, 1992) recruited only people who lived in non-smoking homes and as a consequence, analyses were not adjusted for ETS. Nine more studies (Florey, 1979; Berwick, 1989; Dijkstra, 1990; Garrett, 1998; Magnus, 1998; Venn, 2003; Tavenier, 2005; Raaschou-Nielsen, 2009; Sarnat, 2012) also did not adjust for exposure to smoking and no explanation was given. Two studies that excluded the effect of personal smoking by recruiting only non-smoking women (Triche, 2005) and former smokers (Hansel, 2013) did not adjust for ETS.

Studies that adjusted for other air pollutants will be discussed separately later on in Section 2.3.7 'Studies that adjusted for other air pollutants'.

2.3.4 Meta-analyses

Studies excluded from the meta-analyses

Of the fifty studies which were included in the systematic review only 17 could be incorporated into the meta-analysis. The remaining studies were not included in because:

- There were less than four studies that reported the same outcome. The outcomes are listed below:
  - chronic obstructive pulmonary disease (Osman *et al.*, 2007)
  - bronchial obstruction (Magnus, 1998)

- interaction with endotoxin (Matsui, 2013)
- exhaled nitric oxide (FeNO) (Flamant-Hulin, 2010)
- snoring (Zhang, 2004)
- FVC (Florey , 1979, Brunekreef, 1990)
- FEV_1 (Fischer, 1985; Fischer, 1989) but examining the same study population
- FEV1 % change (Brunekreef, 1990; Djikstra,1990 ; Neas, 1991)
- FEV1 % predicted (Jarvis, 2005; Gillespie-Bennett, 2011; Nitschke,1999)
- PEF (Fischer, 1985; 1989; Kattan, 2007)
- FEV_1/FVC % predicted (Jarvis, 2005).

- There were less than four studies that reported the same form of effect estimate (for the same outcome). The form of effect estimates are listed below:
  - exposure index (Simoni, 2002; Simoni, 2004)
  - duration of symptoms (Braun-Fahrlander, 1992)
  - change in daily symptoms score (Gillespie-Bennett, 2011)
  - incidence rate ratio of symptoms (Hansel, 2008)
  - occurrence of daily symptoms (Quackenboss, 1991)
  - relative symptoms rate (Samet, 1993; Nitschke, 2006).

- Estimates could not be standardised to 10μg/m^3 of NO_2 because the reported effect was not given in a continuous scale (Pilotto, 1997; Sunyer, 2004; van Strien, 2004; Triche, 2005; Kattan, 2007; Raaschou-Nielsen, 2010; Hulin, 2011)

In the following sections I am going to present the meta-analysis results starting with the most frequently reported outcome, 12-month prevalence of wheeze followed by prevalence of wheeze (any period prevalence), life-time prevalence of asthma, cough, shortness of breath (SOB), chest tightness and 'non-specific' respiratory symptoms

Twelve-month period prevalence of wheeze

Ten estimates for the association of 12-month prevalent wheeze with indoor NO_2 were identified in 8 studies (Garrett, 1998; Shima and Adachi, 2000; Belanger, 2003; Belanger *et al.*, 2003b; Mi, 2006; Zhao, 2008; Esplugues *et al.*, 2011; Kim, 2011). Individual strata estimates were entered for Shima and Adachi 2000 (strata - females and males) and for Belanger *et al* 2006 (strata - children with mother who was asthmatic and children whose mother was not asthmatic). Results

are shown in Fig 2.3. Most of the effect estimates were non-significant (P>0.05) and tended to be positive (with the exception of the estimate for boys in Shima and Adachi) but once combined the summary effects was positive and significant (OR for random effect 1.06, 95%CI 1.02, 1.12 per 10μg/m^3 of NO_2). There is no evidence of heterogeneity (I^2 0.0%, P=0.853) thus the fixed and random effects do not differ.

Publication bias was assessed visually with the use of funnel plot (Figure 2.4). The plot shows a cluster of studies on the low-right hand side of the plot. This indicates a cluster of small studies publishing effect estimates that are larger than the combined effect (the line in the middle of the plot). It suggests publication bias as small studies are more likely to be published if they report positive results. The result from the Egger's tests suggests some evidence of publication bias (P=0.032). Egger's test has more statistical power than the Begg's test and this may explain why the Begg's test did not find any evidence in publication bias (P=0.16).

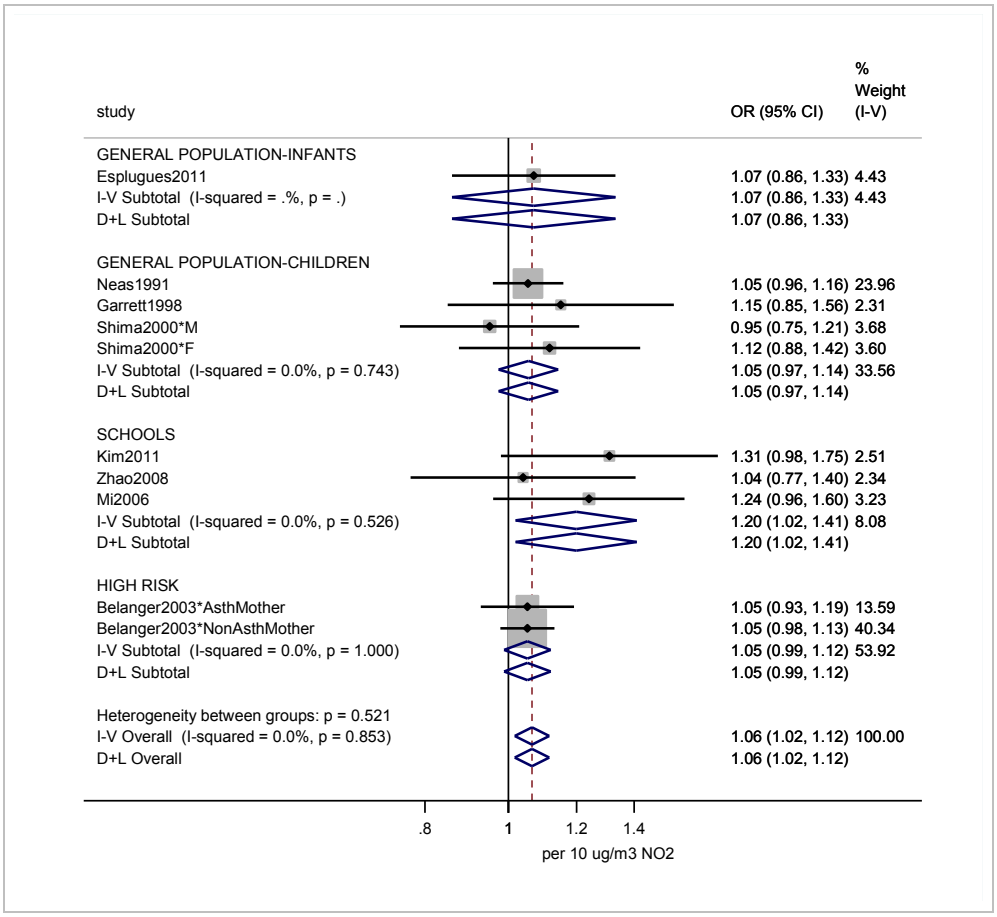


Figure 2.3 Forest plot and meta-analysis of estimates for 12-month period prevalence of wheeze and indoor NO_2 per 10µg/m3 increase stratified by type of study population

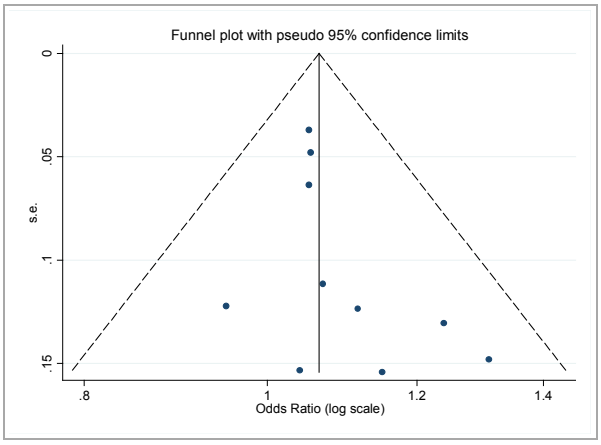


Figure 2.4 Funnel plot showing the estimates for 12-month period prevalence of wheeze and indoor NO_2 per 10 µg/m^3 increase

Prevalence of wheeze (all periods)

Five more studies (Hoek, 1984; Farrow, 1997; Smith, 2000; Emenius, 2003; Belanger, 2006) reported an estimate for prevalent wheeze using a time period other than 12 months. Stratified results were entered for Belanger (strata - living in single-family housing and multi-family housing) and Smith (strata –age groups). After adding the five studies the size of random effect decreased to 1.02 (95%CI 1.00, 1.03) per 10µg/m^3 of NO_2 (Figure 2.5).The estimate arising for the 0 to 14 year age group from Smith *et al*, contributed to more than 50% of random effect (59.6% weight), possibly largely contributing to the heterogeneity in the asthmatics sub-group (I^2 26.5 %, 6 estimates). The funnel plot (Figure 2.6) and the formal statistical tests suggest some publication bias (P value for the Egger's test=0.006; P value for the Begg's test=0.08). The estimates reported by Smith (2000) have very narrow confidence intervals; this could be explained by the increasing statistical power of repeated observations and that NO_2 was measured only when gas appliances were on.

Life-time prevalence of asthma

Seven studies reported an estimate for the lifetime prevalence of asthma and indoor NO_2 that could be standardised per 10µg/m^3 increment and combined for a meta-analysis (Figure 2.7). The random effect was 1.05 (95%CI 0.94, 1.17). The estimate from Hoek study had the largest weight (60.6%). There was moderate heterogeneity between the groups (I^2 42.0%, P=0.098) and within the school sub-group (69.3%). There was no evidence of publication bias (Egger's test P value=0.94 Brigg's test P value=0.92), but again the number of estimates was small (Figure 2.8).

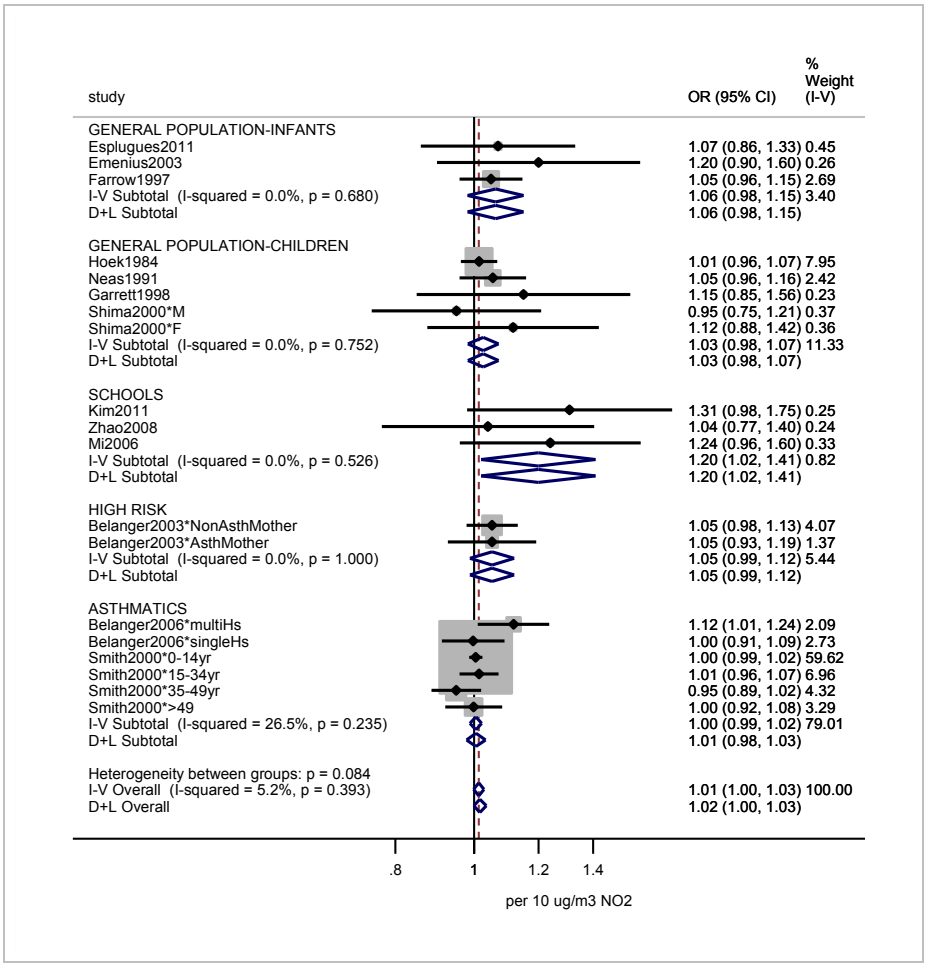


Figure 2.5 Forest plot and meta-analysis of estimates for prevalence of wheeze (all periods) and indoor NO_2 per 10µg/m^3 increase stratified by type of study population

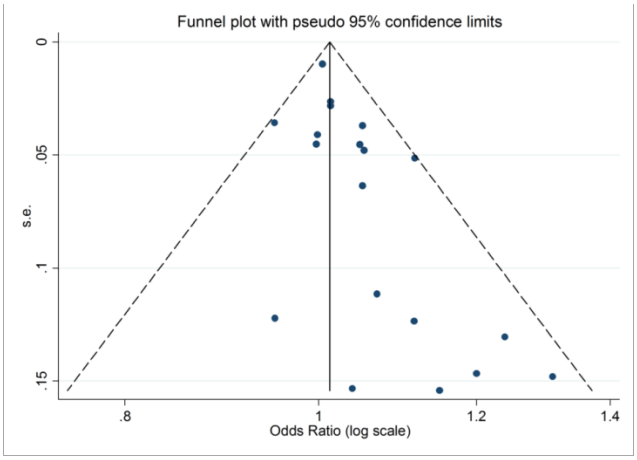


Figure 2.6 Funnel plot for prevalence of wheeze (all periods) and indoor NO_2

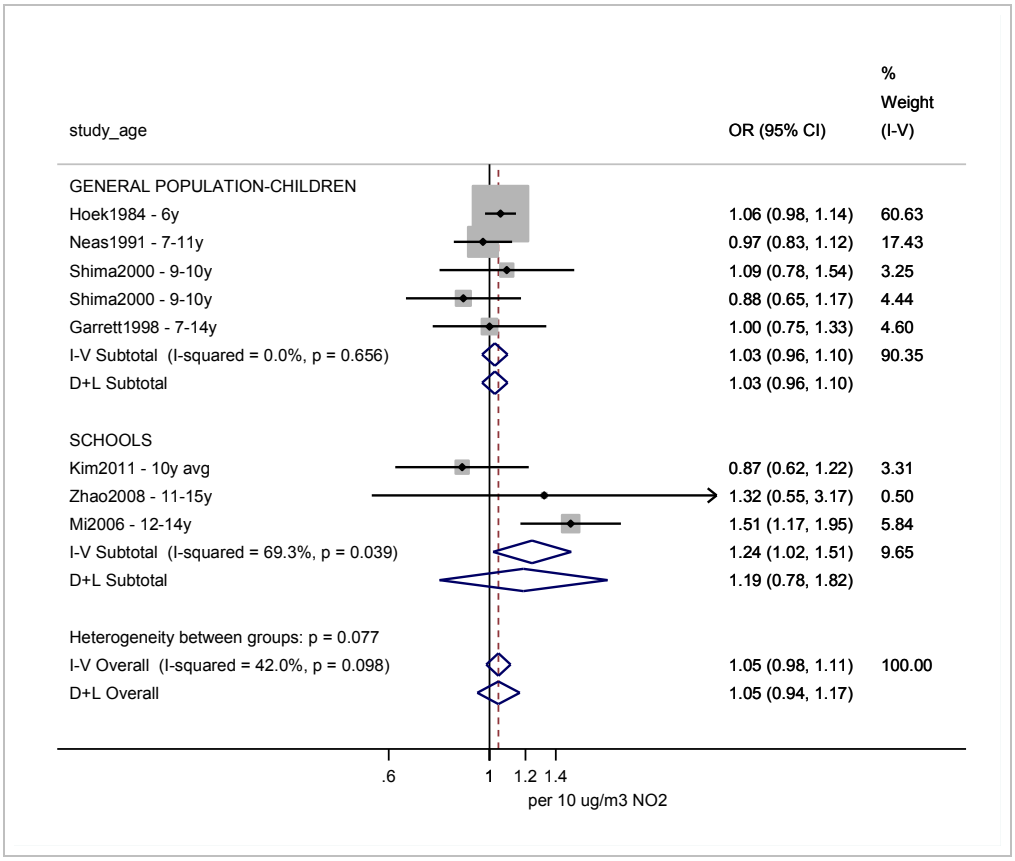


Figure 2.7 Forest plot and meta-analysis of the estimates for life-time prevalence of asthma and indoor NO_2 per 10µg/m^3 increase stratified by type of study population

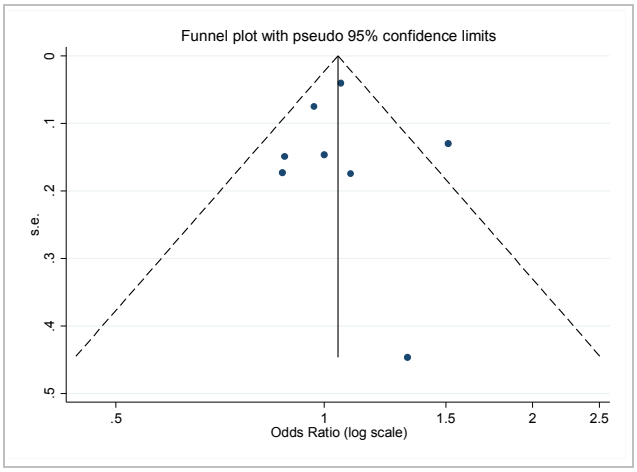


Figure 2.8 Funnel plot for life-time prevalence of asthma and indoor NO_2

Prevalence of cough

Figure 2.9 shows the forest plot of the estimates for prevalent cough (all periods) stratified by type of population study. It includes 8 studies and 13 estimates as 4 studies reported stratified results. The random effect is OR 1.01, 95% CI 0.99, 1.03 per 10μg/m^3 of NO_2. There was some moderate heterogeneity (I^2 29.0%, P=0.15), which was more obvious within some sub-groups (infants, children and high-risk sub-groups). Again, the estimates from the Smith study had a considerable weight in this meta-analysis (53.4% from the estimate in people aged 35-49 and 27.0% from estimate in people aged 0-14).The funnel plot and formal test for bias (P value for the Egger's test=0.20, P value for the Begg's test= 0.10) did not suggest any evidence of publication bias

Figure 2.10).

Prevalence of SOB

Figure 2.11 shows the forest plot of the estimates for SOB within each sub-group. Seven studies were included in the meta-analysis for prevalent SOB; the 11 estimates (three studies had stratified analysis) combined together gave a random effect of 1.01, 95% CI 0.99, 1.03 per 10μg/m^3 of indoor NO_2. There was some heterogeneity (I^2 23.4%, P value=0.22). As in the previous plots, the estimates from Smith's study, (age 35-49) had a large weight (89.65%). The funnel plot (Figure 2.12) suggested some publication bias (P value for Egger's test= 0.081, P value for Begg's test= 0.029).

Prevalence of chest tightness

Figure 2.13 shows the forest plot from 7 estimates arising from 3 studies included in the meta-analysis for the symptoms of chest tightness (prevalence determined over any time frame). The random effect was 1.02, 95%CI 0.99, 1.04 per 10μg/m^3 of NO_2. There was considerable heterogeneity (I^2 71.9%, P value=0.002). The funnel plot of Figure 2.14 does not suggest any publication bias although the number of estimates is too small to be able to draw any conclusions (P value for Egger's test= 0.478; P value for Begg's test= 0.23).

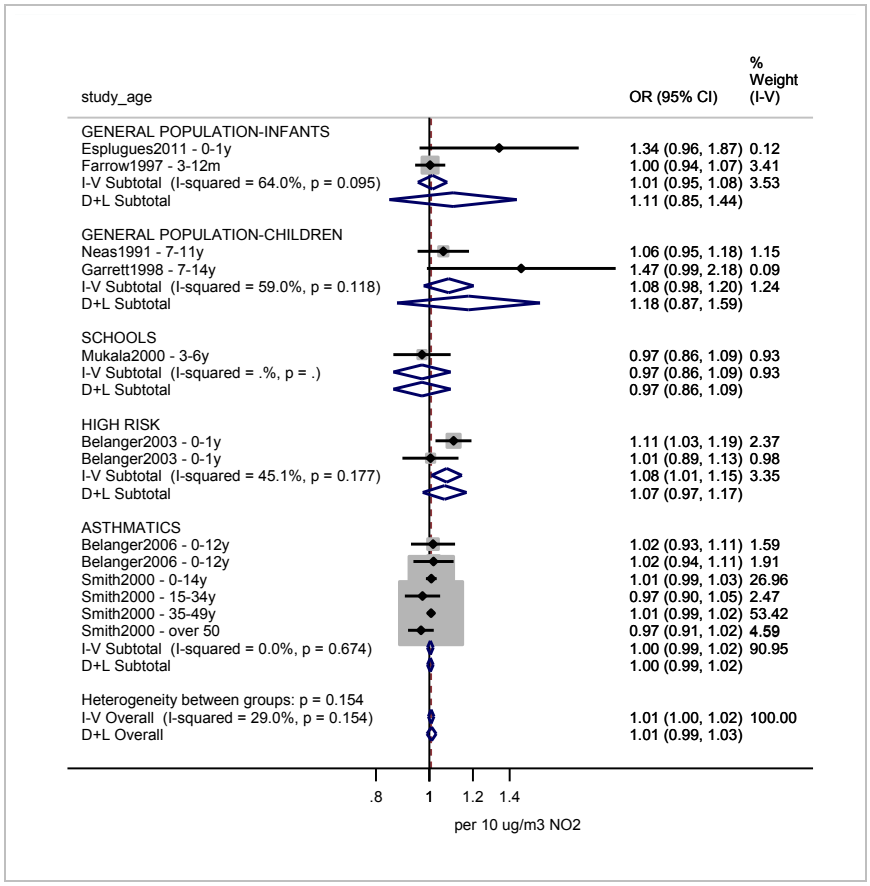


Figure 2.9 Forest plot and meta-analysis of estimates for prevalent of cough (all periods) and indoor NO_2 per 10µg/m^3 increase stratified by type of study population correct spacing

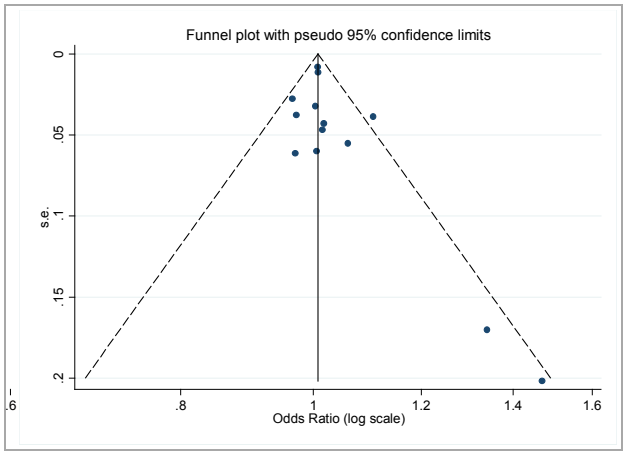


Figure 2.10 Funnel plot for prevalence of cough (all periods) and indoor NO_2

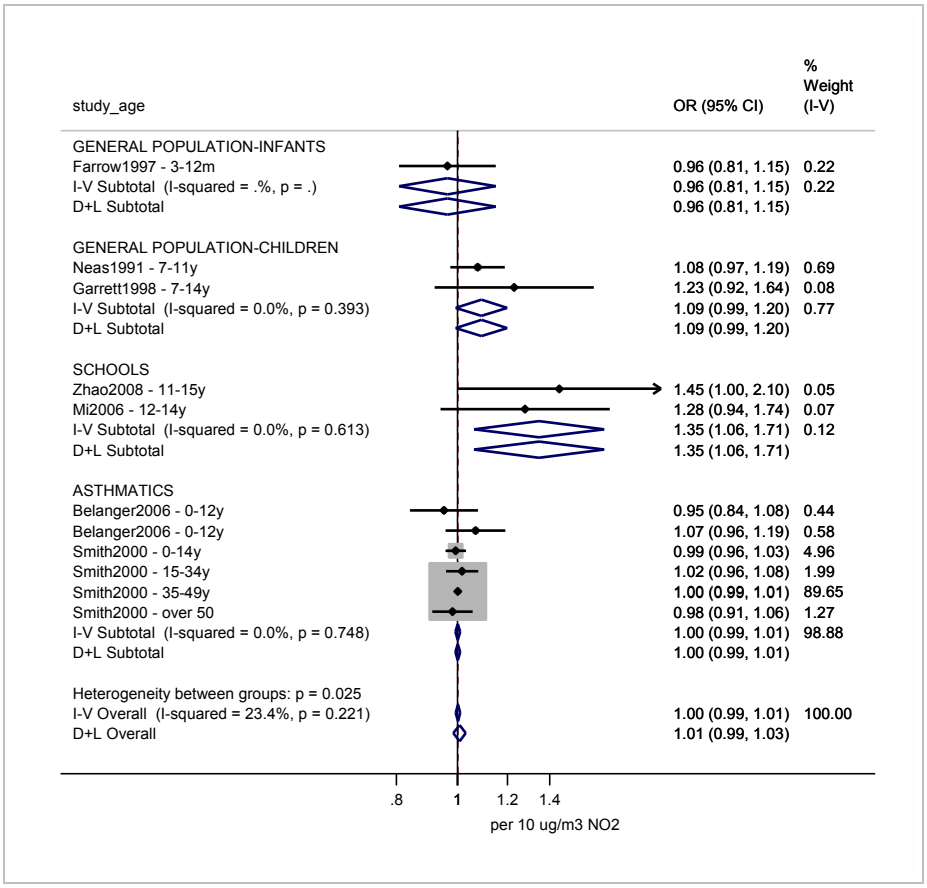


Figure 2.11 Forest plot and meta-analysis of estimates for prevalence of SOB (all periods) and indoor NO_2 per 10µg/m^3 increase stratified by type of study population

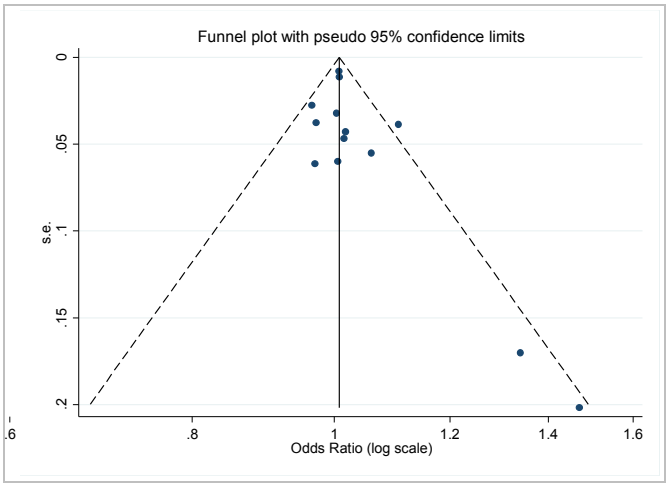


Figure 2.12 Funnel plot for prevalence of SOB (all periods) and indoor NO_2

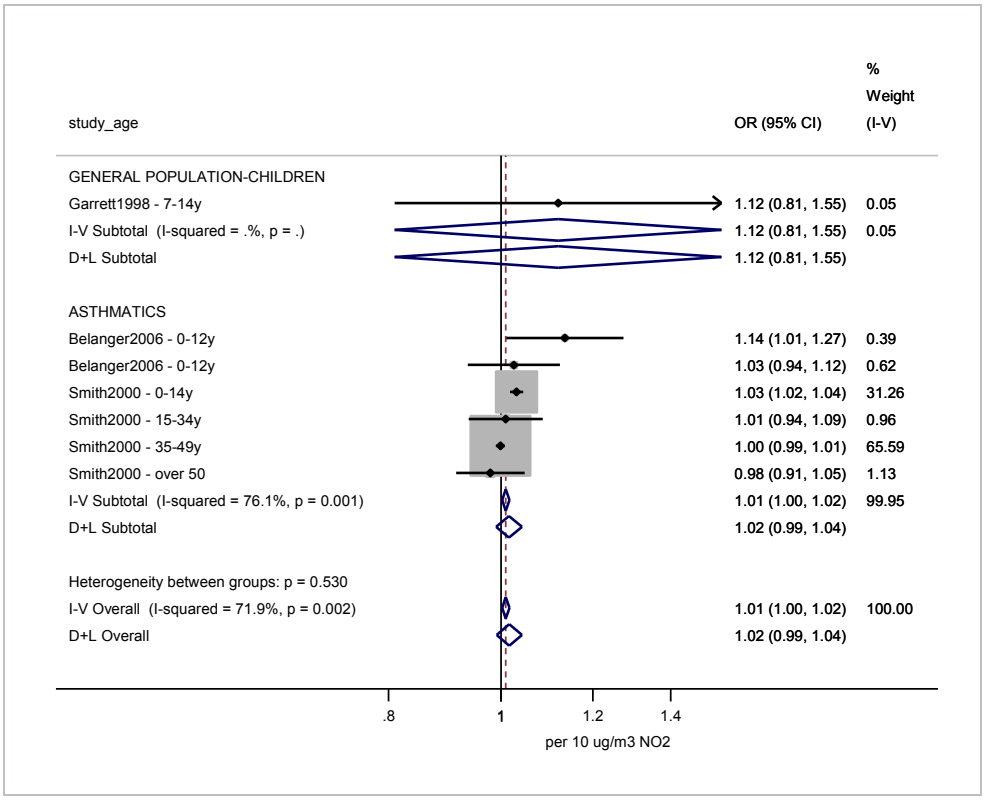


Figure 2.13 Forest plot and meta-analysis of prevalence of chest tightness (all periods) and indoor NO_2 per 10µg/m^3 increase stratified by type of study population

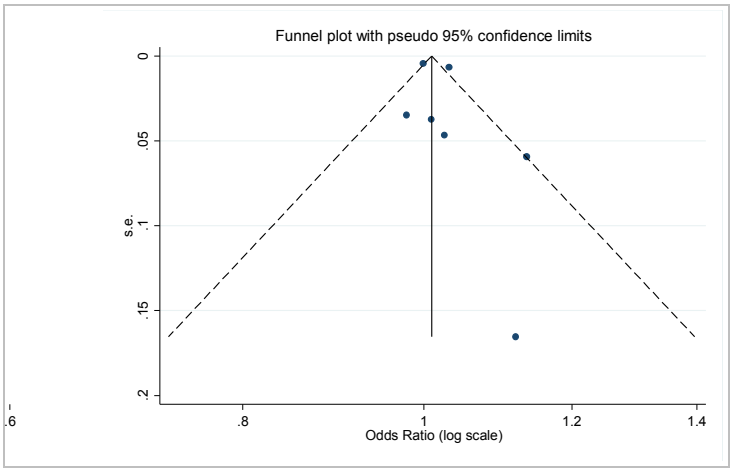


Figure 2.14 Funnel plot for prevalence of chest tightness (all periods) and indoor NO_2

Prevalence of non-specific respiratory symptom

Finally, as in Hasselblad the estimates of the association between 'non-specific' respiratory symptoms or disease and exposure to indoor NO_2 were meta-analysed (Figure 2.15). Eight studies were identified reporting estimates of non-specific respiratory symptoms. These were:

- Berwick (1989) – two or more of the following symptoms reported for one time period (12 weeks): fever, chest pain, productive cough, wheeze, doctor diagnosis of bronchitis, doctor diagnosis of pneumonia and asthma
- Dijkstra (1990) - any of the following: cough, wheeze and asthma
- Espluges (2011) - any episode of bronchitis, bronchiolitis or pneumonia during first year of life diagnosed by a doctor
- Garrett (1998) - presence of at least one of the following symptoms: cough, SOB, waking due to SOB, wheeze, asthma attacks, chest tightness, cough in the morning, chest tightness in the morning
- Melia (1980) - morning cough, day or night cough, or colds going to chest, or whether the child's chest ever wheezy or whistling, asthma attacks or bronchitis
- Melia (1982) - morning cough, day or night cough, or colds going to chest, or whether the child's chest ever wheezy or whistling, asthma attacks or bronchitis
- Neas (1991) - any of the following symptoms: SOB, chronic wheeze, chronic cough, chronic phlegm, bronchitis
- Shima (2000) - any chest illness ever diagnosed as bronchitis by a doctor
- Zhao (2008) - recent respiratory infections defined as either cold, upper respiratory infection, or middle ear infection in the preceding3 months.

The estimate reported by Melia (1980; 1985) and Dijkstra (1990) were tabulated as in Hasselblad (1992) (see section 2.3.1). The overall combined effect was 1.05, 95%CI 1.00, 1.10 per 10µg/m^3 increase indoor NO_2. There was considerable heterogeneity (I^2=41.3%, P=0.066). Visual inspection of the funnel plot (Figure 2.16) and formal statistical test did not suggest any evidence of publication bias (P for Egger's test=0.66, P for Begg's test=0.95).

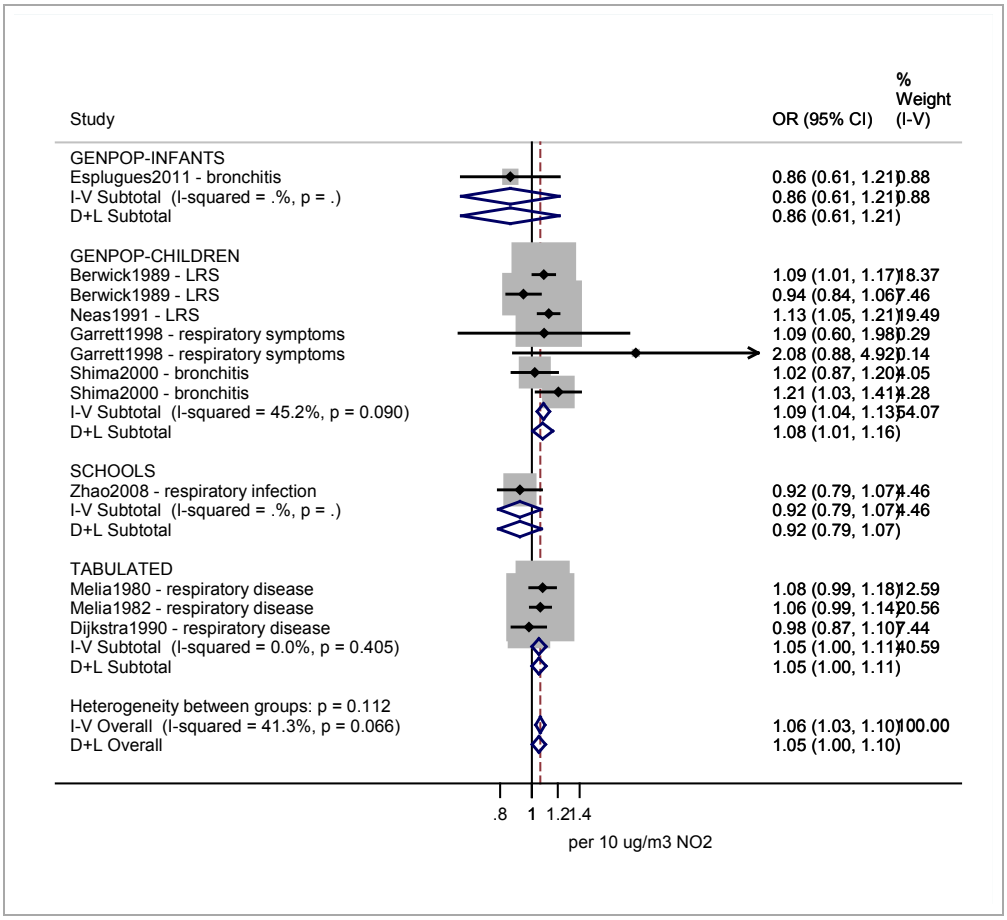


Figure 2.15 Forest plot and meta-analysis of the estimates for the prevalence of 'non-specific' respiratory symptoms and indoor NO_2 per 10µg/m^3 increase stratified by type of study population

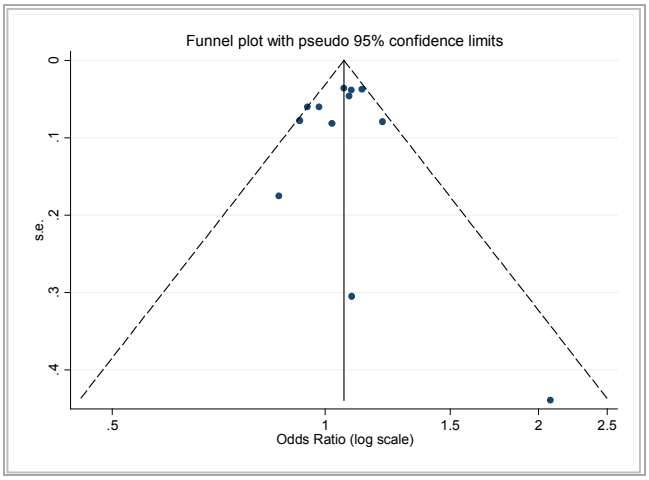


Figure 2.16 Funnel plot for the prevalence of any 'non-specific' respiratory symptoms and indoor NO

Summary of meta-analyses results

The results of each individual meta-analysis have been summarised in Figure 2.17. The results of meta-analysis for wheeze (both 12 months and any period prevalence) and 'non-specific' respiratory symptoms suggest there is a positive significant association of poor respiratory health and average exposure to indoor NO_2. Heterogeneity was present in all of the meta-analyses except for the one relating to the outcome of 12-month period prevalence of wheeze which also had sufficient number of studies to consider a sensitivity analysis worthwhile.

There were a small number of studies included in most of the analyses and so sensitivity analysis was restricted to the meta-analysis of 12-month prevalent wheeze.

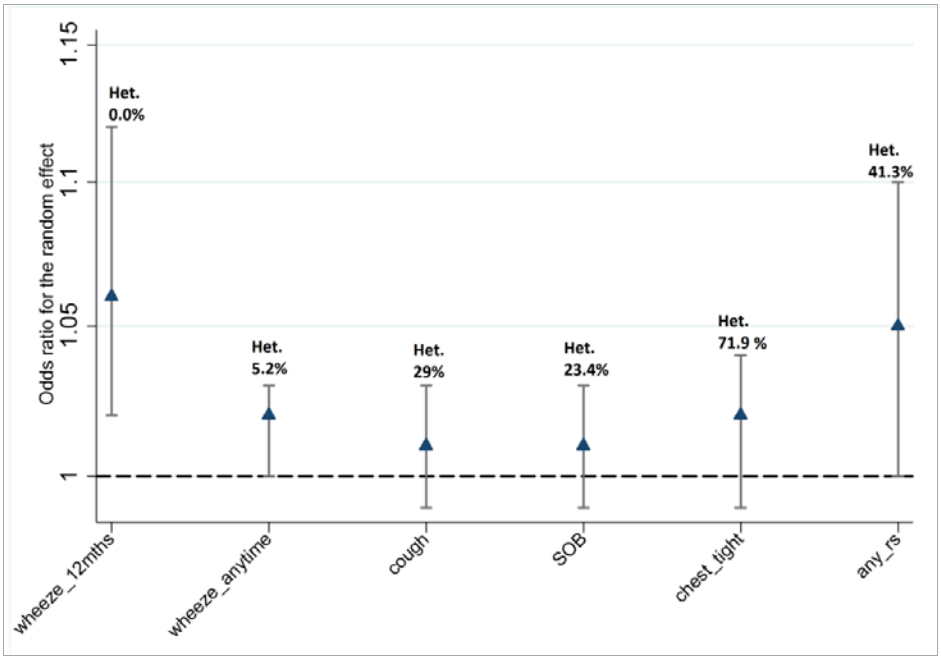


Figure 2.17 Graph plot summarising the random effect (odds ratio per 10 ug/m^3 increase of indoor NO_2) of the meta analyses (Het. = heterogeneity; any_rs=any respiratory symptoms)

Sensitivity analysis

Sensitivity analyses were carried for the meta-analyses of 12-month prevalence of wheeze and investigated the role of gas sources, the effect of indoor NO_2 in the general population and the effect of adjusting for ETS.

The role of indoor gas sources was investigated by removing those studies (Mi, 2006; Zhao, 2008; Kim, 2011) that did not report presence of any gas (or other fossil fuels) appliances

indoors. The random effect slightly decreased from 1.06 (95%CI 1.02, 1.12) to 1.05 (95%CI 1.00, 1.11). There was no heterogeneity between studies (I^2 = 0.0%). On the other hand, meta-analysis of estimates of studies in which there were no indoor gas sources showed a random effect of OR 1.20 (95%CI 1.02, 1.41). This may imply that outdoor NO_2 effect is separate from indoor NO_2 effect and that outdoor air was driving the association rather than emission from indoor gas sources.

The effect of indoor NO_2 exposure on the general population was investigated by removing those studies in high risk-groups, defined as having a family member with asthma (Belanger, 2003). The random effect slightly increased and the precision decreased (OR 1.08, 95%CI 1.01 to 1.16); there was no evidence of heterogeneity (I^2=0.0%, P= 0.73).

The effect of adjusting for environmental tobacco smoking was investigated by removing those studies that did not adjust for it (Garrett, 1998). There was no change in the random effect; the 95% confidence interval slightly decreased (OR 1.06, 95%CI 1.01 to 1.11); there was no evidence of heterogeneity (I^2 =0.0%, P value= 0.81).

2.3.5 Summary of significant findings reported by all studies

Since the majority of the studies could not be included in the meta-analyses a summary of the significance of findings reported by all studies is presented in Table 2.3. The table shows the direction of effect and significance of the reported associations. The studies are listed in alphabetical order by first author in order to facilitate browsing across the table. The table includes:

- Publication year
- The WHO region where the study was conducted
- Type of study population (general population, high-risk group, asthmatics or COPD-diagnosed)
- Age group of study population
- The outcome reported in the paper and the direction of the effect associated with indoor NO_2. The outcomes are divided into ten categories:
  - Asthma (ever asthma, doctor diagnosis of asthma, asthma attack, asthma severity)
  - Wheeze

  - Cough
  - SOB
  - Chest tightness
  - Upper respiratory symptoms (URS)
  - Other (e.g. composite of respiratory symptoms, lower respiratory tract infections (LRTI), asthma medication taken, FEV_1/FVC predicted %)
  - PEF
  - FEV_1
  - FVC.
- Other indoor or outdoor air pollutants which have been assessed for any association with respiratory health in the same publication. The direction of effect of the association is shown (outcome not listed).

The direction of the health effect is represented with the symbol "+", which stands for positive effect or the symbol "-", which stands for negative effect. Note that if there is an effect of indoor NO_2 on lung function (PEF, FEV_1, FVC, FEV_1/FVC %) the association is expected to have a negative direction, i.e. a decrease in lung function. Statistically significant associations ($P<0.05$) are indicated by a double "+" (if positive) or double "-" (if negative). Findings from stratified analysis are listed only if significant and are indicated with an 's' after the "++" or " - - "symbols; the stratum is specified in the footnote. Studies included in one of the meta-analyses are highlighted in grey.

Twenty-seven of the 50 studies reported a significant association of respiratory symptoms, diagnosis or lung function with exposure to indoor NO_2 in the study population, with 11 showing these associations only in a sub-group of the study population. Six of the 24 studies which examined the association of indoor NO_2 with wheeze reported a positive significant association, 4 studies (of 15) reported a positive significant association with cough, 4 studies (of 10) a significant association with SOB, 4 studies (of 7) with chest tightness, 6 studies (of 16) with asthma diagnosis or severity and 4 studies (of 9) reported a negative significant association with FEV_1 and 1 (of 5) a negative significant association with FVC.

There was a geographical variation in the number of publications reporting significant findings. Of the 23 studies conducted in Europe, only 5 reported significant associations between respiratory health and indoor NO_2 while among the 16 studies based in North-America (US and Canada) 13 published some significant findings.

Table 2.3 Table showing the significance of associations of respiratory health and indoor nitrogen dioxide and other air pollutants

Studies included in the meta-analyses are highlighted in grey
WHO regions: E-European region, A= Regions of Americas, SEA=South-East Asia Region, WP=Western Pacific Region (includes Australia, New Zealand, China, Korea, Japan, Hong Kong
Population: GP= general population; COPD= with a diagnosis of COPD; As= with a diagnosis of asthma; HR= at high-risk (i.e. having a relative with asthma)
Age group: C= children ; I= infants; A= adults
Other air pollutants: "ind"=indoor; "out"=outdoor; "pers"=personal
s =stratified results
Outcome: "++"= positive significant (p value<0.05) association; "+" = positive association not significant; "—"=negative significant association; "-"=negative association not significant; "s"=for stratified analysis only (see footnote for details of stratification); N.S.= non- significant (P value ≥0.05)

First Author	Year	WHO region	Population	Age group	Asthma	Wheeze	Cough	SOB	Chest tightness	URS	Other	PEF	FEV1	FVC	Other air pollutants
Annesi-Maesano	2012	E	GP	C	+					-					indPM$_{2.5}$ ++ indCH$_2$O++
Belanger	2003	A	HR	I		+	++s [3]								
Belanger	2006	A	AS	I		++s[4]	+	+	++s						
Belanger	2013	A	As	I	++[5]	++					++[6] ++[7]				
Berwick	1989	A	GP	I							++s[8]				
Braun-Fahrlander	1992	E	GP	I/C						+	+[9]				outNO$_2$++ outTSP++
Brunekreef	1990	E	GP	C								-	-	-	
Carlesten	2011	A	HR	C	+						+[10]				
Dijkstra	1990	E	GP	C	-	-	-					-	-	-	
Emenius	2003	E	GP	I		+									outNO$_2$++
Esplugues	2011	E	GP	I		+	+				+, -[11]				outNO$_2$++
Farrow	1997	E	GP	I		+	+	+	+	+	+				

[3] In children with non-asthmatic mothers;
[4] In multi-family housing;
[5] Asthma severity score;
[6] Night symptoms;
[7] Use of rescue medications;
[8] for lower respiratory symptoms , stratum: age>=7 year;
[9] Respiratory symptoms;
[10] Bronchial responsiveness;
[11] Positive for LRTI, negative for bronchitis;

First Author	Year	WHO region	Population	Age group	Asthma	Wheeze	Cough	SOB	Chest tightness	URS	Other	PEF	FEV1	FVC	Other air pollutants
Fischer	1985	E	GP	A									--	--	
Fischer	1989	E	GP	A								-	--s [12]		
Flamant-Hulin	2010	E	GP	C							++s [13]				indPM$_{2.5}$ ++, indCH$_2$O++
Florey	1979	E	GP	C										-	
Garrett	1998	WP	GP	C	+	+	+	+	+		+ [14]				
Gillespie	2011	WP	As	C		++	++			++	++ [15] + [16]	-	--		outNO$_2$ +
Hansel	2013	A	COPD	A							++ [17]				indPM$_{2.5}$ ++
Hansel	2008	A	As	C		++	++	++	++						
Hoek	1984	E	GP	C	+	+	+				+ [18]				
Jarvis	2005	E	GP	A							- [19]		-		indHONO -- outNO$_2$-
Kattan	2007	A	As	C	++s [20]						+	--s [21]	+		
Kim	2011	WP	GP	C	-	+									outNO$_2$++ out O$_3$ --, outUFP ++
Magnus	1998	E	GP	I							+ [22]				outNO$_2$ –
Matsui	2013	A	GP	C		--s [23]					-- [24]				
Melia	1982	E	GP	C						+					
Mi	2006	A	GP	C	++	+		-			++ [25]				ind/out O$_3$ – outNO$_2$++ out/indCH$_2$O +
Mukala	2000	E	GP	C			+								persNO$_2$++
Neas	1991	A	GP	C	+	+		+	+		++ [26]		-	-	PMn.a [27].

[12] In non-smoking women living in rural areas
[13] for FeNO; significant in non-atopic non-asthmatic
[14] For Respiratory symptoms
[15] Lower respiratory symptoms
[16] Preventer use
[17] COPD severity
[18] For bronchitis and symptoms combined together
[19] FEV_1/FVC %
[20] outcome: morbidity; stratum: non-atopic
[21] Stratum: cold season
[22] Bronchial obstructiveness
[23] Endotoxin interacting with high NO_2
[24] Exercise related symptoms
[25] Asthma medication and asthma attacks
[26] Lower respiratory symptoms
[27] Not reported, but estimates for NO_2 adjusted for PM

First Author	Year	WHO region	Population	Age group	Asthma	Wheeze	Cough	SOB	Chest tightness	URS	Other	PEF	FEV1	FVC	Other air pollutants
Nitschke	2007	WP	As	C	+	+	+	++[28]	++				--s[29]		
Osman	2007	E	COPD	A							+[30]				indPM$_{2.5}$ ++ in smokers
Pilotto	1997	WP	GP	C		+	+			++	++[31]				
Quackenboss	1991	A	As	C								-			indPM +outNO$_2$—
Raaschou-Nielssen	2009	E	HR	I		-									ind(PM$_{2.5}$, BS, NO$_X$, CH$_2$O) all N.S .
Samet	1993	A	GP	I		+	-			+	-[32]				
Sarnat	2012	A	As	C							++[33]				ind/outPM$_{10}$++, indPM$_{10-2.5}$++, indPM$_{2.5}$++, outBC++, outNO$_2$ ++
Shima	2000	WP	GP	C	+	+					++s[34]				outNO$_2$++
Simoni	2004	E	GP	A							++s[35]	++s[36]			indPM$_{2.5}$++
Simoni	2002	E	GP	A							++[37]	+			indPM$_{2.5}$++
Smith	2000	WP	As	C/A	+, ++s[38]	+,-	+, ++s[39]	+,-	+, ++s[40]						
Sunyer	2004	E	GP	C							-[41]				
Tavernier	2005	E	As	C	-										
Trishe	2005	A	GP	A		++s[42]									indSO$_2$++
van Strien	2004	A	HR	C		+	+	++s[43]							HONO
Venn	2003	E	GP	C		-									indCH$_2$0 N.S.

[28] Difficulty breathing
[29] Maximum kitchen exposure
[30] COPD morbidity
[31] For colds and absence from school;
[32] Lower respiratory symptoms duration
[33] eNO
[34] Bronchitis (significant in females but not in males)
[35] ARI (acute respiratory illnesses with fever), WFRI (chronic bronchitis and/or asthmatic symptoms without fever and without ARI, IRR (irritation without fever); significant only in winter in people with asthma or bronchitis
[36] in winter in people with asthma or bronchitis
[37] ARI (acute respiratory illnesses with fever),
[38] age<=14yr
[39] age 35-49 yr
[40] age<=14yr
[41] Lower respiratory tract infections and use of antibiotics
[42] 80ppb vs =<80ppb
[43] (cold season)

First Author	Year	WHO region	Population	Age group	Asthma	Wheeze	Cough	SOB	Chest tightness	URS	Other	PEF	FEV1	FVC	Other air pollutants
Vieira	2012	A	GP	C	++[44]										$indO_3$++ $outO_3$ N.S. $outNO_2$ N.S.
Zhang	2004	WP	GP	C							++[45]				
Zhang X	2011	WP	GP	C							++ [46] ++[47]				$indSO_2$++
Zhao	2008	WP	GP	C	++[48]			++							$indO_3$ ++, $ind/outCH_2O$++ $outNO_2$ N.S. $indSO_2$++

[44] Doctor diagnosis of asthma
[45] snoring
[46] Mucosal symptoms
[47] Symptoms better away from school
[48] Nocturnal attack

2.3.6 Studies not included in the meta-analyses

Studies in adults

Of the 17 studies incorporated in the meta-analyses only one (Smith, 2000) included any adults. The systematic review identified a further 8 studies in adults, but two pairs of studies (Simoni, 2002; 2004; Fischer, 1985; 1989) reported findings from the same study population. Four studies investigated the association of indoor NO_2 with respiratory symptoms (Smith, 2000; Simoni, 2002; Simoni, 2004; Triche, 2005), three studies with lung function (Fischer 1985; 1989; Jarvis, 2005) and two study with COPD severity (Osman, 2007; Hansel *et al.*, 2013).

Fisher (1985 and 1989) found a negative significant association with several lung function parameters and weekly average indoor NO_2 but not with lung function decline over 18 years of follow-up in women.

Smith (2000) examined the respiratory health effect of daily average NO_2 levels measured while gas appliances were on in asthmatics age 2 to 84. The lag effect at day 0 and day 1 were also examined. There was no association for wheeze or cough in adults except for cough at lag 1 in participants aged 35-49.

Simoni (2002) multiplied the average weekly indoor NO_2 level by the length of time spent indoors to estimate a daily index of NO_2 exposure (NO_2 -IndEx) and found a significant positive association with a combination of respiratory symptoms and daily PEF in winter in people with a chronic respiratory disease (bronchitis and asthma) living in a rural area. They went on to include an urban population, and overall having adjusted for area only acute respiratory illnesses with fever but not bronchitis/asthmatic symptoms were found to be significantly associated with daily index of NO_2 (Simoni, 2004).

Triche (2005) found a strong association with wheeze and indoor NO_2 in women exposed to weekly average level above 150µg/m^3(equivalent to the upper quartile) compared to women exposed to weekly average levels below 150µg/m^3 who lived in in Connecticut and Virginia (US).

Jarvis *et al.* (2005) measured NO_2 and nitrous acid (a gas combustion product as well as by-product of NO_2 reaction with water) in the kitchens in a sub- set of an adult cohort and found significant association with lung function decrements and nitrous acid but not indoor fourteen day average NO_2.

For more details on studies on lung functions see section below on 'Studies on lung function'; for details of studies on COPD see section 'Studies in people with COPD'.

Studies in people with asthma

Nine studies were in children with asthma (Quanckenboss, 1991; Nietsche, 1999; Belanger 2006; Kattan, 2007; Hansel, 2008; Gillespie-Bennett, 2011; Sarnat, 2011; Belanger 2013; Matsui, 2013) and one study in children and adults with asthma (Smith, 2000). Most of the studies assessed the association of indoor NO_2 with asthma morbidity by investigating:

- The frequency (i.e. number of days) of symptoms (Smith, 2000; Belanger, 2006; Nitschke, 2006; Kattan, 2007; Hansel, 2008; Gillespie-Bennett, 2011; Belanger, 2013)
- Acute visit to emergency rooms over an extended period (Matsui, 2013)
- Changes in asthma severity score (Gillespie-Bennett, 2011; Belanger, 2013)
- Changes in morning and evening FEV_1 (Gillespie-Bennett);
- Changes in FEV_1% predicted (Nitschke , 2006);
- Changes in FeNO (Sarnat, 2012; Matsui, 2013) over a certain period of time.

Respiratory outcomes were usually assessed at the same time as indoor NO_2 monitoring (Quackenboss, 1991; Smith, 2000; Nitschke, 2006; Hansel, 2008; Gillespie-Bennett, 2011; Sarnat, 2012, Belanger, 2013; Matsui, 2013) or before monitoring. Belanger (2006) assessed the prevalence of symptoms in the 12 months before monitoring and Kattan (2007) in the past 3 months.

Belanger (2006) found a significant association between weekly average indoor NO_2 and the presence and frequency of symptoms in asthmatic children who lived in multi-family housing but not in children who lived in single-family housing. In Connecticut and Massachusetts (US) where the study was conducted, multi-family housing is a characteristic of lower socio-economic status, higher proportion of gas stoves (54.6% compared to 23.5% single-family housing) and lower use of asthma maintenance medication (i.e. inhaled or systemic steroids, cromolyn sodium,long-acting β_2-agonists, leukotriene inhibitors). A more recent study by the same author (Belanger, 2013) reported findings of a longitudinal study with repeated monthly average levels of NO_2 and outcome assessment on a larger and more diverse population than the population in Belanger 2006. After adjustment for other risk factors, a significant dose-dependent increase in severity score, wheeze, night symptoms and rescue medication use was observed above a threshold of 11µg/m^3 of indoor NO_2. Another US study on an inner city children living in Baltimore (Hansel,

2008) found that an increase in 3-day average NO_2 was significantly associated with an increase in the number of days with limited speech, cough and nocturnal symptoms.

Significant associations between daily NO_2 from gas appliances and symptoms (chest tightness, asthma attacks, breathlessness on exertion) were also observed in younger asthmatic participants (age ≤14 years) living in Port Adelaide, Australia (Smith, 2000). No significant associations between respiratory symptoms and NO_2 from gas appliances in the older age group except for cough in participants age 35-49.

Sarnat (2012) examined FeNO in schoolchildren with asthma and found a positive association when considering the percentage change in FeNO and four-day average indoor NO_2 in children's schools.

Kattan (2007) reported a significant increase in the risk for asthma symptoms with increased weekly NO_2 average exposure only in children who did not have positive skin test responses (n=76 i.e. 16% of the whole cohort). Quackenboss (1991) found morning PEFR decreased in those asthmatic children with elevated NO_2 weekly average although the association was significant with outdoor NO_2 but not with indoor NO_2.

Nitschke (2006) investigated the dose-response relationship between asthma symptoms and indoor NO_2 (3-day average measured at school and home) and house dust mite (HDM) allergen (Der p 1) in children. Significant associations were found between school NO_2 levels and difficulty breathing during the day, at night and for chest tightness at night. Kitchen NO_2 levels were significantly associated with FEV_1. Stronger effects were observed in children sensitised to HDM. Matsui (2013) studied the same children population as in Hansel (2008) at an older age and found that endotoxin present indoors was positively associated with asthma morbidity (for maximum days with symptoms, exercise-related symptoms and wheeze/cough/chest tightness) in homes of low NO_2 weekly average exposure (cut off point of 38μg/m^3); the association tended to be 'protective' in homes with high NO_2 exposure suggesting an interaction between household endotoxin and NO_2 (but the interaction was not observed with PM).

Gillespie-Bennett (2011) found that high NO_2 (4-weekly average equal or above 15.9μg/m^3) was significantly associated with greater daily reports of lower an upper respiratory tract symptoms and daily evening and morning FEV_1 (but not PEFR) during winter.

Studies in people with COPD

Two studies examined whether indoor NO_2 exposure exacerbates COPD symptoms in people diagnosed with COPD. Osman (2007) assessed the health status of participants using the St George' Respiratory Health Questionnaire (which assesses symptoms, activity limitation and disease impact) and found significant association with indoor $PM_{2.5}$ but not with weekly average indoor NO_2. Levels of indoor $PM_{2.5}$ were four times higher in smoking households (49% of participants) than in non-smoking households while there was no significant difference of indoor NO_2 levels in smoking and non-smoking households. Hansel 2013 measured averaged 1-week NO_2 at baseline, 3 months and 6 months during which participants completed a spirometry test and a respiratory symptoms questionnaire. Hansel (2013) found that increase in NO_2 concentrations in the main living area was associated with worse dyspnoea and an increase in bedroom NO_2 concentrations was associated with increasing nocturnal symptoms and COPD exacerbations after adjusting for passive smoking. No significant association was found with % predicted FEV_1 and NO_2 concentrations and no effect modification was found when including indoor $PM_{2.5}$ into the model. The potential for these associations to be driven by the ingress of outdoor NO_2 (and other traffic related pollutants) cannot be dismissed as 61% of participants lived 7.6 meter or less away from the kerb.

Studies on lung function

The association between lung functions in children and indoor NO_2 was assessed in 8 studies (Florey, 1979; Brunekreef, 1990; Djikstra, 1990; Neas, 1991; Quanckenboss, 1991; Nitschke, 1999; Kattan, 2007; Gillespie-Bennett, 2011), four of which reported significant findings. Quackenboss (1991) measured peak expiratory flow rate for up to four times a day during two-week study period and found a significant reduction in morning PEFR in children with a current diagnosis of asthma but not in the remaining non-asthmatic cohort. In a secondary analysis of a randomised community trial in asthmatic children Gillespie-Bennett (2011) observed a significant association with a decrease in morning and evening FEV_1 but not with the evening and morning PEFR. A significant negative dose-response relationship between percentage predicted FEV_1 and kitchen NO_2 (0.4% per 18.8µg/m^3 increase) but not in schools was also observed in asthmatic children by Nitschke (1999). Kattan (2007) observed a decrease in PEF associated with high levels of NO_2 in non-atopic children during the cold months.

Morning and evening PEF was tested in a case-control study (Venn, 2003), the cases being the children with persistent wheezing. No significant differences between the two groups were found. In three large studies with more than 800 children aged 5-11 lung functions was measured and

association with weekly average indoor NO_2 assessed. Florey (1979) measured $FEV_{0.75}$ in 808 children of age 6-7; Neas (1991) measured several lung function (FVC, FEV_1, FEV_1/FVC, $FEV_{0.75}$, $FEF_{25-75\%}$, FEV_{25-75}/FVC) in more than 1,500 children aged 7-1; Brunekreef (1990) and Dijkstra (1990) measured FVC, FEV_1, PEF, MMEF in 800 children over a 2-year period. None of the studies found any significant association with lung function.

Five studies assessed the association between lung function in adults and indoor NO_2. In a cross-sectional analyses of a sub-sample of 97 non-smoking women, Fischer (1985) found some significant negative associations between indoor NO_2 exposure and several lung function parameters (IVC, FEV_1, FVC, PEF, MEF_{75}, MEF_{50}, MEF_{25}, MMEF) as measured in 1982 and no significant association with lung function decline since the start of the study in 1965. In a later study (Fischer, 1989) significant associations were found between exposure to NO_2 and lung function (FEV_1, PEF, MMEF, IVC) only among the non-smoking women living in rural area compared to smoking women living in rural area and in both smoking and non-smoking women living in urban areas.

Simoni (2002) found significant associations between daily peak flow and daily indices of exposure to indoor NO_2 only in subjects with chronic respiratory disease (i.e. asthma and bronchitis) and only in winter. When the sample was enriched with adults living in an urban environment (Simoni, 2004) no significant association between PEFR and the daily indices for indoor NO_2 were found.

Jarvis (2005) observed significant decrease in percentage FEV_1/FVC% and FEV_1 predicted % associated with HONO but not with indoor NO_2.

Studies on FeNO

Among the three studies that measured exhaled nitric oxide (FeNO), only one study (Sarnat, 2012) found a positive significant association between raised FeNO and increasing indoor NO_2 levels. Sarnat (2012) measured FeNO every week for 16 consecutive weeks in 58 children with asthma. NO_2 was measured over a 96-hour period inside and outside the 4 children schools. Significant associations were also found between FeNO and outdoor NO_2. The study did not mention the presence of any gas appliances in the school (traffic being the source of NO_2).

Flamant-Hulin (2010) measured NO_2 in children's classrooms and did not find any significant difference in FeNO between the low and the high NO_2 exposed groups (defined with respect to

the third quartile value of 5-days average concentrations) within the asthmatic and non-asthmatic children. In a sub-analysis within the non-asthmatic children stronger associations were found in children who were atopic (defined as being positive to at least one of the most common aeroallergens) but the sample size was quite small (n=17). No presence of gas sources in the classroom was reported.

Matsui (2013) measured FeNO in 150 children aged 5-17 every three months over one-year period. Weekly average indoor NO_2 and airborne endotoxins were sampled within 2 weeks of the clinic visit. The respiratory effect of exposure to household endotoxin was found to be modified by co-exposure to indoor NO_2 (as well as indoor air nicotine) and airborne endotoxin being protective in home with high NO_2 (≥38μg/m^3).

2.3.7 Studies that considered other air pollutants

Twenty-two studies assessed the respiratory health effect of other indoor and outdoor air pollutants. The most common air pollutants to be assessed were outdoor NO_2 (14 studies), indoor PM (8 studies) and indoor formaldehyde (6 studies) (see Table 2.3). Six studies adjusted their analyses for the confounding effects of other air pollutants (PM, outdoor NO_2 or HONO).

PM

Four studies (Simoni, 2004; Hansel, 2008; Flamant-Hulin, 2010; Sarnat, 2011) found that both indoor NO_2 and indoor PM were significantly associated with respiratory health. One study (Annesi-Maesano, 2012) found that levels of $PM_{2.5}$ but not NO_2 in children' classrooms were significantly associated with children's respiratory health. One study in smokers with a COPD diagnosis (Osman, 2007) found significant associations with COPD exacerbation and indoor $PM_{2.5}$ but not indoor NO_2. One study (Raaschou-Nielsen, 2010) did not find any significant association with respiratory symptoms and indoor NO_2 as well as indoor $PM_{2.5}$.

Two studies adjusted the analysis for indoor particles.

After adjusting for $PM_{2.5}$ Hansel (2008) found the association between 3-days average indoor NO_2 concentrations and asthma symptoms was not 'meaningfully' altered; the incidence rate ratio (IRR) for daytime wheezing, coughing or chest tightness changed from unadjusted estimate of 1.05 (95% CI 0.99, 1.12) to 1.03 (95% CI 0.96, 1.11) after adjusting for $PM_{2.5}$ and several

other confounders (season of sampling, age, sex, race, mother's educational level), which makes difficult to interpret the effect of adjusting for $PM_{2.5}$.

One study (Neas, 1991) adjusted for indoor PM_{10} but did not comment on the changes in the estimated effect before and after adjustment for particulate.

Indoor PM_{10} was included in the stepwise regression procedure carried out by Matsui (2013) to identify the 'best' multi-variate model but it was excluded from the final model because no strong association was observed.

Outdoor NO_2

Two studies (Mi, 2006; Sarnat, 2011) found a significant association with respiratory health and outdoor NO_2 as well indoor NO_2. Three studies (Zhao, 2008; Gillespie-Bennett, 2011; Vieira 2012) observed a significant association with indoor NO_2 but not with outdoor NO_2. Six studies (Quanckenboss, 1991; Braun-Farhlender, 1992; Shima, 2000; Emenius, 2003; Kim, 2011, Espluges, 2013) found that outdoor NO_2 was significant associated with respiratory health but not indoor NO_2. Two studies (Magnus, 1998; Jarvis, 2005) found no significant associations with indoor as well as outdoor NO_2.

One study adjusted for outdoor NO_2 but did not comment on the changes before and after adjustment (Shima, 2000). One study (Gillispie-Bennett, 2011) found that the estimated effect size of indoor NO_2 on symptoms and lung function was not significantly reduced after adjustment for outdoor NO_2.

HONO

Two studies adjusted analyses for indoor HONO. After adjustment, one study (VanStrien, 2004) concluded that NO_2 but not HONO was associated with reduced lung function in infants in disagreement with findings of the other study in adults (Jarvis, 2005) that HONO was significantly associated with decrement in lung function (FEV_1 and FEV_1 /FVC) but not indoor NO_2. The study observed a strong correlation between measures of indoor NO_2 and HONO (r = 0.77), likely to be explained by the fact that indoor HONO is formed by the reaction of NO_2 with surface water.

2.3.8 Studies that reported stratified analyses and effect modifications

Atopic status

Eight studies stratified analysis by atopic status. A study (Hansel, 2008) in asthmatic children living in Baltimore (US) found that atopic children were more likely to experience nocturnal symptoms associated with indoor NO_2 than non-atopic children. A Canadian study in a high-risk birth cohort of children (Carlesten) examined the combined effect of sensitisation to Can-f1 (an allergen associated with dog exposure) and indoor NO_2 and found that children sensitised to Can-f1 had an increased risk of asthma when exposed to NO_2 levels above 22μg/m^3 relative to having neither of such exposures. A study (Nitschke, 1999) in asthmatic children living in Adelaide, Australia found that those sensitised to HDM had a higher risk of having asthma symptoms when exposed to indoor nitrogen dioxide but interaction was significant only for wheeze at night.

A large cross-sectional study (Annesi-Maesano, 2012) that included children living in six French cities (The French Six Cities Study) and measured exposure to classroom NO_2 found that the association of asthma in the previous year was stronger in atopic children than non-atopic children. In contrast to this finding, a significant negative association between FeNo levels and classroom NO_2 was observed in non-atopic children (but not in atopic children) in a sub-sample of the same study (Flamant-Hulin, 2010). A stronger and significant association of asthma morbidity with indoor NO_2 in non-atopic children than atopic children was also observed in the National Co-operative Inner-City Asthma Study (NCICAS) that included 8 inner-cities in US (Kattan, 2007).

No significant difference in the risk of having asthma-related symptoms on exposure between atopic and non-atopic children was observed in an Australian study set in Victoria (Garrett, 1998). No difference in risk of having wheeze between atopic and non-atopic children was also found in an English case-control study (Venn, 2003).

Sex

Stronger associations of respiratory symptoms with indoor NO_2 were found in girls than in boys by Melia (1980, 1982 - respiratory illness), Shima (2000 - wheeze, bronchitis, and asthma) and Neas (1991 - annual cumulative incidence of lower respiratory symptoms). Florey (1979)

presented results for boys and girls separately but found no significant associations in boys or girls. Dijkstra (1990) found no consistent pattern in boys as well as girls for lung functions.

Finally of note, in two studies participants were exclusively females. Triche (2005) found that women exposed to concentrations higher equal or higher than 150µg/m^3 (top quartile) of indoor NO_2 had higher risk of wheezing compared to women exposed to levels below 150µg/m^3 and Fisher (1989) found an association with lung function and NO_2 only in non-smoking women living in rural areas.

Seasonality

Some studies investigated whether the effect of indoor NO_2 varied in the cold and warm season. Garrett found that the association with respiratory symptoms was stronger in the summer than in winter but Mukala (2000) and Simoni (2002) showed the opposite. Mukala observed that the risk of cough associated with high NO_2 levels increased significantly in winter compared to summer. Simoni (2002) found that acute respiratory symptoms in adults living in a rural area were significantly associated with daily indices of NO_2 (a combination of spatial and temporal exposures) in winter but not in summer. Kattan (2007) did not find any difference in asthma morbidity between winter and summer with the exception that peak flow was lower in winter than summer when NO_2 levels were high (59.8µg/m^3). Van Strien (2004) found that the association with wheeze was significant only in spring/summer while persistent cough and shortness of breath were significant in autumn/winter but not in summer.

Smoking/ETS

Only one study stratified results by smoking status (Fisher, 1989). Negative associations between pulmonary function and exposure to NO_2 were observed in non-smoking women, particularly those living in rural areas but not in smoking women.

Neas (1991) found that the NO_2 effect on respiratory symptoms appeared to be stronger among children with current domestic exposure to passive cigarette smoke (OR 1.48, 95% CI 1.19 to 1.84) compared to those in non-smoking homes (OR 1.22, 95% CI 0.89 to 1.66) – the implication being that there could be an interaction between smoking and NO_2 or that there could another component in second-hand tobacco smoke that affects respiratory symptoms, for example particulate.

Emenius (2003) observed that the risk of having recurrent wheezing (OR 1.31, 95% CI 0.49 to 3.47) in infants exposed to the highest quartile (>15.6 μg/m^3) of indoor NO_2 compared to those in the lowest quartile (<8.4μg/m^3) increased when they were also exposed to ETS (OR 3.10. 95% CI 1.32 – 7.30).

Of note, Osman (2007) found that indoor $PM_{2.5}$ but not indoor NO_2 levels were higher in smoking households compared to non-smoking households levels (four times higher).

Other

A study (Matsui, 2013) in children living in Baltimore, US found that the effect of indoor NO_2 on asthma was modified by the level of household airborne endotoxin and vice versa, the effect of household airborne endotoxin on asthma was modified by the level of indoor NO_2. It is not clear the direction of the modifying effects.

Contrary to common knowledge that individuals with a family history of asthma are more at risk a birth-cohort study (Bellanger, 2003) with at least one sibling with asthma living in Massachusetts, US found that children were more likely to have persistent cough with increasing weekly average indoor NO_2 but only if the mother did not have a history of asthma compared to those whose mother had a history of asthma. The authors suggested that specific gene-environment interactions might help to explain these differences.

The asthmatic siblings of these children were later assessed for symptoms (Belanger, 2008); those who lived in **multifamily housing** had a significant higher risk of having wheeze and chest tightness than those who lived in single-family housing and being exposed at same levels of indoor NO_2. The authors suggest as possible explanations that in single-family housing indoor NO_2 levels tend to be lower, home size larger and children's bedroom is more likely to be on a different floor than kitchen compared to multifamily housing homes. However, it cannot be ruled out that exposure to indoor NO_2 is simply a marker for poor housing conditions and that any association between NO_2 and respiratory symptoms indicates a risk from poor housing.

2.3.9 Other studies of note mentioning but not included in this systematic review

Three intervention trials (Pilotto, 2004; Howden-Chapman, 2008; Marks, 2010) were excluded from the systematic review as they did not meet the inclusion criteria (study must report a health effect estimate for indoor NO_2 and respiratory health). However, they still deserve to be

mentioned because of their superior study design, i.e. randomised controlled trial of unflued gas heaters, the control which produces high levels of indoor NO_2 against flued gas heaters, the intervention, which produces less NO_2.

One Australian study (Pilotto, 2004) and one New-Zealander study (Howden-Chapman, 2008) conducted heating intervention trials in children's classrooms and children's homes respectively. Both studies observed an improvement in respiratory symptoms in the intervention group but no differences in lung function or airways hyper responsiveness in children with asthma (Pilotto, 2004). The groups were randomised but at baseline intervention homes had significantly lower concentrations of indoor NO_2 (8.5µg/m^3) than control homes (15.7µg/m^3) (Howden-Chapman, 2008). After intervention average indoor temperature was higher (0.57°C) in the intervention homes than in the control homes, likely to be explained by the fact that the unflued heaters are more efficient in warming up a home. A warmer indoor temperature may have also contributed to the health improvement. Another limitation of both studies was that they were not blinded since participants were aware which type of heater had been allocated to and significant improvements were observed for subjective outcomes (self-reported symptoms) but not for objective outcomes (lung function and airways hyper-responsiveness). The authors acknowledged that there could be an element of reporting bias (Howden-Chapman, 2008).

To minimise the risk of reporting bias a double-blind cluster-randomised crossover study was conducted in 22 schools in New South Wales, Australia (Marks, 2010). Classrooms were fitted with both types of heaters. The existing flued heater was left *in situ* and the new low-NO_x flued heater was surrounded with a screen (the blind component of the trial). During the study period either the flued or the unflued heaters were functioning (the cross-over component). Nitrogen dioxide concentrations were, on average, 1.8 times higher during exposure to unflued gas versus flued gas heaters. Results were similar to the previous two studies, that children exposed to unflued gas heaters were more likely to report respiratory symptoms (OR for wheeze reported in the morning 1.38; 95% CI, 1.04–1.83) but no difference in lung function was observed. Besides, results suggested that the association of wheeze with NO_2 was greater in atopic children, defined as those children sensitised to any of the following: house dust mice, cockroach, cat, *Alternaria* , *Aspergillus*, rye grass and a grass mix.

2.4 Discussion

This literature review identified 50 studies that reported some associations between respiratory health and indoor NO_2 (measured). The most common outcome to be assessed was prevalence of wheeze. Studies in asthmatics tended to assess the health effect of indoor NO_2 exposure as the daily frequency or duration of a symptom over a period of time rather than prevalence. Indoor NO_2 was measured in the room where the participants spent most of their time, living area or bedroom. Some studies measured indoor NO_2 in children's classroom because it was considered the place where they spent most of their day. Monitoring was carried out with passive diffusion samplers and indoor NO_2 was reported as an average (mainly weekly). As expected indoor NO_2 levels tended to be high in homes with a gas stove or unflued gas heater. Studies published before 2000 tended to report higher concentrations, mainly determined by the presence of water heating gas appliances (geyser), which were widespread in Dutch homes at that time and use of unflued gas or paraffin or kerosene appliances for heating.

Nearly all studies reported positive associations between respiratory outcomes and indoor NO_2, with over half of the studies reporting at least one significant estimate. Studies were very heterogeneous in design, exposure and outcome assessments and statistical methodology, making it difficult to combine the findings using meta-analysis. Only seventeen studies could be included in any meta-analysis. Results of the meta-analyses for 12 month period prevalence of wheeze (OR for random effect 1.06, 95% CI 1.02 to 1.12 per 10µg/m^3 change of indoor NO_2) confirmed the findings of Hasselblad's meta-analysis (OR for random effect 1.08, 95% CI 1.01 to 1.16 per 10µg/m^3) conducted more than 20 years ago

2.4.1 Meta-analysis of the effect of indoor nitrogen dioxide and gas cooking on asthma and wheeze in children published in 2013

While this work was ongoing a systematic review and meta-analysis of the effect of indoor NO_2 and gas cooking on asthma and wheeze in children was published (Lin *et al.*, 2013). The meta-analysis included 41 studies in children that looked at the effect of indoor NO_2 on current and lifetime asthma and wheeze. It included only studies on indoor NO_2 generated from gas cooking or its surrogate (use of gas for cooking). Ten estimates from 7 studies were included in the meta-analysis for current wheeze as only those studies that measured household levels of indoor NO_2 generated from gas cooking were considered. Studies that measured indoor NO_2 in schools or at

selected times of the day when gas appliances were on or those that combined personal and indoor NO_2 exposure were excluded.

Results from the Lin meta-analysis were concordant with Hasselblad and my findings, i.e. indoor NO_2 is associated with current (i.e. prevalent) wheeze in children (OR for random effect 1.05, 95%CI 1.02 to 1.08 per 10μg/m^3 change). In disagreement with my findings that there was evidence of some publication bias the authors did not find any. In agreement with the results of my meta-analysis for asthma prevalence the combined effect of indoor NO_2 on current and lifetime asthma was not significant (OR for random effect 1.03, 95%CI 0.97 to 1.09), probably due to the small number (n=5) of estimates included in the meta-analysis.

The meta-analysis of the effect of gas cooking included a larger number of studies. Results suggested that there is a significant association between the use of gas cooking and current wheeze (OR for random effect 1.05, 95%CI 1.01 to 1.10) and between gas cooking and current and lifetime asthma (OR for random effect 1.32, 95%CI 1.18 to 1.48).

2.4.2 Publication bias and quality score

Visual assessment of funnel plot and formal test for publication bias of the meta-analysis for 12-months prevalence of wheeze suggested evidence of some publication bias, i.e. results from small studies were more likely to be positive than we would expect by chance.

Publication bias is a widely recognised problem limiting and influencing the results of a meta-analysis. It has been suggested that results from meta-analysis of observational studies should be considered cautiously (Egger *et al.*, 1998). In observational studies confounding and precision of measurements may cause a relation between study size and effect estimates (Sterne *et al.*, 2011). For example, larger observational studies might use self-reported symptoms which are more error prone, while smaller studies may use more precise measuring instruments (e.g. lung functions measures). Sensitivity analyses that investigate the influence studies have on the meta-analysis results are recommended, particularly if any heterogeneity across the studies has been observed.

In order to minimise bias induced by low quality of evidence it is a regular practice in the meta-analysis of clinical trials to assign a quality score to each individual study. A weight, which is directly proportional to the quality score, is often assigned to each study and included in the

meta-analysis. Initially, while assessing the reviewed studies I developed a 6-point quality score derived from Newcastle-Ottawa Quality Assessment Scale (Wells GA), a quality scale to assess case-control and cohort studies. The scale needed to be modified to account for the characteristics of the studies I reviewed. It included an assessment on:

1. Representativeness of cohort - *Was the sample representative of the general population?*
2. Ascertainment of exposure – *Were indoor NO_2 measurements repeated across the year or at least, if only one measurement taken, were the analyses adjusted for seasonality?*
3. Ascertainment of outcome - *Was respiratory health assessed with an objective measured (rather than self-reported)?*
4. Temporal assessment of exposure and outcome: *Was the respiratory health assessed during or after indoor NO_2 measurements were taken (rather than before)?*
5. Adjustment for confounders in the analysis - *Were the analysis adjusted for main confounders: sex, smoking and/or ETS (if children) and age?*
6. Adjustment for confounders in the analysis - *Were the analysis adjusted for other indoor exposures (e.g. mould, damp, allergen)?*

A positive answer to one of the questions above was equal to one point score. None of the studies reached score 6 (positive answer to all questions). The most common 'disqualifying' points for a study were: 1) not adjusting for the season when a single NO_2 measurement was carrying out; 2) subjective assessment of outcome, i.e. self-reported (parental reporting in case of children).

I eventually dropped the quality score system as scoring systems in observational studies lack demonstrated validity. One of the main issues with quality scores applied to observational studies is that there is no standard score for this type of studies and they need to be created *ad hoc*. Observational epidemiological studies are designed and conducted to observe 'real' life situations and this requires a flexible design. A large array of useful assessment tools has been developed but they lack agreement (Sanderson *et al.*, 2007). The use of a score to assess the quality of a study is controversial; findings of a study may not be associated with quality and the assumption that there is a linear relationship between quality score and effect estimate is questionable.

Rather than relying on a quality score MOOSE (Stroup *et al.*, 2000) has recommended conducting sensitivity analysis to explore the characteristics of the studies and investigate

heterogeneity, if present. Unfortunately, because of the small number of studies included in the meta-analysis sensitivity analysis could not be carried out extensively and were limited to the meta-analysis on 12-month prevalence of wheeze.

Sensitivity analysis suggested that outdoor NO_2 may have an independent role from indoor NO_2 in the prevalence of symptoms, that non-asthmatic people are more susceptible to indoor NO_2 pollution and that analysis which do not adjust for environmental tobacco exposure tend to inflate the risk of having respiratory symptoms.

2.4.3 Adults

One striking feature among the identified studies is the paucity of research that has been conducted in adults.

Findings from one study in adults with COPD suggest that elderly people may be particularly at risk to indoor NO_2 exposure. Studies in adults where the association of respiratory health with the use of gas appliances has been examined have reported contradictory results. Eisner *et al* (Eisner *et al.*, 2002; Eisner and Blanc, 2003) found no association with lung function and respiratory symptoms in a cross-sectional study of adult asthmatics living in California. Franklin *et al* (Franklin *et al.*, 2012) examined daily symptoms and lung function in older people with asthma over a 12 week period and observed an increased risk in wheeze and dyspnoea and small reduction in PEF and FEV_1 on the days when an unflued gas heater was used. Jarvis *et al* (Jarvis *et al.*, 1996) found that gas cooking was positively associated with asthma symptoms and a lower lung function in women but not in men – but these findings varied between countries (Jarvis *et al.*, 1998). It has been suggested that women could be at higher risk as they are more likely to spend more time cooking or perhaps because they are more susceptible to pollutants. Using ‘gas cooking’ a proxy measure for NO_2 exposure has considerable utility (cheap, gas appliance use is a major determinant of indoor NO_2) but does not allow to discern whether the pollutant driving the association is NO_2 or a co-pollutant such as particulate matter. This is an important consideration – although in public health terms, if the use of gas for cooking or combustion is associated with poor health – measures would need to be taken to reduce exposure to emission irrespective of the causal pollutant.

2.4.4 Asthma and atopy

Most of the studies that assessed asthma morbidity were in children and only one in adults (Smith, 2000). All studies observed some significant association between exposure to NO_2 and asthma symptoms or some lung function measures or FeNO. Unfortunately, there were not enough studies of similar design to be able to carry out a separate meta-analysis in asthmatics and wherever possible the estimates from these studies were combined with the estimates derived from the general population. Studies on asthmatics tended to assess the frequency of symptoms (rather than the 12-month prevalence), which requires different statistical methodology.

Findings suggested that atopic asthmatics are at higher risk of having asthma-related symptoms when exposed to indoor NO_2 and some studies observed a synergistic effect between NO_2 and allergens (Nitchske, 1999). Evidence was inconsistent as a couple of studies found that non-atopic children were more likely to experience symptoms than atopic children when exposed to same level of NO_2 (Kattan, 2007; Flamant-Hulin, 2010; Matsui, 2013).

2.4.5 Other air pollutants

In studies of respiratory health and ambient pollution there is concern that any observed health effects are confounded by exposure to other highly correlated and sometimes unmeasured pollutants. The few studies that did adjust for other pollutants did not provide strong evidence that this is the case. Several studies adjusted for the confounding effects of other indoor or outdoor pollutants but only a few commented on whether the relationship between asthma and indoor NO_2 changed after adjustments.

Indoor environment affects NO_2 chemistry differently from outdoors. Because of the relative high humidity in kitchen and large surface areas NO_2 generated from gas combustion will react quickly with surface water to produce HONO. It has been suggested that 17% of the measured indoor NO_2 concentrations may correspond to HONO (Lee *et al.*, 2002). There is limited evidence that HONO may cause lung inflammation but findings were inconsistent.

Stronger respiratory health effect associated with outdoor NO_2 than indoor NO_2 were observed in several studies (Braun-Fahlender, 1992; Shima, 2000; Emenius, 2003; Mi, 2006; Sarnat, 2006; Espluges, 2011; Kim, 2011). Results from the sensitivity analysis that removed studies with no gas sources appliances suggest that indoor NO_2 originating from outside is associated with

higher risk of having wheezing in children than indoor NO_2 when originated from gas appliances. Possible explanations are:

1. Indoor measures reflect high exposure to outdoor NO_2;
2. The effect of indoor NO_2 differs from the effect of outdoor NO_2 as they represent surrogates for different pollutants mixtures: gas combustion related pollutants in case of indoor NO_2 and traffic related pollutants with a possible more harmful effect in the case of outdoor NO_2.

Finally, it should be mentioned that only one study (Smith, 2000) considered the confounding effect of temperature even though some did consider season. Temperature has been found to be associated with lung function in children with asthma (Pearce and Douwes, 2013) and is highly correlated with the use of heating and cooking appliances and socio-economic status.

2.4.6 Limitations of systematic review and meta-analysis

This systematic review and meta-analysis had some limitations. PRISMA guidelines recommend that abstracts obtained through literature searches are reviewed by more than one assessor. This was not done here although where I felt uncertainty about interpretations of abstracts or results I discussed these with my supervisor.

The approach I adopted assumes a linear dose-response relationship between exposure to indoor NO_2 and health effects without a threshold. This is generally believed to be the case for health effects of NO_2 (World Health Organization, 2013) and none of the identified studies provided strong evidence to the contrary although a very recent study reported a dose-response effect above a certain threshold level (Belanger, 2013).

All effect estimates were based on an average exposure that varied from a few days to several weeks. The meta-analysis assumed that these exposures are representative of average indoor NO_2 levels. However, indoor levels do vary with season, and time spent indoors also varies with season thus the estimates may not be representative of effects had the assessment been carried out throughout the year (or in a different season).

Participants in Belanger (2003) and Belanger (2006) were related (siblings). This means that they were not totally independent, an underlying assumption in meta-analysis as they lived in the

same household but they were included in the same meta-analysis. Removal of the latter study made little difference to the overall effects (results not shown).

Sensitivity analyses are recommended in meta-analysis of observational studies to investigate heterogeneity and study's influence on meta-analysis (Stroup, 2000) but the number of studies included in the meta-analysis was too small to be able to carry out extensive sensitivity analyses.

2.5 Summary

In conclusion this systematic review shows limited evidence of an association of respiratory symptoms in children with increasing levels of indoor NO_2. There were very few studies on adults and few studies on people with asthma to draw any conclusion. However, concerns regarding the health effects of nitrogen dioxide exposure in both the outdoor and indoor air continue and further attempts to examine these effects in adult populations are indicated. I will address these gaps in the following chapter (Chapter 3) by examining the association between asthma severity and indoor NO_2 in a multi-centre adult cohort.

3. Association of asthma severity with indoor NO_2

3.1 Introduction

The systematic review has shown that exposure to indoor NO_2 may increase the risk of having asthma-like respiratory symptoms. Of note the review showed:

- There was an increased risk of symptoms of wheeze with increasing indoor NO_2
- Studies of almost exclusively conducted in children suggested that asthma severity increase amongst those with increased exposure
- There were few studies in adults.

This chapter will examine the effect of indoor NO_2 on asthma severity in an epidemiological study in adults, the European Community Respiratory Health Survey (ECRHS).

3.1.1 European Community Respiratory Health Survey

The ECRHS is a multi-centre study carried out in response to the global increase in asthma prevalence in the 1980's. It began in 1990 collecting data on the prevalence of asthma and allergic disease and their known or suspected risk factors in young adult populations. It was originally designed to cover most areas of the European Community but has also included other areas outside Europe. It was the first international large multi-centre study to assess the prevalence of asthma and allergic disease in young adults using standardised protocols for interviewer-led questionnaires, assessment of atopy through skin prick tests and serum specific IgE to common allergens, lung function measurements, tests of airway responsiveness, and blood and urine collection.

Fifty six centres from 25 countries across Europe and other parts of the world took part in the first survey between 1991 and 1993 (ECRHS I). In 1999/2001 twenty-nine of the initial 56 study centres performed a follow-up investigation of individuals who took part in the baseline clinical investigations (ECRHS II), and this follow-up included measurements NO_2 inside in the homes of a sub-sample of participants. A second follow-up known as ECRHS III, i.e. the third survey has recently been completed in 22 centres.

3.1.2 ECRHS methods

ECRHS I

Between 1991 and 1993 a community based random sample of young adults aged 20-44 was identified from available population based registers and invited to complete a short postal questionnaires (stage 1). A random sample of responders to this questionnaire was invited to attend a local clinical centre (stage 2) where they provided more detailed information on health status and suspected risk factors for asthma and allergic disease (family size, family history of disease, occupation, childhood and current exposure to pets, exposure to tobacco smoke, dampness, ventilation, use of soft furnishings and use of gas appliances). Blood samples were taken and serum tested for specific IgE to house dust mite, cat, grass and *Cladosporium herbarum*. Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and bronchial reactivity to methacholine were measured. In addition, most centres enriched their random sample cohort with a symptomatic sample of individuals who reported symptoms suggestive of asthma (being woken by breathlessness or an asthma attack in the last 12 months) and/or current use of asthma medication – but who had not been selected as part of the random sample. Around 180,000 participants completed the questionnaire (stage 1) and around 26,000 from 45 centres participated in the clinical stage (stage 2).

ECRHS II

Between 1998 and 2003 twenty-nine of the initial 56 study centres performed a follow-up investigation. All participants (both random and symptomatic) who took part in the clinical stage were asked to take part in ECRHS II and invited to the local clinical centre for a follow-up visit (clinical stage) where detailed information on health status, risk factors, lung functions tests and blood and urine samples were collected. Around 13,000 short screening questionnaires on asthma symptoms were collected and about 10,000 individuals were assessed in clinics. Fourteen centres from 6 European countries also agreed to measure indoor and outdoor NO_2 at participants' homes (n=1906) using passive diffusion samplers (Passam, AG, Switzerland). These measures comprise the exposure data that was used for the analysis in this chapter.

ECRHS III

Twenty-nine centres from 14 countries (mostly European) agreed to participate in a further follow-up survey (ECRHS III). Individuals who took part in the clinical stage of ECRHS I were sent a short screening questionnaire and in 27 centres those who responded, were invited to a

local fieldwork centre for detailed interview on symptoms and lifestyle and clinical assessment of lung function, FeNO, venepunctures for specific and total IgE testing. ECRHS III started in 2011 and has recently been completed with over 6,000 participants from the original random sample and over 900 participants from the original symptomatic sample.

The full research protocol can be found at http://www.ecrhs.org.

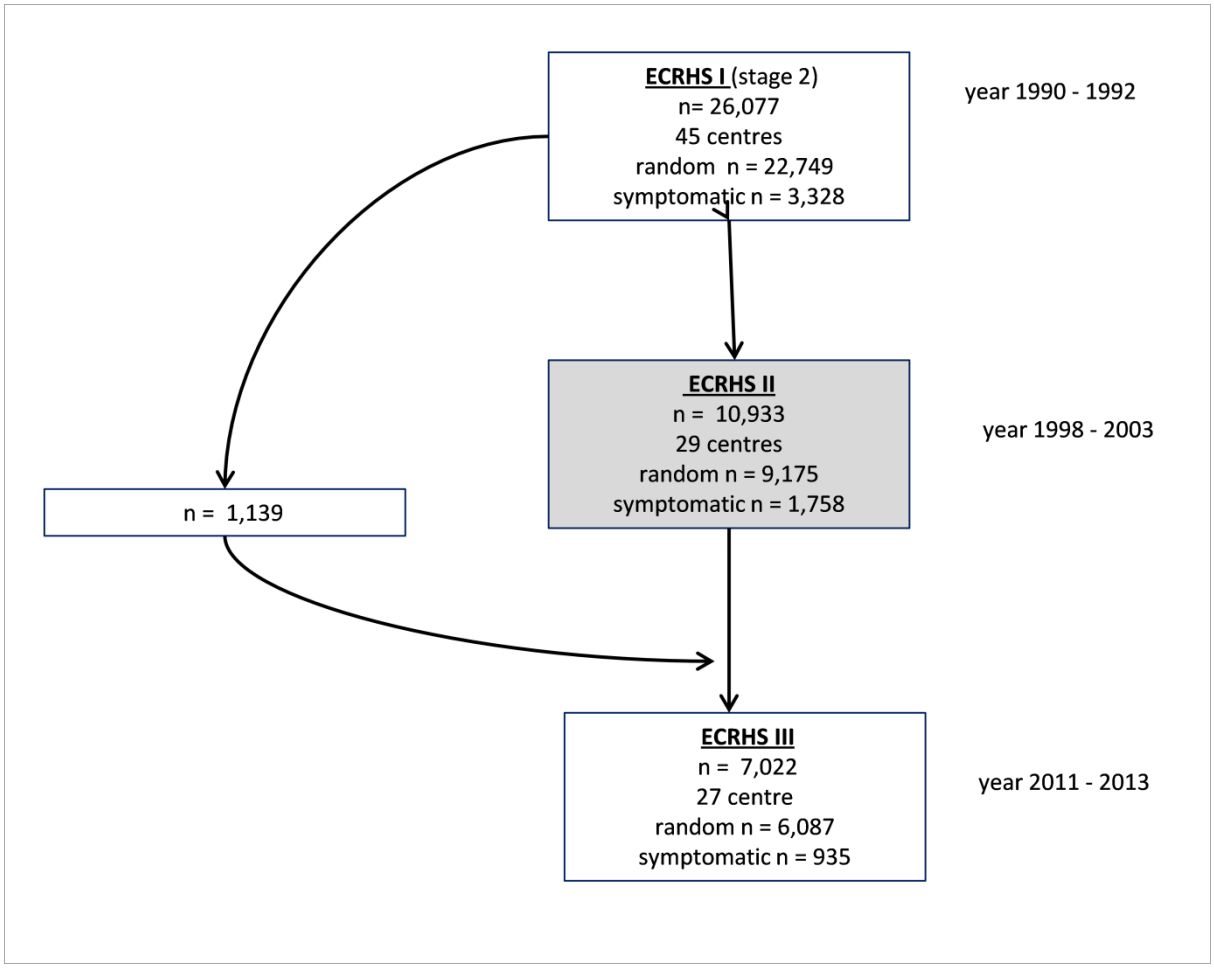


Figure 3.1 Flow chart of participants at each ECRHS survey (n=1,139 are those participants who took part at ECRHS I and ECRHS III but not at ECRHS II)

3.1.3 Hypothesis

Asthma severity in adults is associated with exposure to indoor NO_2.

3.1.4 Objectives

- To investigate the association between asthma severity and indoor NO_2 exposure in a sub-sample of adults with current asthma who participated in ECRHS II.
- To determine whether the observed associations are modified by:
  - Sex;
  - Smoking status;
  - Use of inhaled steroids;
  - Atopy.
- To determine whether associations are also seen between asthma severity and a proxy measure for indoor NO_2 'use of gas for cooking'.
- To include ECRHS II results in the previous meta-analysis of 12-month prevalent wheeze.

3.2 Method

3.2.1 Study population

The main analysis is based on data collected at ECRHS II between 1999 and 2001 in the following14 centres of 6 European countries:

- Belgium: Antwerp South, Antwerp City
- Spain: Barcelona, Galdakao, Albacete, Oviedo, Huelva
- Italy: Pavia, Turin, Verona
- UK: Ipswich, Norwich
- Sweden: Umea
- Switzerland: Basel.

Indoor NO_2 was monitored in the kitchen of 1906 participants, of whom 257 were current asthmatics (Figure 3.2).

Participants who were part of the random sample and the symptomatic sample who fulfilled these definitions were included (n=136 and n=121 respectively). They were defined as current asthmatics if they had:

- A diagnosis of asthma confirmed by a doctor AND
- At least one of the following in the last 12 months:
    a. Any symptom associated with asthma (wheeze, nocturnal chest tightness, attack of breathlessness after exercise, attack of breathlessness at rest, attack of breathlessness at night or woken up by cough).
    b. An asthma attack.
    c. Used inhaled/oral medicines because of breathing problem.

Information on respiratory symptoms in the last 12 months was provided by the participants during an interview led by a field worker at the clinic. At the clinic lung function tests and bronchial challenge were also performed. Blood samples were taken and total and specific IgE for house dust mite, cat, grass and *Cladosporium* was measured in a single laboratory using the Pharmacia CAP System.

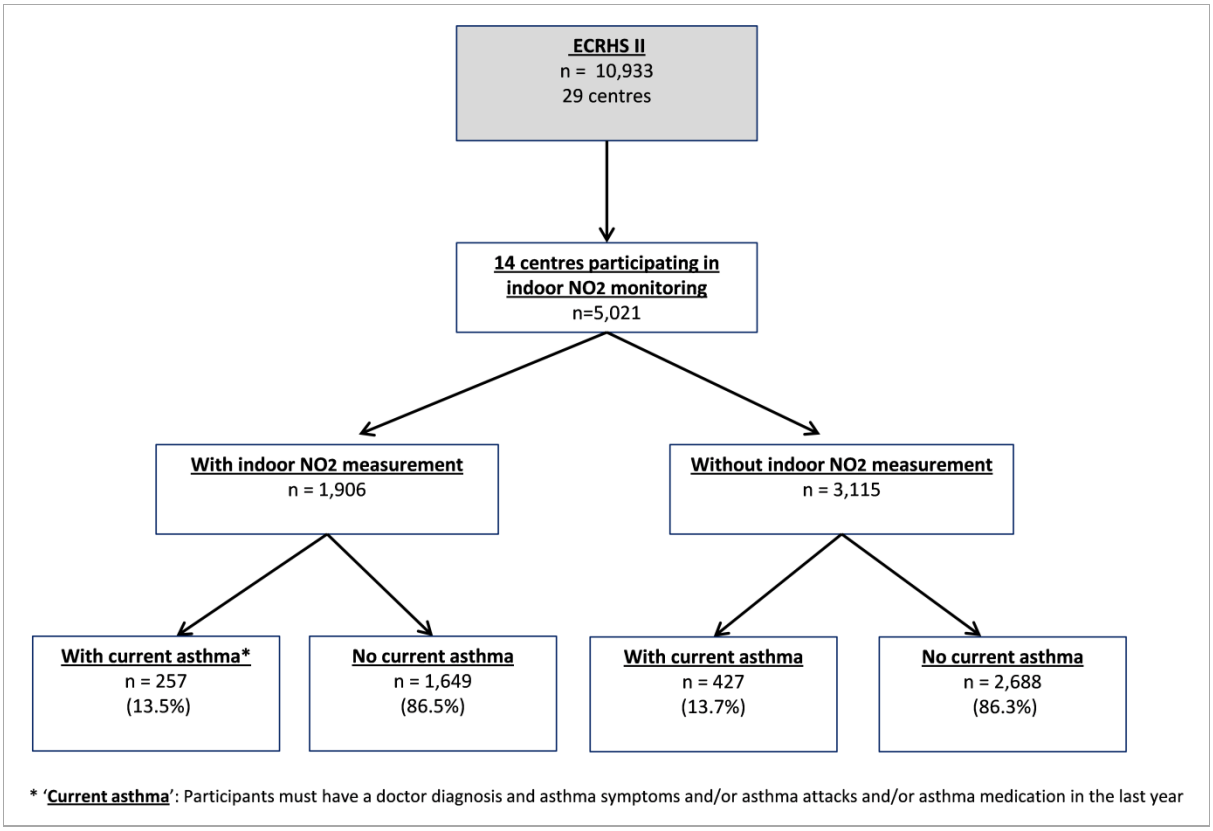


Figure 3.2 Flow chart of ECRHS II participants with current and without current asthma in the 14 ECRHS centres that took part in indoor NO_2 monitoring

3.2.2 Indoor NO_2 monitoring and gas use

Indoor NO_2 was sampled using passive diffusion samplers (Passam AG, Switzerland). Samplers were located in the kitchen away from a window on the opposite side to the cooking appliance (ideally 2-3 metres away from the stove) at 1.5-1.8 metres above ground. Samplers were set up by a trained fieldworker and after 14 days of exposure participants were reminded by fieldworkers to seal the samplers, complete forms detailing opening and closing times, and return them by post in a pre-addressed jiffy bag.

Duplicate and blank samplers were also used to test quality of monitoring. One in 10 participants had two of each sampler installed in their homes ('duplicate'). One in 10 (not the same participants with a duplicate sampler) had two samplers, of which one had the lid left on ('blank'). Within the following 12 months monitoring was repeated in around 850 homes ('replicate').

Exposure to gas cooking was defined by response to the question *'What kind of stove do you mostly use for cooking?'.* Those responding *'Gas from the mains'* or '*Gas from the bottles or other non-mains sources*' were classified as being exposed to gas cooking. Those who answered "electric" were classified as the reference group. Exposure to unflued gas heating (UFGH) sources included open gas fire and portable gas heaters and were identified by participant's reply to the question '*Which of the following appliances do you use for heating or for hot water?'*

In this chapter the term 'indoor NO_2'will mean 'two-week average kitchen NO_2' as passive diffusion samplers measure total exposure over time exposed for, in this case two weeks.

3.2.3 Asthma severity

Asthma severity was assessed with the following four markers:

- **GINA score (range1-4)**
  A four-class asthma severity score (intermittent, mild persistent, moderate persistent, severe persistent in the last 12 months) based on 2002 Global Initiative for Asthma (Global Initiative for Asthma, 2002). The score was developed within the ECRHS cohort by Cazzoletti and colleagues (Cazzoletti *et al.*, 2010). Briefly, the four-class score combines a clinical score (4 classes) derived from frequency of night-time and daytime symptoms reported in the last 12 months and FEV_1 % predicted, with reported daily medication use in the last 12 months (4 classes).

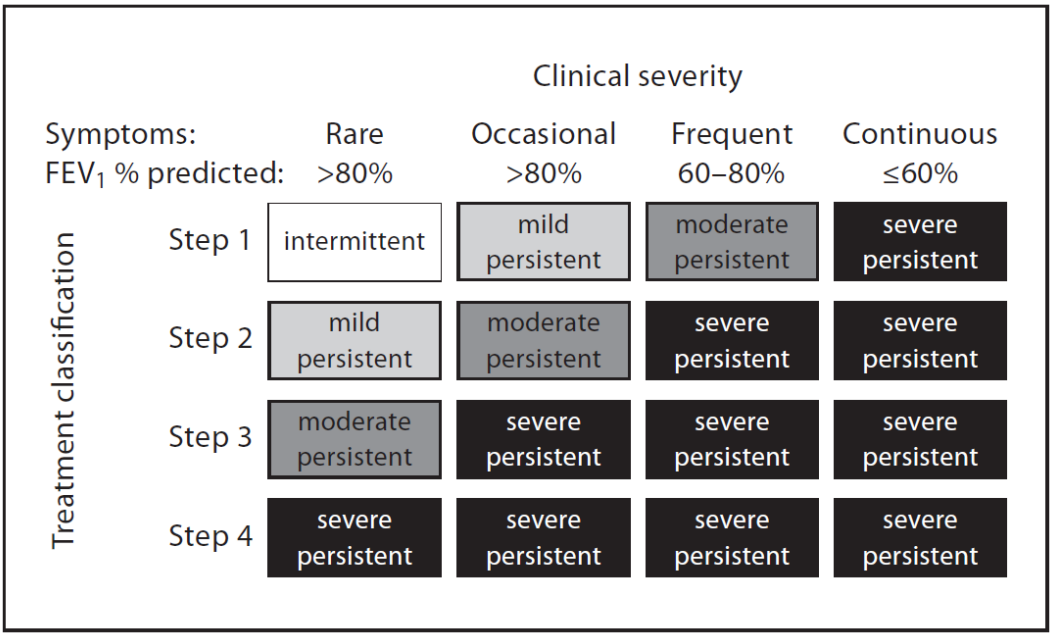


Figure 3.3 GINA severity score based on clinical severity and treatment classification where Step 1= no daily controller; Step 2=low-dose inhaled corticosteroid (ICS), leukotriene modifier, theophylline or cromones; Step 3=low/medium-dose ICS combined with long-acting β2-agonists (LABA), or medium dose ICS combined with leukotriene modifier or theophylline, or high-dose ICS alone; Step 4= high-dose ICS combined with LABA or with leukotriene modifier *(from Cazzoletti 2010)*

- **Asthma score**

  A six-level score (from 0 to 5), first proposed by Sunyer and colleagues (Sunyer *et al.*, 2007) derived as a sum of affirmative questions asking about the presence of any of the following asthma-like symptoms over the previous 12 months:

  1. Wheeze
  2. Woken up by a feeling of tightness in the chest
  3. Attack of shortness of breath at rest
  4. Attack of shortness of breath following strenuous activity
  5. Woken by an attack of shortness of breath.

- **Bronchial responsiveness after methacholine challenge test as a continuous outcome (slope)**

  Participants with FEV_1 at least 70% of predicted, and more than 1.5 litres, underwent bronchial challenge. The challenge was performed by inhalation of increasing amounts of methacholine up to a cumulative dose of 1 mg, with the methacholine solution being administered via a Mefar dosimeter (Mefar, Bovezzo, Italy). Bronchial responsiveness

(BR) was defined by the slope of the dose-response curve obtained with the test and estimated as rate of change of FEV_1 against methacholine dose. The slope was transformed to 100/(log-slope +10) to satisfy the assumption of normality thus a low slope indicates high BR (Chinn *et al.*, 1997).

- **Bronchial responsiveness after methacholine challenge test as a binary outcome (PD20)**
  BR was also assessed as a binary outcome. Participants who had a fall in FEV_1 of more than 20% after inhalation of 1 mg of methacholine we considered have bronchial response compared to those whose FEV_1 change less than 20% after inhalation of 1 mg of methacholine.

3.2.4 Statistical method

Non-response bias

Characteristics of participants who took part in the monitoring campaign and those who did not were compared using chi-square tests for categorical variables and Kruskal-Wallis tests for continuous variables.

Participants' characteristics included sex, age, age left education, age at onset of asthma, smoking status (never, ever or current), smoking at home, socio-economic status (a simplified version of the International Standard Classification of Occupations (ISCO) classification, which includes 6 social classes groups, based on the longest held job during the follow-up period between ECRHS I and ECRHS II) and ever exposed to vapour, gas, dust or fumes (VGDF); sensitization (defined as having a IgE for a specific allergen above 0.35kU/L) to house dust mite (HDM), *Cladosporium,* any common indoor allergen (defined as any specific IgE for HDM or *Cladosporium,* cats and grass above 0.35kU/L), total IgE level, type of cooking and heating appliances, presence of damp and mould at home, asthma severity and asthma symptoms score in the last 12 months, use of inhaled steroids in the last 12 months and BR (methacholine challenge test).

Measures of indoor NO_2

Limits of agreement between duplicate and replicate measures of indoor NO_2 were tested using the Bland-Altman method (Bland and Altman, 1986). Blank readings were checked for accuracy

of the measurements. Analyses were based on indoor NO_2 levels measured on the first sampling only.

Asthma severity

The following clinical characteristics of participants were first examined: GINA severity score, asthma symptoms score, inhaled corticosteroids, BR and atopic status.

Because of the relatively small size of the sample the four categories for GINA score were reduced to two categories: intermittent (i.e. GINA score= 1; 51.5% of participants with NO_2 data) versus persistent (GINA score = 2, 3 and 4; 48.5% of participants with NO_2 data), which included mild persistent, moderate persistent and severe persistent as classified in the original GINA classification. This categorisation divided the sample into two broadly equally sized groups. The association between indoor NO_2 and GINA score was examined using logistic regression comparing current asthmatics with intermittent asthma severity (baseline) against current asthmatics with persistent (mild, moderate or severe persistence) asthma severity.

As the asthma score was an ordered score without zero inflation, its association with indoor NO_2 was examined using an ordered logistic model (also known as proportional odds model) as in previous literature (Rage *et al.*, 2009) .The crude association between asthma score and indoor NO_2 was tested for proportionality of odds and if proportionality did not hold, a multinomial regression model was then adopted. Odds ratios were given for one class difference in the score.

The BR slope was examined using linear regression; a lower slope is indicative of bronchial hyper-responsiveness. The binary outcome (PD20) was examined with logistic regression as in previous literature (Chinn, 1997).

The effect of NO_2 was explored on a continuous scale standardised to 10μg/m^3 increase and as a binary variable with a cut-off point of 40μg/m^3 (the maximum value for annual average exposure recommended by WHO).

In the first instance univariate analyses were conducted, and then multivariate analyses adjusted for the effects of main confounders (main model) selected *a priori*:

- Age
- Sex

- Smoking status
- Country where the study centre was located.

To explore variation of effect between countries, analyses were also conducted within country and effect estimates combined using meta-analysis (DerSimonian and Laird, 1986). Heterogeneity between countries was tested using the I^2 statistic (Higgins, 2003) which describes the percentage of total variation across studies that is due to heterogeneity rather than chance. For some analyses the sample size was too small for this approach.

In addition to the main *a priori* confounders, a larger number of suspected confounders associated with asthma and indoor NO_2 were included in secondary analyses (secondary multivariate model). These were:

- Anybody smoking at home (y/n), which comprises both participants smoking at home as well as family members (as reported in the questionnaire)
- Lifetime exposure to VGDF (y/n), which was merged into 2 categories (no and sometimes/often)
- Presence of mould in the house (y/n)
- Presence of damp in the house (y/n), which was merged into 2 categories (no/don't know and yes)
- Season of when NO_2 monitoring took place: spring, summer, autumn and winter.

Throughout statistical significance was defined as having a P value < 0.05 while interaction terms (effect modifiers) were considered significant at 10% level (P value <0.1).

Statistical analyses were conducted using Stata 12.1.

Effect modifications

The following effect modifications were tested by inclusion of an interaction term in the analyses adjusted for the main confounders (sex, age, smoking status):

- *Sex*
- *Smoking status* (never, ever) at ECRHS II
- *Atopy,* defined as present if an individual had serum specific IgE at ECRHS I less than 0.35kU/L to at least an allergen to the following: HDM, timothy grass, cat or *Cladosporium*

- *Use of inhaled steroids* in the last 12 months (but not on GINA score as the score includes information on inhaled steroid use).

Association of asthma severity with use of gas for cooking

Further analyses were conducted on those participants (n=1571) who had current asthma at ECRHS II and reported the main type of fuel used for cooking was gas or electricity (baseline).

3.2.5 Derivation of estimate for inclusion in meta-analysis of Chapter 2

Finally, a logistic regression model was carried out to assess the association of wheeze in the last 12 months and indoor NO_2 in all ECRHS participants (irrespective of their asthmatic status) from the random sample with NO_2 measurements adjusted for sex, age, smoking status (y/n) with random effect by country. The effect estimate was then incorporated in the meta-analysis of 12-month prevalent wheeze conducted in the previous Chapter 2.

3.3 Results

3.3.1 Participants characteristics

Among the 5021 participants in the 14 centres that took part in the indoor NO_2 measurement protocol, 684 (13.6%) were current asthmatics. Of these, 37.6% (n=257) had indoor NO_2 measures.

Compared to those participants with current asthma and without NO_2 monitoring data those with measures of indoor NO_2 tended to be older (median age: 42.6 vs 41.0 years), more likely to be ex-smokers (30% vs 22%) and more likely to have a gas fired boiler (55% vs 43%) (Table 3.1).

Table 3.1 Characteristics in current asthmatics with and without NO_2 measurements in ECRHS 14 centres in which NO_2 was measured

	Without NO_2 sampling (n=427)		**With NO_2 sampling** (n=257)		**P value for difference** *
Age in years median (IQR)	41.0 (34.7-47.1)		42.6 (37.6-48.8)		0.0024^
Age left education in years (median, IQR)	19 (16-23)		18 (16-22)		0.15^
Age of asthma onset in years (median, IQR)	18 (6-28)		18 (7-29)		0.62^
	n	%	n	%	
Female	256	60.0	145	56.4	0.36
Smoking status					0.033
Never	206	48.5	111	44.1	
Ex	96	22.6	77	30.6	
Smoker	123	28.9	64	25.4	
Exposure to smoking at home	172	40.4	90	35.0	0.16
Occupation					0.40
Managers and professionals	93	21.8	56	21.8	
Technicians & associate professionals	65	15.2	38	14.8	
Other non-manual	111	26.0	79	30.7	
Skilled manual	30	7.0	23	9.0	
Semi-skilled or unskilled manual	58	13.6	32	12.5	
Unclassifiable or unknown#	70	16.4	29	11.3	
Cooking appliance					0.57
Gas (mains/bottled)	183	44.5	123	48.1	
Electricity	222	54.05	128	50	
Other	6	1.5	5	2.0	
(Sources of heating)					
Open gas fire or portable gas heater	102	23.89	60	23.35	0.872
Solid fuel (Open coal, coke, wood fires)	59	16.62	32	13.97	0.39
Paraffin heater	16	4.51	4	1.75	0.073
Gas fired boiler	153	43.22	125	54.59	0.007
Presence of damp	93	22.91	61	23.92	0.76
Presence of mold	115	27.98	86	33.46	0.13
Country					<0.001
Belgium	10	3.9	44	10.3	
Spain	93	36.2	121	28.3	
Italy	27	10.5	21	4.9	
UK	62	24.1	99	23.2	
Sweden	37	14.4	85	19.9	
Switzerland	28	10.9	57	13.4	

*χ^2test unless stated; ^ Kruskal-Wallis test for continuous variables

Clinical characteristics

Clinical characteristics at ECRHS II of current asthmatics with indoor NO_2 measures are reported in Table 3.2. Around half of the current asthmatics were classified as having intermittent asthma according to the GINA score classification (GINA score=1); half of them had one or two asthma-related symptoms (asthma score=1/2) in the previous 12 months; a small percentage (7.8%) reported they did not have symptoms used in the asthma score over the 12 months and 11.7% had 5 different types of symptoms (asthma score=5) in the last 12 months. Around 40% of individuals used inhaled steroids. Nearly half of them were sensitised to HDM and 68.0% were sensitised to at least one of the most common allergens. Clinical differences between current asthmatics with and without NO_2 measures were tested for significance; there was no difference in markers of asthma severity except in the use of inhaled steroids (P=0.044) as current asthmatics without NO_2 measures were more likely to use inhaled steroids (48.7%) than those with NO_2 measures (40.7%) (Results not shown).

Table 3.2 Table showing clinical characteristics at ECRHS II of current asthmatics with indoor NO_2 measures

Clinical characteristics	**% of current asthmatics with NO_2 measures** (n=257)
GINA 1	51.46
GINA 2	14.23
GINA 3	12.55
GINA 4	21.76
Asthma score 0	7.78
Asthma score 1	25.29
Asthma score 2	24.12
Asthma score 3	15.18
Asthma score 4	15.95
Asthma score 5	11.67
IgE for HDM allergen >0.35kU/L	47.6
IgE for *Cladosporium* allergen >0.35kU/L	6.6
IgE >0.35kU/L for any allergens (HDM, cats, grass or *Cladosporium*)	68.0
Using inhaled steroids in the last 12 months	40.71
PD20<1mg (data available for n=163)	50.9
Bronchial responsiveness slope, mean (SD)*	5.4 (2.3)

*data available for n=163

3.3.2 NO_2 measurements

The determinants of indoor NO_2 in the ECRHS sampling programme are examined in greater depth in the next chapter (Chapter 4). However the main patterns of exposure are described here to improve understanding of the analyses presented in this chapter.

Indoor NO_2 was monitored continuously for two weeks between 1999 and 2001. Figure 3.4 shows the distribution of indoor NO_2 (first sampling), skewed to the right with median value of 29.3µg/m^3 (IQR 15.8µg/m^3 to 50.5µg/m^3). Two homes had NO_2 levels above 200µg/m^3.

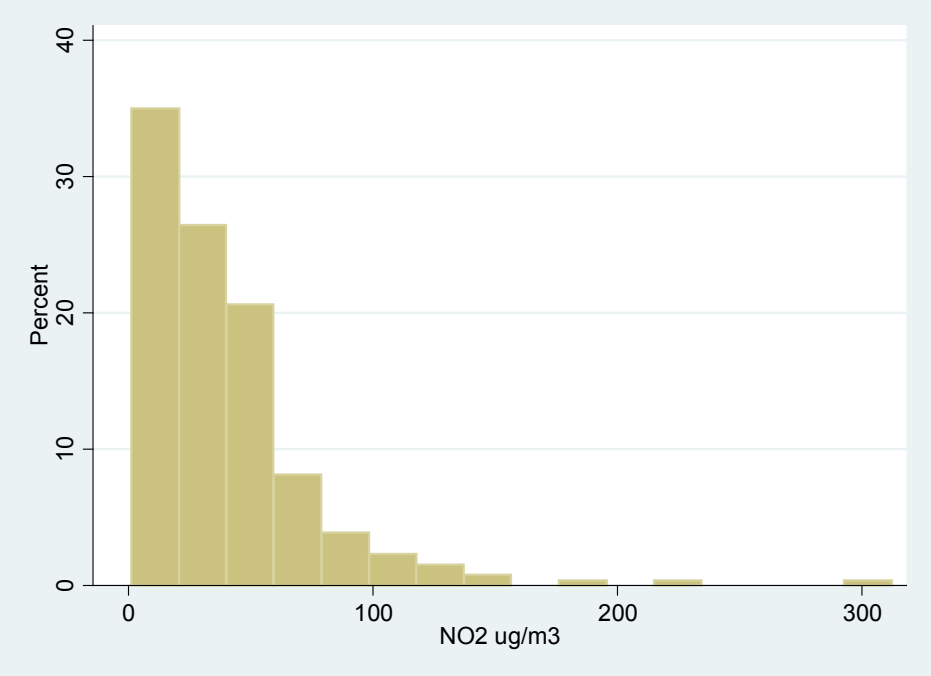


Figure 3.4 Histogram showing distribution of indoor NO_2 measured in homes of current asthmatics at the first sampling

Levels of NO_2 tended to be low (median 19.9µg/m^3, IQR 8.9-34.4µg/m^3) during the summer months (July, August, September) and high (40.1µg/m^3) during the spring months (March, April and June) and winter (median 38.5µg/m^3) months (January, February and March) (Figure 3.5).

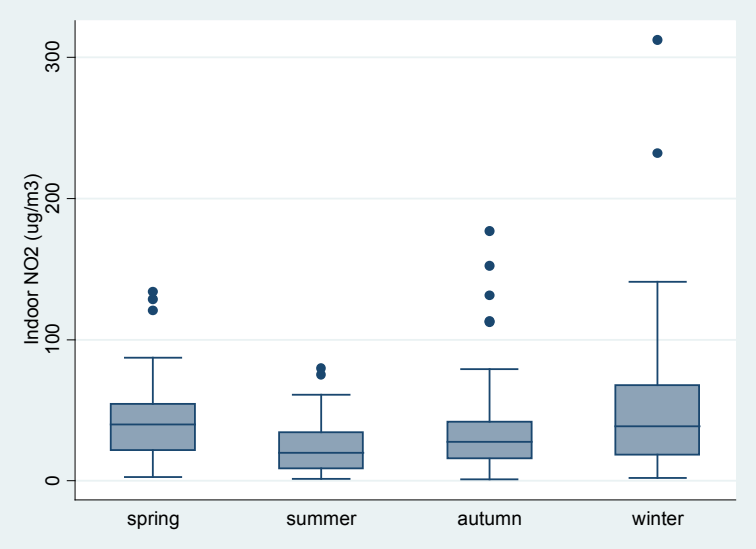


Figure 3.5 Box plots showing indoor NO_2 levels measured in homes of current asthmatics by season of sampling

The level of indoor NO_2 varied across countries. Spain and Italy tended to have the highest levels with a median of 44.5 µg/m^3 (IQR 32.0-74.8µg/m^3) and 45.3 µg/m^3 (IQR 34.3-79.9µg/m^3) respectively. Sweden had the lowest concentration of indoor NO_2 (median 4.2 µg/m^3 IQR 2.8-7.6µg/m^3) (Figure 3.6).

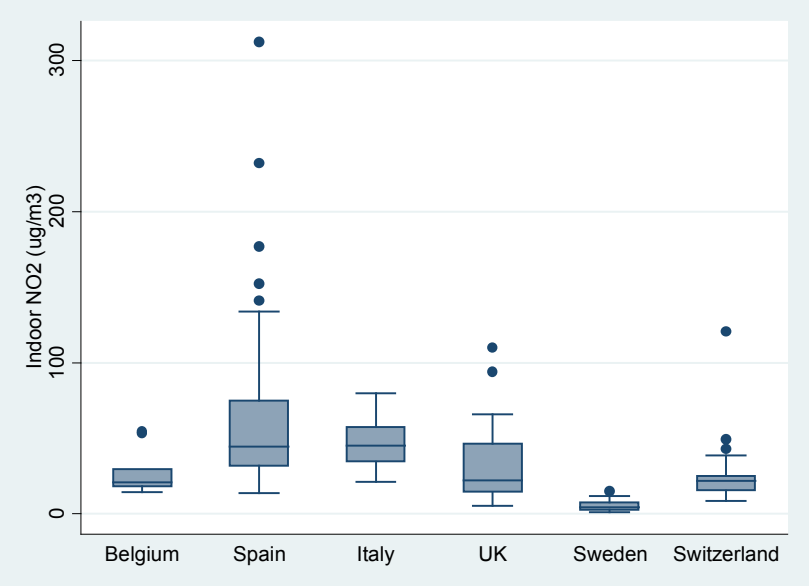


Figure 3.6 Box plot showing indoor NO_2 measured in homes of current asthmatics by country

NO_2 concentrations tended to be higher in those countries with higher proportion of participants who used gas for cooking or UFG for heating (only Spain and UK). None of the Swedish current asthmatics reported using gas for cooking and only one Italian current asthmatic reported using an electric stove for cooking (Table 3.3).

Table 3.3 Distribution of indoor NO_2 (µg/m^3) and use of gas for coking by country

COUNTRY	Use of gas for cooking %	Use of UFGH* %	Indoor NO_2 (µg/m^3)				
			Min	25th centile	50th centile	75th centile	Max
Sweden (n=37)	0.0	0.0	1.1	2.8	4.2	7.6	15
Belgium (n=10)	20.0	0.0	14.5	18.3	20.9	29.7	54.4
Switzerland (n=28)	21.4	0.0	8.7	15.45	21.7	24.95	120.7
Spain (n=93)	55.9	40.9	13.9	32	44.5	74.8	312.2^
UK (n=37)	61.3	35.5	5.4	14.5	22.3	46.3	109.9
Italy (n=27)	96.2	0.0	21.2	34.3	45.3	57.3	79.9

*UFGH= unflued gas heaters: include open gas fire and portable gas heater; ^ in Barcelona.

Overall, homes that mostly used a gas stove for cooking tended to have higher NO_2 levels (median 49.3 µg/m^3 IQR 34.4-71.4 µg/m^3) compared to homes that mostly used an electric stove (median 16.85 µg/m^3 IQR 8.15-27.1 µg/m^3) (P for Kruskal-Wallis test = 0.0001).

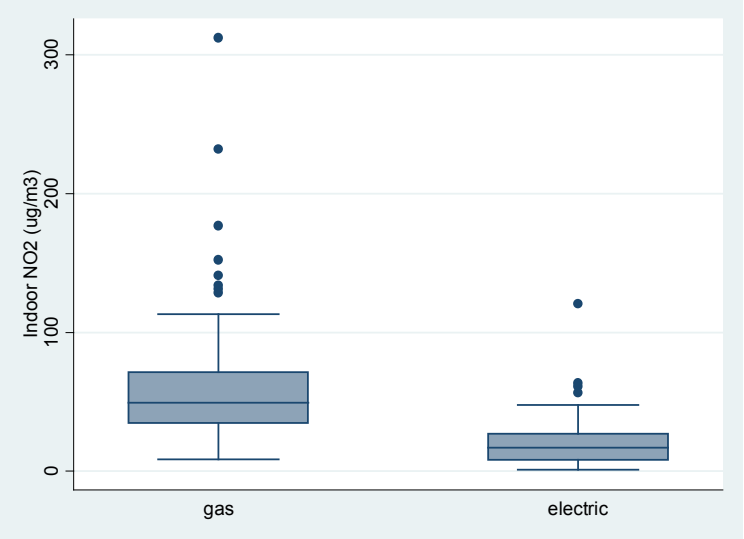


Figure 3.7 Box plot showing indoor NO_2 measured in homes of current asthmatics by gas and electric appliances

Duplicate, blank and replicate measurements

Duplicate and blank measurements were carried out in 33 and 27 homes of current asthmatics respectively. Monitoring was repeated in 125 homes within the following 12 months (Table 3.4).

Table 3.4 Distribution of indoor NO_2 ($\mu g/m^3$) with duplicate, replicate and blank samplings

	Indoor NO_2 ($\mu g/m^3$)				
	Min	25th centile	Median	75th centile	Max
First sampling (n=257)	1.1	15.8	29.3	50.5	312.2
Duplicates (n=33)	3.8	15.3	27.3	56.5	141.8
Replicates (n=125)	1.3	8.8	22.0	37.7	131.5
Blanks (n=27)	-1.0	-0.3	-0.1	0	12

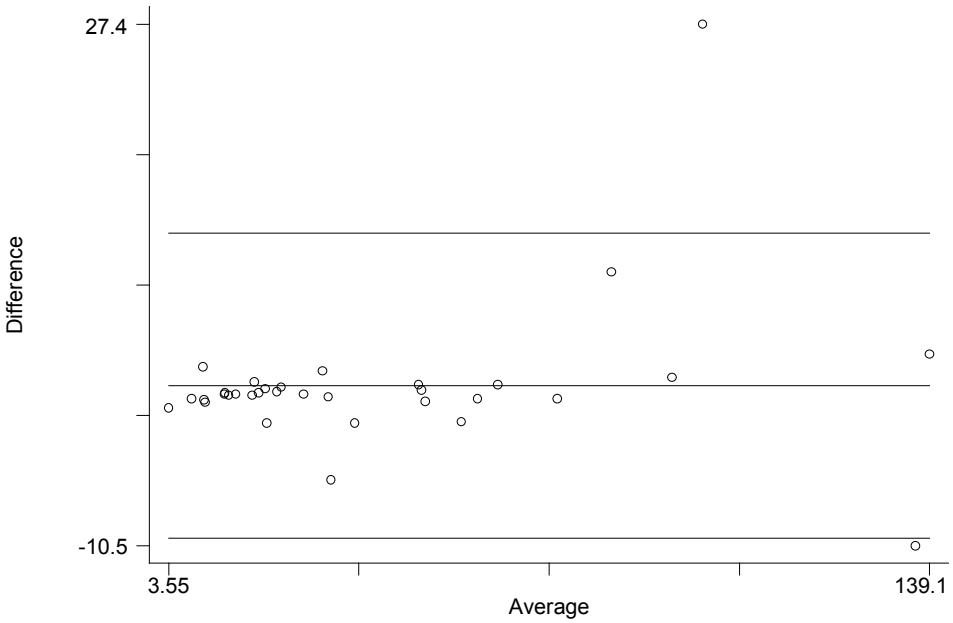


Figure3.8 Bland-Altman plot comparing indoor NO_2 measurements against duplicate measurements (Average = average between first + duplicate measurement; Difference = difference between first - duplicate measurement)

Table 3.5 Results of the Bland-Altman test comparing first NO_2 measurements against duplicate and replicate measurements

	Median (range) difference with first sampling)	95% limits of agreement for difference
Duplicate (n=33)	0.50 (0.10, 1.20)	-9.98 to 12.20
Replicate (n=125)	-0.10 (-4.70, 4.20)	-35.2 to 34.3

The Bland-Altman test (Figure3.8) suggested a good agreement between the first and duplicate samplings with limits of agreement ranging from -10.0 to 12.2. The limits of agreement for difference between first and replicate show large limits (-35.2 to 34.3); replicate measurements were carried out at different time of the year and the large difference is indicative that indoor NO_2 levels is affected by seasonal variations. Because of the limited availability of data replicate measures were not considered in the analysis.

3.3.3 Association of asthma severity with indoor NO_2

Associations of GINA score with indoor NO_2

Table 3.6 shows the odds of having persistent asthma (GINA score = 2/3/4) rather than having intermittent asthma (GINA score = 1) per 10µg/m^3 increase in two-week average indoor NO_2 before and after adjustment for relevant confounders. There was no evidence of an association between severity of GINA score and indoor NO_2 whether NO_2 was considered as linear or binary exposure (with the annual WHO guidelines of 40µg/m^3 as the cut-off point). There was insufficient data to carry out separate analysis by country.

Table 3.6 Unadjusted and adjusted odds ratios for having 'persistent' rather than 'intermittent' GINA score per 10µg/m^3 increase in indoor NO_2 and when exposed to indoor NO_2 concentrations above 40µg/m^3 compared to when exposed to 40µg/m^3 or less

Analysis	Intermittent asthma (n=123)	Persistent asthma (n=116)
per 10µg/m^3 NO_2	OR	OR (95% CI)
unadjusted	1.00	0.97 (0.90, 1.04)
multivariate adj ^	1.00	0.98 (0.90, 1.07)
multivariate adj ^^	1.00	0.95 (0.85, 1.06)
>40µg/m^3 NO_2		
unadjusted	1.00	1.10 (0.65, 1.85)
multivariate adj ^	1.00	1.35 (0.72, 2.56)
multivariate adj ^^	1.00	1.16 (0.58, 2.35)

^Main model: Adjusted for sex, age at interview, smoking status (never, ever) and country; ^^ Adjusted as above and for smoking at home, presence of damp at home, presence of mould at home, age left education, ever exposed to VGDF, season of NO_2 sampling.

Associations of asthma score with indoor NO_2

Table 3.7 shows the association of asthma score (three steps) and exposure to two-week mean indoor NO_2 before and after adjustment for relevant confounders. The assumption of

proportionality of odds across response categories was not violated (P=0.59 for unadjusted model) and an ordinal logistic model was carried out. There was no evidence suggesting that asthma score increased as exposure to indoor NO_2 increased (adjusted OR 1.04, 95%CI 0.95 to 1.12 per 10µg/m^3 NO_2 increase). Adjustment for confounders did not change the size of the effect estimate.

Table 3.7 Unadjusted and adjusted odds ratio of having one higher class difference in the asthma symptoms score per 10µg/m^3 increase in indoor NO_2

Analysis	OR (95% CI) *
per 10µg/m^3 NO_2	
unadjusted	1.04 (0.97, 1.11)
multivariate adj ^	1.04 (0.97, 1.14)
multivariate adj ^^	1.05 (0.97, 1.15)
>40µg/m^3 NO_2	
unadjusted	1.26 (0.81-1.97)
multivariate ad ^	1.30 (0.76-2.21)
multivariate adj^^	1.16 (0.67-2.03)

*Odds ratios for the increase in one point of asthma score; ^Adjusted for sex, age at interview, smoking status (never, ever) and country; ^^Adjusted as above and for smoking at home, presence of damp at home, presence of mold at home, age left education, ever exposed to VGDF, season of NO_2 sampling.

The association was further investigated by considering a random effect by country. First, separate ordered logistic regression analyses adjusted for sex, age and smoking status were separately carried out for each country and then a combined effect calculated. After taking into account the random effect of country the odds ratio increased to 1.12 (95% CI 0.92 to 1.3 per 10µg/m^3 NO_2 increase) but did not reach statistical significance (Figure 3.9). There was some evidence of moderate heterogeneity between countries (I^2=40.9%, P=0.13). The estimate for Spain had considerable weight in the meta-analysis while the estimate for Sweden had very wide confidence intervals.

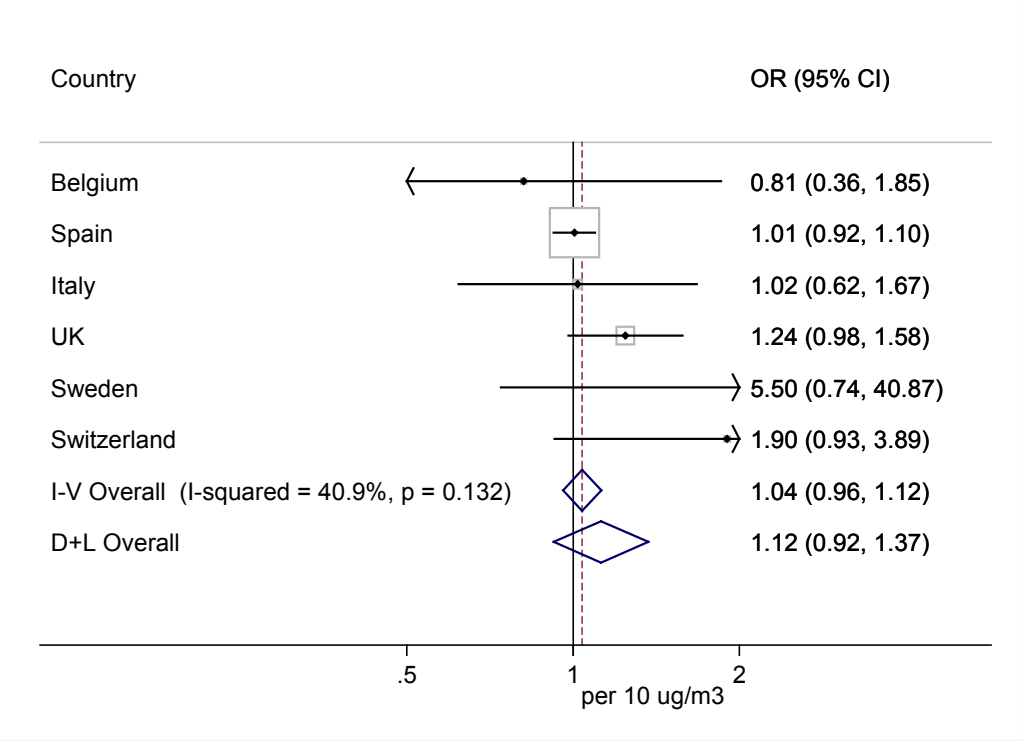


Figure 3.9 Forest plot showing odds ratios of having one higher class difference in the asthma symptoms score per 10µg/m^3 increase of indoor NO_2 in current asthmatics at ECRHS II by country adjusted for sex, age and smoking status

Associations of BR with indoor NO_2

One hundred sixty-three people with current asthma and indoor NO_2 measures also had BR to methacholine assessed; of these 50.9% had a PD20 less than 1mg. In two centers in Belgium no BR testing was conducted and 10 people with asthma living in the Belgian centers were excluded from this analysis.

There was no evidence that increasing BR (as shown by a decreasing slope) was associated with indoor NO_2 exposure (Table 3.8). BR slope tended to increase with increasing exposure (beta 0.06, 95% -0.07 to 0.19 per 10µg/m^3 increase in indoor NO_2). The association with PD20 was in the opposite direction (adjusted OR 0.94, 95% CI 0.84 to 1.07 per 10µg/m^3 increase in indoor NO_2) suggesting that indoor NO_2 is protective but failed to reach conventional levels of statistical significance. There was no evidence of heterogeneity between countries (I^2=0.0%, P=0.43 for slope and I^2=2.8% P=0.39 for PD20).

Table 3.8 Unadjusted and adjusted estimates of having higher bronchial responsiveness per 10µg/m^3 increase in indoor NO_2

	BR slope	PD20
Analysis	β (95% CI)	OR[#] (95% CI)
Unadjusted (n=162)	0.04 (-0.07, 0.14)	0.95 (0.86, 1.04)
multivariate adj ^ (n=161)	0.06 (-0.07, 0.19)	0.94 (0.84, 1.07)
multivariate adj ^^ (n=154)	0.08 (-0.06, 0.22)	0.93 (0.82, 1.06)

[#] odds ratios compare participants who experienced more than 20% fall in FEV_1 after inhalation of 1 mg of methacholine (as an indication of bronchial hyper-responsiveness with those who did not; ^Adjusted for country and sex, age at interview, smoking status (ever, never); ^^ Adjusted as above and for smoking at home, presence of damp at home, presence of mold at home, age left education, ever exposed to VGDF, season of NO_2 sampling.

3.3.4 Effect modifications by sex, smoking status, use of inhaled steroids and atopic status

Effect modification by sex, smoking status in the last 12 months and atopic status with exposure to indoor NO_2 on GINA score, asthma score and bronchial responsiveness (BR slope and PD20) were tested for significance with the Wald test. Effect modification by use of inhaled steroids in the last 12 months was also tested on asthma score and bronchial responsiveness - but not on GINA score as the score includes information on inhaled steroid use. The Wald test was used to assess the effect modifications for significance. Table 3.9 shows the results of the test for each effect modifier. None of the effect modifications reached significance ($P_{interaction}$ >0.10) except for smoking status and asthma score ($P_{interaction}$=0.0078). Stratified analysis by smoking status suggested current asthmatics who were current smokers at ECRHS II had a significant increased risk of having one higher class difference in the asthma symptoms score (adjusted OR for sex, age and country: 1.20, 95% CI 1.06 to 1.36) per 10µg/m^3 increase in indoor NO_2. In current asthmatics who never smoked there was no association (adjusted OR 0.95, 95% CI 0.86 to 1.04 per 10µg/m^3 increase in indoor NO_2).

Table 3.9 Results of significance test for effect modification by sex, smoking, use of inhaled steroids and atopic status with indoor NO_2 on GINA score, asthma score and bronchial responsiveness

	P value for significance test for effect modification*			
Outcome	Sex	Smoking status	Inhaled steroids	Atopic status^
GINA	0.76	0.45	n/a^^	0.23
Asthma score	0.49	0.0078	0.97	0.98
BR slope	1.00	0.32	0.26	0.90
PD20	0.27	0.62	0.31	0.26

*Wald test; ^ defined as having any specific IgE for HDM, *Cladosporium,* cat or grass allergens above 0.35kU/L;^^ GINA score includes use of inhaled steroids

3.3.5 Association of asthma severity with gas cooking within the whole ECRHS II cohort

The association of the use of gas for cooking with asthma severity was examined in the complete group of participants with current asthma (n=1556) within the 29 centres from 11 European (Iceland, Norway, Sweden Estonia, UK, Belgium, Germany, Switzerland, France, Italy and Spain) and 2 non-European (Australia and US) countries that took part in ECRHS II. No information on use of gas for cooking or for heating was available for participants in The Netherlands.

Overall, gas appliances (from mains or from gas bottles) for cooking were used by 40% of current asthmatics at ECRHS II, while UFGH (which included open gas fires and portable gas heaters) were used only by 14.6 % of this population. In some countries the use of gas for cooking was common (81.0% in Australia, 65.5% in France, 59.4% in UK, 59.6% in Spain and 52.2% in Belgium) or overwhelmingly predominant (95.7% in Italy). People living in the Nordic countries tended to use electric cooking appliances (100% in Norway, 97.1% Sweden and 93.8% in Iceland). The use of UFGH was quite low across the countries (14.6%) with the exception of Spain (45.3%), Australia (33.9%), Estonia (30.8%) and UK (26.6%) where open gas fire were used for heating or hot water.

Table 3.10 Frequency of gas use for cooking and heating in current asthmatics within ECRHS II by country

Country	Current asthmatics (n)	Use of electric appliance for cooking (%)	Use of gas appliance for cooking (%)	Use of UFGH* (%)
All	1556	58.1	40.2	14.6
Iceland	81	93.8	6.2	1.2
Norway	64	100.0	0.0	0.0
Sweden	350	97.1	1.7	0.3
Estonia	13	46.2	46.2	30.8
UK	229	38.7	59.4	26.6
Belgium	54	45.7	52.2	1.9
Germany	48	81.3	18.8	0.0
Switzerland	85	78.6	21.4	0.0
France	160	30.0	65.6	1.3
Italy	48	2.1	95.7	4.2
Spain	214	38.0	59.6	45.3
Australia**	174	17.2	81.0	33.9
US	36	66.7	30.6	5.6

*UFGH=unflued gas heaters, includes open gas fire and portable gas heater; **no information available on GINA score

Table 3.11 shows the associations of persistent asthma (compared with intermittent asthma), asthma score, bronchial responsiveness (slope and PD20) with exposure to gas cooking compared with electric cooking after adjustment for main confounders (sex, age, smoking status) in the group of current asthmatics with indoor NO_2 measurement and in the whole sub-set of current asthmatics within ECRHS II. There is a consistent direction of effect suggesting that cooking with gas may be associated with an increase in asthma severity although none of the associations are significant. In some countries the use of gas for cooking (Italy) or the use of electricity (Sweden) was universal and this prevented comparison of the effect of cooking with gas versus the effect of cooking with electricity within these countries (no comparison group); in technical terms this is called lack of exposure contrast. As a consequence heterogeneity between countries could not be assessed.

Table 3.11 Adjusted odds ratios and beta coefficient for markers of asthma severity when cooking mostly with gas compared with cooking mostly with electricity in current asthmatics at ECRHS II

		Current asthmatics with NO_2 measurements (n=257)	All current asthmatics (n=1556)
GINA score	OR (95% CI)	1.23 (0.65, 2.36)	1.13 (0.82, 1.57)
Asthma score	OR (95% CI)	1.65 (0.94, 2.89)	1.10 (0.86, 1.41)
BR slope	β (95% CI)	-0.61 (-1.47, 0.25)	-0.19 (-0.64, 0.25)
PD20	OR (95% CI)	0.53 (0.25, 1.12)	0.95 (0.65, 1.40)^^

^ Odds ratios adjusted for sex, age at interview, smoking status (ever, never) and country; ^^number of current asthmatics with bronchial responsiveness data= 799

3.3.6 Association of wheeze with indoor NO_2 within the whole ECRHS II cohort

Finally, the association between 12-month prevalence of wheeze at ECRHS II and indoor NO_2 was assessed in the random sample who had indoor NO_2 measurements at ECRHS II (n=1527). The logistic regression model was adjusted for sex, age at ECRHS II, smoking status (never, ever) and month (October as a baseline) when NO_2 measurement was carried out with random effect by country. Figure 3.10 shows the effect estimate by country and the combined random effect. There was some evidence of significant association between wheeze and indoor NO_2 in the general population (OR 1.05, 95% CI 1.01 to 1.09 per 10μg/m^3 increase of indoor NO_2, I^2= 0.0%, $P_{heterogeneity}$=0.75).

A sensitivity analysis was carried out by adjusting for two-week average outdoor NO_2 exposure at address level (as well as for the usual confounders). Outdoor NO_2 was measured outside participants' homes at the same time as indoor NO_2. The random effect did not change (OR 1.05, 95%CI 1.00 to 1.09 per 10μg/m^3 increase of indoor NO_2. There was no heterogeneity across country (I^2=0.0%, $P_{heterogeneity}$=0.81).

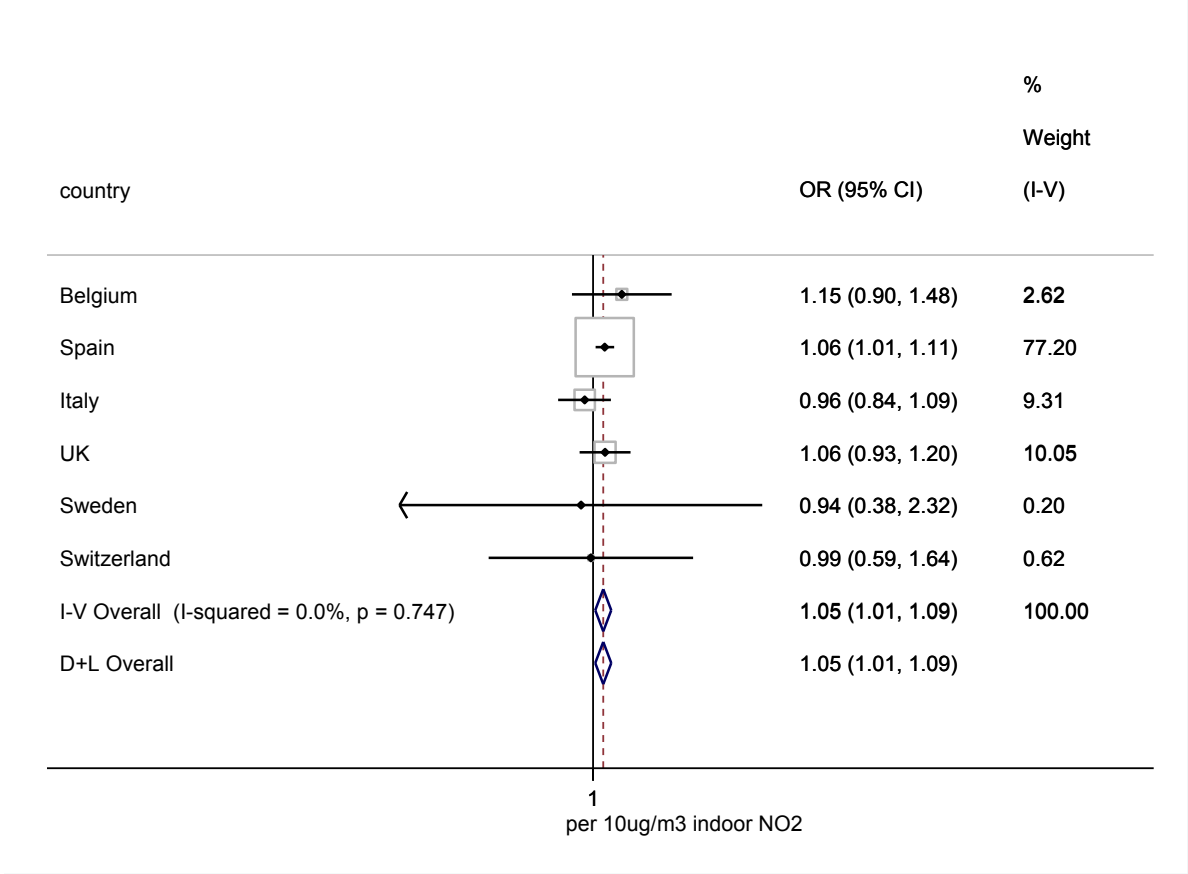


Figure 3.10 Forest plot showing the odds ratios of having wheeze in the last 12 months per 10µg/m^3 increase in indoor NO_2 by country at ECRHS II in the random sample adjusted for sex, age, smoking status (never, ever) and month when indoor NO_2 measurement was carried out

Incorporation of ECRHS II estimate into meta-analysis of 12-month prevalent wheeze and indoor NO_2 presented in Chapter 2

Inclusion of the effect estimate of ECRHS II into the meta-analysis for the association of 12-month prevalent wheeze and indoor NO_2 presented in the previous Chapter 2 decreased the size of the overall effect estimate from 1.06 (95% CI 1.02 to 1.12) 1.05 (95% 1.02 to 1.08) per 10µg/m^3 increase of indoor NO_2. The forest plot with the updated results is presented in Figure 3.11.

The ECRHS estimate had a considerable weight in the meta-analysis (59.4%) but this did not affect the heterogeneity between studies (I^2=0.0%, P=0.88). There was some weak evidence of publication bias (Egger's test P value=0.105, Begg's test P value=0.062).

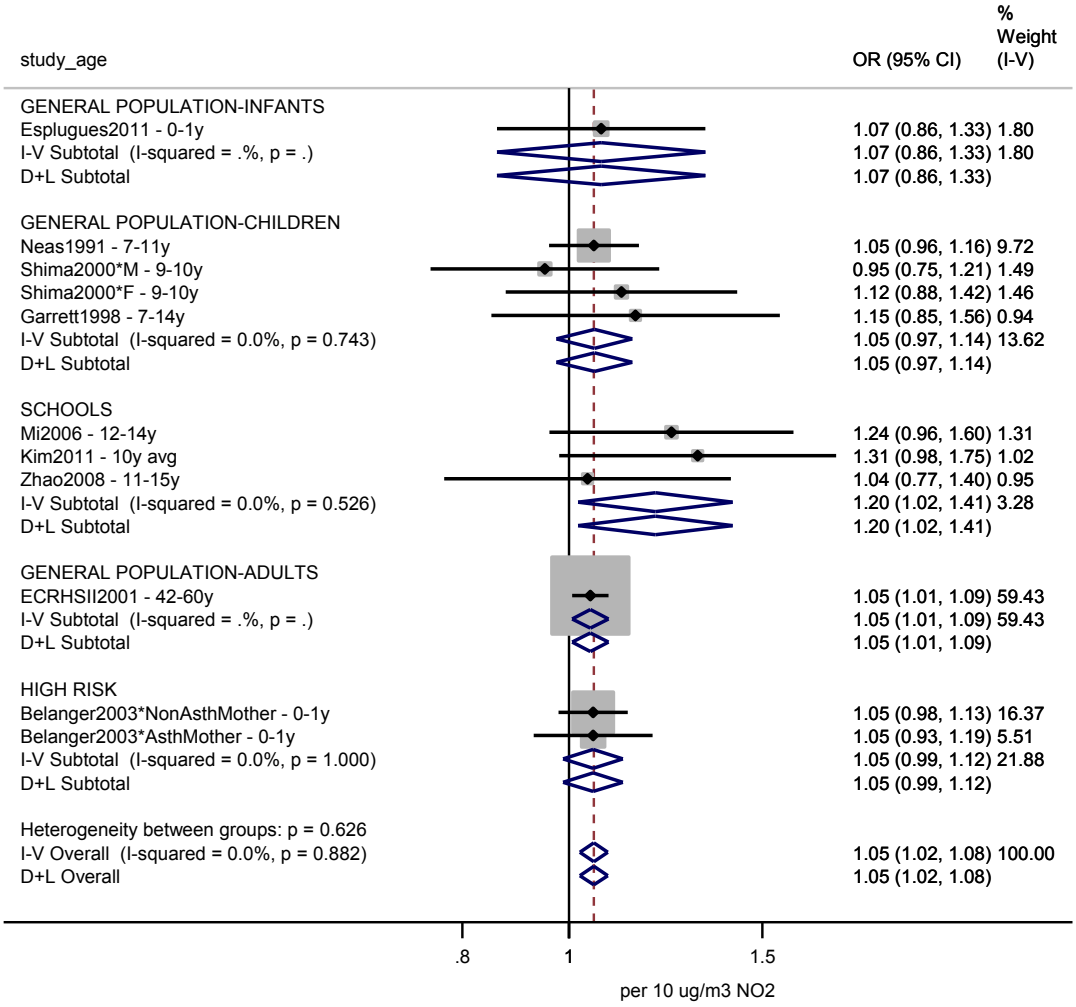


Figure 3.11 Forest plot and meta-analysis of estimates for 12-month period prevalence of wheeze and indoor NO_2 (per 10µg/m^3) stratified by type of study population and updated with the effect estimate of the ECRHS II cohort

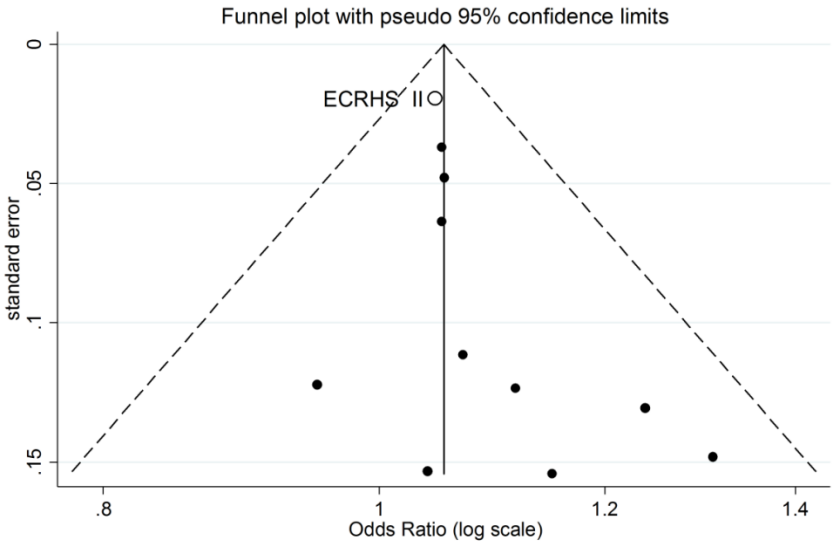


Figure 3.12 Funnel plot of the estimates for 12-month period prevalence of wheeze and indoor NO_2 (per 10µg/m^3) updated with the effect estimate of the ECRHS II cohort (indicated with a hollow circle)

3.4 Discussion

Within a multi-centre, international study (the ECRHS) there was no evidence that a range of markers of asthma severity increased with increasing two-week average kitchen NO_2. Similarly, there was no evidence that asthma severity increased with reported exposure to gas for cooking (electric cooking as a baseline). The direction of effect of the various associations tended to suggest there may be an effect – although associations with bronchial responsiveness were not consistent with this. Cross-sectional analysis of the association of wheeze with indoor NO_2 within the ECRHS II cohort suggested that there is an effect and incorporation of the estimate into meta-analysis of 12-month prevalent wheeze and indoor NO_2 presented in Chapter 2 did not affect the overall combined effect.

In this study asthma severity was determined by the presence of asthma-related symptoms (asthma score) and medication regime in the previous 12 months. This did not take into account the frequency or the duration of each symptom over the 12 months period, which may be more informative. To my knowledge there is only study (Smith, 2000) on the health effects of indoor NO_2 exposure in asthmatic adults (and children). The study investigated the association with asthma by asking participants to keep a daily diary of their asthma- related symptoms for 6

weeks. No significant associations between respiratory symptoms and NO_2 from gas appliances were found in asthmatic adults except for cough in participants age 35-49.

The population of the study presented in this chapter was an asthmatic sub-set of a large multicentre international study. Previous analyses of the association between respiratory symptoms and use of gas for cooking in all participants at ECRHS I observed some variations in the health effects between countries (Jarvis, 1998). In my analysis of the association of asthma score with indoor NO_2 I also observed some mild heterogeneity between countries. The score is based on several self-reported symptoms; it is a relatively novel approach that defines asthma phenotypes on an ordinal scale rather than as a dichotomous entity. However, the score is based on self-reporting of symptoms, which may be influenced by cultural attitudes towards illnesses and differences in health service provision (Burney *et al.*, 1991). Of note, no heterogeneity between countries was seen for bronchial responsiveness, an objective marker of asthma severity. The effect of indoor NO_2 may also differ between countries as gas appliances and cooking style may vary leading to different patterns in NO_2 peak exposures or different pattern of co-pollutants.

I repeated my analyses by replacing indoor NO_2 with the use of gas for cooking. It has been hypothesised that the health effects of combustion products such as NO_2 from gas appliances may depend on repeated exposure to peaks thus individuals who cook with gas are particularly at risk since they are regularly exposed to these peaks. Use of gas for cooking was not associated with asthma severity in agreement with findings from a US based cross-sectional study, the NHSE III (Eisner and Blanc, 2003) where no association with prevalence of wheeze and other respiratory symptoms in the previous 12 months or FEV_1 were observed in a population of over 400 adult asthmatics. Contradictory findings were observed in two US panel studies that followed adult asthmatics over a certain period of time. Increased morbidity in individuals who cooked with gas was observed in a panel of 164 adults with moderate to severe asthma who were followed for 3 months (Ostro *et al.*, 1994). In another panel of 349 non-smoking adults no associations were observed between asthma severity (defined as the frequency of current asthma symptoms (daytime or nocturnal), use of systemic corticosteroids, use of other asthma medications (besides systemic corticosteroids), and history of hospitalisations and intubations and gas stove use (Eisner and Blanc, 2003). In sub-sample of a large UK cohort (Moran *et al.*, 1999) a decrement in FEV_1 was observed in current asthmatics who used gas for cooking compared to those who used electricity but no association were found when considering the frequency of asthma attacks or reporting of sleep disturbed by wheezing in the previous year.

The modifying effects of sex, atopy and smoking status and use of inhaled steroid were tested but none was significant with the exception of smoking suggesting that asthmatics who smoke were at a greater risk of having a worse asthma if exposed to indoor NO_2, in agreement with findings of a Dutch study that found an association of decline in lung function with indoor NO_2 in smoking women but not in non-smoking women who lived in rural[1] areas (Fischer, 1989). No significant association was observed in non-smokers. Further adjustments for pack-years of smoking or current intensity of smoking were not considered because of the time constraint of this thesis. However, the effect of some residual confounding by amount smoked should not be ruled out.

Effect modification of genotype has also been considered in a recent study (Amaral *et al.*, 2014). Findings suggested that increased bronchial responsiveness was associated with gas cooking among individuals with the *GSTM1* null genotype, which may reflect the oxidant effects on the bronchi of exposure to NO_2. In my study I considered stratified analyses by genotype but I decided *a priori* that the small sample size restricted the power of statistical calculations. Of note, the test for interaction of *GSTM1* and indoor NO_2 on asthma score in the asthmatic group suggested some evidence P=0.07 but not for BR or GINA severity) (results not shown).

In this investigation the strength of the ECRHS was the large breadth of the study. Standardised questionnaires and standardised monitoring protocols were applied across all ECRHS centres and analysis could be carried out in over 250 current asthmatics with indoor NO_2 measurements. Unfortunately, the sample size of participants with current asthma was relatively small and influenced the precision of the results. A larger sample size may have increased precision although calculating the 'observer' statistical power is rather pointless. It has been claimed that calculation of power after a study is inappropriate (Goodman and Berlin, 1994) and fundamentally flawed (Hoenig and Heisey, 2001).

However, increasing the sample size by using a proxy measure for indoor NO_2 would give the opportunity of assessing the health effects over a larger population sample. The use of gas cooking as a proxy measures can be useful when no other information is available but can be imprecise in assessing the health effect as cooking and ventilation which affect the dispersion rate of combustion gases may vary by individual and household. The ECRHS has information on the presence of gas appliances and some relevant household characteristics – but only a subsample of participants had direct measures of indoor NO_2.

[1] There was no difference between smoker and non-smoker women who lived in urban areas

One of the main limitations of this study was the assumption that a two-week measurement of indoor NO_2 is representative of long-term exposure to indoor NO_2 ignoring that indoor NO_2 levels may fluctuate between summer and winter. An adjustment for season of monitoring was included in the secondary analyses and this did not alter the results. A further limitation of the two-week monitoring was that it does not provide any information on peaks exposure (Franklin, 2006). Gas cooking combustion can generate short-term peaks of NO_2 as high as 1880µg/m^3 (Dennekamp, 2001). Although some studies have shown that average and peak exposures may be correlated (Ross, 1996) exposure to indoor NO_2 peaks may affect respiratory health. Asthmatics may be particularly at risk as findings from chamber studies have suggested. In the next chapter I will assess the feasibility of conducting a panel study that examined the association of respiratory health with exposure to indoor NO_2 peaks generated from gas combustion.

3.5 Summary

Within a multi-centre international study (ECRHS) there was no evidence that a range of markers of asthma severity increased with increasing two-week average kitchen NO_2 or with the reported use of gas for cooking (electric cooking as a baseline). Cross-sectional analysis of the association of wheeze with indoor NO_2 within the whole random sample of participants at ECRHS II suggested that there is an effect. The incorporation of the estimate into meta-analysis of 12-month prevalent wheeze and indoor NO_2 presented in Chapter 2 did not affect the overall combined effect.

4. A pilot study on asthma and exposure to indoor NO_2 peaks

4.1 Introduction

Evidence from clinical studies suggests that asthmatics could be at higher risk when exposed to short-term high concentrations of NO_2, which do normally occur while cooking with gas. In this chapter I am going to describe a pilot study I designed and conducted to assess the feasibility of conducting a panel study that examined the association of respiratory health in asthmatic people with exposure to indoor NO_2 peaks generated from gas combustion.

4.1.1 Background

It has been shown that cooking on a four-ring gas hob can produce 5 minute peaks of about 1,880μg/m^3 NO_2 (Dennekamp, 2001). These peaks may be associated with adverse respiratory health effects although the evidence has been inconsistent.

Controlled human exposure studies have observed that 30 minutes exposure to 500μg/m^3 of NO_2 can cause alterations in bronchial reactivity and increase sensitivity to inhaled allergens in asthmatics (Tunnicliffe, 1994; Strand, 1997). Orhek et al (1976) observed an increase in bronchial sensitivity to bronco-constrictors agents when people with asthma were exposed at concentrations as low as 188 μg/m^3 for one hour. Bylin et al (1985) exposed asthmatics at higher concentration (910μg/m^3) for a shorter period of time (20 minutes) and also noted an increase in bronchial reactivity. Bauer (1986) observed changes in forced expiratory flow rate after exercise in asthmatics who were exposed at 564μg/m^3 for 30 minutes.

In contrast, no changes in lung function were observed in asthmatic adolescents when exposed at 226μg/m^3 and 338μg/m^3 for an hour with intermittent exercises (Koenig, 1987). Similarly, more recent studies have observed no decrease in lung function after NO_2 exposure but at same time they have noticed an activation of eosinophils in lung fluid and sputum and an increase in allergic and inflammatory responses (Barck *et al.*, 2002). A recent double-blind crossover study (Ezratty *et al.*, 2014) has found that peak exposure to NO_2 (376μg/m^3, 1128μg/m^3) for 30 minutes

repeated on two consecutive days performed without allergen exposure were associated with airway eosinophilic inflammation in asthmatic in a dose-related manner.

Current WHO guidelines (World Health Organization, 2006) recommend that 1-hour exposure should not exceed an average of 200µg/m^3. The original guidelines were set in 1997 (Graham, 1997) based on findings of a meta-analysis of 20 clinical studies in asthmatics (Folinsbee, 1992). Although no individual studies had shown clearly significant effects on airway responsiveness at 190µg/ m^3 for 60 minutes the meta-analysis suggested that increased airway responsiveness may occur at concentrations as low as 200µg/m^3 of NO_2 (Graham, 1997). Inconsistencies in findings may be explained by studies testing participants with different degrees of asthma severity, variations in the exposure protocol (oral or oral-nasal exposure, participant is sitting or standing) and different outcomes (airway hyper-responsiveness, changes in lung function).

Evidence from epidemiological studies on the health effects associated with high peaks exposure to indoor NO_2 is scarce. Few epidemiological studies have attempted to measure indoor NO_2 peaks, mainly because the monitors (chemiluminescence analysers) are expensive, bulky and impractical for the purpose of larger scale epidemiological studies. Some studies have tried to mimic real life exposure by measuring associations when indoor NO_2 is generated from gas combustion. Goldstein (Goldstein *et al.*, 1988) measured spirometric lung function in 11 people with and without asthma, before, during and after cooking with gas and found that FVC in people with asthma dropped when exposed at levels above 564µg/m^3 of NO_2 (measured with a chemiluminescence analyser) for at least 5 minutes. Ng (2001) measured peak flow in 16 asthmatic women before and after cooking with gas and found that PEFR fell (3.4%) significantly immediately after an episode of gas cooking, which generated on average 121µg/m^3 (range: 0 µg/m^3 - 491µg/m^3) of NO_2.

Most of the epidemiological studies on respiratory health and indoor NO_2 have traditionally measured NO_2 with passive diffusion samplers. These provide average concentrations thus underestimating peak levels (Franklin, 2006). Some studies have attempted to measure short-term exposure to NO_2 generated from gas combustion by keeping the passive diffusion samplers open only when the gas appliance is on (Pilotto, 1997; Smith, 2000). Pilotto (1997) found that exposure to NO_2 at hourly levels of the order to 150µg/m^3 compared with background levels of 38µg/m^3 was associated with a significant increase in sore throat, colds and absences from school in Australian school children. Another Australian study (Smith, 2000) examined daily respiratory symptoms (wheeze, breathlessness, chest tightness, cough, breathlessness on exertion, daytime asthma attacks and night time asthma attacks) on the same day (lag 0) and following day (lag 1) of exposure to short-term gas generated NO_2 (median range 7- 276µg/m^3)

in people of any age living in 125 homes; significant associations were observed only in the young age group (≤14 years) and in the 35-49 age group for cough (lag 1).

Many panel studies have traditionally relied on proxy measures to assess the health effects of short-term exposure to NO_2 such as the daily use of gas cooking (Ostro, 1994) or the daily use of unflued gas heater (Franklin, 2012). Ostro (1994) investigated the occurrence of several respiratory symptoms (cough, wheeze, shortness of breath, chest tightness and sputum production), nocturnal asthma, medication use and restriction in activity following the daily exposure to gas and wood stoves and fireplaces in a panel of 164 adult asthmatics. Among the indoor sources gas stove use was found to be most strongly associated with moderate or severe cough and moderate or severe shortness of breath. Franklin (2012) investigated daily lung function (PEF, FEV_1) and respiratory symptoms (wheeze, cough, dyspnoea) in a panel of 71 asthmatic patients above the age of 55 and the daily use of their primary source of heating, an unflued gas heater (UFGH). Same and previous day exposure to UFGH was associated with significantly increased odds ratios for wheeze and dyspnoea compared with days when no heating was used, and significant increases in the average odds of reported wheeze and dyspnoea per hour of UFGH heater use.

It has been suggested that peaks in exposure to NO_2 of indoor origin is associated with acute asthmatic responses to NO_2 and that these peaks may be more closely associated with acute asthmatic responses to NO_2 than weekly average exposure in susceptible individuals but there is little conclusive evidence from clinical studies and few epidemiological studies have been conducted. There is a need to conduct new studies that can objectively assess the short term health effect of exposure to indoor NO_2 peaks from gas combustion. Women, who in many families may do most of the cooking, could be particularly at risk from exposure to NO_2 peaks.

In this chapter I am going to describe a pilot study I have designed and conducted. The purpose of the pilot study was to assess the feasibility of conducting a panel study that examined the association of respiratory health in people with exposure to indoor NO_2 peaks generated from gas combustion. The study could not be completed because of recruitment problems. This chapter will cover details of the study design and its evaluation.

4.1.2 Nitrogen dioxide monitors

This pilot study used a new-to-market portable, low-cost monitor that could measure short-term exposure to indoor NO_2, the Aeroqual Series 500 (http://www.aeroqual.com/indoor-air-quality/portable-monitors). Short-term measurements of NO_2 are normally carried out with a

chemiluminescence analyser, the 'gold standard' for NO_2 monitoring but they are bulky and expensive to use and impractical for the purpose of an epidemiological study. In the recent years new NO_2 monitors that can measure short-term exposure have become available on the market and one of the main objectives of this pilot study was to assess whether these instruments were suitable for large scale epidemiological investigations.

The following two short-term exposure monitors with a fast uptake rate, suitable for detecting NO_2 peaks were initially considered for this project:

1. Gradko (http://www.gradko.com) Rapid Air Monitor (RAM), a passive diffusion sampler but with a quick diffusion rate that allows short period of monitoring
2. Aeroqual^{TM} Series 500 electro-chemical gas sensor.

In addition, the study measured weekly average exposure and the following traditional passive diffusion samplers were also considered:

1. Ogawa passive diffusion sampler (badge design)
2. Gradko long-term passive diffusion sampler (tube design)
3. Passam long-term passive diffusion sampler (tube design).

Table 4.1 (adapted from Yu, 2008) describes the various features of the five monitors listed above. The estimated costs are given per 10 participants each followed for 8 week (costs are as on June 2013).

Table 4.1 Evaluation of NO_2 monitors considered in the pilot study (*adapted from Yu 2006*)

	Known or potential interferents	Temperature influence	Relative humidity influence	Dimension	Sampling rate(a)	Duration of sampling	Working concentration	Detection limit	Uncertainty	Cost per 10 person x 8 week (b)
Short-term monitors										
Gradko RAM (Rapid Air Monitor) – badge(c)	nitrous acid, peroxy acetyl nitrate	operable from -5°C to 30°C	operable from 20% to 70%	44mm x18mm X28 mm	28.6 ml/min	from 1hour to 7 days	n/a(d)	<0.2ug/m^3 over 1 week exposure period	±13.0% uncertainty at 40 µg/m^3	£2,8180(e)
Aeroqual GSE (Sensitive Electrochemical) (f)	chlorine, ozone	operable from 0° to 40°C	operable from10% to 90%	195 x 122 x 54 mm	n/a	can log up to 4,300 data point(g)	0-1,880 µg/m^3	9.4 µg/m^3	<±37.6 µg/m^3 between 0- 376 µg/$m^{3;}$ < ±10% between 376-1810 µg/m^3(h)	£1,528
Long-term monitors										
Gradko long-term passive –tube	ozone, nitrous acid, peroxy acetyl nitrate	<10% operable from 2°C to 30°C	<10% from 20% to 80%	7.1 cm x 0.95 cm^2	1.2 cm^3/min	2-4 weeks	1.9-18,800 µg/m^3	<2 µg/m^3 over 4 week exposure period	<30% uncertainty above 19.2 µg/m^3 ; <5% precision	£476
Passam long-term passive –tube	oxidants	No influence from 5°C to 40°C	operable from 20% to 80%	7.4cm x 0.75 cm^2	0.85 cm^3/min	1-4 weeks	0.9-199 µg/m^3	0.36 µg/m^3 for weekly; 0.72 µg/m^3 for fortnight	23.4% uncertainty between 19.9-38.0 µg/m^3	£1,008
Ogawa sampler –badge	n/a(d)	operable between from -10°C to 40°C	operable from 50% to 80%	0.6cm x 0.79 cm^2	12.1 cm^3/ min	24- 168 hours	0-47,000 µg/m^3 for 24h; 0-6,768 µg/m^3 for 168h	4.2 µg/m^3 for 24h; 0.54 µg/m^3 for 168h	<±10%	£1,856

(a)manufacturer's specifications; (b)as on June 2013; (c) manufacturer's specifications http://www.gradko.com/environmental/products/no2-rapid-air-monitor.shtml; (d)not reported in manufacturer's specification or from HoYu (2006); (e) samplers need to be replaced every day for short-term exposure measurements, the cost is for 8 samplers replaced daily per 10 persons; (f) manufacturer's specifications http://www.aeroqual.com/wp-content/uploads/Series-500-Brochure.pdf ; (g) one point is equivalent to 1 or 5 minute average exposure depending on manual settings; (h) data refers to accuracy of calibration.

All monitors shared similar characteristics in terms of uncertainty (10% or less), detection limit being proportional to exposure period (except for Aeroqual) and sensitivity to oxidant species, such as ozone, temperature above 30° C (40°C for Aeroqual) and relative humidity above 80% (70% for Gradko RAM).

The small size and easy-to-use features made Gradko RAM (Rapid Air Monitor) an attractive monitor to use in participants' homes but it was quite expensive when considering that the sampler had to be replaced daily to monitor short-term exposure. Aeroqual (Figure 4.1 on the left) had a finer monitoring resolution than Gradko RAM, could be used repeatedly for multiple sequential participants and measurements were carried out in real-time.

Among the three long-term passive diffusion samplers, Passam (Figure 4.1 on the right) was chosen because it could measure one-week average rather than two-week average (e.g. Gradko long-term sampler) and was cheaper than Ogawa badges. Both monitors had been used in large scale studies in the past.

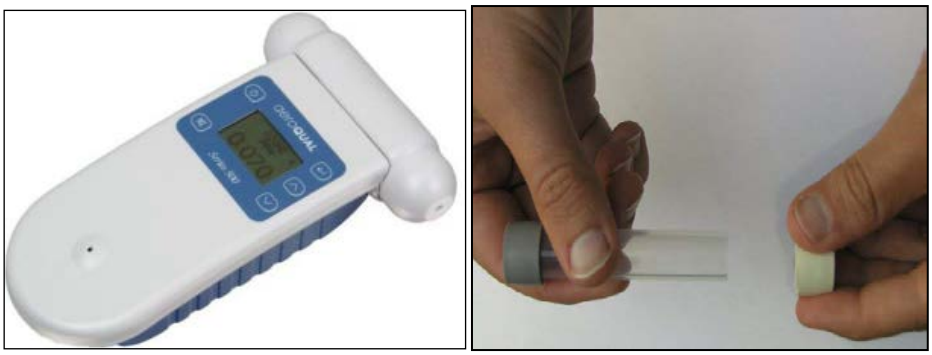


Figure 4.1 NO_2 monitors for the pilot study: Aeroqual Series 500 with NO_2 gas sensor *(left)* and Passam tube *(right)*

Aeroqual NO_2 monitor

The Aeroqual Series 500 is an electro-chemical gas sensor. It comprises of a monitor with a display window and a battery case and interchangeable gas (in this case NO_2) sensor head. It has an internal fan that pulls air across the sensor at a specified flow rate for accurate gas detection. A stream of air is pulled inside the sensor every 60 seconds providing a new reading of NO_2 level and resulting in a 60 second response time (manufacturer's specification). Nitrogen dioxide values can be logged (up to 4,300 data points) and easily downloaded and converted into an Excel file. The sensor head uses Gas Sensitive Electrochemical (GSE) technology to detect NO_2 from 0- 1,880µg/m^3 with 1.88µg/m^3 resolution and <±37.6µg/m^3 accuracy (in concentrations

from 0-37.6µg/m^3) or <± 10% accuracy (in concentrations from 376- 1,880µg/m^3) (see Table 4.2 for list of manufacturer's specifications).

Table 4.2 Aeroqual Series 500 with NO_2 sensor head manufacturer's specifications*

Features	Specifications
Manufacturer	Aeroqual
Gas	Nitrogen Dioxide (NO_2)
Sensor Type	GSE (Gas Sensitive Electrochemical)
Range	0-1ppm (0-1880µg/m^3)
Maximum Exposure	2 ppm (3,760 µg/m^3)
Minimum Detection Limit	0.005 ppm (9.4 µg/m^3)
Accuracy	< +/- 0.02 ppm (37.6µg/m^3) from 0-0.2 ppm (0- 376µg/m^3); < +/- 10% from 0.2-1 ppm (376-1880µg/m^3)
Resolution	0.001ppm (18.8µg/m^3)
Response Time	<60 seconds
Sampling Method	Fan
Operational Temperature Range	0 to 40°C
Operational Relative Humidity Range	10% to 90%

*specifications for NO_2 are usually given in ppm or ppb as these metrics are not affected by atmospheric pressure or temperature; I have calculated the conversions from ppb to µg/m^3 using the WHO conversion factor of 1.88 that assumes temperature is 25°C and pressure 1013mb.

As Aeroqual is an electro-chemical gas sensor it operates quite differently from a passive diffusion sampler. Briefly, an electrochemical gas sensor comprises of three electrodes (a working electrode, a counter electrode and a reference electrode) separated by a thin layer of electrolyte and enclosed in plastic housing. Air that comes in contact with the sensor first passes through a smaller capillary-type opening and then diffuses through a hydrophobic barrier (gas permeable membrane), eventually reaching the electrode surface. The gas is allowed to react at the sensing electrode to produce a sufficient electrical signal while preventing the electrolyte from leaking out of the sensor. The current generated is proportional to the concentration of gas present outside the sensor, which gives a direct measure of the gas (NO_2) present. This current is limited by the rate of diffusion of the gas which in turn is proportional to the concentration gradient across the cell (Fine *et al.*, 2010).

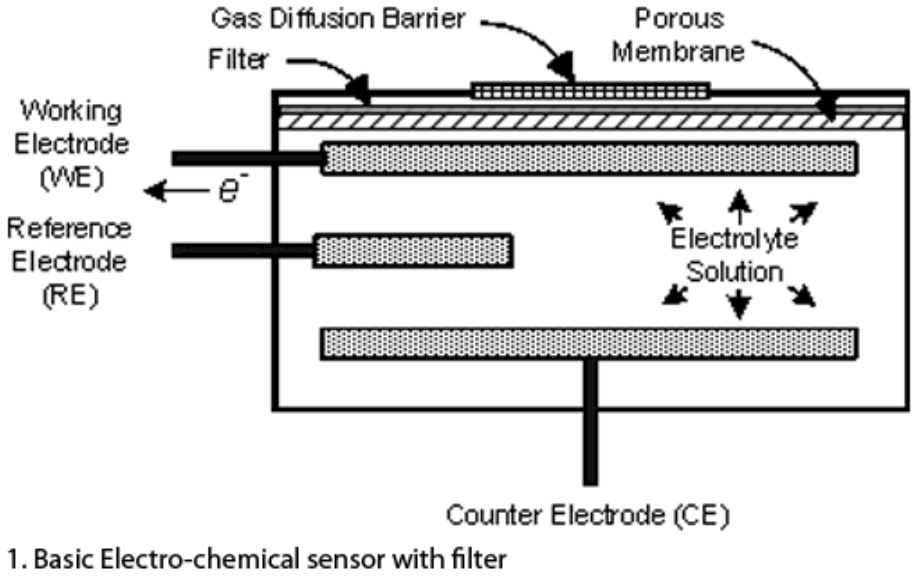


Figure 4.2 Schematic of an electrochemical cell (*from http://electronicdesign.com/components/sensible-sensors-it-s-control-thing*)

The choice of the components materials and their arrangement will affect their performance of a gas sensor. Aeroqual manufacturers use Tungsten (VI) oxide (WO_3) as the oxide material of the electrodes. WO_3 is very sensitive to NO_2 gas and shows large resistance-increase signals in response to traces of the oxidizing gases (Williams *et al.*, 2009).

The selection of the correct capillary pore size is also important; it must allow the proper amount of gas molecules to enter the electrolyte area but at same time must be able to prevent electrolytes from leaking out or drying out the sensor too quickly. Some cross-sensitivity with other gases may occur, which interfere with the monitoring. A 'scrubber' filter is commonly installed in the sensor to filter out unwanted gases. Ozone is the main gas that interferes with NO_2 monitoring and Aeroqual manufacturers use a thermal scrubber that maximises the removal of the ozone interference (Williams, 2009).

Overall, electrochemical sensors are commonly sensitive to temperature and need to be internally temperature-compensated.

Evaluation of the monitor response against gas cooking records

A very recent work carried out by Delgado-Saborit (Delgado-Saborit, 2012) suggested that the high resolution of the Aeroqual monitor would allow identification of short-term peak exposures.

I tested the Aeroqual response to detect NO_2 peaks in my kitchen for 8 days. The kitchen had a cooker connected to the gas mains (four gas hobs, gas oven and gas grill). Any time the cooker was on it I recorded it in a diary. I also kept record of the type of appliance used (hob, number of hobs, oven, grill). Aeroqual was place 0.5 meter away from the cooker and 2 meters high. Data were logged into Aeroqual every 5 minutes (one data point is the average of five 1-minute measurements). The time of the peak occurrence logged by Aeroqual was checked against the time of cooking recorded in the diary.

The 8-day average exposure to indoor NO_2 that Aeroqual measured was 19µg/m^3 (IQR 11-32µg/m^3) but during this period high levels lasting for a short time were recorded by Aeroqual any time the gas appliances were on. The highest level was recorded when the gas oven was on. Table 4.4 shows the number and frequency of peaks and their concentrations recorded by Aeroqual. During the 8 days there were 7 peaks above 600µg/m^3; six lasting for 5 minutes and one for 20 minutes.

Table 4.3 NO_2 levels (5-minutes average) measured in the author's kitchen when gas appliances were on using the Aeroqual monitor

	median (IQR) (µg/m^3)	**min - max** (µg/m^3)
over 8 days (any time)	19 (11-32)	0-998
when only 1 gas hob is on	197 (129-290)	13-485
when only 1 small gas hob is on	117(85-130)	38-164
when only 1 large gas hob is on	303(198-414)	17-485
when only gas oven is on	644(363-948)	28-998
when only gas grill is on	478(203-632)	88-684

Table 4.4 Length and frequency of NO_2 peaks ($\mu g/m^3$) measured in the author's kitchen for 8 days using the Aeroqual monitor

Peaks	Lasting for	no. events*	Max level reached ($\mu g/m^3$)
>=200 $\mu g/m^3$	5 min	15	667
	10 min	7	684
	15 min	1	335
	20 min	1	758
	25 min	1	541
	65 min	1	998
>=600 $\mu g/m^3$	5 min	6	790
	20 min	1	998

* One event = 5 minutes.

Figure 4.3 and Figure 4.4 show the level of NO_2 measured by Aeroqual from 07/07/2013 to 15/07/2013 and as an example, the peaks which were generated in the afternoon of 10/07/2013 when the gas oven was on. This was the only occasion when NO_2 level exceeded 200$\mu g/m^3$ for longer than one hour (i.e. the current WHO guidelines for 1-hour daily maximum exposure to NO_2). This small 'in-house' evaluation suggested the Aeroqual sensor could detect NO_2 peak generated from cooking with gas as shown by the extensive work by Delgado-Saborit.

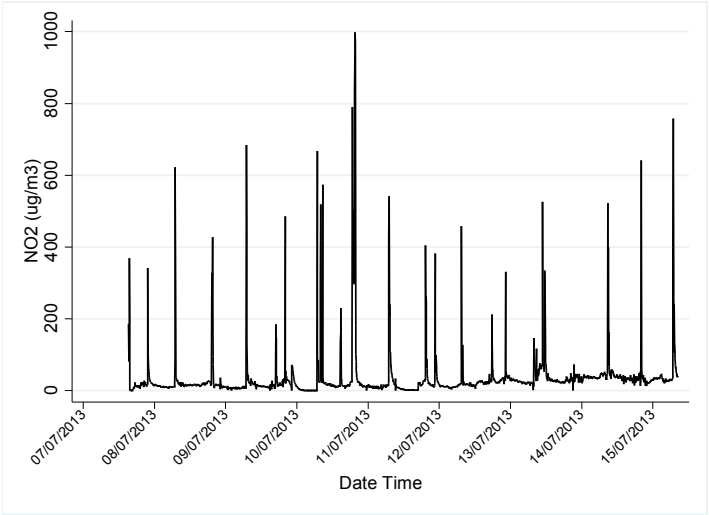


Figure 4.3 Graph showing 5-minutes average concentrations of NO_2 measured in a kitchen with a gas cooker for 8 days (from 07/07/2013 to 15/07/2013)

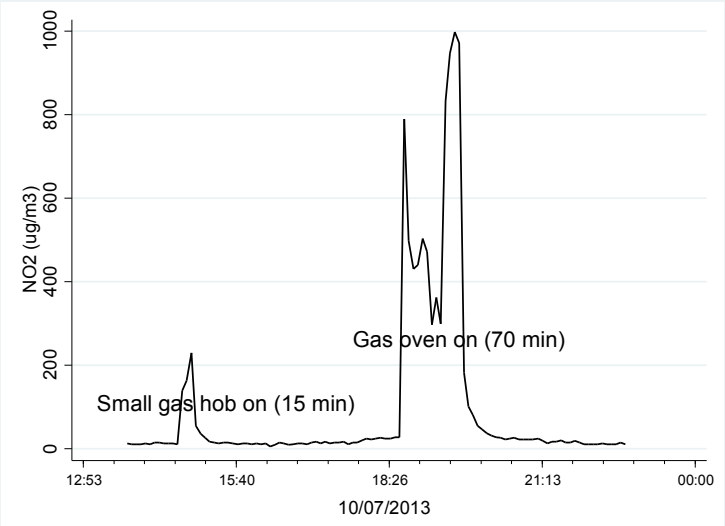


Figure 4.4 Graph showing NO_2 peaks when gas hob and gas oven were on

4.1.3 Aim

The aim of this pilot study was to assess the feasibility of a panel study to assess the association of respiratory health with exposure to indoor NO_2 peaks from gas combustion.

4.1.4 Objectives

The **pilot** study intended to assess the feasibility of a panel study by evaluating the following study features:

- Recruitment rates
- Participants compliance to study procedures
- Outcomes assessment
- Use of diary and problems arising from
- Data collection
- Instrument monitoring and in particular, problems arising from NO_2 sensor use.

Ultimately, the main objectives of the **panel** study were:

- To assess the association of daily morning and evening peak flows and daily changes in respiratory symptom severity score with short-term exposure to indoor NO_2 peaks

- To compare the associations of morning and evening peak flows and daily changes in respiratory symptoms severity score with peak indoor NO_2 with those observed for weekly average exposure.

4.2 Design of the pilot study

4.2.1 Overview

The pilot study intended to recruit 20 women with asthma who cook with gas for a period of 8 weeks during which their respiratory health and NO_2 kitchen levels were assessed (Figure 4.5). During the study period weekly average exposure to indoor NO_2 was measured (for 8 weeks) in the participant's kitchen using passive diffusion samplers and NO_2 peaks monitored (for 8 consecutive days) using the Aeroqual monitor. Concomitantly, participants recorded their morning and evening PEF, daytime and night-time asthma symptoms and timing of cooking (Figure 4.6 and Figure 4.7).

In the next sections I will explain in more detail participants' selection criteria, recruitment strategy, exposure and outcome assessments and proposed statistical methodology to analyse collected data.

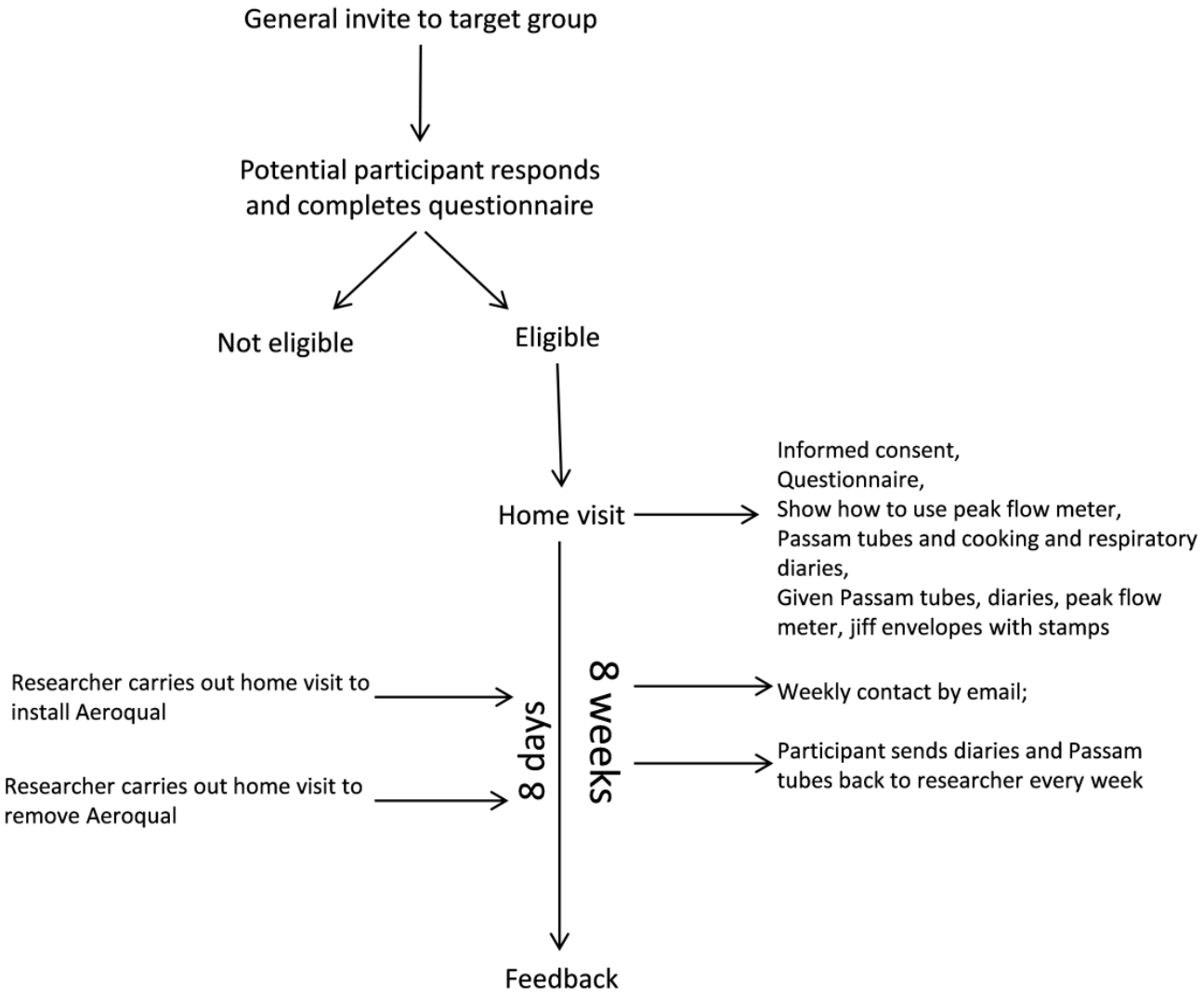


Figure 4.5 Flow chart of study from recruitment to home visit

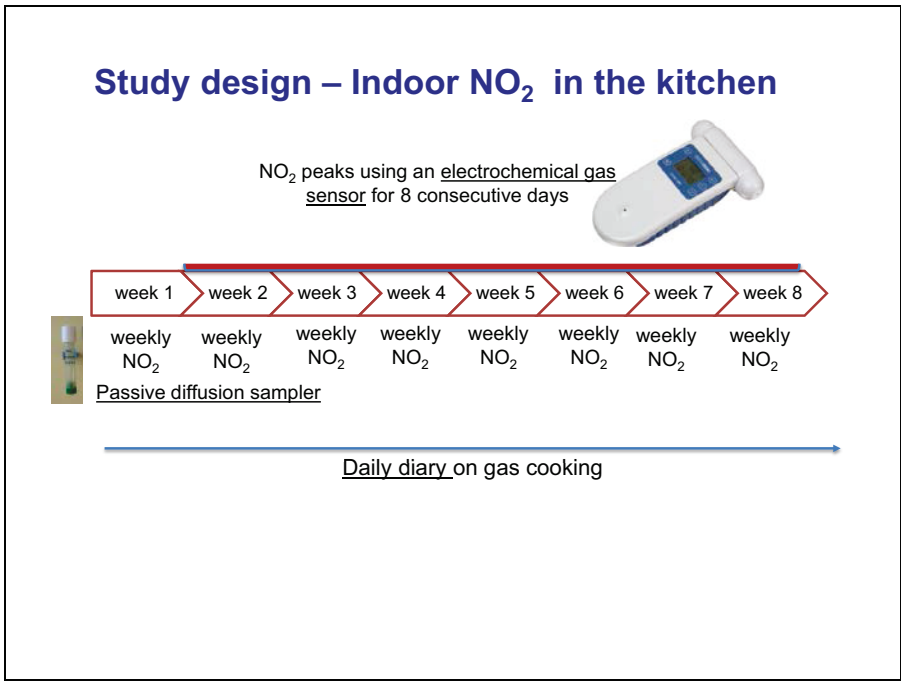


Figure 4.6 Exposure assessment during the 8-week study period

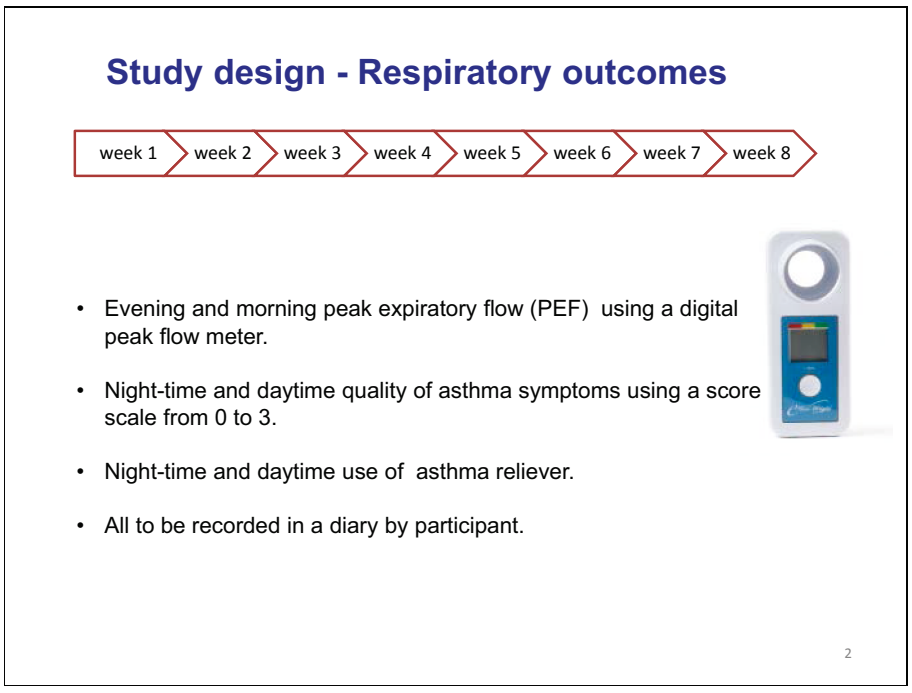


Figure 4.7 Outcome assessment during the 8-week study period

4.2.2 Selection criteria

The pilot study aimed to recruit 20 women with current asthma aged 20-65 who lived in London and cooked at home on at least four occasions (lunch or dinner) a week. Women were defined as current asthmatics if they had been diagnosed by a doctor as having asthma and had used β-agonists for the treatment of asthma in the last 12 months. The study aimed to recruit 75% of women who cooked with gas and 25% who cooked with electricity.

Women who were current smokers, pregnant, been diagnosed with a heart condition (congestive cardiac failure, angina, myocardial infarction, hear attack), with any other long-term limiting illness that required hospitalisation in the last year or had planned to go on holiday for more than 4 consecutive days during the 8 weeks of observation period were excluded.

4.2.3 Recruitment

Several recruitment strategies were attempted through different agencies:

- Asthma UK (http://www.asthma.org.uk/), a leading UK asthma charity and patients' organisation
- South East London Community Air Pollution, a network of individuals and community-based organisations who are interested or involved in improving outdoor air pollution in London
- Imperial College
- GP practices (directly contacted)
- GP practices (contacted via the National Institute for Health Research Clinical Research Network (NIHR-CRN) for North West London. The NIHR-CRN (http://www.crn.nihr.ac.uk/north-west-london/) is one of the 15 networks across England working with NHS Trusts to fund and deliver clinical research studies.

The following advertising strategies were attempted in the following order:

1. On the newsletter of Research and Policy volunteers of Asthma UK
2. On Asthma UK Facebook
3. On the newsletter of South East London Community Air Pollution group
4. On the staff noticeboard of Imperial College web site
5. By sending an invitation letter to patients with asthma in collaboration with a GP practice, which had been contacted by NIHR-CRN for North West London.

Women who were interested in the study were asked to complete a short questionnaire for eligibility assessment (Figure 4.8). If eligible women were invited to participate in the study and provided with a Participant Information Sheet and an Informed Consent Form (see Appendix of Chapter 4 for more details). Potential participants were given the choice on how to communicate with the researcher (email, post, face to face or phone). All women preferred communicating by email.

4.2.4 Home visit

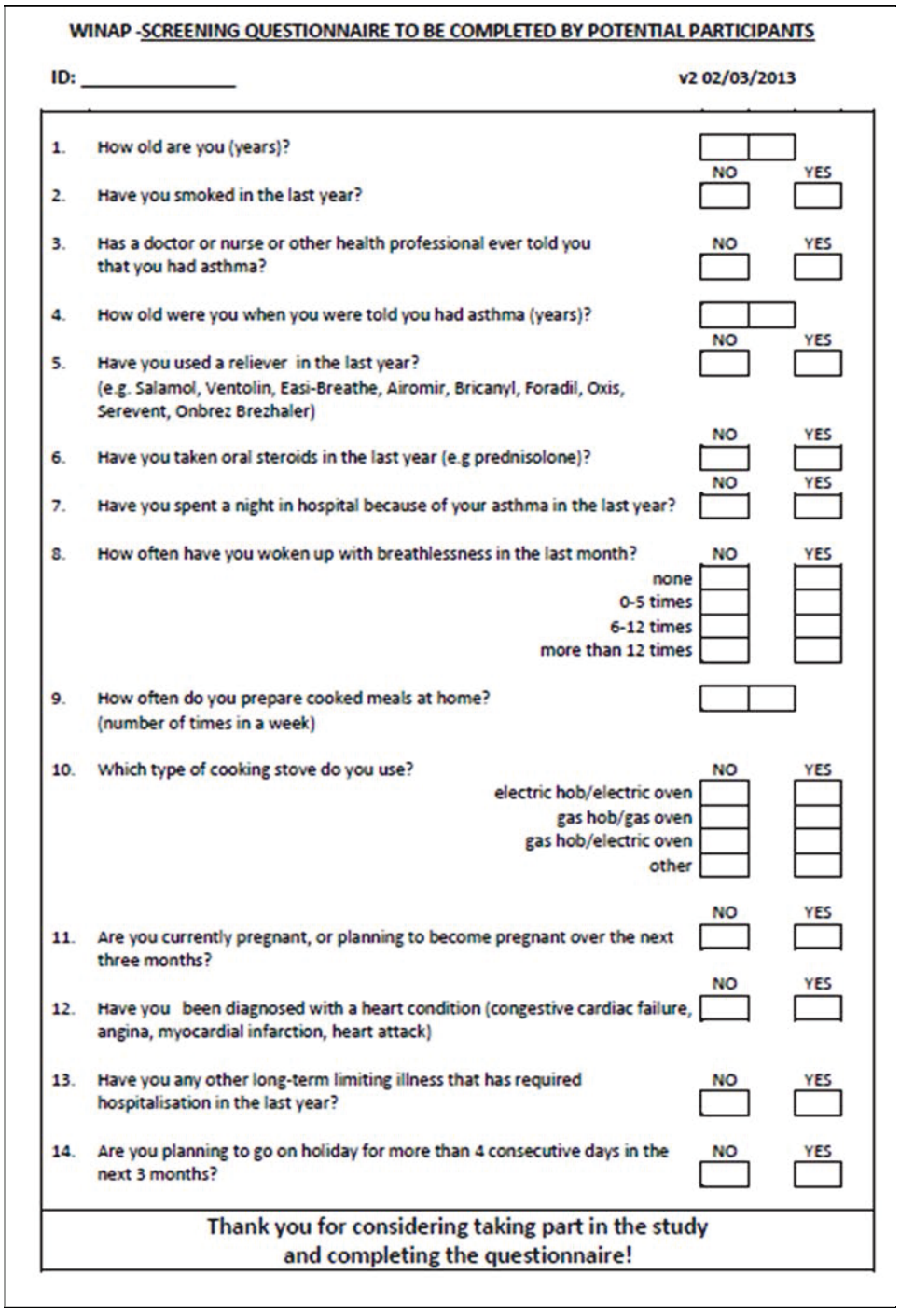
Once the invitation to take part in the study was accepted, the participant was visited at home. She was asked to sign the informed consent form and complete a questionnaire about herself (e.g. age, occupation), her asthma symptoms and her kitchen (See Appendix of Chapter 4 for more details). She received instructions on how to handle the Passam tubes, measure her own peak flow, score her asthma symptoms and keep a health and cooking diary for 8 consecutive weeks. Home visits were carried out to install the Aeroqual and again, after 8 days, to remove it.

4.2.5 Assessment of exposure to NO_2

Exposure to NO_2 peaks was continuously monitored with the Aeroqual Series 500 for 8 consecutive days during the study period. Five-minute average exposure was used for analysis.

NO_2 was also measured weekly for the full study period (8 consecutive weeks) using Passam tubes. Participants were asked to replace the tube every week and send it back to me. Duplicate sampling was carried out three times and a blank testing twice. Aeroqual monitor and Passam tubes were both placed in the kitchen between 1.5m and 2m high and at least 0.5m away from cooker and 1m away from window.

As part of the exposure assessment participants were also asked to keep a diary about their cooking activities in a weekly diary (Figure 4.9).



WINAP -SCREENING QUESTIONNAIRE TO BE COMPLETED BY POTENTIAL PARTICIPANTS

ID: _____ **v2 02/03/2013**

		NO	YES
1.	How old are you (years)?		
2.	Have you smoked in the last year?	☐	☐
3.	Has a doctor or nurse or other health professional ever told you that you had asthma?	☐	☐
4.	How old were you when you were told you had asthma (years)?		
5.	Have you used a reliever in the last year? (e.g. Salamol, Ventolin, Easi-Breathe, Airomir, Bricanyl, Foradil, Oxis, Serevent, Onbrez Brezhaler)	☐	☐
6.	Have you taken oral steroids in the last year (e.g prednisolone)?	☐	☐
7.	Have you spent a night in hospital because of your asthma in the last year?	☐	☐
8.	How often have you woken up with breathlessness in the last month?	NO	YES
	none	☐	☐
	0-5 times	☐	☐
	6-12 times	☐	☐
	more than 12 times	☐	☐
9.	How often do you prepare cooked meals at home? (number of times in a week)		
10.	Which type of cooking stove do you use?	NO	YES
	electric hob/electric oven	☐	☐
	gas hob/gas oven	☐	☐
	gas hob/electric oven	☐	☐
	other	☐	☐
11.	Are you currently pregnant, or planning to become pregnant over the next three months?	☐	☐
12.	Have you been diagnosed with a heart condition (congestive cardiac failure, angina, myocardial infarction, heart attack)	☐	☐
13.	Have you any other long-term limiting illness that has required hospitalisation in the last year?	☐	☐
14.	Are you planning to go on holiday for more than 4 consecutive days in the next 3 months?	☐	☐

Thank you for considering taking part in the study and completing the questionnaire!

Figure 4.8 The screening questionnaire

WINAP - COOKING DIARY - MONDAY date: _____ / _____ / _____ ID: _____

Please, tick where the answer is 'YES'

TIME	Were any hob on? How many?	Was the oven on?	Was the grill on?	Was the fan on?	Any window to the outside open?	Any door to the outside open?	Anybody smoking in the kitchen?	Which style of cooking did you do? (e.g frying, boiling, stewing, roasting)	Any comment?	TIME
		✓	✓	✓	✓	✓	✓			
6:00am										6:00am
6:30am										6:30am
7:00am										7:00am
7:30am										7:30am
8:00am										8:00am
8:30am										8:30am
9:00am										9:00am
9:30am										9:30am
10:00am										10:00am
10:30am										10:30am
11:00am										11:00am
11:30am										11:30am
12:00pm										12:00pm
12:30pm										12:30pm
1:00pm										1:00pm
1:30pm										1:30pm
2:00pm										2:00pm
2:30pm										2:30pm
3:00pm										3:00pm
3:30pm										3:30pm
4:00pm										4:00pm
4:30pm										4:30pm
5:00pm										5:00pm
5:30pm										5:30pm
6:00pm										6:00pm
6:30pm										6:30pm
7:00pm										7:00pm
7:30pm										7:30pm
8:00pm										8:00pm
8:30pm										8:30pm
9:00pm										9:00pm
9:30pm										9:30pm
10:00pm										10:00pm
10:30pm										10:30pm
11:00pm										11:00pm

Please, write if any cooking was done outside the hours listed in the diary.

Figure 4.9 The cooking diary for Monday

4.2.6 Assessment of respiratory health

Respiratory health was assessed by examining daily variations in peak flow and respiratory symptoms.

Participants were asked to take three peak flows in the morning and three in the evening. The highest peak flow was chosen for analysis. The peak flow was measured using a digital peak flow meter (http://www.clement-clarke.com/). This PEF meter has the advantage of being small (95mmx40mmx45 mm), light (43 grams), with a resolution of 5L/min and accuracy of 5% (or 10L/min). Readings and timing of reading could be saved in the memory (as many as 240 tests) to be downloaded later on. It has a 6-month battery life. The eMiniWright had only recently been introduced on the market and the pilot study intended to test its use and reliability.

WINAP - WEEKLY DIARY

Start of week _____ / _____ / _____

Please score in the ***morning*** *the symptoms and medication you had during the night and in the evening the symptoms and medications you had taken during the day.*
Use a score 0-3 **(0= no symptom, 1=mild, 2=moderate, 3=severe)** *to describe the quality of your symptoms*

	Any wheezing and/or breathlessness? Score 0-3	Did you feel any chest tightness? Score 0-3	Any attack of short-of-breath at rest? Score 0-3	Any attack of short-of-breath after exercise? Score 0-3	Were you woken by attack of short-of-breath? Score 0-3	Did you use any asthma reliever ? Tick if YES.		First reading	Second reading	Third reading	Time when PEF was measured
MONDAY											
At daytime					XXXXXXXX		Evening PEF				
TUESDAY											
At night-time			XXXXXXXX	XXXXXXXX			Morning PEF				
At daytime					XXXXXXXX		Evening PEF				
WEDNESDAY											
At night-time			XXXXXXXX	XXXXXXXX			Morning PEF				
At daytime					XXXXXXXX		Evening PEF				
THURSDAY											
At night-time			XXXXXXXX	XXXXXXXX			Morning PEF				
At daytime					XXXXXXXX		Evening PEF				
FRIDAY											
At night-time			XXXXXXXX	XXXXXXXX			Morning PEF				
At daytime					XXXXXXXX		Evening PEF				
SATURDAY											
At night-time			XXXXXXXX	XXXXXXXX			Morning PEF				
At daytime					XXXXXXXX		Evening PEF				
SUNDAY											
At night-time			XXXXXXXX	XXXXXXXX			Morning PEF				
At daytime					XXXXXXXX		Evening PEF				
MONDAY											
At night-time			XXXXXXXX	XXXXXXXX			Morning PEF				

Use this space if you would like to add any comment.

Figure 4.10 The health diary

Participants were also asked to record and score (on a 0-3 scale) the quality of their daytime and night-time respiratory symptoms in a weekly diary (Figure 4.10). The symptoms included:

- Wheezing and or/SOB
- Chest tightness
- Attack of SOB at rest
- Attack of SOB after exercise
- Being woken by attack of SOB
- (Use of asthma reliever)

At the end of each week participants were reminded by email to send the completed diary (on health and cooking) and the passive diffusion tube back to the researcher.

4.2.7 Statistical methodology

The **primary outcome** of the panel study was the daily difference between morning and evening peak flow, as a percentage, over the study period mean of the daily difference within the same individual (after Franklin, 2012) as follows

$$\frac{(\text{Morning PEF} - \text{Evening PEF})}{\text{mean}_{\text{within individual}}(\text{Morning PEF} - \text{Evening PEF})}\% \qquad \textbf{(Equation 4.1)}$$

Secondary outcomes were:

- Severity score as the sum of the score of five symptoms (wheeze, chest tightness, SOB at rest, SOB after exercise, woken up by SOB), adapted from Ostro (1994)
- Use of asthma reliever medication.

The focus of the **primary analysis** was:

- To assess the association of the primary outcome (see Equation 4.1) with same day exposure (lag 0) and previous day (lag 1) to NO_2 peaks (Franklin, 2012).

A peak was defined as a 15-minute average NO_2 concentration equal or above 600μg/m^3. Due to the inherent variation of peak flows within each participant statistical analysis considered a mixed model with the fixed part of the model reflecting NO_2 exposure and the random part of the model reflecting the variations within individual.

The study was powered at 90% with α at 0.05 to detect 5% reduction in geometric mean PEF within the same individual assuming that an individual will be exposed to an indoor NO_2 peak at least on 2 of the 8 days of continuous NO_2 monitoring. The power calculation was complex because of repeated measures within same individuals and I received advice from the Medical Statistics Lecturer in my department, Roger Newson (personal correspondence, 14 December 2012) (see Appendix of Chapter 4 for details of calculation).

The study was approved by the Imperial College Research Ethics Committee (ICREC_13_2_10) and NHS REC (13/SC/0560). The Principle Investigator of the study was the PhD supervisor, Prof Debbie Jarvis.

4.3 Evaluation of the pilot study

This section discusses the issues arising from the study design in detail as follows:

- Recruitment
- Participants compliance to study procedures
- Outcomes assessment
- Use of diary and problems arising from
- Data collection
- Instrument monitoring and problems arising from the use of NO_2 sensor.

4.3.1 Recruitment

Recruiting participants proved to be very difficult and different strategies to advertise the study were implemented throughout the period the pilot study was conducted. The pilot study was planned to last for 12 months, the first 4 months spent on recruitment and the remaining months on following the participants. However, recruitment proved challenging and the period dedicated to this was extended to the whole 12 months. Table 4.5 summarises the history of recruitment strategies and response. Overall only two participants were recruited.

Table 4.5 Table showing history of recruitment strategies and response

Agencies through recruitment was attempted		Response	Reply rate[1]	Recruitment rate[2]
Asthma UK	Policy and Research Volunteering group (120 members) newsletter - June 2013)	1 person interested but did not leave in London.	1/120[3]	
	Facebook (around 17, 000 friends) - July 2013	22 people commented (see Appendix of Chapter 4) 5 persons interested in study but were not recruited because they did not live in London; 2 people did not reply back and reasons were not given.	7/17,000[4]	0/7
South East London Community Air Pollution group	Newsletter to members (include individuals and organisations, see below) – July 2013	Three organisations further advertised study in their own newsletter (see below); 1 individual interested in study and recruited.	1/100[5]	
	Sustrans (number of members unknown) – July 2013	Study advertised in the newsletter (19/08/ 2013) ; 1 person interested.	1/3500 [6] (in UK)	
	London Sustainability Exchange (number of member unknown) – July 2013	Study advertised in the newsletter (01/07/ 2013) ; 2 people interested.	2/possibly 1,000[7]	
	CleanAir London (9, 000 followers)	Interested in advertise study in Twitter account but because study does not have a web link they eventually refused to do so because of Internet security.	n/a	
Imperial College online staff board	From November 2013 to May 2014	2 people interested, 1 recruited.	2/~10,000 [8]	1/2
NHS GP practices	8 Chelsea and Battersea GP Practices - January 2013 10 South London GP practices – August 2013	Wrote to GP practices asking if they are interested in helping by sending invitation letter to their patients ; 3 GP replied, 1 interested in displaying a poster at reception.	3/18[9]	0
NIHR-CRN North West London[10#]	Advertise study to their GP members – February 2014	4 GP surgeries interested, 1 agreed to send invitation letters to patients (see below)	4/?[10]	0
	Gladstone GP – May 2014	Invitation letters sent to their 122 eligible patients 1 replied asking for information irrelevant to the study	1/122[11]	0/1

[1]no. people who replied to advert/ estimated population target number ; [2]no. people recruited/no. people replied to advert ; [3]personal communication with Asthma UK; [4] number of Facebook friends; [5] includes organisation and individuals (personal communication with group manager); [6] http://www.charitycommission.gov.uk; [7] as estimated target number as figure not available http://www.charitycommission.gov.uk; [8] http://www3.imperial.ac.uk/communications/resources/factsandfigures, includes IC staff and post-graduate students who both have access to noticeboard, figure for 2011;; [9] number of GP surgeries; [10] number of GP surgeries and patients not available; from their web site between 2013 and 2014 1,685 patients were recruited in any study; [11] personal communication with GP surgery.

Advertising for recruitment was carried out through the following agencies:

Asthma UK

Initially, it was thought that it would be sufficient to advertise the study through Asthma UK Asthma UK agreed to advertise the study to a special group of members, the Policy and Research Volunteering group. This group comprises of around 120 women and men living in the UK, who are active within the charity and are willing to consider whether to take part in studies on asthma. One person replied to the advert but lived in Berkshire rather than London and cooked with an electric stove.

The study was subsequently posted on Asthma UK Facebook, which is mainly followed by asthmatic adults or parents of asthmatic children. Twenty-five people liked the post and 27 people commented on it on the same or following day it went 'live'. No further comments or request for participating in the study have been received since then. Some people were interested in the study and would like to participate because they believed their asthma was affected by indoor air quality; some people instead accused the study of being 'sexist' as men were not recruited claiming that many men cooked too. Comments suggested that nobody was aware that exposure to gas cooking may affect asthma symptoms.

South East London Community Air Pollution group

The study was advertised in the group newsletter. Two women showed an interest in the study but were not asthmatic. Representatives of two organisations saw the advert and agreed to advertise the study in their own newsletter. A further organisation, CleanAir London, offered to advertise the study on Twitter as long as the study had a web page to which Twitter users could be directed to. There was not study web page so this was not possible. One person was eventually recruited via South East London Community Air Pollution newsletter.

Imperial College staff noticeboard

The study was advertised on the staff noticeboard website in November 2013 and stayed on the website until May 2014. Two people expressed an interest: the first could not commit herself to participate as she spent a considerable amount of time outside London for work; the second filled the eligibility criteria and agreed to participate in the study.

GP surgeries

Eighteen GP surgeries in South-West London were initially contacted by post enquiring whether the surgery would be interested in helping with the study. The location (South-West London) was chosen because of being easily accessible. Most of the GP surgeries (n=15) did not reply. Those who did reply (n=3) apologised for not being able to advertise the study because of their current workload.

The National Institute for Health Research (NIHR) Clinical Research Network (CRN) for North West London

The network asked surgeries in North West London whether they were interested in advertising the study to women with asthma registered with their practice (see Appendix of Chapter 4 for copy of letter). A few surgeries replied to the call but were too busy to help or asked for financial compensation. One surgery offered to send an email to their patients as long as the study had a web site (which it did not). Finally, one surgery agreed to participate and posted an invitation letter to their patients (n=122) who met most of the eligibility criteria (non-smoker, age 20-65, female, current asthmatic, no other long-term illnesses). One response was received.

4.3.2 Reasons for low recruitment rates

Recruitments rates were affected by:

Personal reasons

Inclusion criteria were very stringent. Women had to live in London (London was chosen because of easy accessibility to participants' home), cook at least 4 times a week (in the same house where the monitoring occurred) and not go away for more than 4 consecutive days during the 8-week study period. However, women who expressed an interest in participating in the study tended to be mobile as they travelled for work or holidays, spend the night outside London and often cooked in their partner's home.

Advertising coverage (type of media, the length and frequency of advertising, population target)

The highest response came from the post on Asthma UK Facebook (7 people but none recruited). The lack of a web page for this study prevented some social media and GP surgeries from advertising the study as they needed a web link to do so (Table 4.6). Although the number of respondents was relatively low, the most successful strategies for recruitment were those

which targeted a selective population somehow related to academic research, i.e. Imperial College staff noticeboard and South East London Community Air Pollution group.

Table 4.6 Reasons for not recruiting

Reasons for not participating in the study	
Personal	
Living outside London	6 persons
Not asthmatic	2 persons
Spend 3 days/week with boyfriend	1 person
Partner cooks	1 person
Work commitment outside London	2 persons
Personal reasons	1 persons
Other interests (wanted reference for participating in the study)	1 person
Staying away from London home during the study period	4 persons
Advertising coverage	
Could not advertise in Twitter because web site of study needed for link	1 organisation
GP practice asking for financial compensation	1 surgery
GP practice too busy	4 surgeries
GP willing to send a text to patients but web site needed for link	1 surgery

4.3.3 Ethical approval for conducting the study

Any time there was a change in recruitment strategy an amendment to the relevant ethics committee had to be requested. The first request for ethical approval was to the Imperial College Research Ethical Committee in March 2013. Approval was received in May 2013. Because of delay the advertising deadline for expression of interest in the study was no longer appropriate and Asthma UK requested an ethical amendment. Further amendments had to be requested as the study progressed to relax the eligibility criteria (upper age limit extended from 50 to 65) and to permit advertisement of the study on social networking media (Facebook, Twitter, other newsletters groups).

Later, NIHR-CRN for North West London agreed to help with the recruitment of GP surgeries and a new application, this time through IRAS (Integrated Research Application System), which

approves any study conducted within the NHS had to be filed. Overall, applying for ethical approval proved lengthy and slowed down the recruitment process.

During the application process issues related to field worker safety were raised by Imperial College Health and Safety. New protocols to ensure fieldworker (i.e. me) safety were requested and had to be dealt by ensuring that safety procedures were carried out any time I visited a participant at home. (This included informing other Imperial College staff about home visit and not travelling in the evening alone in secluded areas.)

4.3.4 Participants compliance to study procedures

Questionnaires

Women who were interested in participating in the study received a screening questionnaire which assessed their eligibility. Fifteen screening questionnaires were sent out accompanied by a Participants Information Sheet (PIS). Four were returned. There is a possibility that the questionnaire was too long (11 questions) which must have stopped some women from completing it. One woman found the question on oral steroid confusing as she assumed that the question was on inhaled steroid. '*Oral'* should have been underline or put in bold to make it more noticeable. (N.B. Women on oral steroids were excluded)

Outcome assessment: peak flow meters

Participants monitored their own peak flow with a downloadable eMini-Wright. They quickly learned how to use the digital peak flow meter and enjoyed it. One meter stopped working the day after the study had started. The battery went dead even though the meter had never been used before. The participant contacted me for a replacement but only a manual one was available (Mini-Wright Peak Flow Meter, Clement Clarke). The peak flow meters complied with European regulations on peak flow standardised measurements (EN 13286).

Participants were asked to measure their peak flow three times every morning and evening for 8 weeks and record the measurement in a diary which had been handed in at the beginning of the study so that data downloaded from the digital peak flow meter could be tested for reliability.

One participant had some unusually high peak flow readings (above 700 mL), which were not entered in the diary but recorded by the digital meter. These high readings are typical for a young male adult. The time when PEF measurements were taken and recorded by the digital meter did

not coincide with the self-completed diary, even though digital meter time settings were checked before handing it to the participant. A likely explanation is that another person (i.e. a young adult male) had used the digital meter but we cannot exclude the fact that the digital meter may not be reliable. Unfortunately, it was not clearly explained to the participants that data of the digital meter was downloadable and the pilot study meant to test its reliability.

Exposure assessment: NO_2 weekly monitoring

Participants were asked to replace the Passam tube every week and send it back to me. They were given clear instructions when and how to do it. It is difficult to confirm whether participants complied with the procedures on how to use the Passam tubes, e.g. keeping them in the fridge when not being used, recording the time when the tubes were open and closed. The tubes I received back appeared to have been handled with care and the dates and timing of opening and closing had been recorded in the diary. The laboratory results confirm that the blank tubes were kept closed.

Participants attitude towards monitors

Although the Aeroqual monitor came with a battery it needed to be powered by electricity as the battery lasts for 15 hours only. In one household the cable was not long enough and I had to use an extension cable. There was some buzzing from the gas monitor but none of the participants reported being annoyed by this.

Feedback from participants

The feedback from one participant was quite positive. She enjoyed the study and did not find it too long. She asked to be informed about the results at the end of the study. The other participant did not complete the 8-week study period (she participated in it up to week 7) and did not provide any reason for stopping. However, the digital peak meter was returned at the end of the study and PEF readings were regularly taken until the end of week 8. She never gave any feedback and/or reasons for not completing the study. When asked, participants preferred communicating with the researcher by email rather than telephone.

4.3.5 Participants use of the diaries

Participants were asked to fill in a daily diary regarding their asthma symptoms, recording their PEF and time of cooking. One week diary consisted in 9 pages and needed to be completed by hand.

One participant found the health diary confusing as night symptoms were on the same row as morning PEF. She asked for clarification about the question: '*Did you have any attack of SOB after exercise today?*'. Since the participant did not take any exercise that day she did not know how to answer. This needs to be modified to take into account that people may not exercise (in some cases because they are limited by their asthmatic symptoms).

The cooking diary consisted of a table of 30 minutes slots (from 7 to 7:30, from 7:30 to 8:00 and so on). If the participant cooked during that time she was asked to tick the relevant slot. Although this design aimed to minimise the time participant completes the diary the use of the slots is not very precise (e.g. somebody may start cooking at 7:15 and finish at 8:15). The cooking diary was created to examine whether NO_2 peaks were correlated with gas cooking but the slot design is not the most appropriate solution.

Some NO_2 peaks occurred when participant had not recorded having cooked. The participant explained that her partner did some of the cooking but she did not record it in the diary as she understood the diary was about ***her*** cooking activities. For a future panel study we need to clarify whether a cooking diary should consider **any** cooking or only cooking by the participant.

4.3.6 Instrument monitoring

Aeroqual was delivered with a certificate that calibration and validation had already been carried out by the manufacturer. According to the manufacturer's specifications the accuracy of the calibration is around ±10%. However, while the pilot study was ongoing a paper was published suggesting that the monitor needs independent re-calibration by complex laboratory methods (Mead *et al.*, 2013). The Environmental Research Group (ERG) at King's College London offered use of their laboratories and access to their air monitoring station data to evaluate potential problems.

Briefly, the following steps to evaluate the monitor were carried out:

- Co-location testing against a chemiluminescence analyser (the 'gold standard') at an air monitoring station in London managed by ERG ;
- Calibration and validation against a chemiluminescence analyser under laboratory conditions using ERG facilities at King's College.

Co-location testing

Method

The co-location testing with a chemiluminescence analyser of an air monitoring station was conducted outdoors on the kerbside of a busy road, Putney High St, London during rush hours on 15/08/2013 from 8:45am to 10:15am. Aeroqual was placed as close as possible to the chemiluminescence analyser on top of the cabin that encloses the analyser (

Figure 4.11). The location and timing were chosen as NO_2 peaks as high as 150ppb (282μg/m^3) for 15 minutes or longer during rush hour. These peaks are similar to the peaks produced during gas cooking. The day was chosen because there were no rain and wind. The chemiluminescence analyser records outdoor NO_2 concentrations every 1 minute thus the Aeroqual monitor was set to log data every one minute too.

Data from the air station monitoring were supplied by King's College as an Excel file and then compared with the Aeroqual logged data using Stata 12.1. Data were visually compared with the Bland-Altman plot and by regressing the Aeroqual logged data against the chemiluminescence logged data.

Figure 4.11 Putney High St air monitoring station with Aeroqual positioned on top of station cabin near the inlet of the chemiluminescence analyser (north wind view)

Results

During the 90 minutes of testing both monitors detected very high peaks of NO_2 reaching nearly 600µg/m^3 but peaks were detected at different time and lasted for different lengths of time (Figure 4.12).

The agreement between the two instruments was very poor (Figure 4.13). The limits of agreement ranged between -171ppb (-321.5µg/m^3) to 106ppb (193.6µg/m^3) and the mean difference was -32ppb (equal to 60.2µg/m^3 with CI -88.4µg/m^3 to -33.8µg/m^3). In other words, the mean difference between all pairs of measurements from the two monitors was around 60µg/m^3 and there were large variations with the Aeroqual monitor more likely to underestimate NO_2 concentrations (compared to chemiluminescence analyser).

The scatter plot with regression line comparing the two instruments is shown next (Figure 4.14) only for the purpose of illustrations. This method is regularly used in analytical chemistry and has been used to examine Aeroqual against other monitors performance. (For epidemiological research the Bland-Altman method is the most appropriate method).

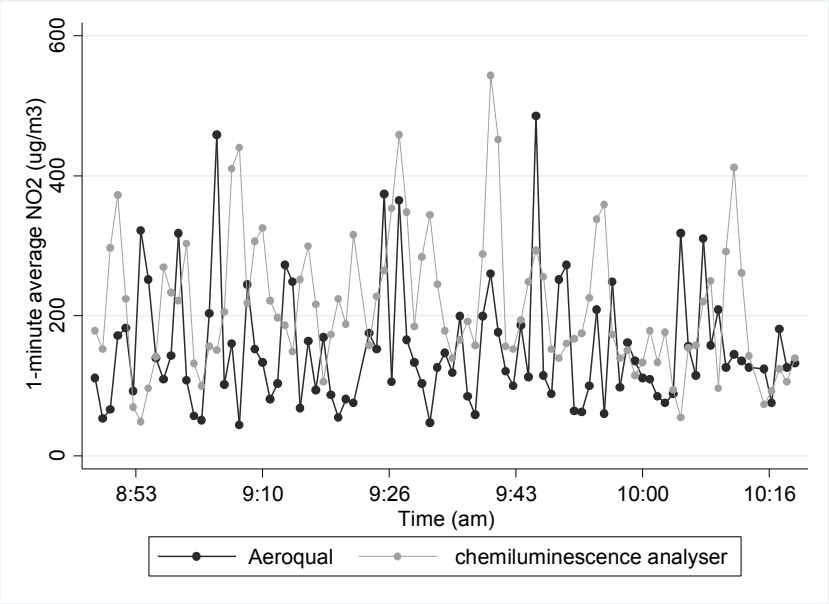


Figure 4.12 One-minute NO_2 concentrations (µg/m^3) measured concomitantly with Aeroqual and chemiluminescence analyser in Putney High St on 15/08/2013

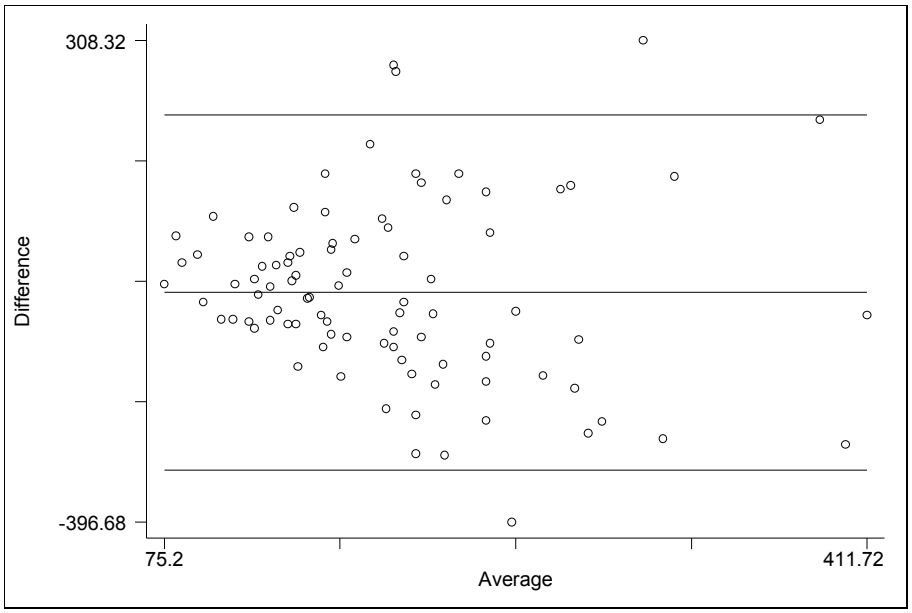


Figure 4.13 Bland-Altmann plot showing difference against the average in measurements (ppb) carried out with Aeroqual and chemiluminescence analyser of the air monitoring station at Putney High St on 15/08/2013

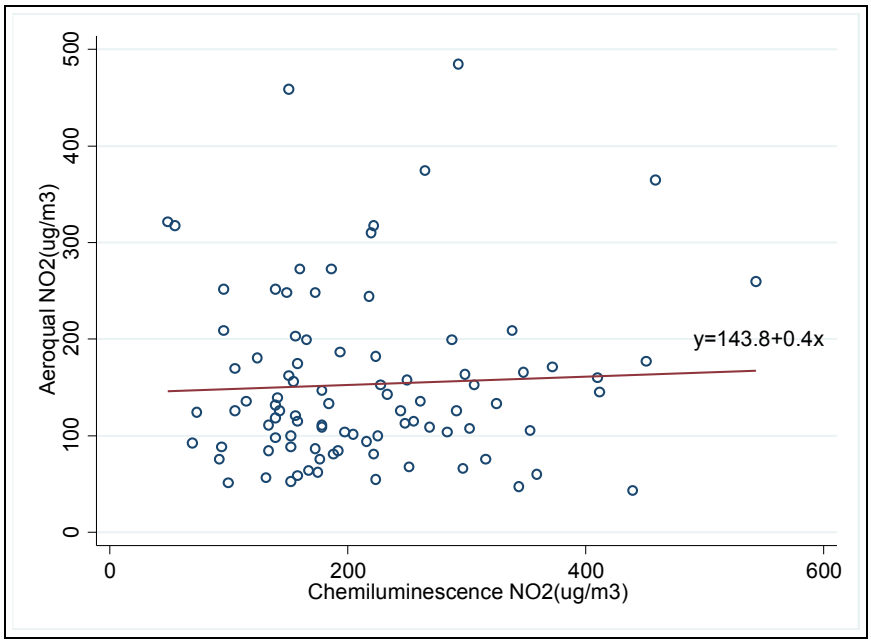


Figure 4.14 Scatter plot of measurements carried out with Aeroqual against the measurement carried out with the chemiluminescence analyser of the air monitoring station at Putney High St on 15/08/2013

Validation

Method

Validation of the Aeroqual at low and high exposures had already been carried out in the manufacturer's laboratory and a further validation was conducted under laboratory settings following a method proposed by Delgado-Saborit, University of Birmingham, who had previously tested the Aeroqual (Delgado-Saborit, 2012). The monitor was placed in an enclosed cabinet and exposed to known levels of NO_2 generated by an NO_2 cylinder of 500ppb and pure air both connected to mass flow meters.

Technical issues arising from calibration

During the validation procedures the Aeroqual monitor stopped working and NO_2 readings stayed at 0ppb while NO_2 concentrations were at 500ppb. Laboratory and technical staff at King's College were unable to fix the Aeroqual and the sensor head was returned to the manufacturer. They found no fault and re-calibrated it in their own laboratory. The sensor head was returned

and since then has been 'working'. No further attempts at the above procedures were made and the scientist who developed this method was not available (maternity leave) for further guidance.

4.4 Some results

In this section I am going to present some of the results collected from the participants (ID2 and ID16) just for the purpose of illustration.Figure 4.15 and Figure 4.16 show the NO_2 measurements recorded with Aeroqual in the kitchens of both participants. No occurrence exceeded a peak of 600µg/m^3 lasting for 15 minutes. Indoor NO_2 exceeded 200µg/m^3 in 12 occurrences lasting 30 minutes or less in the kitchen of participant ID2 and 14 occurrences in the kitchen of ID16.

The study was powered to detect a reduction in PEF within the same individual assuming that an individual will be exposed to an indoor NO_2 peak at least on 2 days of the 8 days of continuous NO_2 monitoring. A peak was defined as a 15-minute average NO_2 concentration equal or above 600µg/m^3 but within this limited data exposure to this concentration never happened.

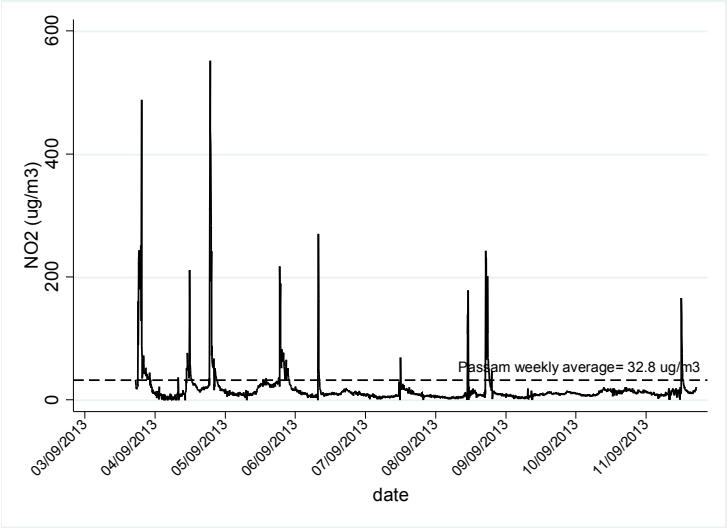


Figure 4.15 Graph showing 5-minutes average concentrations of NO_2 and weekly average concentration measured with Passam tube measured in the kitchen of participant ID2 (from 03/09/2013 to 11/09/2013)

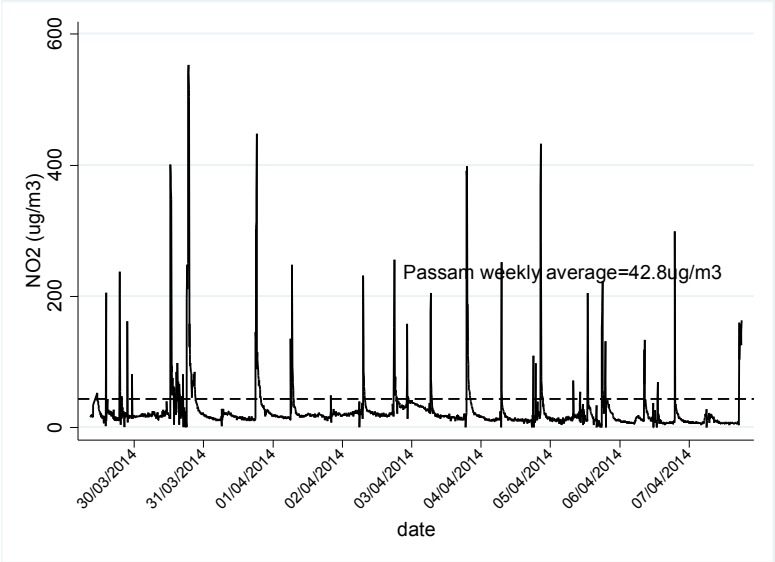


Figure 4.16 Graph showing 5-minutes average concentrations of NO_2 and weekly average concentration measured with Passam tube measured in the kitchen of participant ID 16 (from 29/04/2014 to 07/04/2014)

4.5 Discussion

4.5.1 Recruitment issues

This pilot study was designed to test the feasibility of a panel study to assess the effect of exposure to NO_2 peaks generated from gas cooking on asthma morbidity. It aimed to recruit 20 women with asthma but succeeded in recruiting only 2 participants.

Several strategies were used for advertising recruitment in the study. The highest recruitment rate came from advertising the study with those organisations that have close links with academic research (Imperial College and South East London Community Air Pollution group) or are involved with campaigning for better air quality in London (South East London Community Air Pollution, London Sustainability Exchange). These organisations reflect a selective group of women, employed, educated, involved with work within the local community. It has been shown that this particular group of people are more likely to participate in epidemiological studies as

they tend to put greater trust in scientific research and have greater rates of volunteerism (Galea and Tracy, 2007). They are not representative of the general population although it has recently been argued that non-representative populations produce only weak bias in exposure-disease associations (Ebrahim and Smith, 2013).

Social media such as Facebook proved to be a place for ephemeral comments lasting the day when the post went alive but not a place to develop an enduring commitment to an 8-week study. Twitter was not tested, although I wonder whether the response would be similar to the one received from Facebook's users. The advert on the Imperial College web site was in place for 7 months; only two people responded but one of them became a participant. Posting an advert in sites people are familiar with and perceive them as reliable (i.e. staff noticeboard) and leaving it for several months may be a better option than Facebook.

The study did not have its own web site or a link to the host academic department. Having a study website may legitimise the study and give confidence to potential participants that the study is genuine and valid.

Recruitment through GP practices did not prove successful. Only one person out of the 120 who were invited to the study replied asking for something unrelated (whether she could get a job reference if she participated in the study). It is possible that people do not like receiving unsolicited mail from the GP practice and may perceive it as an intrusion to their medical records. An invitation letter to a study could be perceived as an unsolicited 'telemarketing' practice (Groves, 1992; Galea and Tracy, 2007).

In addition to this, our invitation letter came with a screening questionnaires and a Participant Information Sheet, which due to ethical stipulation altogether made up to 5 A-4 sized pages. The amount and complexity of papers with inappropriate lengthy language to disclaim study insurance and liability (as requested by the various Ethical Committees) may have discouraged participation and contributed to the low recruitment rate.

Another underlying reason for poor recruitment may be that people do not like home visits by strangers (the researcher) to have some 'unusual' equipment installed in the house. Ideally, there should be an opportunity for the researcher to familiarise with the potential participant. It may be easier to recruit people personally by approaching them individually face-to-face in the out-patients asthma clinic of a local hospital or at a GP asthma clinic. Possibly the asthma clinic staff may act as an informal guarantor to the researcher and the study. Other work suggests face-to-

face recruitment has higher participation rates in contrast with studies that rely on less personal forms of contact between study recruiter and potential participant (Galea and Tracy, 2007).

A financial reward may increase the recruitment rate. Sexton (Sexton, 2005) reported on three major exposure-monitoring projects, the School Health Initiative: Environment, Learning, Disease (SHIELD), the Minnesota Children's Pesticide Exposure Study (MNCPES) and the National Cooperative Inner-City Asthma Study (NCICAS). The latter study measured indoor NO_2 in more than 600 homes of asthmatic children for 7 days (Kattan, 2007). These studies managed to achieve high recruitment (between 40% and 64%) and retention rates (between 85% and 95%) by using financial incentives. Financial incentives were between $5 and $50 for completing a questionnaire, $20 for a urine sample, between $20 and $30 for a blood sample, $20 for end-of-study completion, and so on. Full participation to the study and follow-up could result to a total of $130 and $195. However, financial incentives did not work with the collection of peak flow data. All three studies targeted US inner-cities children, mainly from poor minority communities. It is reasonable to wonder whether a financial incentive would work in less poor groups.

Since both participants were interested in the new digital peak flow meter a future study should consider whether give the digital meter to participants as a reward at the end of the study.

4.5.2 Instrument monitoring and use of gas sensors

Finally, there were a few concerns about the performance of the Aeroqual. The monitor could detect NO_2 peaks generated from gas cooking combustion; it was calibrated and validated by the manufacturer but recent published work recommended that calibration and validation should be carried out independently.

Lin and colleagues (Lin *et al.*, 2015) tested the Aeroqual Series 500 at an urban background site in central Edinburgh. The gas sensor measurements were very poorly correlated with the reference chemiluminescence analyser but concluded that calibration against the reference analyser would improve performance. Similarly, Mead and colleagues (Popoola, 2012) tested an NO_2 electrochemical sensor manufactured by Alphasense (Saffell and Dawson, 2004; Saffell *et al.*, 2010) and concluded that the sensor needs to be corrected for temperature. Both tests were carried out outdoors

My co-location testing was also carried out outdoors on a busy road (Putney High Street, London). However, outdoor NO_2 sources and environmental conditions are different from those that we normally encounter indoors. Ozone, which is involved with the oxidation of NO_x and also

interferes with NO_2 monitoring, tends to be higher outdoors (especially in the summer when the test was carried out) than indoors (where it is virtually non-existent). The principle sources of outdoor NO_2 in an urban environment are mobile (lots of travelling vehicles) while indoors the main source of NO_2 is fixed (a gas stove). During the co-location testing cars were constantly accelerating and decelerating at traffic lights next to the air monitoring station leading to very quick changes in NO_2 levels. This can be a problem with instruments such as Aeroqual as they tend to have a slow sensor response, which means that rapid changes in the atmosphere is not tracked by the Aeroqual as fast as the chemiluminescence analyser (Williams, 2009). On the other hand combustion gases generated from gas cooking are released at a constant rate from a fixed hob.

While an electrochemical sensor may not be recommended for outdoor monitoring we cannot rule out that it performs well indoors. So far all the published work on testing has been carried out outdoors. Unfortunately, because of the lack of department laboratory infrastructures (my department is medical oriented) I did not have many opportunities to test the gas sensor with a chemiluminescence analyser under laboratory conditions, ideally close to a Bunsen burner flame.

In this study there has been emphasis of one product of gas combustion, i.e. NO_2. However, there is evidence that some of the health effects observed with gas cooking may be related to particles, another product of gas combustion. Particles are grouped in size and special attention has recently been given to ultrafine particles (UFP), which have a diameter less 100nm. High level of UFP have been observed while cooking with gas at high temperature (Dennekamp, 2001; Zhang *et al.*, 2010). Real time monitoring of particles has already been tested out for several years and a large range of reliable and portable instruments is available on the market. Any future panel study that aims to assess the health effects of gas cooking should calibrate any electro-chemical NO_2 sensor and carry out continuous NO_2 and particles simultaneously.

4.5.3 Recommendation for a future panel study

After evaluating the results of the pilot study the recommendations for conducting a panel study are given in Table 4.7 with related estimated costs listed in Table 4.8.

Table 4.7 Recommendations for conducting a panel study

Study features	Recommendations
Participants' characteristics	20 men and women, 18-65 years old; with current asthma; cook with gas (75%) or electricity (25%) at least four times a week; live in London or South-East England.
Length of study	18 months: 3 months for ethical approval, 3 months for recruitment, 6 months of monitoring participants, 6 months for analysis.
Recruitment strategies	(1) through NIHR-CRN as tested in the pilot study; they will advertise recruitments to the GPs clinics linked to the network and their asthmatic patients living in London and South-East England; this will be done electronically (i.e. by sending an email and/or text to patients and advertising on GP practices web site and electronic information(board) and by displaying poster in GP practices; (2) at outpatient asthma clinic at Brompton Hospital, London: field worker to advertise study and recruit patients at face to face level; (3) social media of community–based groups; (4) financial incentives.
Aeroqual	Two monitors and 4 NO_2 sensor heads so that they can be used at the same time in more than one participants house; Calibration: to be carried out independently from manufacturer in an accredited laboratory which may possibly collaborate in the study.
Passam samplers	As in the pilot study, to be replaced weekly and sent to Passam laboratory for analysis.
e-mini Wrights download	25 e-Mini Wrights (20 for the participants and 5 spare ones to be used for demonstration by field workers); Ensure that participant knows that peak flow data are stored in the digital meter and will be downloaded for analysis; Leave the digital meter with participant as a reward for participating in the study.
Field workers	Two field workers are needed to visit participants' home together because of Health and Safety requirements.
Travel	To be prepared to extend the area of recruitment outside London
Use of electronic resources	To use social media and electronic resources to full extent; To create electronic versions of diaries and questionnaires and apps to be installed on phones for diaries, questionnaires, etc. To consider use of Quartix software (freely available at Imperial College) to create, distribute, store and manage survey, questionnaire and diaries data
Researcher	A researcher is needed to manage data
Postage	Participant needs to send Passam tubes by post back to Imperial College after sampling
NIHR-CRN London and South-East England	at moment the network provides free support to academic health-related researcher and it is expected that they would contact GPs and their patients for free
Web page, online questionnaire	Freely available through Imperial College web site
Calibration	Collaboration with an academic department specialised in environmental monitoring is recommended but calibration may involve some laboratory and staff cost

Table 4.8 Estimated cost for a panel study aimed to recruit 20 participants and lasting for 18 months

	Estimated cost	**Quantity**	**Total estimated cost**
Aeroqual			
Monitor	£996.00	2	£1992.00
Sensor head	£355.00	2	£710.00
Spare sensor heads	£177.00	2	£354.00
Delivery	£35.00		£70.00
Passam samplers			
Samplers + Delivery + Laboratory analysis (including postage)		8x20	£1,600.00
Laboratory cost for calibration	£500		£500
Real-time portable PM monitor/counter (Casella)	£1,500.00	2	£3,000.00
e-mini Wrights download	£14.00	20+5 spare	£350
Field workers to deal with recruitment and carry out home visit			
FT 0.5*12month	£25,000	2 (for safety)	£50,000.000
Travel costs for home visits	£20	20participants x3visits x2field workers	£2,400
Researcher/study co-ordinator/database manager			
FT0.4*18month	£40,000	1	£40,000
Postage for participants to send back Passam tubes to department			
jiffy bags		8x20	£32.00
stamps		8x20	£160.00
Email to patients	free service[TBC		
Web page	free[TBC]		
Online questionnaires	free[TBC]		
NIHR-CRN	free patients database searching[TBC]		
Miscellaneous	£300		£300
Total estimated cost			**£101,468.000**

4.6 Summary

This pilot study aimed to assess the feasibility of conducting a panel study that examined the association of respiratory health with exposure to indoor NO_2 peaks generated from gas combustion. The study piloted a new-to-market portable, low-cost monitor that could measure short-term exposure to indoor NO_2 but studies published while this work was ongoing recommended that independent calibration should be carried out. Several strategies for recruitments were attempted: asthma patients associations, GP practices, local communities and staff noticeboard at the academic institution where I am based but the study could not be completed because of poor recruitment. A future study should consider laboratory cost for calibration and validation of the NO_2 monitors and face-to-face people recruitment, for example, in an asthma outpatient clinic. Financial incentive should also be considered.

Monitoring indoor exposure is quite often a difficult task in any epidemiological study because of the cost and time consuming that monitoring involves. Besides, some people do not like being visited at home by some ‘stranger’ and having ‘unusual’ instrument installed. Some studies have relied on the use of proxy measures, such as the use of gas cooking but this can be imprecise. Predicting indoor NO_2 average exposure based on questionnaire information has been previously attempted. The ECRHS has information on the presence of gas appliances and some relevant household characteristics, which may determine indoor NO_2 levels. In the next chapter (Chapter 5) I will assess whether such exposure modelling can be applied to the ECRHS data and be used for respiratory health assessment of indoor NO_2.

5. Modelling indoor

5.1 Introduction

Monitoring indoor exposure is a demanding task in any large epidemiological study because of the cost and time consuming. It is common for such studies to be based on relatively small numbers of household measurements. For example, of the 50 studies included in the systematic review of Chapter 2, 22 studies were based on fewer than 200 households. The small sample size can affect the statistical power to conduct health assessment analyses and studies often rely on the use of proxy measures, such as the use of gas cooking. However, this can be imprecise. Modelling the exposure by regressing available information on determinants of exposure on a much larger sample of people may provide an affordable alternative to exposure monitoring.

In this chapter I will develop a model suitable for the estimation of two week average indoor concentration of NO_2 using questionnaire based information available for all participants in ECRHS.

5.1.1 Background

It has long been recognised that gas cooking is the major determinant of indoor NO_2 in homes where gas is used for cooking. One of the earliest studies (Dockery et al., 1981) measured indoor NO2 in nine families living in Kansas (US) to determine the variability of indoor concentrations. The study found that homes where gas was used for cooking had twice the indoor NO_2 levels than homes where electricity was used and three times the outdoor level. Another early study (Sexton et al., 1983) identified gas stove and outdoor NO_2 as the main determinants of indoor NO_2 but air-exchange rates and strength of indoor sources also contributed to some of the variability of the concentrations. Unvented gas heaters and gas stoves with a constant burning pilot light were also identified as strong predictors of indoor NO_2 by other early studies (Spengler, 1983; Ryan et al., 1988; Quackenboss et al., 1986; Wilson et al., 1986; Ryan, 1988).

One of the most extensive exposure assessment of indoor NO_2 was carried out by Spengler and colleagues (Spengler et al., 1996) as part of a large study on the health effect of indoor NO_2 (Samet et al., 1987). Indoor NO_2 was monitored in 1,400 houses in Albuquerque, New Mexico,

on multiple occasions throughout the year and information on household characteristics collected. Several determinants of indoor NO_2 were identified by regressing indoor NO_2 against the following: building characteristics (e.g. age, size, attached garage); type of cooking stove (e.g. with burning pilot light, electric, gas); presence of gas or kerosene space heaters, fireplace, humidifier; use of cooking stove (e.g. time spent cooking and whether stove was used for space heating); meteorological factors (season, temperature, rainfall). Findings from this study (Spengler, 1996) showed that elevated levels of NO_2 were associated with the presence of gas cooking appliances with continuously burning pilot lights, use of gas stove or burners.

Smaller house size (measured in squared feet, although how this was measured is not specified), decreasing outdoor temperature and increasing daily rainfall were also found to be associated with elevated indoor NO_2 levels. It was suggested that the house size affects the level of indoor NO_2 because as the volume of a house increases the degree by which NO_2 dilutes indoors increases too and that during cold and wet days people are more likely to increase the use of heating and cooking appliances and less likely to open doors and windows thus minimising the exchange of indoor air with outdoor air.

Further monitoring was carried out in a sub-set of households for up to 36 months to assess year-to-year variability. Findings suggested that the indoor NO_2 levels were affected by seasonal and annual variations.

5.1.2 Characteristics of studies on indoor NO_2 models

A large array of studies on indoor NO_2 modelling has been published since then. Studies have relied on indoor NO_2 measurements, information on household characteristics and data on outdoor environmental factors to develop the models. Predictors have been assessed with the use of univariate or multivariate regression analyses and kept in the model if they are statistically significant. Statistical significance has been assessed by the size of P value ($P< 0.05$) and model performance by the proportion of variability the model explains in terms of R^2.

Measurements of indoor NO_2 have mainly been conducted over 7 to 14 days but occasionally only a couple of days (Lai *et al.*, 2006; Baxter *et al.*, 2007). Some studies have measured indoor NO_2 in more than one season (Lee *et al.*, 1998; Baxter, 2007; Lawson *et al.*, 2011). Household characteristics have normally been collected with the use of questionnaires.

The most common significant predictors of indoor NO_2 to be identified are: having a gas stove (for example by Lee *et al.*, 1998; Cyrys *et al.*, 2000; Algar *et al.*, 2004: Esplugues *et al.*, 2013;

Lawson *et al.*, 2011; Heroux *et al.*, 2010; Valero *et al.*, 2009; Monn *et al.*, 1998); a gas fire (Algar, 2004); an unflued gas heater (Sakai *et al.*, 2004); having a gas appliance with a constant burning pilot light (Lee *et al.*, 1998); outdoor NO_2 (for example by Lee *et al.*, 1998 Zota *et al.*, 2005; Heroux, 2010; Valero, 2009; Monn, 1998); proximity to road (Roorda-Knape *et al.*, 1998; Lawson *et al.*, 2011).

Indoor smoking has also been identified by some studies (Monn, 1998; Algar, 2004; Lai, 2006, Heroux, 2010; Levy, 1998) but the presence of tobacco smoke may have only a small impact on indoor NO_2 levels and only significant in the absence of other indoor NO_2 sources (Leaderer *et al.*, 1986). Some other minor sources of indoor NO_2 that have been identified are candle burning (Sorensen *et al.*, 2005) and if an attached garage is present, moving a car in and out of the garage (Yang, *et al*. 2004). The number of people living in the household (occupancy) and occupant density have also been often considered as indicative of indoor NO_2-producing activities such as cooking, heating and smoking (Baxter, 2007).

As ventilation influences the rate at which indoor NO_2 disperses and/or escapes outdoors and the rate at which outdoor NO_2 penetrates inside, some studies have attempted to include ventilation in their model but defining ventilation has proven difficult. Some authors have measured air exchange rates using perfluorocarbon (PFC) tracer techniques[1] (Lee, 1998; Zota et al, 2005; Gilbert et al., 2006). Others have used proxy measures, for example: open windows (Heroux, 2010); home size (Spengler, 1996) or whether the household is a flat or a house. It has also been suggested that large homes may also provide more surface area onto which NO_2 may be absorbed thus providing a 'sink' into which NO_2 is converted to nitrous acid in the presence of water (Spicer *et al.*, 1993). Of note, US literature uses the term 'multi-family' or 'multi'-unit' building and 'single-family' or 'single-unit' building instead of the terms 'flat' and 'house'.

Seasonal variations

Meteorological factors have also been found to influence levels of indoor NO_2 such as outdoor temperature and rainfall (Spengler, 1996), wind (Roorda-Knape, 1998) and seasonality.

As part of the Infancia And Media Ambiente (INMA) study indoor and outdoor NO_2 levels were measured in 352 Spanish homes. The study found that season was a significant determinant (along with type of cooking stove and water heater and outdoor NO_2) and that the relative

[1] The PFC technique is based on a continual release of a non-toxic tracer gas (perfluoromethyl cyclohexane) and its uptake by diffusion samplers (capillary absorption tubes or CATs).

contribution of outdoor NO_2 to indoor NO_2 levels was higher in summer than winter (Esplugues, 2013). Some studies have developed separate models by season. For example, Gilbert (2006) considered a model only for the cold months (January to April), a time of the year when people in Quebec City, the location where the study was carried out, usually keep their windows closed. One-week average NO_2, relative humidity and temperature were measured in the living room of 97 homes. PFC trace techniques were used to measure infiltration rate; air change rates were calculated by including house volumes and number of occupants to infiltration rates. Air change rate together with the presence of a gas stove and gas-powered main heating system explained nearly half of the variance of indoor NO_2 during the cold months (January to April). In another Canadian study (Heroux, 2010) a multivariate model was developed for the summer months only. The strongest predictors were gas stove, smoking inside (yes/no), keeping windows open and outdoor NO_2.

A study in Boston, US (Zota, 2005) observed a strong collinearity between season and AER and because of this, season was not included in the model. Indoor NO_2 measurements were carried out in the kitchen, living room and outdoors for three sampling sessions in 77 flats. Air exchange rate (AER) was measured using a PFC tracer technique. Several predictors were initially considered but only outdoor NO_2 and AER remained significant in the multivariate models. All 77 flats had a gas stove, making it impossible to quantify the contribution of gas stove to indoor NO_2.

Assessment of predictors of indoor nitrogen dioxide levels in multicentre studies

Some models have included more than one geographical location and observed a degree of 'unexplained' variability in exposure across cities or countries.

Cyrys (Cyrys, 2000) assessed the contribution of indoor sources and outdoor sources (traffic exhaust emissions) to indoor weekly average NO_2 in 385 living rooms in Erfurt (former East-Germany) and Hamburg (former West-Germany) by running a linear regression model that included place of residence as well as determinants of indoor NO_2. Outdoor NO_2 contributed more to indoor NO_2 concentrations in Hamburg (where outdoor NO_2 levels were higher) than Erfurt.

Algar (2004) examined weekly or bi-weekly indoor NO_2 average exposures in 1421 homes in Ashford (UK) and Menorca Island and Barcelona (Spain). The contribution of the main determinants (gas cooker, gas fire, parental cigarette smoking and season of measurements) to indoor NO_2 differed across locations.

Lai (2006) modelled indoor NO_2 for 4 European cities as part of the EXPOLIS study. Models were developed at city-level by including an interaction term for city. As a result the contribution and significance of the predictors varied between cities and sometimes the effect had opposite directions. For example, wind speed was negatively associated in Basel and Prague but positively associated in Oxford.

5.1.3 Personal exposure

This overview of major studies on indoor NO_2 modelling highlights the major findings contributing to the field. Studies that model personal exposure to NO_2 have not been included although many studies of personal exposure agree that residential indoor concentrations are better predictors of personal exposure than are residential outdoor concentrations (Levy, 1998; Monn, 1998; Levy *et al.*, 2010). Breysse et al (2005) and others (Rotko *et a*l. 2001; Lai 2004) found that the presence of gas stove at home was significantly associated with personal NO_2 exposure. Levy et al (1998) calculated that the use of a gas stove in the home was associated with 67% increase in mean personal NO_2 exposure.

5.1.4 Predictors of indoor NO_2

In the next page Figure 5.1 displays the predictors that have been identified by previous studies on monitoring and modelling and their direct and indirect relationship with indoor NO_2.

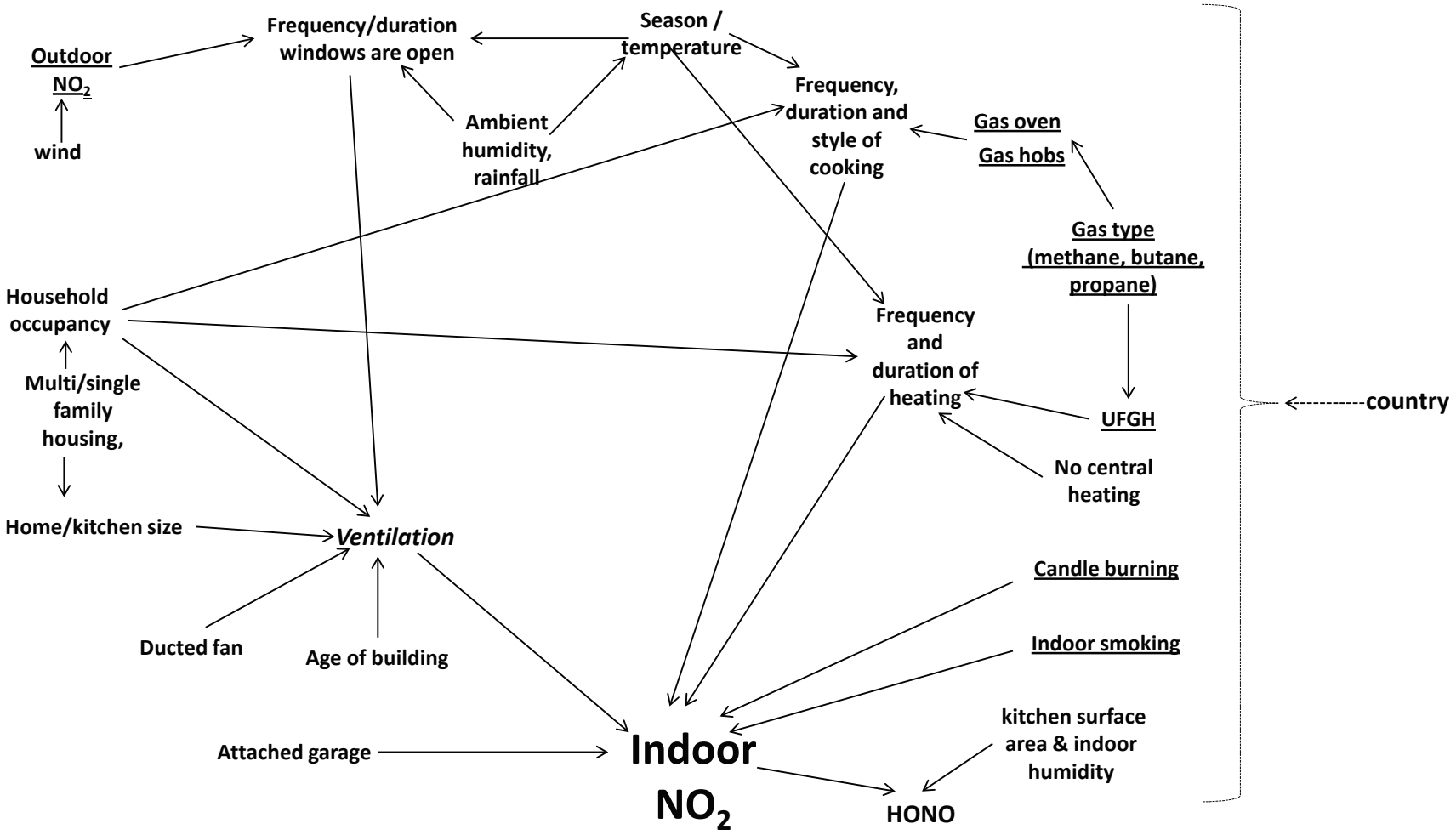


Figure 5.1 Potential predictors of indoor NO_2 levels

To summarise, elevated indoor NO_2 levels can be affected by:

- The presence of generating NO_2 indoor sources
- The amount generated from the source
- The degree by which a building retains NO_2
- Outdoor NO_2 penetrating inside the building.

The main sources of indoor NO_2 are:

- Gas appliances: hobs, oven, unflued gas heaters (UFGH).

Minor sources include indoor smoking and candle burning. Combustion from other fossil fuels (e.g. coal, coke, wood) can also generate NO_2 but have not been considered in this project.

The amount of NO_2 generated by a gas appliance is influenced by several factors, some of them difficult to quantify:

- Type of gas appliances (gas ovens generate more NO_2 than gas hobs, (Dennekamp, 2001), unflued gas heaters generate more indoor NO_2 than externally flued gas heaters (Gillespie-Bennett *et al.*, 2008).
- Type of gas – the gases butane and propane are used in gas bottles and tend to produce more NO_2 than methane, the main component of natural gas from the mains. It has been estimated that 1 kg of propane or butane emits 2.3 g of NO_x during combustion while 1 kg of natural gas (methane) emit 1.0g of NO_x but application temperatures and air/fuel ratios may vary these emissions. In general higher air/fuel ratios increase NO_x emission (http://www.engineeringtoolbox.com/nox-emission-combustion-fuels-d_1086.html).
- The frequency and duration of gas appliance use (the longer an appliance is on the more NO_2 is generated).
- Style of cooking - e.g. frying generates more NO_2 than boiling water, four gas rings on generate more NO_2 than one ring on (Dennekamp, 2001).
- The number of people living in a house as high occupancy is more likely to be associated with a heavier use of heating and cooking appliances and if occupants smoke, higher levels of indoor tobacco smoking.
- Time of the year as the use of heating and cooking appliances tend to be heavier during the cold season.

The degree by which a building retains NO_2 that is emitted indoors is affected by:

- Size of the house as large houses have more indoor air in which indoor NO_2 can disperse and provide more surface area onto which NO_2 may be absorbed and converted to nitrous acid in presence of water. Thus flats with a gas cooker will tend to have higher indoor NO_2 than a house with a gas cooker.
- Building ventilation such as the presence of ducted fans or ducted hoods above gas stoves to let cooking fumes out and window and doors that open to the outside to let indoor air out.
- Human activity affecting the degree of ventilation (e.g. frequency and duration of keeping a window to the outside open).
- Time of the year as people are more likely to keep windows and doors open during the warm season.

The amount of outdoor NO_2 that penetrates inside is affected by:

- The residential level of outdoor NO_2, which in turn depends on other factors such as proximity to road, traffic intensity and landscape characteristics.
- Building ventilation such as the presence of windows and doors that open to the outside and allow outdoor NO_2 to penetrate in.
- Time of the year as people are more likely to keep windows and doors open during the warm season.

The above characteristics may be influenced by differences between countries. For example, emissions from gas appliances may vary because of different cooking styles (Zhang, 2010), gas appliances design or chemical mixture used to add smell to the gas (Jarvis, 1998). Ventilation may vary because of housing characteristics, outdoor temperature and proximity to busy roads.

5.1.5 Regression model for predicting NO_2 exposure

Systematic approaches to predict individual exposures in cohort studies have been developed for assessment of health impact associated with variability in outdoor air pollution. Regression mapping or, as later became known as Land Use Regression (LUR) was introduced by Briggs (Briggs *et al.*, 1997) in the Small Area Variations In Air quality and Health (SAVIAH) study. It combines monitoring of air pollution at a relatively small number of locations and development of stochastic models using predictor variables usually obtained through geographic information systems (GIS).The model is then applied to a large number of unsampled locations (e.g.

addresses) in the study area. After the successful pioneering work in SAVIAH LUR methods have been increasingly used in outdoor air pollution epidemiological studies over the past decade.

In recent years the European Study of Cohorts for Air Pollution Effects (ESCAPE, http://www.escapeproject.eu) has developed a flexible methodology for assessment of long-term population exposure to air pollutants using the LUR methodology in order to apply the exposure assessment methodology on existing cohort of mortality and chronic diseases studies in Europe (ESCAPE, 2010). This manual has been used as a guide to develop the model for indoor NO_2 exposure assessment described in this chapter.

To date no standardised approach to model indoor NO_2 has been proposed.

5.1.6 Aims

To explain variability in indoor concentration of NO_2 using variables likely to be available for all participants taking part in a large cohort study (ECRHS).

5.1.7 Objectives

1. To develop a model that explains the variation of two-week average indoor NO_2 using data collected in a sub-sample of ECRHS participants with indoor NO_2 measurements.
2. To evaluate whether the model is suitable to estimate indoor NO_2 levels in ECRHS homes.
3. To predict two-week indoor NO_2 average exposure in people who participated in ECRHS II and ECRHS III.

5.2 Methods

5.2.1 Indoor NO_2 measurements

Two-weekly average monitoring data of indoor NO_2 from 1,906 homes and related information on household characteristics (i.e. type of cooking and heating appliances and ventilation) were collected using a standardised questionnaire within the ECRHS II survey in 14 ECRHS centres of 6 European countries (Belgium, Italy, Spain, Sweden, Switzerland and UK) between July 2000 and June 2002 at different times of the year.

For details of the measurement protocol see Chapter 3, section 3.2.2.

5.2.2 Predictors included in the development of the model

Participants provided information on housing characteristics and use of gas appliances at ECRHS II during a standardised interviewer administered questionnaire.

Data on annual average outdoor NO_2 at their residential address was generated in work conducted during the ESCAPE project, a European project that modelled outdoor air pollutant exposure of ECRHS and other European study cohorts using LUR. Briefly, a standardised protocol was applied in the cohort study areas between 2008 and 2011. Outdoor NO_2 (and other traffic pollutants) measurements were carried out for a 14-day period during each of three season (cold, warm and intermediate) and annual average concentrations for each monitoring site were computed by averaging the three measurements and combining them with measurements collected from a central reference site to adjust for seasonal variability. An annual average estimated NO_2 concentration was allocated to each cohort participant's place of residence. This model will use the predicted exposure for the year 2010 and back-extrapolated exposure for the year 2002 (http: //www.escapeproject.eu/ manuals/ Procedure_for_extrapolation_back_in_time.pdf).

Meteorological data were provided from the ECRHS meteorological database (ECRHS meteo) and Weather Underground website (http://www.wunderground.com/). ECRHS meteo provided monthly average temperature and relative humidity at city level for all ECRHS centres except UK. Weather Underground provided historical meteorological data for the UK (Norwich and Ipswich). As no weather data were available for Ipswich Wattisham was chosen because it was the closest location to Ipswich (16 miles away). Daily average temperatures and relative humidity data of the

two UK locations (Norwich and Wattisham) for the year 2000 and 2001 were downloaded as an Excel file and then transferred to Stata 12.1 for data manipulation. Average monthly temperature for each location was calculated from the mean daily temperature for the period 2000-2001.

Table 5.1 lists the potential predictors available from the ECRHS main questionnaire and their expected direction of effect, defined *a priori*, which were considered in the development of the model. Because of missing or incomplete data for some predictors only some of the predictors in Figure 5.1 could be included in the development of the model.

The following indoor sources of indoor NO_2 were included in the model and treated as binary variable ('yes' for if present and 'no' if not present) as follows:

- Gas hob (including hobs connected to the mains and those connected to bottled gas).
- Gas oven (including ovens connected to the mains and those connected to bottled gas).
- Use of bottled gas.
- Open gas fire or portable gas heater or paraffin heaters (considered as one category of unflued gas heaters – UFGH - because of the relatively small number of each type of appliance, which could cause overfitting[1] in the model development).
- Indoor smoking if participants reported that at least one person smoked inside the home.

The following variables were included to describe ventilation and housing characteristics which affect the degree by which indoor NO_2 disperses:

- The presence (y/n) of a kitchen ducted fan ('vent that takes fumes out') based on the answer to the question: '*If you have an extractor fan over the cooker, does the fan take the fumes outside the house*?'.
- Keeping a window or door open while cooking (y/n) ('kitchen door or window that opens to the outside') based on the answer to the question '*Over the last four weeks when you were cooking did you have a door or window to the outside air open?*'. A response of *'always'* or '*most of the time'* was considered 'yes' and a response of '*some time'*, '*never'* or '*there are no windows or doors to the open air in the kitchen*' as 'no'. The direction of the effect of keeping the window open was not assumed '*a priori*' as the direction of effect may vary depending on indoor/outdoor (I/O) exchange rate.

[1] Overfitting generally occurs when a model has too many parameters relative to the number of observations. A model that has been overfit can exaggerate minor fluctuations in the data leading to poor predictive performance, as it.

- Flat, i.e. smaller home (yes) versus house, i.e. larger home (no) – 'flat' included any housing unit that was part of a larger building made up of more than one unit and 'house' included detached, semi-detached or terraced houses. (Of note, in US the terms 'multi-unit dwellings' or 'multi-family housing' are used instead of the term 'flat').
- Reported age of building in years. Older buildings are more likely to have bad ventilation and 'unflued' cooking and heating appliances.

Further variables that may affect the amount of NO_2 generated indoors were included:

- The 'average time (in minutes) spent cooking per day' in the month previous to the questionnaire was included in the model to quantify the use of gas cooking appliances.
- The absence of central heating at home ('no central heating') was treated as a proxy for recurrent use of heating appliances as people are more likely to use UFGH or an oven to warm up the kitchen if no central heating is present.

Outdoor NO_2 sources and other environmental variables that may affect ventilation, cooking and heating habits were included:

- Annual average outdoor NO_2 (from ESCAPE model)
- Outdoor monthly average temperature
- Outdoor monthly average relative humidity.

Table 5.1 Predictor variables extracted from the available datasets and applied in the regression model

Predictors	expected direction of effect	Specification / Impact	Information available in dataset
Gas hob (Y/N)	+	Indoor NO_2 source; high	ECRHS II main questionnaire
Gas oven (Y/N)	+	Indoor NO_2 source; high	ECRHS II main questionnaire
Gas bottle (Y/N)	+	Indoor NO_2 source; high	ECRHS II main questionnaire
UFGH (open gas fire, portable gas heater, paraffin heater) (Y/N)	+	Indoor NO_2 source; high	ECRHS II main questionnaire
Indoor smoking (Y/N)	+	Indoor NO_2 source; impact is related to the amount of smoking indoors but information not available from questionnaire.	ECRHS II main questionnaire
Vent that takes fumes outside (Y/N)	-	A well-functioning ducted fan lower levels of indoor NO_2 as cooking fumes are fanned outside	ECRHS II main questionnaire
Keeping a window/door open in the kitchen while cooking (yes=most of the time or sometimes, no=rarely or there is no window/door) (Y/N)	+/-	Indoor NO_2 decreases because it escapes from the building. On the other hand, outdoor NO_2 may penetrate inside the building. Ozone and UV light can also penetrate indoors and react with NO_2	ECRHS II main questionnaire
Flat as a type of building (against houses) (Y/N)	+	May indicate smaller home and kitchen size hence NO_2 is less diluted in the air	ECRHS II main questionnaire
Absence of central heating (Y/N)		The absence of central heating is a proxy measure to indicate a more frequent use of unflued heating appliances	ECRHS II main questionnaire
Age of building (years)	+	Older buildings tend to have worse ventilation and heating *–	ECRHS II main questionnaire
Daily average spent on cooking the last 4 weeks (self- reported in min/day)	+	Longer cooking period more NO_2 produced	ECRHS II main questionnaire
Annual average modelled outdoor NO_2 at address level (measured in $\mu g/m^3$)	+	Outdoor NO_2, the most common source being fumes exhaust from vehicles ; it can penetrate inside the house and increase indoor NO_2 concentrations	ESCAPE
Monthly average temperature (measured in Celsius)	-	Affects the rate people open windows (during warm weather people are more likely to keep window open) and time of cooking (during cold weather people are more likely to spend more time indoors cooking); also NO_2 decays faster at higher temperatures	Weather Underground (for UK dataset) ECRHS_meteo dataset (for the other countries)
Monthly average relative humidity (measured in %)	-	Affects the rate people keep windows open; at higher relative humidity NO_2 is more reactive	Weather Underground (for UK dataset) and ECRHS_meteo dataset
Country (Belgium, Spain, Italy, UK, Sweden)	unknown	The effect of country is unknown, probably higher if gas appliances used	ECRHS II main questionnaire

* However, Sakai 2004 found that older Japanese houses have lower indoor NO_2 compared to more recent build houses, the main reasons being that new houses tend to be built with concrete rather than wood which increases insulation and retention of NO_2.

5.2.3 Development of the regression mode

Data required for the potential predictors listed in Table 5.1 were extracted from their original datasets (ECRHS II, ESCAPE and Weather Underground/ECRHS meteo) and transferred to Stata. Variables were created using standard data techniques and merged (e.g. use of open gas fire, portable gas heater and paraffin heater were merged together into 'UFGH'), re-categorised (e.g. the 4-category variable 'keeping the window/door open' was transformed into a binary variable) or re-scaled (e.g. cooking time was rescaled from 1 minute to a 30-minute scale, outdoor NO_2 from a scale per 1 $\mu g/m^3$ increase to 10 $\mu g/m^3$ increase) as necessary. Their relation with indoor NO_2 was first investigated with simple cross-tabulation and scatter plots.

In order to model indoor exposure using a standardised approach I adapted the protocol the ESCAPE project developed to model annual average exposure to outdoor air pollution. Briefly, the development of the model can be summarised into the following steps carried out using the training set:

1. Univariate regression of predictors against indoor NO_2.
2. Stepwise regression to maximise adjusted R^2 starting from predictor with highest R^2.
3. Removal of predictors from multivariate regression if adjusted R^2 does not increase by 1%.
4. Inclusion of the variable country in the model.
5. Removal of predictors with P>0.10 one by one starting with the predictor having the largest P value.

Finally, the model was:

- Tested for normality using standard regression diagnostic test.
- Validated on the independent set (hold-out validation).
- Cross-validated for sensitivity analysis.

The dataset was divided at random into a training set (75% of the data) and a test set (the remaining 25% of data). The model was developed using the training set and then validated using the independent test set.

Stata 12.1 was used for regression analyses, validation of model and plots.

Development of model using the training set

The relation of each predictor with indoor NO_2 was visually investigated with the use of scatter plots and box plots. Standard linear regression was carried out to develop a model that best predicts the measured concentrations, i.e. a model that maximizes the percentage explained variability (R^2). The predictors were identified with a forward stepwise procedure as in ESCAPE. Each predictor variable was regressed against indoor NO_2 and the computed R^2 of each individual univariate regression was ranked in decreasing order. The univariate regression model with the highest R^2 was regarded as the 'start model'. To this 'start model' the remaining variables were added separately, and the effect on the adjusted R^2 recorded. The predictor variable was maintained in the model if three criteria were satisfied (as in ESCAPE project):

1. The increase in adjusted R^2 is greater than 1%
2. The coefficient conforms to the pre-specified direction
3. The direction of effect for predictors already included in the model does not change. This ensures that models involving counter-intuitive associations be avoided, even if they give a stronger basis for prediction as indicated by adjusted R^2 value.

Co-linearity in the multiple regressions was tested using the Variance Inflation Factor (VIF) that measures how much the variance of the estimated regression coefficient has increased because of co-linearity following subsequent variables being added to the model. It was defined that any variable with a VIF larger than 3 would be removed to avoid multicollinearity (Wang *et al.*, 2012).

At this stage 'country' was forced into the model and predictors with a P value larger than 0.10 were removed starting with the predictor having the largest P value until all predictor variables had a P valued equal or smaller than 0.10. Each time a predictor was removed the P value of the remaining predictors was checked as the removal of one predictors may affect the P value of the predictors included in the model.

In contrast to ESCAPE where each centre had its own model it was not possible to develop separate models by country because of the lack of variable contrast in Italy and Sweden (99% of households cooked on gas hobs in Italy and 0% in Sweden). As the study was multi-centre and there are likely to be unconsidered predictors correlated with country, the variable 'country' was forced into the model even if VIF was higher than 3.

Testing for normality and influential observations

Standard diagnostic tests for ordinary least squares regression were applied to the final model as follows:

- Normality of the residuals were checked with the use of plots.
- Influential observations, i.e. those that have a large influence on the parameter estimates were identified using Cooks' *D*, a measure of the influence of an observation proportional to the sum of the squared differences between predictions made with all observations in the analysis and predictions made leaving out the observation in question. An observation with a value of Cook's *D* over 4/N where N is sample size was considered to have excess influence. Cook's distance can be used to indicate data points that should be checked for validity and provides information on the effect of deleting a given observation. As far as possible no data was excluded.
- Homogeneity of variance (heteroscedasticity).

Hold-out validation

To assess how accurately the predictive model performed in practice a validation assessment was carried in the independent set (test set). Validation was done by regressing the predicted concentrations against the observed concentrations. The observed and modelled values in the validation set were also visually assessed with the Bland-Altman plot (Bland and Altman, 1986), which examines the agreement between a pair of two different measurements by plotting the difference of paired variables versus their average.

Sensitivity analyses

The cross-validation test required division of the complete dataset (i.e. training and test datasets) at random into four sub-set of equal size (sub-set 1, sub-set 2, sub-set 3 and sub-set 4). A model was run using the data of three sub-sets to predict NO_2 levels in the fourth set (the left-out set); predicted concentrations were then plotted against the observed NO_2 levels to examine the performance of the model. This procedure was carried out four times so that all sub-sets were tested.

5.3 Results

Of the 1906 households with indoor NO_2 measures, 26 were excluded because the date of sampling was missing. Thirteen households had NO_2 concentrations above 200µg/m^3 and were excluded as the values appear to be implausibly high and it was considered likely that closing times reported were incorrect (Jarvis, 2005). All participants from the ECRHS Swiss centre were excluded (n=132) as data were missing for ECRHS III because of ongoing work to complete data harmonisation (Basel used a different questionnaire). Households cooking with biomass, coal, coke (n=11) or paraffin (n=3) were excluded because of the small number of homes using these types of fuel. Thirteen more homes were excluded because the cooking fuel was unknown or 'other'. Fifteen participants reported cooking for more than 240 minutes a day and because the time seemed implausible, it was replaced with a missing value.

The final dataset included 1,574 households in 13 centres in 5 countries with measurements on indoor NO_2 and information on gas cooking and modelled annual average exposure to outdoor NO_2 for the year 2002.

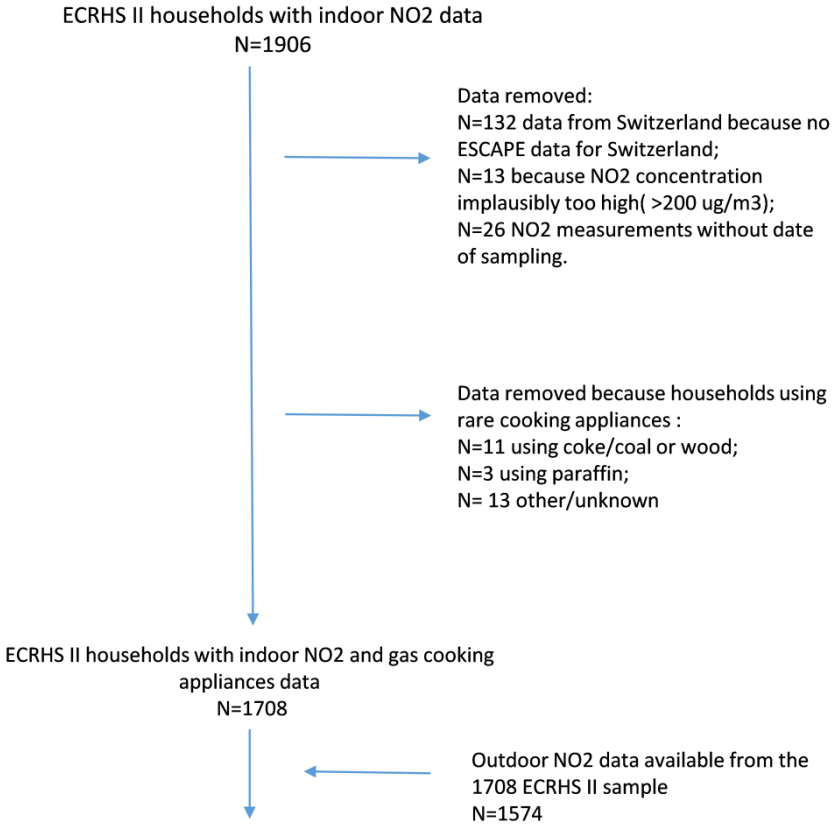


Figure 5.2 Data removal procedures and the number of observations available for modelling

5.3.1 Univariate associations of measured two week average indoor NO_2 with housing characteristics as determined from questionnaire data

First, the relationship of indoor NO_2 and predictors was examined by cross-tabulating the distribution by each predictor and testing if the difference between categories within predictors was significant. The difference between categories was significant for all predictors except for 'time spent on cooking' (Table 5.2). As expected households with gas appliances, without central heating, without a ducted fan over the cooker, where at least one person in the house smoked or where window or doors were rarely or never kept open while cooking, flats compared to houses and older buildings (more than 28 years, i.e. the median value) had higher indoor NO_2 levels. Households in Spain and Italy tended to have the highest concentrations of indoor NO_2 and those in Sweden had the lowest.

Table 5.2 Distribution of indoor NO_2 concentrations by predictors

Predictors	%*	Median (IQR) µg/m^3 YES	Median (IQR) µg/m^3 NO	Test for difference**
Gas hob	56.7%	52.7 (36.7-71.9)	20.6 (8.8-32.7)	<0.001
Gas oven	19.1%	57.3 (42.1-79.3)	32.2 (17.9-53.0)	<0.001
Bottle gas for cooking	12.5	62.8 (43.4-91.7)	34.2 (18.9-54.5)	<0.001
UFGH	27.6%	48.6 (30.3-72.1)	33.8 (17.6-54.4)	<0.001
No central heating	29.9%	33.9 (17.9-54.0)	46.8 (27.5-72.2)	<0.001
Keeping a window/door open in the kitchen while cooking	68.9%	42.3 (25.5-63.6)	21.1 (5.75-39.2)	<0.001
Flat (as a type of building)	59.5%	47.1 (29.9-67.6)	22.7 (8.8-40.6)	<0.001
Smoking indoors	40.2%	44.9 (27.7-63.9)	32.2 (15.8-54.0)	<0.001
Vent that takes fumes out	66.8%	36.3 (19.4-59.2)	39.5 (24.1-59.7)	0.0044
Age of building (less or 28 years and more than 28 years)	50%	23.0 (19.6-53.3)$^+$	41.4 (22.1-64.1)$^{++}$	<0.001
Time spent cooking (less or 30 minutes, more than30 minutes)#	50%	36.4 (20.3-57.3)	38.3 (21.3-59.7)	0.20
Belgium (n=157)	9.2%	29.7 (20.7-39.8)	-	-
Spain (n=796)	46.6%	46.4 (21.3-69.5)	-	-
Italy (n=309)	18.1%	50.8 (37.3-66.9)	-	-
UK (n=248)	14.5%	23.6 (14.4-41.4)	-	-
Sweden (n=198)	11.6%	4.8 (3.1-6.8)		-

* percentage of households having the predictor; **Kwallis test; ***percentage refers to the proportion of households without central heating; $^+$ the median and IQR of indoor NO_2 refers to the proportion of homes that were built 28 years ago or less; $^{++}$ the median and IQR of indoor NO_2 refers to the proportion of homes that were built more than 28 years ago; # 30 minutes is the median.

Housing characteristics by country

There was a large variation in the use of gas appliances and household characteristics by country (Table 5.3). The presence of a gas hob varied greatly from nearly 100% in Italy to 0.0% in Sweden. Around half of the households in Belgium, Spain and UK had a gas hob but the proportion of households with a gas oven was lower. The use of UFGH was relatively common in Spain and UK. Spain had also the largest proportion of households without central heating. The use of gas bottle was nearly exclusive to Spain.

More than 80% of Swedish, Belgium and Spanish kitchens had a fan to extract fumes which was regularly used (according to participants' self-reporting) while in Italy and UK the percentage was

less than 40%. In Spain, Italy, Belgium and UK over 60% of participants reported that they kept a door or window open to the outside always or most of the time while cooking while in Sweden only 14% of participants did this. Indoor smoking was the most common in Spain and Italy and the least common in Sweden. Frequency of gas appliances and housing characteristics by country

Table 5.3 Frequency of gas appliances and housing characteristics by country

Housing characteristics	**Belgium** (n=157) N (%)	**Spain** (n=796) N (%)	**Italy** (n=309) N (%)	**UK** (n=248) N (%)	**Sweden** (n=198) N (%)	**P value of test for difference ***
Gas hob (Yes)	78 (49.7)	449 (56.4)	306 (99.0)	135 (54.4)	0.0 (0)	<0.001
Gas oven (Yes)	32 (20.4	124 (15.6)	87 (28.2)	83 (33.5)	0.0 (0)	<0.001
Bottle gas (Yes)	0.0 (0)	211 (26.5)	1 (0.3)	1 (0.4)	0.0 (0)	<0.001
UFGH (Yes)	2 (1.3)	360 (45.2)	10 (3.2)	87 (35.1)	13 (6.6)	<0.001
No central heating in the house	24 (15.3)	412(51.8)	18 (5.8)	16 (6.5)	41 (20.7)	<0.001
Keeping a window/door open in the kitchen while cooking (Yes)	88 (61.5)	523 (88.5)	223 (75.1)	154 (62.9)	27 (13.7)	<0.001
Flat(Yes)	30 (19.3)	691 (87.3)	247 (82.6)	6 (2.5)	29 (14.6)	<0.001
Smoking indoors (Yes)	42 (27.0)	437 (55.6)	121 (39.4)	46 (18.6)	36 (18.2)	<0.001
Vent that takes fumes out (Yes)	133 (84.7)	624 (80.0)	84 (30.6)	93 (37.7)	163 (89.1)	<0.001
Average time spent on cooking per day in minutes - median (IQR)	40 (30-60)	30 (0-90)	30 (10-60)	30 (15-60)	30(20-60)	0.56^
Age of the building in years - median (IQR)	41 (23-71)	24(15-31)	31 (18-46)	46 (29-71)	26(21-41)	<0.001^

*Chi-square test unless stated; ^Kruskal-Wallis test.

Indoor NO_2 and outdoor NO_2

Two-week average indoor NO_2 was compared with: (1) the annual average outdoor NO_2 at residential level predicted by ESCAPE; (2) two-week average outdoor NO_2 measured outside participants' home at the same time as indoor NO_2 (Table 5.4)

The Italian centres had the highest levels of outdoor NO_2 (both modelled and measured) and the highest levels of indoor NO_2; Sweden had the lowest levels for both. Measured outdoor NO_2 tended to be higher than modelled outdoor NO_2 excepting UK. Indoor NO_2 levels tended to be lower than outdoor NO_2 levels in Belgium, UK and Sweden while the reverse occurred in Italy and Spain.

Table 5.4 Modelled annual average for outdoor NO_2 and measured two-week average for outdoor NO_2 and indoor NO_2 by country

	Belgium	Spain	Italy	UK	Sweden
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
ESCAPE annual average outdoor NO_2 at address level ($\mu g/m^3$)	37.6 (34.5-41.4)	35.8 (25.8-47.3)	42.0 (15.9-62.0)	27.6 (25.0-30.0)	7.1 (6.3-9.2)
Measured outdoor* NO_2 ($\mu g/m^3$)	38.8 (30.4-46.0)	43.1 (30.7-57.1)	56.1 (43.6-69.5)	27.0 (19.5-33.6)	5.7 (3.0-8.3)
Measured indoor NO_2 ($\mu g/m^3$)	29.7 (20.7-39.8)	46.4 (21.3-69.5)	50.8 (37.3-66.9)	23.6 (14.4-41.4)	4.8 (3.1-6.8)

* Outdoor NO_2 measured at same time as indoor NO_2 outside participants' home

Figure 5.3 shows scatter plots for indoor NO_2 against modelled annual average outdoor NO_2 by country. Indoor NO_2 concentrations tended to be positively correlated with outdoor NO_2. The correlation was the strongest in Italy ($r=0.32$, P value<0.001) and the weakest in Belgium ($r=0.08$, $P=0.41$). Correlation between outdoor and indoor NO_2 in the UK was comparatively strong ($r=0.31$, P value<0.001) but unexpectedly there was some evidence that these correlations were weaker in homes with fewer gas appliances (for example, the correlation decreased to 0.098 in households that cooked with an electric hob). There were no major differences in the other countries when data were divided by type of gas hob (results not shown).

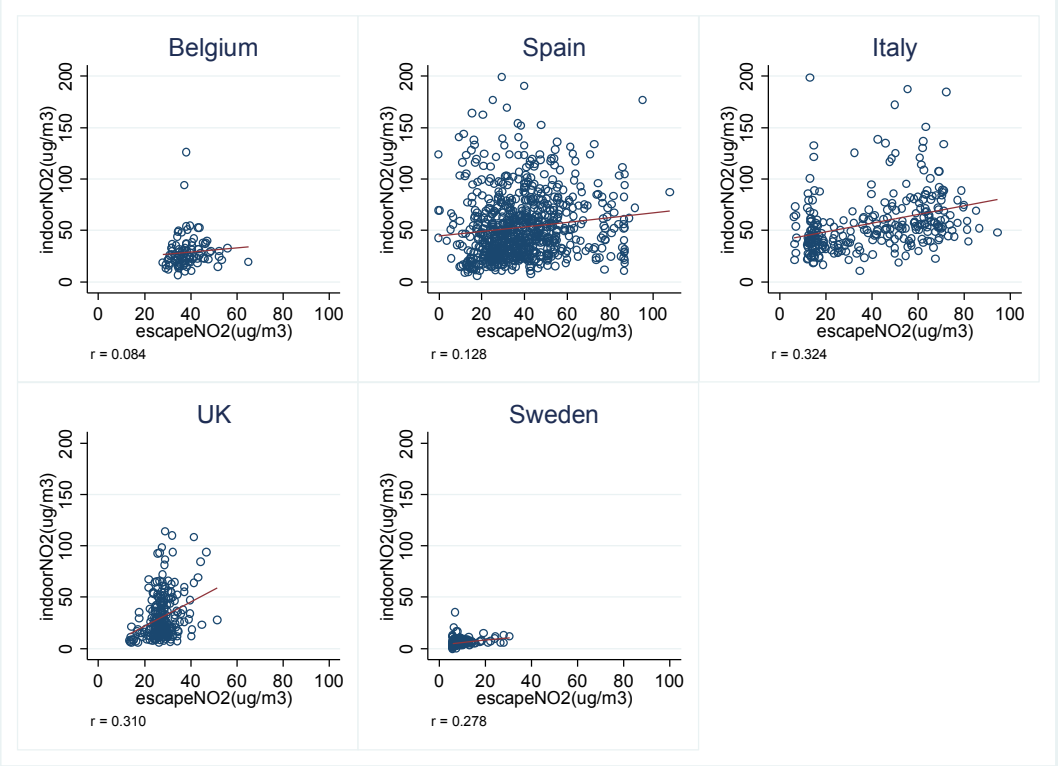


Figure 5.3 Relationship of two-week average indoor NO_2 and modelled annual average outdoor NO_2 by country

Indoor NO_2 and outdoor temperature and relative humidity

Over the period of monitoring, Sweden tended to have the highest monthly outdoor relative humidity and the lowest average monthly outdoor temperature. Correlation between temperature and indoor NO_2 was negative suggesting that as outdoor temperature increases indoor NO_2 decreases (Figure 5.4). The correlation was relatively strong in all countries except for Sweden where the correlation was very weak and not significant.

Table 5.5 Monthly average outdoor temperature and relative humidity by country

	Belgium	Spain	Italy	UK	Sweden
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Monthly average outdoor relative humidity	75.8 (68.1-81.2)	73.6 (67.2-78.5)	75.2 (68.4-88.1)	83.9 (78.7-86.7)	84.5 (76.2-89.7)
Monthly average outdoor temperature (^{o}C)	15.4 (9.9-16.7)	13.6 (9.5-17.1)	10.2 (7.1-15.0)	10.4 (6.5-14.3)	1.78 (0.9-6.9)
Warm season temperature (^{o}C)	17.8 (16.7-19.3)	21.0 (18.1-25.0)	21.9 (20.8-21.9	14.3 (14.3-17.1)	12.6 (9.4-13.4)
Cold season temperature (^{o}C)	6.1 (4.3-7.9)	9.4 (8.1-9.6)	5.9 (5.5-7.5)	5.9 (5.9-7.4)	-3.8 (-4.8-0.9)

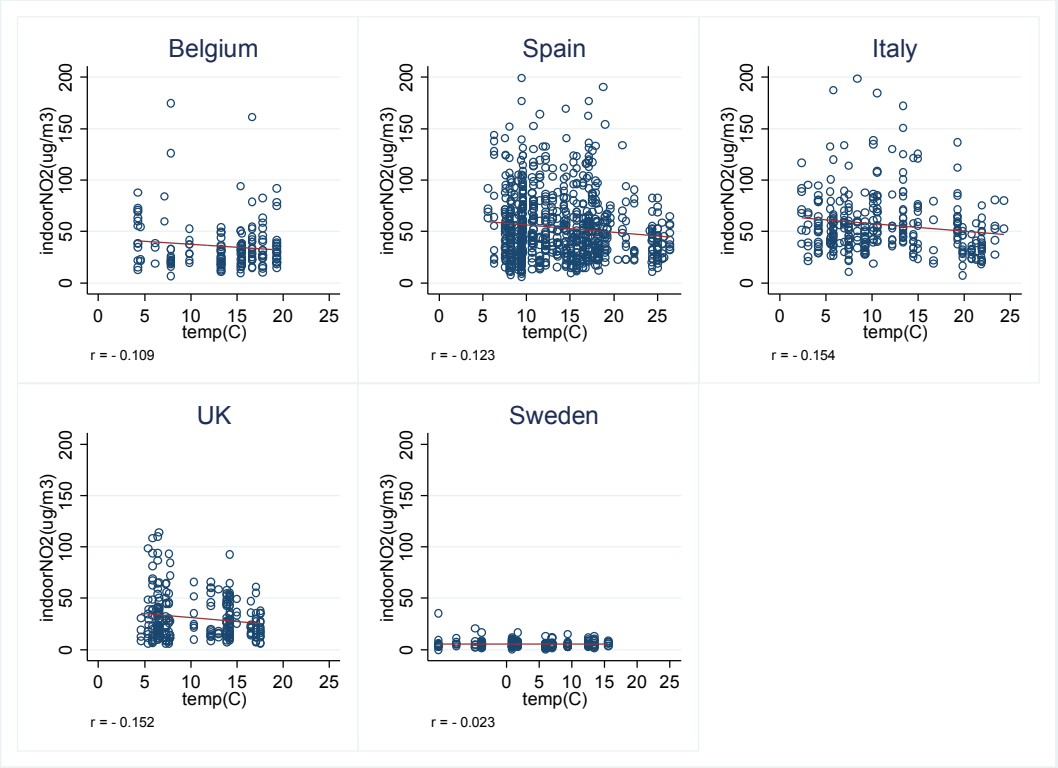


Figure 5.4 Relationship of indoor NO_2 and monthly average temperature by country

Indoor NO_2 and seasonality

The relationship of indoor NO_2 and temperature was further explored by examining indoor NO_2 concentrations during the warm season (June to September) and cold season (November to February). Indoor NO_2 levels were significantly higher in the cold season than the warm season in Spain and Italy. There was no significant difference between summer and winter levels measured in the Belgium, UK and Swedish centres.

Table 5.6 Indoor NO_2 concentrations by warm and cold seasons and by country

Country	Warm season (Jun-Sep)		Cold season (Nov-Feb)		P value **for Test for difference***
	N (%)*	Indoor NO_2 (µg/m^3) Median (IQR)	N (%)	Indoor NO_2 (µg/m^3) Median (IQR)	
Belgium	70 (44.6)	30.1 (22.7-42.1)	34 (21.7)	29.2(19.9-55.4)	0.85
Spain	175 (22.0)	39.3 (27.6-55.3)	312 (39.2)	51.6 (29.6-74.4)	0.0002
Italy	35 (11.3)	34.3 (27.0-50.3)	145 (46.9)	50.8(37.7-64.7)	0.0001
UK	102 (41.1)	24.5(14.7-35.7)	60 (24.2)	22.9(11.5-46.7)	0.87
Sweden	49 (24.7)	5.3 (3.5-7.1)	75(37.9)	4.3(2.8-7.1)	0.20
Total	431 (25.2)	30.9 (18.6-46.3)	626 (36.7)	41.7 (21.7-64.6)	0.0001

* Kwallis test; **percentage over the total number of measurements by country

5.3.2 Development of the model using the training set

Univariate regression

Each predictor was individually regressed against monitored indoor NO_2 concentrations using the training dataset. Results are listed in Table 5.7 and presented in decreasing order by the size of R^2.

All the predictors were significant (P<0.05) in the univariate models with the exception of 'age of building' (P=0.15). Having a gas hob was the strongest predictor of increasing indoor NO_2 level (34.2µg/m^3) and gave the highest R^2 (0.28). In these univariate analyses outdoor NO_2 and living in a flat were the next strongest predictors explaining 17% and 13% respectively. The remaining predictors explained less than 10% within their univariate models.

Effect estimates were in the direction predicted *a priori* with the exception of temperature (coefficient = 0.81), most likely because of the lack of adjustment for country. The variable 'Keeping a window open while cooking' predicted an increase of indoor NO_2 of 21.1µg/m^3 and explained 9% of the model variability.

Table 5.7 Univariate regression results of each predictor against indoor NO_2 ($\mu g/m^3$) and ranked by R^2 using the training dataset (n=1281)

Predictor	Exposure unit	Expected direction of effect	β	SE	R^2	RMSE	P value
Gas hob	Y/N	+	34.20	1.54	0.28	27.23	<0.001
Annual average outdoor NO_2	per 10$\mu g/m^3$	+	6.72	0.44	0.17	29.41	<0.001
Flat	Y/N	+	23.84	3.06	0.13	29.95	<0.001
Keeping a window/door open in the kitchen while cooking	Y/N	?	21.09	2.00	0.09	30.86	<0.001
Bottled gas used for hob+/-oven	Y/N	+	28.94	2.58	0.09	30.58	<0.001
Gas oven	Y/N	+	23.07	2.17	0.08	30.72	<0.001
Monthly average outdoor relative humidity	per 1%	-	-0.71	0.09	0.05	31.30	<0.001
UFGH	Y/N	+	14.91	1.95	0.04	31.34	<0.001
No central heating	Y/N	+	13.08	1.93	0.04	31.48	<0.001
Monthly average outdoor temperature	per 1Celsius	-	0.81	0.14	0.03	31.62	<0.001
Smoking indoors	Y/N	+	10.24	1.82	0.02	31.74	<0.001
Average time spent on cooking per day	30min	+	1.90	0.56	0.01	31.96	<0.001
Vent that takes fumes out	Y/N	-	-4.69	1.94	0.005	32.14	0.016
Age of the building	per 10years	-	0.36	0.25	0.002	32.13	0.15

Stepwise regression using the training set

First, the predictor with the highest R^2, 'gas hob' was regressed against measured indoor NO_2 and then the remaining predictors were sequentially added in order of decreasing R^2 and the adjusted R^2 checked at each point. If the adjusted R^2 increased by at least 1% the predictor was retained in the model. Inspection of the variance inverse factor (VIF) suggested that there was no strong multi-collinearity (defined VIF≥3) and no variables were excluded for this reason. The decisions made at each addition of each variable are shown below in Table 5.8.

The final result of the stepwise regression identified by the following nine predictors:

1. Presence of a gas hob
2. Annual average outdoor NO_2
3. Living in a flat
4. Keeping a window/door open in the kitchen while cooking
5. Use of bottled gas
6. Presence of a gas oven
7. Presence of an unvented gas appliance (UFGH)
8. Monthly average outdoor temperature
9. Age of building.

Table 5.8 Results of stepwise regression using the training set

Predictors (in decreasing order of R^2)	coefficient when predictor included in the stepwise regression	VIF	Adj R^2 when predictor included in the stepwise regression	Adj R^2- adj R^2 of previous accepted regression	% increase in adj R^2 from previous accepted regression	Predictor accepted if % increase in adj R^2 ≥1%
Step 0 - univariate regression with highest R^2						
Gas hob	34.2	1.00	0.2781	-		
Step 1 Gas hob + outdoor NO_2						
Gas hob	29.77	1.12	0.3518	0.3518-0.2781	26.8	new predictor accepted
ESCAPE outdoor NO_2	4.25	1.12				
Step 2 - Gas hob + outdoor NO_2 + flat						
Gas hob	28.4	1.13	0.3884	0.3884-0.3518	10.2	new predictor accepted
ESCAPE outdoor NO_2	2.9	1.29				
Flat	14.1	1.21				
Step 3 - gas hob + outdoor NO_2 + flat+ Keeping a window/door open in the kitchen while cooking						
Gas hob	26.8	1.18	0.4043	0.4043-0.3884	4.1	new predictor accepted
ESCAPE outdoor NO_2	2.8	1.41				
Flat	13.3	1.30				
Keeping a window/door open	5.58	1.18				
Step 4 - gas hob + outdoor NO_2 + flat+ Keeping a window/door + Bottled gas						
Gas hob	24.6	1.27	0.4162	0.4162-0.4043	2.9	new predictor accepted
ESCAPE outdoor NO_2	3.1	1.42				
Keeping a window/door open	11.9	1.26				
Flat	5.1	1.32				
Bottled gas (hob+/-oven)	12.0	1.14				
Step 5 - gas hob + outdoor NO_2 + flat+ Keeping a window/door + Bottled gas + Gas oven						
Gas hob	21.5	1.49	0.4263	0.4263-0.4162	2.4	new predictor accepted
ESCAPE outdoor NO_2	2.9	1.43				
Flat	12.8	1.34				
Keeping a window/door open	5.1	1.26				
Bottled gas (hob+/-oven)	12.7	1.21				
Gas oven	9.3	1.14				
Step 6 – gas hob + outdoor NO_2 + flat+ Keeping a window/door + Bottled gas + Gas oven + humidity						
Gas hob	21.5	1.50	0.4299	0.4299-0.4263	0.8	new predictor dropped
ESCAPE outdoor NO_2	3.0	1.54				
Flat	13.4	1.42				
Keeping a window/door open	5.2	1.27				
Bottled gas (hob+/-oven)	13.9	1.17				
Gas oven	9.2	1.21				
Monthly average	0.09	1.31				

Predictors (in decreasing order of R^2)	coefficient when predictor included in the stepwise regression	VIF	Adj R^2 when predictor included in the stepwise regression	Adj R^2- adj R^2 of previous accepted regression	% increase in adj R^2 from previous accepted regression	Predictor accepted if % increase in adj R^2 ≥1%
outdoor relative humidity						
Step 7 – gas hob + outdoor NO_2 + flat+ Keeping a window/door + Bottled gas + Gas oven + UFGH						
Gas hob	21.7	1.49				
ESCAPE outdoor NO_2	2.8	1.43				
Flat	12.7	1.34				
Keeping a window/door open	4.6	1.27	0.4306	0.4306-0.4263	1.0	new predictor accepted
Bottled gas (hob+/-oven)	10.9	1.21				
Gas oven	8.8	1.22				
UFGH	5.4	1.10				
Step 8 - gas hob + outdoor NO_2 + flat+ Keeping a window/door + Bottled gas + Gas oven + UFGH + No central heating						
Gas hob	22.4	1.52				
ESCAPE outdoor NO_2	2.8	1.43				
Flat	12.0	1.36				
Keeping a window/door open	4.4	1.28	0.4335	0.4335-0.4306	0.7	new predictor dropped
Bottled gas (hob+/-oven)	9.3	1.29				
Gas oven	8.7	1.22				
UFGH	4.1	1.19				
+ No central heating	4.8	1.26				
Step 9 - gas hob + outdoor NO_2 + flat+ Keeping a window/door + Bottled gas + Gas oven + UFGH + temperature						
Gas hob	21.8	1.49				
ESCAPE outdoor NO_2	2.9	1.45				
Flat	13.5	1.36				
Keeping a window/door open	5.6	1.32	0.4364	0.4364-0.4306	1.3	new predictor accepted
Bottled gas (hob+/-oven)	11.9	1.20				
Gas oven	8.8	1.23				
UFGH	5.6	1.12				
Monthly average outdoor temperature	-0.3	1.21				
Step 10 – gas hob + outdoor NO_2 + flat+ Keeping a window/door + Bottled gas + Gas oven + UFGH + temperature + smoking indoors						
Gas hob	21.9	1.49				
ESCAPE outdoor NO_2	2.9	1.45				
Flat	12.9	1.40				
Keeping a window/door open	5.2	1.33	0.4386	0.4386-0.4364	0.5	new predictor dropped
Bottled gas (hob+/-oven)	11.7	1.22				
Gas oven	8.7	1.23				
UFGH	5.2	1.13				
Monthly average	-0.3	1.21				

Predictors (in decreasing order of R^2)	coefficient when predictor included in the stepwise regression	VIF	Adj R^2 when predictor included in the stepwise regression	Adj R^2- adj R^2 of previous accepted regression	% increase in adj R^2 from previous accepted regression	Predictor accepted if % increase in adj R^2 ≥1%
outdoor temperature						
Smoking indoors	3.5	1.09				
Step 11 – gas hob + outdoor NO_2 + flat+ Keeping a window/door + Bottled gas + Gas oven + UFGH + temperature + time on cooking						
Gas hob	21.7	1.50				
ESCAPE outdoor NO_2	3.0	1.46				
Flat	13.3	1.37				
Keeping a window/door open	5.1	1.37				
Bottled gas (hob+/-oven)	11.4	1.22	0.4376	0.4376-0.4364	0.2	new predictor dropped
Gas oven	8.9	1.23				
UFGH	5.3	1.14				
Monthly average outdoor temperature	-0.3	1.21				
Average time spent on cooking per day	0.8	1.12				
Step 12 – gas hob + outdoor NO_2 + flat+ Keeping a window/door + Bottled gas + Gas oven + UFGH + temperature + vent						
Gas hob	21.2	1.56				
ESCAPE outdoor NO_2	2.9	1.44				
Flat	14.4	1.39				
Keeping a window/door open	5.7	1.34				
Bottled gas (hob+/-oven)	11.8	1.23	0.4357	0.4357-0.4364	-0.1	new predictor dropped
Gas oven	9.9	1.24				
UFGH	5.3	1.12				
Monthly average outdoor temperature	-0.3	1.22				
Vent that takes fumes out	-0.03	1.07				
Step 13 – gas hob + outdoor NO_2 + flat+ Keeping a window/door + Bottled gas + Gas oven + UFGH + temperature + age of building						
Gas hob	21.5	1.50				
ESCAPE outdoor NO_2	2.8	1.48				
Flat	14.4	1.42				
Keeping a window/door open	5.6	1.32				
Bottled gas (hob+/-oven)	12.3	1.21	0.4410	0.4410-0.4364	1.1	new predictor accepted
Gas oven	8.9	1.24				
UFGH	5.8	1.12				
Monthly average outdoor temperature	-0.3	1.21				
Age of the building	0.4	1.08				

*Variance Inflation Factor = $1/(1-R^2)$

Inclusion of country and exclusion of predictors with P value >0.10

At this stage the predictor ‘country’ was forced into the model and the P value of the predictors was checked for significance (see Table 5.9) for results at each step. Inspection of VIF showed that there was some multi-collinearity when the variable ‘country’ was included in the model. Spain and Italy had VIF≥3.0 but were kept in the model because of *a priori* rule that all countries would be kept in the model.

After including ‘country’ in the model the explained variability increased to 47% and the coefficient size of most predictors decreased. In particular, the coefficient ‘gas hob’ decreased from 21.5 to 17.7, ‘living in a flat’ from 14.4 to 4.20 and ‘keeping a window/door open’ from 5.6 to 1.4 suggesting an influence of country on these variables. ‘Living in a flat’ and ‘keeping a window/door open’ had a P value above 0.10 and were sequentially removed from the model.

Table 5.9 Results of regression model before and after country adjustment, after removing predictors with P value>0.10 and final model

	Step 13 (model without country)				Step 14 (model with country)				Step 15				Step 16 and final model			
Predictors	β	se	P value	VIF	β	se	P value	VIF	β	se	P value	VIF	β	se	P value	VIF
Gas hob	21.47	1.91	<0.001	1.50	17.74	2.21	<0.001	2.10	18.93	2.00	<0.001	2.00	18.83	1.98	<0.001	2.00
ESCAPE outdoor NO_2 per 10 µg/m^3	2.83	0.49	<0.001	1.48	2.04	0.50	<0.001	1.68	1.88	0.45	<0.001	1.59	2.03	0.44	<0.001	1.52
Living in a flat	14.38	1.90	<0.001	1.42	4.20	2.58	0.1038	2.87	3.48	2.32	0.1330	2.62	-	-	-	-
Keeping a window/door open in the kitchen while cooking	5.59	1.95	0.0042	1.32	1.37	2.01	0.4973	1.52	-	-	-		-	-	-	-
Bottle gas (hob+/-oven)	12.29	2.67	<0.001	1.21	9.68	2.86	<0.001	1.45	9.39	2.54	<0.001	1.44	8.89	2.51	<0.001	1.43
Gas oven	8.89	2.17	<0.001	1.24	10.00	2.14	<0.001	1.25	9.46	1.99	<0.001	1.24	9.53	1.97	<0.001	1.24
UFGH	5.82	1.84	0.0016	1.12	5.15	1.94	0.0081	1.31	5.76	1.76	<0.001	1.28	5.94	1.75	<0.001	1.28
Outdoor temperature	-0.30	0.13	0.0205	1.21	-0.62	0.14	<0.001	1.50	-0.59	0.13	<0.001	1.48	-0.57	0.13	<0.001	1.47
Age of building (per 10 years)	0.43	0.23	0.066	1.08	0.67	0.23	0.0042	1.11	0.71	0.22	<0.001	1.11	0.68	0.22	0.002	1.11
Sweden	-	-	-		0	-	-	-	0	-	-	-	0	-	-	-
Belgium	-	-	-		15.08	4.43	<0.001	1.89	15.80	4.10	<0.001	1.88	14.97	4.05	<0.001	1.87
Spain	-	-	-		29.04	3.95	<0.001	6.66	30.20	3.46	<0.001	6.08	32.28	3.12	<0.001	5.03
Italy	-	-	-		25.39	4.18	<0.001	4.99	26.04	3.83	<0.001	4.43	27.84	3.53	<0.001	3.91
UK	-	-	-		10.68	3.40	0.0018	2.84	10.63	3.17	<0.001	2.55	9.81	3.12	0.0017	2.54
constant	5.59	2.08	0.007		2.14	2.19	0.33		2.21	2.15	0.304		2.51	2.13	0.238	
RMSE	24.45				23.82				23.76				23.69			
Adj R^2	0.44				0.47				0.46				0.46			

* n=1163 as some of the variables had missing data.

The final model included 7 predictors (gas hob, outdoor NO_2, bottle gas, gas oven, UFGH, outdoor temperature and age of building) and country (Belgium, Spain, Italy, UK and Sweden) explaining 46% of the variability.

The model predicts that on average having a gas hob increases the indoor NO_2 levels by 18.8μg/m^3, having a gas oven by 9.5μg/m^3, using bottled gas by 8.9μg/m^3 and having an open gas fire or portable gas heater or a paraffin heater by 5.9μg/m^3. Each 10μg/m^3 increase in outdoor NO_2 corresponds to a 2.0μg/m^3 increase in indoor NO_2. Compared to Sweden, a household in Belgium has an additional 15.0μg/m^3 of indoor NO_2, Spain an additional 27.8μg/m^3, Italy an additional 27.8μg/m^3 and UK an additional 9.8μg/m^3.

For example, let us predict the two-week average concentration of indoor NO_2 of a Swedish household in Umea, 30 years old, with no gas appliances (no gas hob, no gas oven, no UFGH) and an annual average outdoor NO_2 of 9μg/m^3 in October when the average monthly outdoor temperature is 6° Celsius. The predicted two-week indoor NO_2 concentration in the month of October will be 2.98μg/m^3, that is:

2.03μg/m^3 * 0.9μg/m^3 outdoor NO_2*10 - 0.57μg/m^3 *6 °C outdoor monthly temperature (October) + 0.68μg/m^3 *3.0 age of building (per 10 years) + 0 μg/m^3*Sweden + 2.51 μg/m^3(constant) = 2.98μg/m^3

Let's take an example of an UK household in East Anglia at the same month of the year, October. A 15-years old home with a gas hob and gas oven, an annual average outdoor NO_2 of 40μg/m^3 and a monthly average outdoor temperature of 13° Celsius will have a predicted two-week average concentration in the month of October of 42.45μg/m^3 , that is:

18.83μg/m^3 *gas hob (yes=1, no=0) + 2.03μg/m^3 * 4 μg/m^3 outdoor NO_2* 10 + 9.53μg/m^3 * gas oven (yes=1, no=0) + -0.57μg/m^3 *13 °C outdoor monthly temperature (October) + 0.68μg/m^3 *1.5 age of building (per 10 years) + 9.81μg/m^3 *UK + 2.51μg/m^3 (constant) = 42.45μg/m^3

5.3.3 Testing the assumptions of normality

For each household of the training set (n=1,301) a predicted level of NO_2 was determined and compared with the observed levels. Residuals were standardised and visually assessed for normal distribution (see Figure 5.5). The histogram of the distribution of residuals is slightly skewed to the left. However, as in ESCAPE (ESCAPE, 2010, pg. 30) concentrations were not transformed into log because they are more readily interpretable.

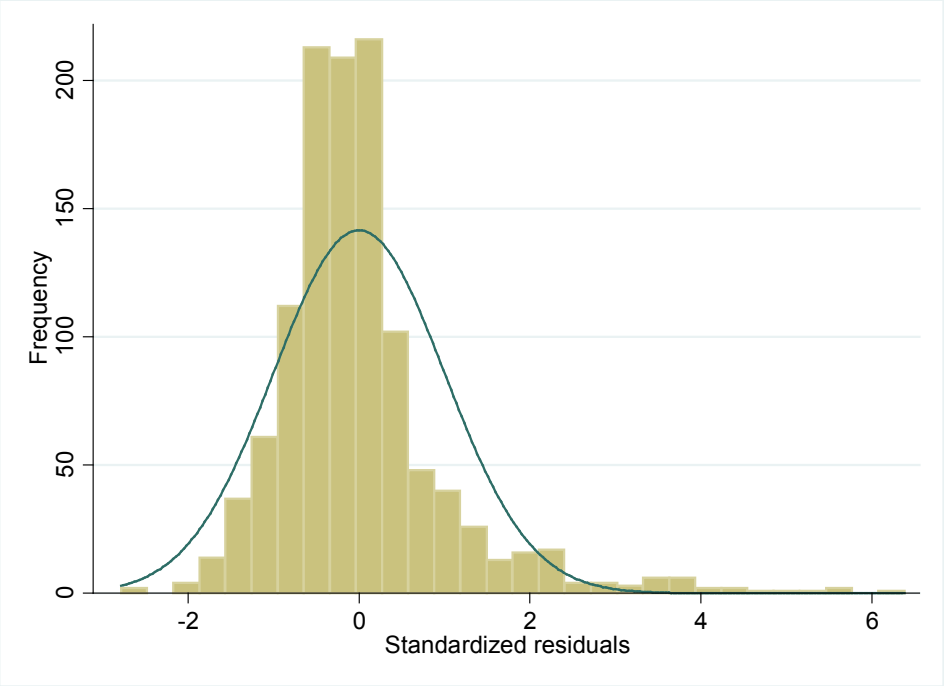


Figure 5.5 Histograms showing distribution of standardised residuals of the final model using the test dataset

Twenty-five outliers were identified as having a standardised residual greater than 3 or smaller than -3. These homes had very high indoor NO_2 levels (between 111µg/m^3 and 199µg/m^3) – and the model as built is unable to explain the reason for this. Removal of these outliers increased the adjusted R^2 from 0.47 to 0.56. There was little change in the size and precision of the coefficients except for Spain and Italy, possibly due to the fact that most of the outliers were from these two countries (15 observations from Spain and 8 from Italy). By default all outliers were kept in the model.

Table 5.10 Model after removing outliers * (n=1138)

Predictor	β	Se	P value
Gas hob	18.58	1.59	<0.001
ESCAPE outdoor NO2 per 10 μg/m^3	2.31	0.35	<0.001
Bottle gas (hob+/-oven)	7.81	2.02	<0.001
Gas oven	10.60	1.58	<0.001
UFGH	5.96	1.40	<0.001
Outdoor temperature	-0.50	0.10	<0.001
Age of building (per 10 years)	0.55	0.18	0.0020
Sweden	0	-	-
Belgium	12.05	3.23	<0.001
Spain	28.00	2.49	<0.001
Italy	22.73	2.83	<0.001
UK	8.17	2.48	0.0010
constant	2.58	1.69	0.13
Adj R^2	0.56		
RMSE	18.71		

*Outliers defined as having a standardised residual bigger than 3 or smaller than -3 (n=25); ** P value for country as a variable with 5 categories.

The formal test for homogeneity of variance (Cook, 1977; Breusch and Pagan, 1979) as well as the scatter plot showing the distribution of residual variance (see Figure 5.6) suggests that variance is not constant (P<0.001) and that it tends to increase as indoor NO_2 values increase in size.

The Cooks test identified 71 (5%) influential observations. Fifty-eight of these influential observations had a predicted NO_2 lower than the observed value; all of them but one had had high levels of indoor NO_2 measurements (median 124.3 (IQR 117-139) μg/m^3), tended to be homes with a gas hob (81%) and were mainly in Spain (63.8%) and Italy (25.9%). All observations (n=13) with a predicted value higher than the observed value had a gas hob and were mainly in Spain (84.6%).

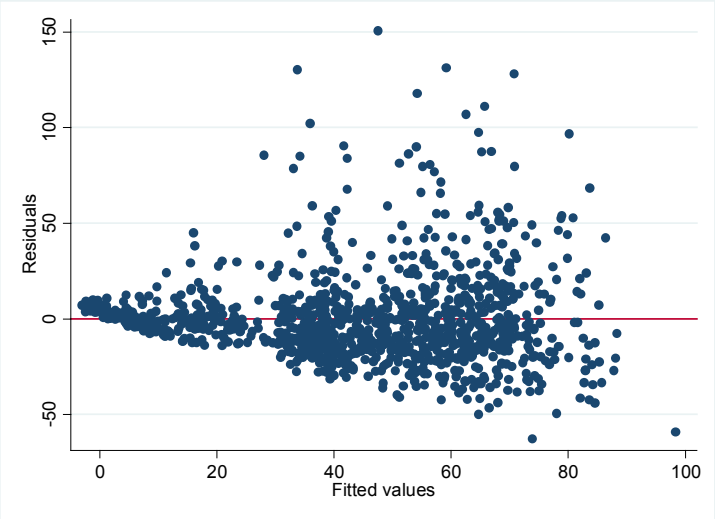


Figure 5.6 Distribution of residual variance

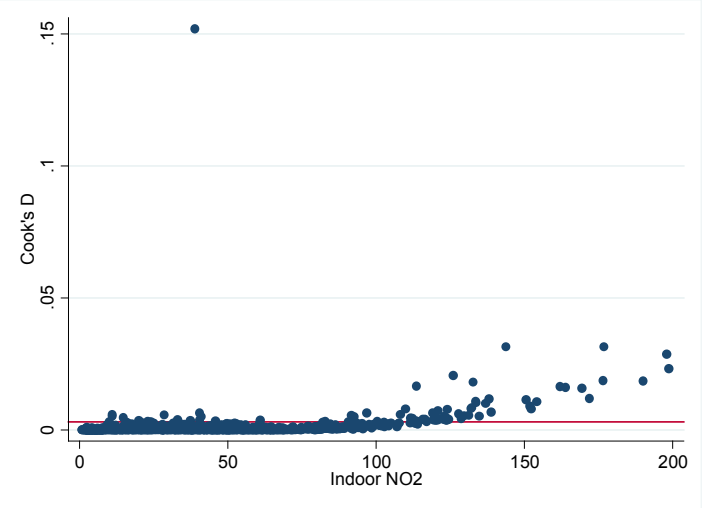


Figure 5.7 Scatter plot showing influential observations identified with *D* Cooks (above line)

5.3.4 Validation of model

The model was validated by first predicting the values of indoor NO_2 in the test set and then comparing the predicted values against the observed values with a regression plot (Figure 5.8). There were no changes in the explained variability (46% for the training set and 47% in the test set), intercept and slope suggesting that the model was quite 'robust' when applied to the test

dataset. The scatter plot suggests that the model tends to underestimate higher concentrations and overestimate lower concentrations.

In the same way a visual inspection of the Bland-Altman plot (Figure 5.9) suggests that the model tends to underestimate the observed values, particularly at high concentrations. The higher average values tend to lie above the upper limit of agreement (limits of agreement ranged between -45.9µg/m^3 to 46.5µg/m^3). The mean difference was 0.28µg/m^3 (CI -2.0 to 2.6).

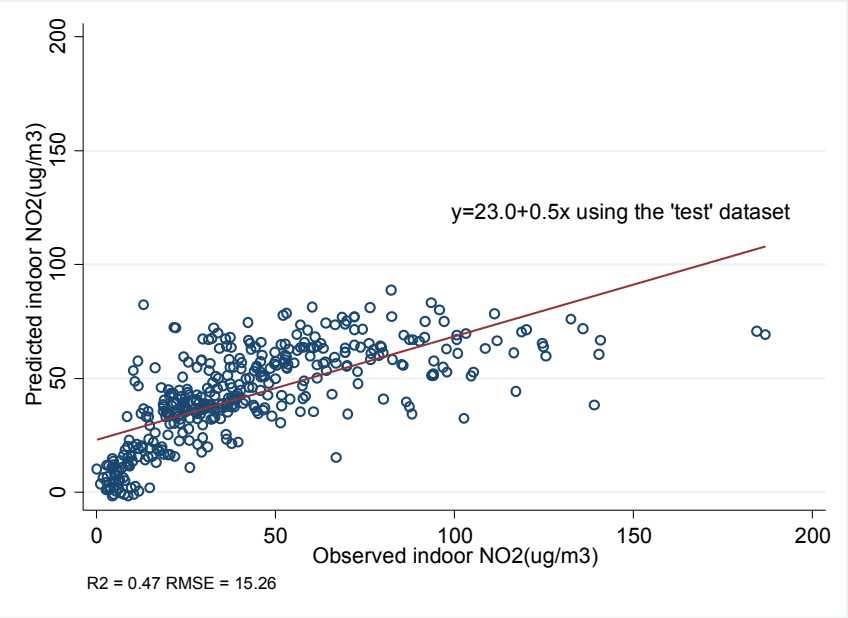


Figure 5.8 Predicted levels of indoor NO_2 against the observed levels using the observations of the test set

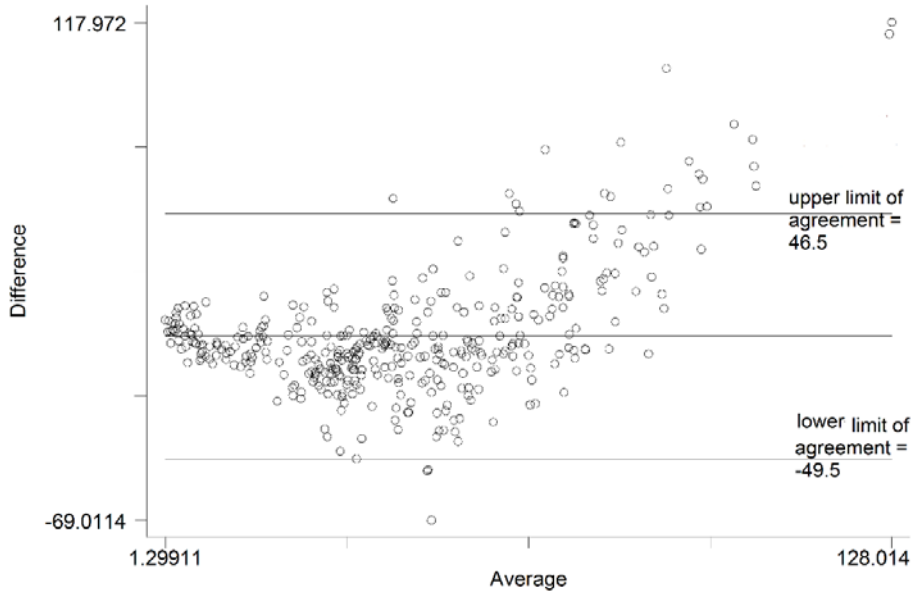


Figure 5.9 Bland-Altman plot showing agreement between observed indoor NO_2 and predicted indoor NO_2 in the test set (average = average of the sum of observed value + predicted value; difference = observed value – predicted value)

5.3.5 Sensitivity analyses

Cross-validation

A sensitivity analysis was conducted by carrying out a cross-validation test. The full dataset (training set and test set merged together) was divided into 4 sub-set at random. Table 5.11 shows the results for each sub-set model. The size of the coefficients and their statistical significance, the adjusted R^2 slightly changed any time the model was run in different sub-sets. There was little change in the proportion of explained variability, which varied between 46% and 48%. There was a slightly variation in the coefficient size and P value, which stayed below 0.05 for all predictors except for 'age of building'. The coefficients for 'age of building' varied from 0.38 to 0.82 (0.68 in the main model) and the coefficient for 'bottled gas' varied from 5.41 to 11.05 (8.89 in the main model) suggesting that these two predictors may be unstable in the indoor NO_2 model.

Table 5.11 Table showing changes in coefficients size, adjusted R^2 and RMSE in the cross-validation tests using the four sub-sets split at random

	Main model			Model using sub-set 2+3+4 and tested on sub-set 1			Model using sub-set 1+3+4 tested on sub-set 2			Model using sub-set 1+2+4 tested on sub-set 3			Model using sub-set 1+2+3 tested on sub-set 4		
Predictor	β	se	P value	β	se	P value	β	se	P value	β	Se	P value	β	se	P value
Gas hob	18.83	1.98	<0.001	17.90	1.95	<0.001	17.86	1.90	<0.001	17.55	1.96	<0.001	17.06	1.92	<0.001
ESCAPE outdoor NO2 per 10 μg/m^3	2.03	0.44	<0.001	2.02	0.45	<0.001	1.91	0.43	<0.001	2.44	0.44	<0.001	2.43	0.43	<0.001
Bottle gas (hob+/-oven)	8.89	2.51	<0.001	10.05	2.56	<0.001	5.41	2.51	0.031	8.14	2.56	0.0015	11.05	2.43	<0.001
Gas oven	9.53	1.97	<0.001	10.43	2.04	<0.001	11.63	1.91	<0.001	12.43	1.97	<0.001	11.59	1.97	<0.001
UFGH	5.94	1.75	<0.001	7.37	1.74	<0.001	6.23	1.66	<0.001	6.51	1.74	<0.001	6.34	1.69	<0.001
Outdoor temperature	-0.57	0.13	<0.001	-0.65	0.13	<0.001	-0.64	0.12	<0.001	-0.56	0.13	<0.001	-0.52	0.13	<0.001
Age of building (per 10 years)	0.68	0.22	<0.001	0.53	0.21	0.013	0.38	0.20	0.057	0.57	0.22	0.0091	0.82	0.22	<0.001
Sweden	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
Belgium	14.97	4.05	<0.001	15.79	3.91	<0.001	16.44	3.86	<0.001	15.00	3.98	<0.001	11.33	3.97	0.0044
Spain	32.28	3.12	<0.001	33.57	3.05	<0.001	32.81	2.95	<0.001	31.49	3.06	<0.001	30.34	3.05	<0.001
Italy	27.84	3.53	<0.001	29.02	3.50	<0.001	30.60	3.37	<0.001	28.54	3.55	<0.001	28.52	3.49	<0.001
UK	9.81	3.12	0.0017	11.07	3.09	<0.001	11.01	2.97	<0.001	7.43	3.11	0.017	8.06	3.08	0.0089
Constant	2.51	2.13	0.24	3.64	2.12	0.86	3.76	2.05	0.067	2.42	2.19	0.27	1.92	2.18	0.38
Adj R^2	0.46			0.46			0.46			0.46			0.48		
RMSE	23.69			23.80			22.74			23.76			23.37		

Figure 5.10 shows the predicted values against the observed values in the left-out sub-set. On average the model explained 46.2% of variability and RMSE was 16.1. As previously noted the scatter plots suggest that the model tends to underestimate the highest observed concentrations and overestimated the lowest concentrations.

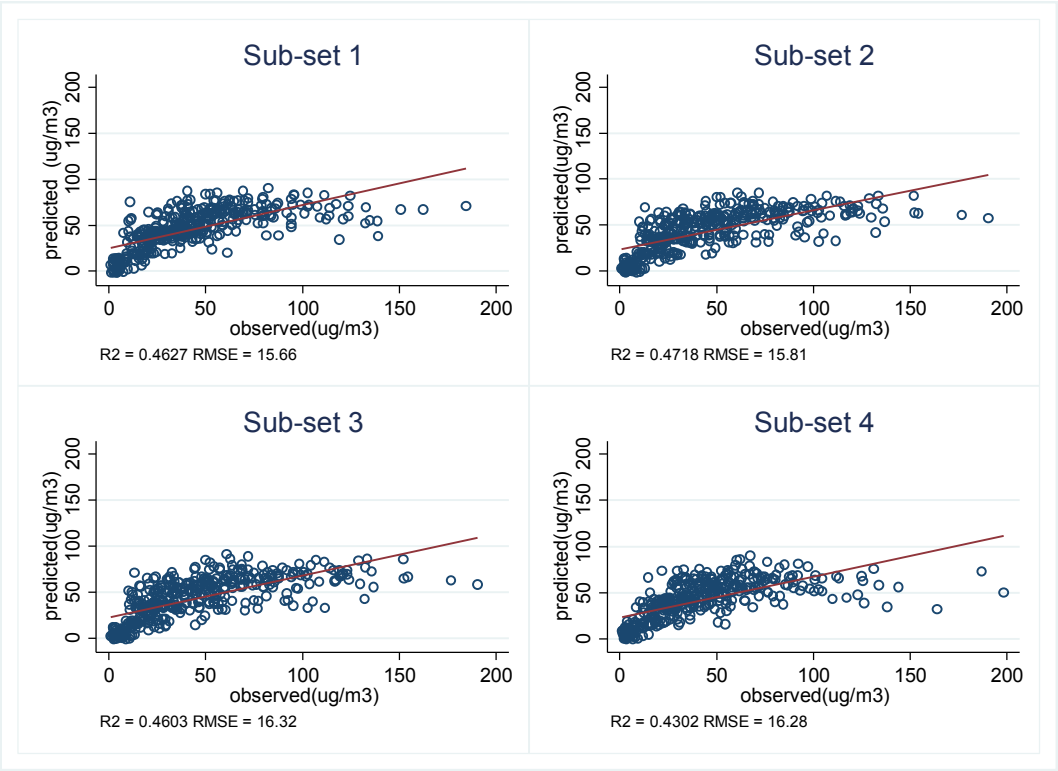


Figure 5.10 Predicted indoor NO_2 vs monitored indoor NO_2 in the cross-validation using four sub-sets

5.4 Application of model

5.4.1 Results applied to ECRHS dataset

Finally, the model was applied to predict two-week average indoor NO_2 exposure for the month of October in ECRHS participants for whom the necessary information was available. Information on household characteristics (i.e. gas hob, gas oven, gas bottle, UFGH, age of building) collected at ECRHS II and ECRHS III, monthly average temperature at centre level and modelled outdoor

NO_2 annual average at address level for year 2002 and 2010 were used to predict indoor NO_2 at ECRHS II and ECRHS III respectively. Indoor NO_2 levels at ECRHS III could not be predicted for Italian centres because information on gas appliances was not collected at ECRHS III. In order to predict indoor NO_2 exposure the following assumptions were made:

- The amount of indoor NO_2 emitted from a gas appliance (gas hob, gas oven, UFGH) or from bottled gas did not change over the 10-year period between ECRHS II and ECRHS III.
- The annual average exposure to outdoor NO_2 modelled by ESCAPE for the year 2010 was the same as for the year 2010-2013 when ECRHS III was conducted and the annual average exposure to outdoor NO_2 modelled (back-extrapolated) by ESCAPE for the year 2002 was the same as for the year 2000-2002 when indoor NO_2 was measured in the houses of ECRHS participants.
- Monthly average temperature at ECRHS II was the same as at ECRHS III.

Since the predicted concentrations varied according to monthly average temperature a two-week average for the month of October was predicted. October is a month of less extreme temperature in all 5 countries included in this model.

The predicted October concentrations at ECRHS II and ECRHS III by country and annual outdoor NO_2 concentrations are shown in Table 5.12. Modelled indoor NO_2 tended to be higher than outdoor NO_2 in Spain, Italy and UK at ECRHS II and ECRHS III. The reverse was seen in Belgium and Sweden where outdoor NO_2 concentrations tended to be higher than indoor NO_2 concentrations.

Modelled indoor NO_2 concentrations decreased from ECRHS II to ECRHS III in all 5 countries, likely to be affected by the fall in the annual average concentrations of outdoor NO_2 and the use of gas for cooking appliances (see next Chapter 6). The decline was particularly strong in Spain where median concentrations for modelled indoor NO_2 declined from 53.4 (IQR 36.4-64.6) $\mu g/m^3$ to 39.5 (IQR 36.7-46.80) $\mu g/m^3$. In Sweden the decline of indoor NO_2 was relatively small, likely to be influenced by the small decline in outdoor NO_2.

The model was also tested to predict two-week average concentrations at different time of the year, i.e. the month when the ECRHS interview was carried out. Under this scenario a proportion (13% at ECRHS II and 24% at ECRHS III) of predicted values for Sweden was negative if the interviews were carried out during the warmest months of the year (June, July and August). This is explained by the fact that the model predicts a fall in indoor NO_2 concentrations as the

temperature increases but in the absence of gas appliances and in the presence of low levels of outdoor NO_2 (both conditions apply to Sweden) the predicted value is bound to become negative.

Table 5.12 Modelled two- week average indoor NO_2 concentrations for the month of October at ECRHS II and ECRHS III and annual average outdoor NO_2 concentrations predicted by ESCAPE at ECRHS II and ECRHS III by country

Country	Average temperature in October*	ECRHS II		ECRHS III	
		Modelled ** outdoor NO_2 ($\mu g/m^3$)	Modelled indoor NO_2 ($\mu g/m^3$)	Modelled** outdoor NO_2 ($\mu g/m^3$)	Modelled indoor NO_2 ($\mu g/m^3$)
	°C	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Belgium	13.3 (0)	37.6 (34.5-41.4)	22.2 (19.3-38.8)	38.3 (33.1-45.7)	21.6 (18.9-37.0)
Spain	16.5 (1.4)	35.8 (25.8-47.3)	53.4 (36.4-64.6)	28.4 (21.9-44.1)	39.5 (36.7-46.8)
Italy***	15.8 (0.7)	42.0 (15.9-62.0)	53.5 (46.4-59.7)	30.7 (19.1-52.1)	-
UK	12.6 (0.4)	27.6 (25.0-30.0)	33.1 (17.4-44.0)	24.9 (22.7-26.9)	30.1 (15.1-40.8)
Sweden	6.0 (0)	7.1 (6.3-9.2)	3.2 (2.0-5.0)	4.3 (3.7-5.7)	3.1 (2.1-4.5)

* mean (standard deviation) based on monthly average temperature at each centre; **annual; *** indoor NO_2 could not be predicted at ECRHS III because Italian centres did not collect data on gas use.

5.5 Discussion

5.5.1 The main determinants of indoor NO_2

The study presented in this chapter has shown that it is possible using data available within ECRHS to develop a model that includes variables to predict indoor NO_2 concentrations. Using a stepwise regression approach the model identified the presence of gas hob, gas oven, bottled gas, annual average exposure to outdoor NO_2, presence of UFGH, age of building and monthly outdoor temperature as the determinants of indoor NO_2. It was decided *a priori* to force the variable 'Country' into the model; the variable proved to have a strong influence in the model as each country contributed to an additional 'background' level of indoor NO_2, which differed across countries. Different cooking and heating practices, housing characteristics, ventilation and indoor smoking habits may explain some of these differences but it proved difficult to qualify them.

The model predicts that a gas stove (hob and oven included) is associated with two-week average of about 28.3μg/m^3 of indoor NO_2 (gas hob=18.8μg/m^3 + gas oven= 9.5μg/m^3), a concentration very close to the estimation used in the Hasselblad meta-analysis (28.3μg/m^3 for gas stove). This coefficient is slightly higher than the coefficients derived from previous studies, (Monn, 1998; Lee, 1998; Baxter, 2007; Valero, 2009; Levy, 2010) but in these reports it is not clear whether 'gas stove' includes both a gas hob and a gas oven.

Outdoor NO_2 was a main determinant in the model. The model predicts that for every 10μg/m^3 of outdoor NO_2 2.0μg/m^3 are present indoors, a concentration lower than the one predicted by other studies (Valero, 2009). Other models have included outdoor NO_2 but the choice of parameter and NO_2 transformations make the predicted coefficients difficult to compare. Lee (1998) predicted that indoor NO_2 was associated with 0.6*mean annual outdoor NO_2 concentration, similar to Monn (1998) that predicted 0.5 times outdoor NO_2 concentrations; Espluges (2013) that 1μg/m^3 of outdoor NO_2 determined an increase of 0.06 of the square root of indoor NO_2, which varied by season and degree of urbanisation. Heroux (2010) predicted an increase of 0.031 of the natural log of indoor NO_2 per each ppb (1.88μg/m^3) increase of outdoor NO_2, Cyrys (2000) a 33% increase when associated with 17μg/m^3 of outdoor NO_2 and Garcia-Algar (2003) an 1.01 increase of the geometric mean ratio. These estimates demonstrate the difficulty of interpreting models based on log transformation of the indoor NO_2 variable.

5.5.2 The regression model

The model explains 46% of variability. Compared to outdoor NO_2 models which may reach as far as 90% of explained variability (Hoek *et al.*, 2008), 46% of explained variability may appear quite a small proportion but is comparable to the highest proportions achieved by other NO_2 indoor models. For example, the models developed by Heroux et al (2010), Lee et al (1998), Algar et al (2004) explained 44%, 52% and 52% respectively. Other indoor NO_2 models have reached a lower proportion of explained variability, such as those developed by Clougherty et al (2011), Baxter et al (2007), Valero et al (2009), Loo et al (Loo *et al.*, 2010) and Cyrys (2000), which explained 16%, 20%, 32%, 33% and 38% of variability respectively. On the other hand, Lai et al (2006) generated a model that explained 67% of the variability but this model included a log transformation of indoor NO_2. Such log transformations tend to improve the explained variability of the model. A log transformation of the indoor NO_2 variable of the model developed in this chapter would increase the explained variability from 46% to 75% but at the cost of not being able to predict exposures in absolute terms.

As with any regression model the predicted values tended to regress towards the average (Bland and Altman, 1994). As a consequence the model tended to over predict and under predict low and high exposures respectively. In particular, visual inspections of the scatter plot of the predicted values against the observed values and the Bland-Altman plot suggest that the model under-predicted observations with high levels of exposure.No predicted values exceeded 100µg/m^3; on the other hand, 77 observations of the training set (n=1,301) and 28 observations of the test set (n=434) were equal or above 100µg/m^3; the highest observed exposure was equal to 198µg/m^3. Even though a small percentage (around 6% of the complete dataset) of people are exposed to such high levels of indoor NO_2 the model cannot predict their exposure. This may lead to exposure measurement error when conducting a health assessment.

Some multi-collinearity was observed for Italy and Spain. Correlation between determinants is a major problem of stepwise regression, the approach adopted in this model to select potential predictors. Some predictor variables are highly correlated and once a variable has entered the equation in a step-up analysis, the other may not enter, even though it is related to the outcome and as a consequence, it will not appear in the final equation (Bland, 2000). The *a priori* decision to force the variable 'country' into the model ensured that Spain and Italy were included in the model in spite of having a VIF above 3 - but at a cost of results being driven by Italian and Spain. The Cook's *D* test indicated that most of the influential observations were from Spain or Italy. Ideally, country-specific models should have been developed but this would have decreased the power of the model and led to imprecision due to the lack of contrast for the main predictor (gas hob) in Italy and Sweden (the use of gas hob was virtually universal in Italy and absent in Sweden).

5.5.3 Ventilation

Several predictors related to ventilation and consequently to housing characteristics (type of building, windows/door to the outside kept open while cooking, presence of a ducted fan in the kitchen, absence of central heating) were initially considered but only 'age of building' was retained in the final model. This predictor was sensitive to cross-validation.

Ventilation is a main determinant of indoor NO_2 levels. The most appropriate way of measuring ventilation is measuring AER and then including the parameter into the model. However, AER measurements tend to perform better in winter than summer and some models have included these data only for the winter season (Zota, 2005). No AER measurements were carried out in ECRHS participants' homes and self-reported information was used as proxy indicators for ventilation instead. Self-reported information is generally affected by recall bias and random

error. For example, it has been found that people who rent their homes are less likely to know whether their kitchen vent extracts fumes (Seltenrich, 2014), a proxy indicator for ventilation. Besides, the model was based on information supplied by the household member participating in ECRHS (e.g. keeping window open, average time spent cooking) and did not take account of other household members who may also cook or keep a window open.

Once the variable 'country' was included in the model 'keeping the window open' was no longer held in the model because its P value was above 0.10. This suggests that there may be some activity types that may determine NO_2 and vary by country. Transferability of models to other locations could be problematic unless information on predictor variables was collected in a standardised way and locations were nearby (Hoek, 2008). In the model developed in this study, data on predictors were collected using the ECRHS standardised questionnaire but there was some considerable heterogeneity across centres.

5.5.4 Limitations of the model

The main limitations of this model were:

- The model was not country-specific and Spain and Italy tended to drive the model.
- Contribution of ventilation to indoor NO_2 concentrations could not be quantified. Proxy indicators were used instead. They relied on self-reporting information, which tended to be correlated and lacked precision.
- The model used the annual average exposure to outdoor NO_2 to predict the indoor exposure for a specific month (October).

5.5.5 Recommendations

Future research on indoor NO_2 modelling should consider:

- To develop country-specific models
- To consider whether to carry out air exchange rate (AER) measurements
- To explore alternative methodologies to identify predictors in order to minimise problems related to stepwise regression.

5.6 Summary

The model developed in this chapter has shown that it is possible to predict average indoor NO_2 exposure for a group of ECRHS participants for which the necessary information to develop the model was available. Predictors identified by the model included the presence of gas appliances, outdoor $NO_{2,}$ outdoor temperature, age of building and country. They explained 46% of the model variability, a proportion comparable to the highest achieved by other indoor NO_2 models.

6. Long-term associations of respiratory symptoms with exposure to gas for cooking and modelled indoor NO_2

6.1 Introduction

Previous work in the ECRHS showed an association of asthma and asthma-like symptoms with the use of gas for cooking in European adults (Jarvis, 1998). The ECRHS study is one of the very few adult cohorts that have prospectively collected information on symptoms and exposure to gas for cooking over a twenty year period.

In this chapter I am going to use this prospective information to examine the association between long-term asthma-related symptoms and use of gas for cooking. I am also going to apply the modelled exposure to indoor NO_2, which I developed in the previous chapter, to assess the association between long-term asthma-related symptoms and modelled indoor NO_2.

6.1.1 Background

There are relatively few cohort studies that have examined the health effects of the use of indoor gas appliances or exposure to indoor NO_2 over a prolonged period of time. A Dutch longitudinal study (Fischer, 1985) which followed women over 18 years found no association between lung function decline and exposure to current indoor NO_2 levels associated with the use of gas water heating appliances in a sub-sample of non-smoking women (n=97). A longitudinal analysis of 1449 young adults who participated in the British 1958 birth cohort (Moran, 1999) examined the evolution of respiratory symptoms from childhood to the age of 35 in relation to the reported use of gas for cooking at age 35 and, as recalled, at the age of 11. This showed that individuals who currently used gas for cooking had a significantly reduced FEV_1 but being exposed to gas cooking in childhood or adulthood was not associated with incidence or prognosis of asthma and wheeze or severity of respiratory symptoms. A British retrospective cohort study examined all-cause mortality patterns in those who had taken part in a housing survey in 1936 and were followed through the National Health Service Central Register from 1951 to 1989. In over 15,000 adults there was no evidence that having a gas cooker was associated with increased mortality (Coggon *et al.*, 1993).

A small number of studies have followed adult participants over much shorter periods of time. For example, Keller et al (1979b) followed over 400 families and observed mothers (and children) in homes that used gas for cooking had lower incidence of respiratory illness compared to those with electric cookers. No association of asthma severity with use of a gas stove was found in a panel study of 349 adults with asthma followed over a period of 18 months (Eisner, 2002).

6.1.2 ECRHS methods

The methodology of ECRHS has been described in Chapter 3. Briefly, in 1992/3, participating centres in ECRHS I recruited random population based samples of adults aged 20-44 years to answer a brief postal questionnaire on asthma and asthma-like symptoms. A random sub-sample of responders were invited for further assessments including an interviewer-administered questionnaire, lung function tests, venesection for blood sample (IgE) and skin prick tests. Serum specific IgE to house dust mite, Timothy grass, cat and *Cladosporium* was tested using the Pharmacia CAP system in a single laboratory in Uppsala, Sweden. In 2002 all those taking part in this clinical assessment were invited to undergo exactly the same investigations (ECRHS II) and this was repeated between 2011 and 2013 (ECRHS III).

In 1992/3 the cohort was enriched with a further sample with symptoms highly suggestive of asthma (but who had not been recruited into the random sample). This sample is referred to as the 'symptomatic' sample. The analyses presented here have been conducted on information collected from the random sample only.

6.1.3 Hypothesis

Adults who cook with gas are at higher risk of having asthma-like symptoms than those who cook with electricity over 20-year period and the effect is modified by sex, smoking, atopy and asthma.

6.1.4 Objectives

- To determine whether prevalence of wheeze is more common in adults who cooked with gas compared to adults who cooked with electricity in a cohort followed up for 20 years.
- To use the predictive model for indoor NO_2 developed in Chapter 5 to determine whether prevalence of wheeze is associated with an increase in exposure to indoor NO_2 (modelled).
- To determine whether the observed association is modified by:

  - Sex
  - Smoking
  - Atopy
  - Asthma.
- To determine whether in the same cohort a higher asthma score is associated with the use of gas cooking and/or an increase in exposure to indoor NO_2 (modelled).

6.2 Method

6.2.1 Study population

Study centres

The main analysis of this chapter was restricted to centres in which information on type of fuel used for cooking was collected at all three surveys (ECRHS I, ECRHS II and ECRHS III) and where prevalence of use of gas for cooking was greater than 5% and less than 95% at baseline (ECRHS). The following 17 centres from 7 countries were included in the analyses:

1. Antwerp South (Belgium)
2. Antwerp City (Belgium)
3. Erfurt (Germany)
4. Hamburg (Germany)
5. Bordeaux (France)
6. Grenoble (France)
7. Montpellier (France)
8. Paris (France)
9. Barcelona (Spain)
10. Galdakao (Spain)
11. Albacete (Spain)
12. Oviedo (Spain)
13. Huelva (Spain)
14. Ipswich (UK)
15. Norwich (UK)
16. Tartu (Estonia)

17. Melbourne (Australia).

The following centres were excluded:

- Pavia, Turin and Verona (Italy) - They did not collect information on type of fuel used for cooking at ECRHS III and the prevalence of use of gas cooking was more than 95% at ECRHS I.
- Reykjavik (Iceland), Bergen (Norway), Goteborg, Umea and Uppsala (Sweden) - Prevalence of use of gas cooking was less than 5% at ECRHS I.
- Dublin (Ireland), Groningen, Bergen-op-zoom and Geleen (The Netherlands), Cambridge and Caerphilly (UK), Winnipeg, Vancouver, Hamilton, Montreal and Halifax (Canada), Prince Edward Island Wellington Christchurch and Hawkes-Bay (New Zealand), Mumbai (India) and Wroclaw (Poland) - They all participated at ECRHS I only.
- Aarhus (Denmark) - It did not participate at ECRHS II.
- Portland (Australia) - It did not participate at ECRHS III.
- Basel (Switzerland) - Data for ECRHS III were missing because of ongoing work to complete data harmonisation (Basel used the SAPALDIA III questionnaire).

Participants

In the 17 centres included in the analysis a number of participants did not participate in all three surveys (Figure 6.1). To be included in the main analysis a participant must have had complete information on 12-month prevalence of wheeze and mode of cooking (gas/electric) at ECRHS I but not necessarily at ECRHS II or ECRHS III ('all cases'). All participants must have cooked with gas or electricity.

Over the 20-year period some participants were lost to follow-up, and some, even though they took part, provided incomplete or inconsistent questionnaire information. 'Complete' cases were those participants who had complete information on 12-month prevalence of wheeze and mode of cooking at ECRHS I, II and III. 'Incomplete' cases were those participants with information on 12-month prevalence of wheeze and mode of cooking at ECRHS I but not at ECRHS II or/and ECRHS III.

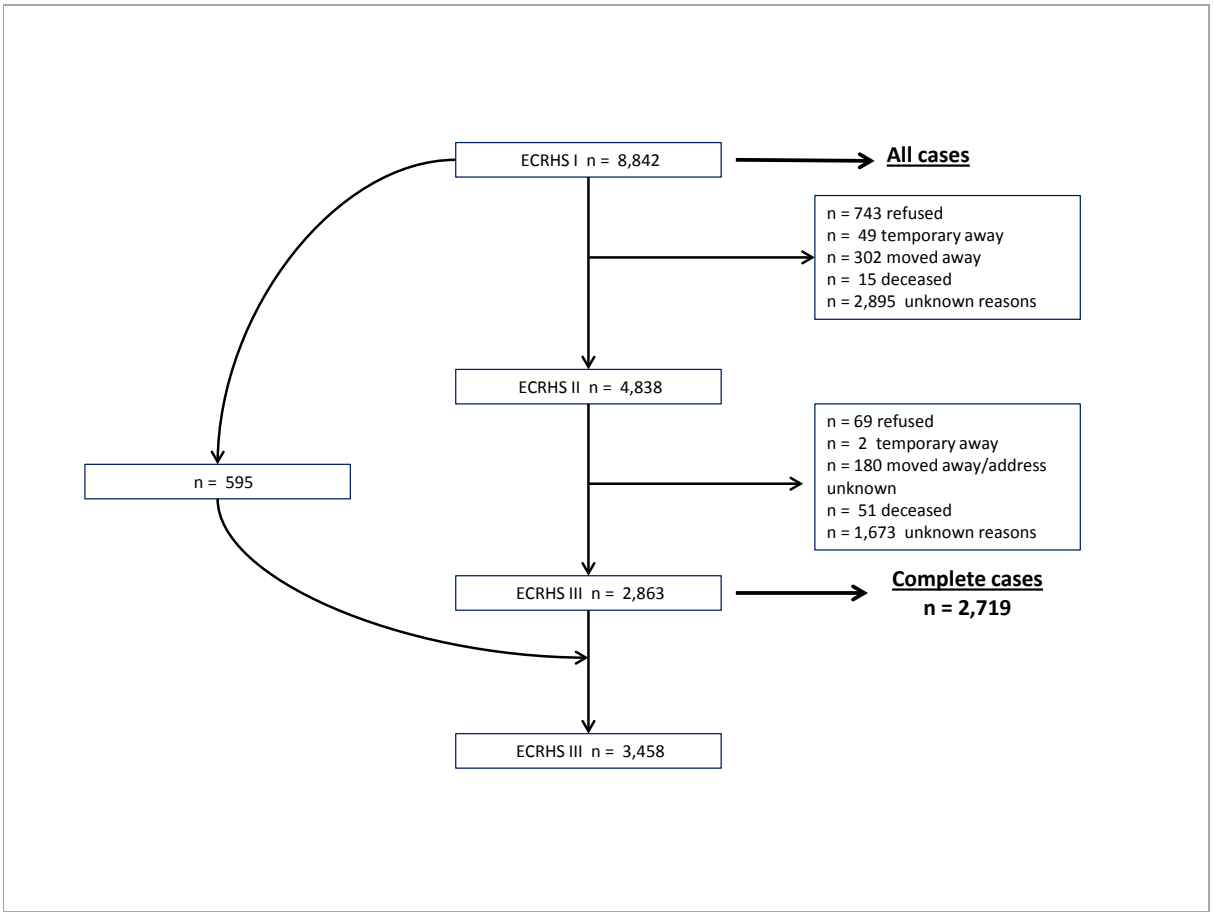


Figure 6.1 Flow diagram of participants included in the analysis of the association of respiratory symptoms and gas use at each survey (n=595 are those participants who took part in ECRHS I and ECRHS III but not ECRS II)

6.2.2 Use of gas for cooking and modelled indoor nitrogen dioxide

At each survey participants were asked: *'What kind of stove do you mostly use for cooking?*'. At ECRHS I the options for response included '*Gas'* , '*Electricity*', '*Paraffin',* '*Coal*, *coke or wood*' or '*Other'*. At ECRHS II participants were asked to state the source of the gas used for cooking i.e. '*Gas from mains*' or '*Gas from bottles'*. At ECRHS III options included '*Gas from mains'* and '*Gas from bottles or other non-main sources'*. For this analysis reported gas (irrespective of source) was the exposure of interest, and electricity was the reference group. Those who reported they used other fuels for cooking were excluded.

In Chapter 5 I developed a model to predict indoor NO_2 levels in homes of participants in ECRHS II and ECRHS III. Briefly, a two-week average indoor NO_2 for the month of October was modelled using ECRHS II and ECRHS III questionnaires based information, monthly average temperature and available annual average outdoor NO_2 level supplied by the ESCAPE project for the year

2002 and 2008. All predicted values were calculated for October, a month, which in all centres temperature tends to be mild lying in between the warm and cold seasons.

6.2.3 Wheeze and asthma score

At each survey participants were asked a series of questions relating to their respiratory health. For this analysis the primary outcome was the reporting of wheeze in the last 12 months.

The secondary outcome was a six-level asthma score (Sunyer, 2007), which has already been presented in Chapter 3. Briefly, the score is derived as a sum of affirmative questions asking about the presence of any of the following asthma-like symptoms over the previous 12 months: (1) wheeze; (2) woken up by a feeling of tightness in the chest; (3) attack of shortness of breath at rest; (4) attack of shortness of breath following strenuous activity; (5) woken by an attack of shortness of breath.

6.2.4 Statistical method

Non-response bias

Characteristics of participants with full data at all three surveys ('complete' cases) and those with incomplete data ('incomplete' cases) were compared. The following characteristics were considered: age (categorical), sex, smoking status at ECRHS I, presence of wheeze at ECRHS I, age left full time education, main fuel used for cooking at baseline and centre Differences in these characteristics at baseline (i.e. ECRHS I) between 'complete' cases and 'incomplete' cases (see 6.2.1 for definition) were compared. A multi-variate logistic regression adjusted for all these characteristics was carried out to find the odds ratio of being a complete case rather than an incomplete case.

Association of wheeze with the use of gas for cooking across the three surveys

A population-averaged generalised estimating equation (GEE) approach (Zeger and Liang, 1986) with exchangeable correlation matrix was used to estimate the odds ratio of having wheeze when cooking with gas compared with electricity over the three ECRHS surveys. This provides a marginal model for the probability of having symptoms and applies a working exchangeable correlation between observations (i.e. within an individual any two observations are equally correlated). Robust standard errors were used to correct for model misspecification.

To account for the variation in effects between centres analyses were conducted within centres and the effect estimates combined using random effect meta-analysis (DerSimonian and Laird, 1986). Heterogeneity between centres was assessed using the I^2 statistic (Higgins, 2003), which describes the percentage of total variation across studies that is due to heterogeneity rather than chance. Cross-sectional analyses of the association of wheeze and gas cooking at each survey were conducted to assist interpretation of the GEE estimate.

To account for the missingness of data a sensitivity analysis was carried out by using the Inverse Probability Weight (IPW) (Hernán and Robins, 2006). Briefly, an inverse weight for factors associated with response (cooking with gas, wheezing, age, age left education, sex, centre) was assigned to the 'complete' cases. This weight relates to the probability of having a datum being observed if a participant has one or many of the factors associated with response.

Association of wheeze with modelled indoor NO_2 across two surveys

Analyses for the associations of wheeze and asthma score with modelled indoor NO_2 across ECRHS II and ECRHS III (modelled data were available only for these two surveys) were repeated using the same approach as above. To assist interpretation of the GEE estimate the association of wheeze with modelled indoor NO_2 at each surveys was further examined by conducting cross-sectional analyses at each surveys. All results are reported per 10μg/m^3 increase in exposure to modelled indoor NO_2.

Association of asthma score with the use of gas for cooking across the three surveys

Analyses that examined the associations of asthma score with use of gas cooking and indoor NO_2 (modelled for the month of October) were carried out using the same methodological approach as above. A GEE approach with robust standard error that took into account the correlation of observations within same individual was used to estimate: (1) the association with asthma score and gas cooking over the three surveys; (2) the association with asthma score and modelled indoor NO_2 over ECRHS II and III. Heterogeneity between centres was assessed using the I^2 statistic. Random effect meta-analyses were carried out to take into account the variations of effect between centres.

Since in the general population asthma score shows a distribution with a majority of zeroes, a negative binominal regression model (with *'loglink'*) that allows for extra-Poisson variation was applied within the GEE. Results are expressed as the geometric mean ratio (GMR) of the asthma scores. This equates to the percentage increase of the geometric mean score in people who use gas for cooking compared to those who use electricity.

To further investigate the association between asthma score and gas cooking the association of other asthma-related symptoms and gas cooking was examined using the same approach (GEE and random effect). For certain outcomes the sample size in some centres was too small to conduct a meta-analysis with random effect by centre and analyses were only adjusted for centre.

Adjustment for confounders

All analyses were adjusted for the following confounders selected *a priori*:

- Sex
- Age at time of survey, included in the analysis as a time- dependent variable
- Smoking status at time of survey, included in the analysis as a time-dependent variable and defined as:
  - Never; if a negative response was given to the question: '*Have you ever smoked for as long as a year?* '
  - Ex: if a positive response was given to the question '*Have you ever smoked for as long as a year?*' and negative response to *'Do you now smoke (as of one month ago)?'*
  - Current: if positive responses were given to both questions '*Have you ever smoked for as long as a year?*' and *'Do you now smoke (as of one month ago)?'*
- Age left full-time education, which was self-reported at ECRHS I (as an indicator of social-economic status). If in full time education at ECRHS I, the age was based on the answer given to the same question at ECRHS II. The variable was classified into four groups based on quartiles of the distribution (<17 years, 17-18, 19-22 and ≥ 23).

As all participants with modelled indoor NO_2 had their exposure predicted for the same month of the year (October) analyses of the health effects of indoor NO_2 were not adjusted for seasonality.

Effect modifications

Effect modifications were included in the GEE analyses on wheeze and gas cooking and on wheeze and modelled indoor NO_2 and tested for significance. The following effect modifiers were considered:

- *Sex*
- *Smoking status* (never, ex, current) at ECRHS I (for the analysis on gas) and at ECRHS II (for the analysis on indoor NO_2)

- *Atopy*, defined to be present if an individual has serum specific IgE > than 0.35kU/L to at least one allergen to house dust mite, timothy grass, cat or *Cladosporium* at ECRHS I

Effect modification by asthmatic status was tested on the association of asthma score with gas cooking and modelled indoor NO_2. An asthmatic individual was identified as previously defined in Chapter 3:

a) Participant must have a diagnosis of asthma confirmed by a doctor and
b) At least one of the following in the last 12 months:
   - Any symptom associated with asthma (wheeze, nocturnal chest tightness, attack of breathlessness after exercise, attack of breathlessness at rest, attack of breathlessness at night or woken up by cough)
   - An asthma attack
   - Used inhaled/oral medicines because of breathing problem.

The Wald test was used to assess the statistical significance of the interactions. A P value threshold of 0.10 (equal or smaller) was used to provide suggestive evidence of an interaction.

As the asthma score in the asthmatic group was an ordered score without zero inflation, its association with gas cooking was examined using an ordered logistic model and the effect estimate is expressed as an odds ratio. Ordinal data models cannot be fitted by GEE in Stata (Stata 12.1) and a model with a robust variance estimator that considers the cluster effect of repeated observation within the same participant was instead applied. (http://www.stata.com/statalist/archive/2009-04/msg00369.html last visited on 14/01/2015).

Ad hoc sensitivity analyses

Ad hoc sensitivity analyses were carried out to explore the difference in results between:

- The association of wheeze with modelled indoor NO_2 vs the association of wheeze with gas cooking
- The association of wheeze with modelled indoor NO_2 vs the association of wheeze with measured indoor NO_2.

All analyses were carried out in Stata 12.1.

6.3 Results

6.3.1 Participants response

From the original random sample of 9,160 participants living in the selected 17 ECRHS centres and for whom there was information on wheeze at ECRHS I the following participants were excluded:

- 8 who did not provide information on type of fuels used for cooking
- 168 who cooked with a fuel other than gas or electricity at ECRHS I
- 103 who reported cooking with a fuel other than gas or electricity at ECRHS II
- 39 who reported cooking with a fuel other than gas or electricity at ECRHS III.

This left a total of 8,842 individuals who met the following criteria:

a. At ECRHS I answered the question on '*Have you had wheeze at any time in the last 12 months?*'
b. At ECRHS I provided information showing they cooked with gas or electricity
c. If they had taken part in further follow-ups, reported that they cooked with either electricity or gas.

Of these participants 4,838 (54.7%) took part in ECRHS II and 3,458 (39.1%) in ECRHS III (Figure 6.1); 2,719 participants had information at all three surveys (i.e. were 'complete cases'); 595 took part in ECRHS I and III but not in ECRHS II.

At ECRHS II 15 people who took part in ECRHS I were known to have died, some had moved out of the area (n=302), some refused (n=743) and 2,895 did not participate at ECRHS II for reasons that remain unknown. At ECRHS III 51 further people who took part in ECRHS II were known to have died, 69 refused, some moved away to unknown address (n= 180) and 1,673 did not participate at ECRHS III for unknown reasons.

The characteristics of 'complete cases' were compared with the characteristics of those who had incomplete data (Table 6.1). At ECRHS I (baseline), complete cases were more likely to be older, less likely to be current smokers and more likely to have left full time education at an older age when compared to incomplete cases.

Table 6.1 Characteristics at ECRHS I of complete and incomplete cases

Risk factors at ECRHS I	Incomplete cases n = 6,123	Complete cases n = 2,719	P value*	Adjusted** OR for being a complete case
	%	%		
Female	52.5	52.0	0.62	0.97 (0.88; 1.07)
Age at ECRHS I			<0.001	
median(IQR) years	32.9 (27.0-39.4)	35.2 (28.9 -40.8)	<0.001^	
<30 years	38.0	29.9		1.00
30<40 years	38.6	41.1		1.44 (1.29;1.62)
>=40years	23.4	29.0		1.69 (1.48; 1.93)
Smoking status at ECRHS I			<0.001	
Never	38.7	43.4		1.00
Ex	20.7	22.1		0.90 (0.79; 1.03)
Current	40.6	34.6		0.74 (0.67; 0.83)
Wheezing at ECRHS I	22.3	20.2	0.023	1.01 (0.89; 1.14)
Cooking mostly with gas at ECRHS I	59.4	66.0	<0.001	0.92 (0.82; 1.03)
Age left education			<0.001	
<17 years	21.6	20.7		1.00
17<19 years	24.9	20.1		1.15 (0.98; 1.34)
19<23 years	32.2	33.8		1.60 (1.38; 1.86)
≥ 23 years	21.3	25.4		1.75 (1.49; 2.06)
Nasal allergy (hay fever)	26.8	26.7	0.87	1.03 (0.92; 1.15)

*P value for Chi-square; ** odds ratios adjusted for the risk factors listed in the table and for centre; ^P value for Kruskal-Wallis rank test

6.3.2 Prevalence of use of gas cooking and wheeze across ECRHS surveys

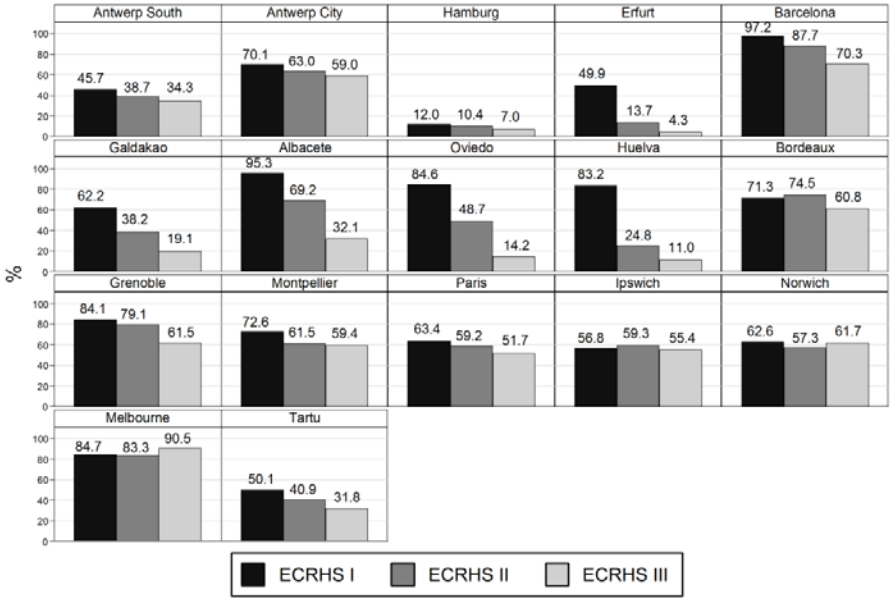


Figure 6.2 Prevalence of use of gas cooking by centres and survey

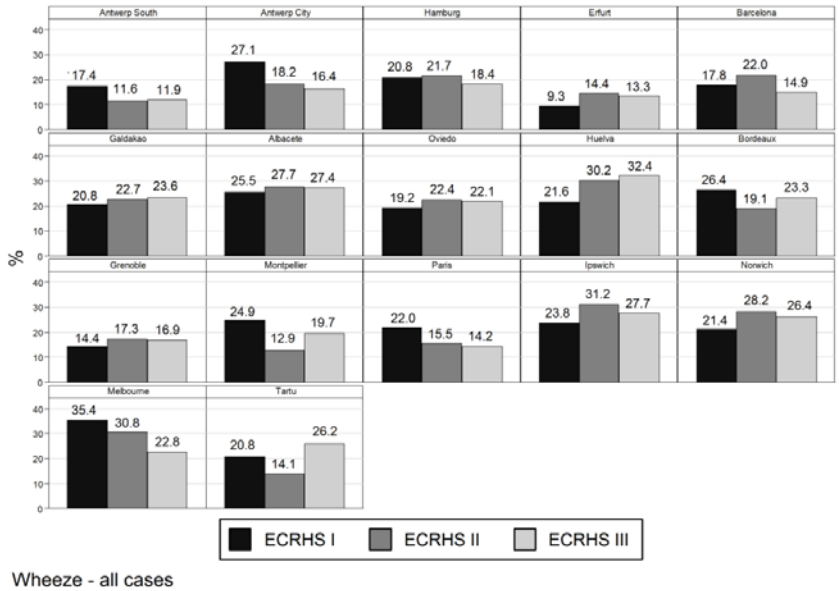


Figure 6.3 Prevalence of wheeze in the previous 12 months by centres and survey

Over the three surveys the use of gas for cooking fell in most ECRHS centres (Figure 6.2). The largest fall occurred in Huelva and Oviedo, Spain and in Erfurt, former East Germany. In Huelva the percentage of participants cooking with gas fell from 83.2 % at ECRHS I to 24.8% at ECRHS II and 11.0% at ECRHS III; in Oviedo it fell from 84.6% to 48.7 at ECRHS II and to 14.2% at ECRHS III; in Erfurt from 49.9% to 13.7% at ECRHS II and to 4.3% at ECRHS III. These figures changed little if only complete cases were considered and if participants who used fuels other than electricity and gas were included in the denominator (data not shown). Overall the prevalence (unadjusted) of wheeze tended to decrease (Figure 6.3). On average across all centres prevalence of wheeze at ECRHS I was 21.7% and at ECRHS III was 19.8%. The changes in prevalence of wheeze varied across centres and ranged from 9.3% in Erfurt to 35.4% in Melbourne. At ECRHS III prevalence of wheeze ranged from 11.9% (Antwerp South) to 32.4% (Huelva).

6.3.3 Associations of wheeze with gas cooking across ECRHS surveys

The forest plot of Figure 6.4 shows the GEE estimated effect of cooking with gas on 12-month prevalence of wheeze by centre and the combined random effect estimate (D+L Overall). Over 20 years across the three ECRHS surveys there was no evidence of an association between wheeze and gas cooking (OR 1.05, 95% CI 0.96 to 1.15) and no evidence of heterogeneity (I^2=0.0%, $P_{heterogeneity}$=0.75).

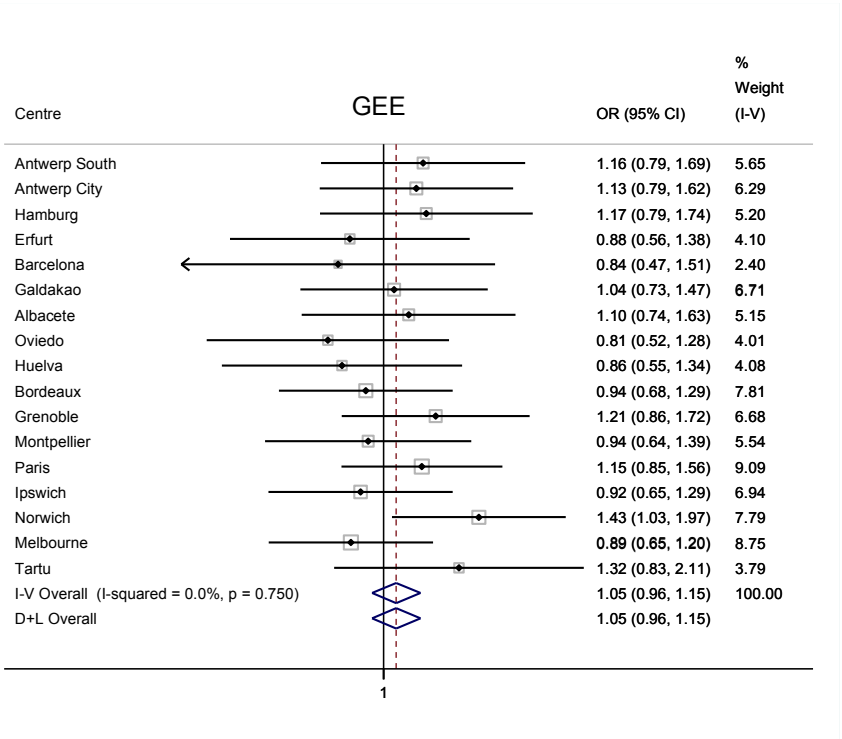


Figure 6.4 Forest plot showing GEE odds ratio of having wheeze when cooking with gas (versus electricity) by centre overall the three surveys and adjusted for sex, age, smoking status and age left education

Results of the cross-sectional analyses for the prevalence of wheeze and gas cooking at each survey (Table 6.2) show a positive significant association at ECRHS II (OR 1.22 95% CI 1.00, 1.47 I^2=16.1%, $P_{heterogeneity}$=0.26). There was some heterogeneity between centres at ECRHS I and II. See Appendix of Chapter 6 for cross-sectional results by centre at each survey.

Table 6.2 Adjusted odds ratio of having wheeze when cooking with gas (versus electricity) with random effect by centre at each surveys and overall (GEE)

	OR* (95% CI)	I^2 (P value)**
ECRHS I (n=8,842)	1.09 (0.94, 1.26)	16.1% (0.26)
ECRHS II (n=4,840)	1.22 (1.00, 1.47)	15.9% (0.27)
ECRHS III (n=3,458)	1.12 (0.89, 1.41)	0.0% (0.78)
ECRHS I, II and III (GEE)	1.05 (0.96, 1.15)	0.0% (0.75)

*Adjusted for sex, age at survey, smoking status at survey, age left education with random effect by centre; ** I^2 and P value for heterogeneity test

Sensitivity analyses with Inverse Probability Weighting

An Inverse Probability Weighting (IPW) was assigned to complete cases and analyses for wheeze were repeated as above (i.e. GEE with random effect by centre). After adjusting for IPW there was little change in the effect estimate of the association between wheeze and gas cooking (OR 1.02, 95% CI 0.93 to 1.12) (Table 6.3).

Table 6.3 IPW adjusted odds ratio of having wheeze when cooking with gas (versus electricity) with random effect by centre overall the three surveys (GEE)

Wheeze	OR* (95% CI)	I^2 (P value) **
Adjusted for IPW	1.02 (0.93, 1.12)	0.0% (0.79)
Not adjusted for IPW	1.05 (0.96, 1.15)	0.0% (0.75)

*GEE odds ratios adjusted for sex, age at survey, smoking status at survey, age left education and IPW and with random effect by centre; ** I^2 and P value for heterogeneity between centres

Associations in complete cases versus all cases

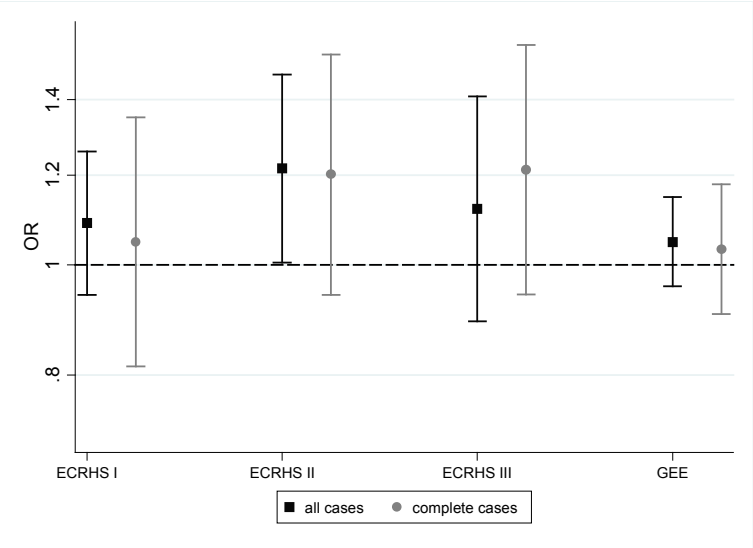


Figure 6.5 GEE odds ratios in all cases and complete cases of having wheeze when cooking with gas (versus electricity) at each survey and on average at each surveys Modelled indoor NO_2 at ECRHS II and ECRHS III

The association of wheeze with gas cooking at each survey in 'complete' cases and 'all' cases were also visually compared (Figure 6.5). Estimates arising from 'complete' cases are similar to the estimates arising from 'all' cases but tend to have larger confidence intervals reflecting the smaller sample size.

6.3.4 Associations of asthma score with gas cooking across ECRHS surveys

GEE analysis with random effect by centre (Figure 6.6) did show no evidence of an association between asthma score and gas cooking across the three surveys (OR 1.02, 95%CI 0.96 to 1.09). There was some evidence of heterogeneity between centres (I^2=33.2%, $P_{heterogeneity}$=0.090); asthma score was positively associated with use of gas cooking in Norwich (OR 1.29, 95%CI 1.05 to 1.60) and negatively associated in Oviedo (OR 0.73, 95%CI 0.56 to 0.95).

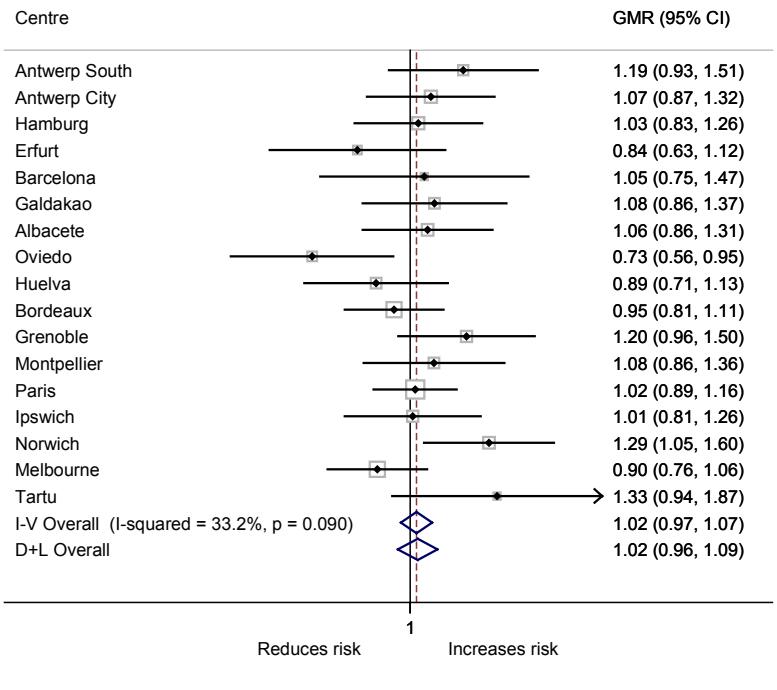


Figure 6.6 Forest plot showing GEE geometric mean ratio of having a higher asthma score when cooking with gas (versus electricity) by centre overall the three surveys and adjusted for sex, age, smoking status and age left education

Cross-sectional analyses for the association of asthma score with gas cooking at each surveys (Table 6.4) showed a significant association at ECRHS II. There was some heterogeneity across centres, particularly at ECRHS III (I^2 40.3%, $P_{heterogeneity}$=0.044). See Appendix of Chapter 6 for cross-sectional results by centre at each survey.

Table 6.4 Adjusted geometric mean ratio of having a higher asthma score when cooking with gas (versus electricity) with random effect by centre at each surveys and overall (GEE)

	GMR* (95% CI)	I^2 (P value)**
ECRHS I (n=8,842)	1.07 (0.96, 1.20)	7.1% (0.37)
ECRHS II (n=4,840)	1.13 (1.00, 1.27)	29.9% (0.12)
ECRHS III (n=3,458)	1.13 (0.96, 1.32)	40.3% (0.044)
ECRHS I, II and III (GEE)	1.02 (0.96, 1.09)	33.2% (0.090)

*Geometric mean ratio adjusted for sex, age at survey, smoking status at survey, age left education with random effect by centre; ** I^2 and P value for heterogeneity between centres.

Associations of other asthma-related symptoms with gas cooking

There was no evidence of an association of any asthma-related symptoms with gas cooking across the three surveys (Table 6.5). There was some evidence of heterogeneity across centres for the symptom 'Woken up with chest tightness'. Having an asthma attack or taking asthma medication was negatively associated with the use of gas cooking but the association was not significant and there was not enough data to carry out analysis by centre (nobody who cooked with electricity had an asthma attack or took asthma medication in Barcelona).

Table 6.5 Adjusted odds ratio of having asthma-related symptoms when cooking with gas (versus electricity) with random effect by centre overall the three surveys (GEE)

Asthma-related symptoms^	OR* (95% CI)	I^2 (P value) **
Wheeze	1.05 (0.96, 1.15)	0.0% (0.75)
Wheeze with SOB	1.08 (0.97-1.21)	n/a~
Wheeze with no cough	1.07 (0.95-1.21)	5.0% (0.40)
Woken up with chest tightness	1.05 (0.91-1.20)	24.6% (0.077)
Woken up by cough	1.03 (0.94-1.13)	15.5% (0.27)
Woken up by SOB	1.00 (0.86-1.56)	0.0% (0.95)
Asthma attack	0.98 (0.80-1.20)	n/a~
Taking asthma medication	0.87 (0.72-1.05)	n/a~

^In the past 12 months; *GEE odds ratio adjusted for sex, age at survey, smoking status at survey and age left education with random effect by centre (unless stated); ~adjusted by centre.

6.3.5 Association of wheeze with modelled indoor NO_2 across ECRHS surveys

Using the model developed in Chapter 5 indoor NO_2 exposure at ECRHS II for the month of October was predicted for 2,849 individuals living in 12 centres in Belgium (Antwerp South), Spain (Barcelona, Galdakao, Albacete, Oviedo and Huelva), Italy (Pavia, Turin and Verona), UK

(Ipswich and Norwich) and Sweden (Umea); modelled exposure at ECRHS III was predicted for 1,313 individuals (9 centres). Italian centres did not collect data on gas cooking at ECRHS III and exposure at ECRHS III could not be predicted (Table 6.6).

Table 6.6 Two-week average modelled indoor NO_2 concentrations and temperature for the month of October by centre at ECRHS II and ECRHS III

		ECRHS II (n=2,849)	**ECRHS III (n=1,313)**
Country	**Centre**	median (IQR) µg/m^3	median (IQR) µg/m^3
Belgium	Antwerp South	22.2 (19.2-39.0)	21.6 (18.9-37.0)
Spain	Barcelona	62.5 (57.8-69.2)	62.2 (53.2-67.6)
	Galdakao	34.9 (31.8-58.4)	37.9 (35.4-40.3)
	Albacete	60.6 (42.5-69.3)	39.3 (37.2-47.8)
	Oviedo	43.0 (35.8-60.2)	41.6 (38.7-44.2)
	Huelva	38.8 (36.9-42.5)	36.3 (34.9-38.1)
Italy	Pavia	45.2 (43.8-53.3)	n/a
	Turin	60.9 (55.4-66.0)	n/a
	Verona	52.1 (48.5-57.0)	n/a
UK	Ipswich	34.7 (18.5-44.1)	28.8 (14.6-40.3)
	Norwich	31.9 (14.9-43.5)	32.6 (16.7-43.3)
Sweden	Umea	3.1 (2.0-4.9)	3.2 (2.1-4.4)

GEE analysis with random effect by centre was conducted to examine the association of wheeze with modelled indoor NO_2 across ECRHS II and ECRHS III (Figure 6.7). There was evidence of an association of wheeze with modelled indoor NO_2 (OR 1.10, 95%CI 1.03 to 1.18 per 10 µg/m^3 increase of modelled indoor NO_2) and no evidence of heterogeneity between centres (I^2 0.0% $P_{heterogeneity}$= 0.70)

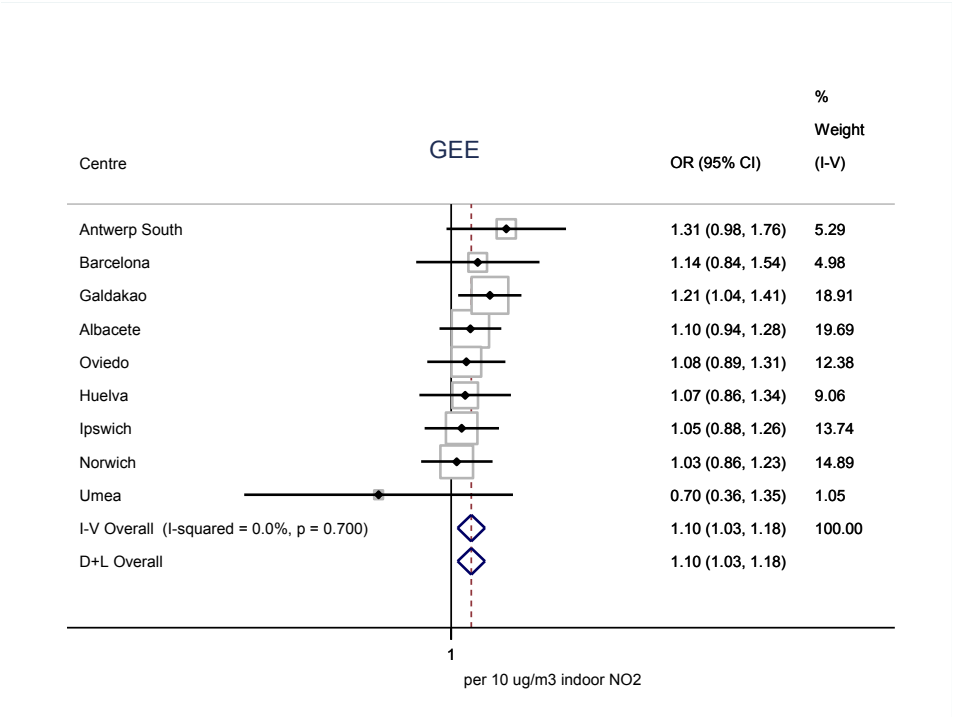


Figure 6.7 Forest plot showing GEE odds ratio of having wheeze per 10µg/m^3 increase in modelled indoor NO_2 by centre overall ECRHS II and III and adjusted for sex, age, smoking status and age left education (Italian centres excluded)

Cross-sectional analyses at each survey (Table 6.7) showed that the association of wheeze with modelled indoor NO_2 was significant at both surveys. There was no heterogeneity between centres. See Appendix of Chapter 6 for cross-sectional results by centre at each survey.

Table 6.7 Adjusted odds ratios of having wheeze per 10µg/m^3 increase of modelled indoor NO_2 with random effect by centre at each surveys and overall (GEE)

	OR* (95% CI)	I^2 (P value)**
ECRHS II (n=2,849))	1.17 (1.08, 1.27)	0.0% (0.81)
ECRHS III (n=1,313)	1.18 (1.01, 1.38)	1.3% (0.42)
ECRHS II and III (GEE)^	1.10 (1.03, 1.18)	0.0% (0.70)

*adjusted for sex, age at survey, smoking status at survey, age left education with random effect by centre; ** I^2 and P value for heterogeneity between centres; ^ n=2,376 because Italian centres were not included in the GEE analysis.

6.3.6 Association of asthma score with modelled indoor NO_2 across ECRHS surveys

GEE results for association of asthma score with modelled indoor NO_2 by centre are shown in Figure 6.8. The combined GEE result suggests that there was an association between asthma score and modelled indoor NO_2 (GMR 1.06, 95%CI 1.01, 1.11 per 10µg/m^3 of modelled indoor NO_2). Cross-sectional analyses at each survey (Table 6.8) showed that the association of asthma score with modelled indoor NO_2 was significant at ECRHS II. There was some heterogeneity between centres, particularly at ECRHS II (I^2 54.8% $P_{heterogeneity}$=0.011). See Appendix of Chapter 6 for cross-sectional results by centre at each survey.

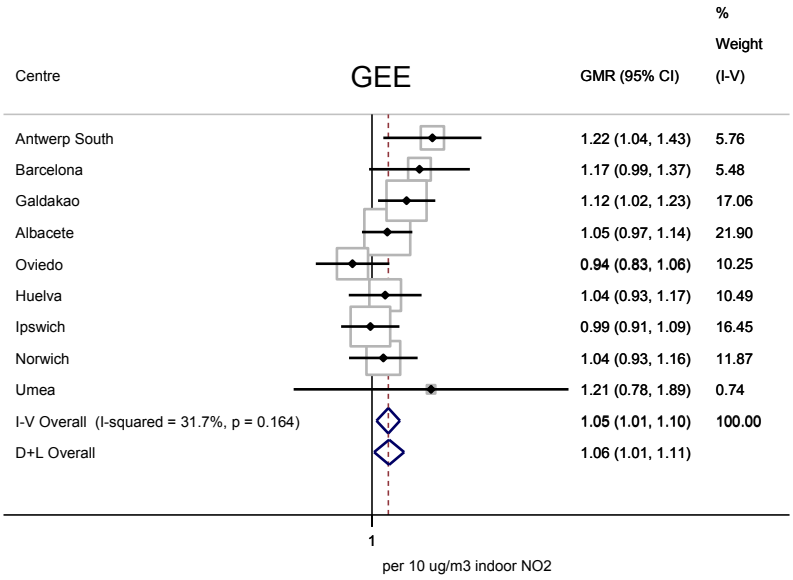


Figure 6.8 Forest plot showing GEE geometric mean ratio of having a higher asthma score per 10µg/m^3 increase in modelled indoor NO_2 by centre overall ECRHS II and III and adjusted for sex, age, smoking status and age left education (Italian centres excluded)

Table 6.8 Adjusted geometric mean ratio of having a higher asthma score per 10μg/m^3 increase of modelled indoor NO_2 with random effect by centre at each surveys and overall (GEE)

	GMR* (95% CI)	I^2 (P value)**
ECRHS II	1.09 (1.01, 1.17)	54.8% (0.011)
ECRHS III	1.07 (0.97, 1.18)	29.6% (0.18)
ECRHS II and III (GEE)	1.06 (1.01, 1.11)	31.7% (0.16)

*Geometric mean ratio adjusted for sex, age at survey, smoking status at survey, age left education with random effect by centre per 10μg/m^3 increase in modelled indoor NO_2; ** I^2 and P value for heterogeneity between centres

6.3.7 *Ad hoc* sensitivity analyses

Association of wheeze with modelled indoor NO_2 vs association of wheeze with gas cooking

The analysis of the association of wheeze with gas cooking showed no evidence of an effect. Contrarily, the analysis of the association of wheeze with modelled indoor NO_2 suggested some evidence. In order to understand this inconsistency an a*d hoc* sensitivity analysis was carried out. The analysis was limited to those participants for whom indoor NO_2 could be modelled. As previously the analysis used a GEE approach and took into account the random effect of centre.

Within this sub-set of participants with modelled indoor NO_2 gas cooking (versus electric cooking) was also significantly associated with wheeze (OR 1.20, 95% CI 1.00 to 1.46).

Table 6.9 Sensitivity analysis of the effect of gas cooking on wheeze within the sub-set of participants for which indoor NO_2 was modelled

GEE analysis across ECRHS II and III for the long-term association of wheeze with	OR* (95% CI)	I^2 (P value)**
Use of gas for cooking (n=2,361)^	1.20 (1.00, 1.46)	0.0% (0.84)
Modelled indoor NO_2 (per 10 μg/m^3)	1.10 (1.03, 1.18)	0.0% (0.71)

*GEE (ECRHS II and III) estimate adjusted for sex, age at survey, smoking status at survey, age left education with random effect by centre; ** I^2 and P value for heterogeneity between centres; ^ Italian centres excluded.

Association of wheeze with <u>modelled</u> indoor NO_2 vs association of wheeze with <u>measured</u> indoor NO_2

Results for the association of wheeze with modelled indoor NO_2 were compared with those for wheeze and measured indoor NO_2 in sub-set of participants whose exposure to indoor NO_2 had both being measured and modelled.

Indoor NO_2 monitoring was only carried out at ECRHS II and at different time of the year. To be able to make a proper comparison sensitivity analyses were based on:

- Cross-sectional analysis at ECRHS II
- Analysis were adjusted for seasonality (i.e. month of the year when measurements were carried out) as well as the usual confounder (age, sex, smoking, age left education)
- Indoor NO_2 was also modelled for the same month indoor NO_2 measurement was carried out (rather than October).

Results presented in Table 6.10 show that the health effect estimated using the modelled exposure tend to be higher than the health effect estimated using the measured exposure and that the size of the health is affected by adjustment for season.

Table 6.10 Sensitivity analysis for the association of wheeze with indoor NO_2 using a modelled exposure (all estimate for NO_2 are per 10μg/m^3) at ECRHS II (n=1271)

Cross-sectional analysis at ECRHS II for the association of wheeze with:	OR* (95% CI)	I^2 (P value)**
<u>Measured</u> indoor NO_2 (+ adjusted for month of monitoring)	1.14 (1.05, 1.24)	0.0%(0.63)
<u>Modelled</u> indoor NO_2 for the month of measurement (+ adjusted for month of monitoring)	1.21 (1.02, 1.43)	12.5% (0.32)
<u>Modelled</u> indoor NO_2 for the month of monitoring (not adjusted for month of monitoring)	1.15 (1.00, 1.33)	13.2% (0.32)
<u>Modelled</u> indoor NO_2 for the month of October (as main analysis)	1.20 (1.05, 1.37)	0.0% (0.59)

* adjusted for sex, age at survey, smoking status at survey, age left education with random effect by centre; ** I^2 and P value for heterogeneity between centres .

6.3.8 Effect modifications on the association of symptoms with gas cooking and modelled indoor NO_2 by sex, smoking, atopy and asthmatic status

Effect modifications were tested by inclusion of an interaction term in the GEE analyses for wheeze (adjusted for sex, age at survey, smoking status at survey, age left education and centre) (Table 6.11).

There was some evidence of an effect modification by smoking on wheeze and gas cooking as well as on wheeze and indoor NO_2 but the effect was inconsistent. Never smokers had a higher risk of having wheeze when cooking with gas than when cooking with electricity (OR 1.17, 95% CI 0.99 to 1.40) compared to current smokers (OR 0.99, 95% CI 0.90 to 1.08). Contrarily, current smokers were at higher risk of having wheeze (OR 1.16, 95%CI 1.04 to1.29) compared to never smokers (OR 1.10, 95%CI 0.98 to1.24) per same increase in modelled indoor NO_2 (10 $\mu g/m^3$).

There was some evidence of effect modification by atopy on the association of wheeze (P=0.020) and modelled indoor NO_2. Non-atopic individuals had a higher risk of wheeze per 10 $\mu g/m^3$ increase of modelled indoor NO_2 (OR 1.13, 95%CI 1.02 to 1.25) compared to atopic individuals (OR for wheeze 1.03, 95% CI 0.91 to1.17).

People who cooked with gas or were exposed to higher levels of indoor NO_2 were more likely to have a higher asthma score if they had asthma rather than if they had not but results were not significant.

Table 6.11 P value for significance test for effect modification across ECRHS surveys by sex, smoking, atopy and asthma with use of gas cooking and modelled indoor NO_2 on wheeze and asthma score (asthmatic status only)

		P value* for effect modification by			
		Sex	Smoking	Atopy^^	Asthma#
Effect modification of gas cooking across ECRHS I, II and III~	On wheeze	0.28	0.011	0.69	-
	On asthma score	-	-	-	<0.001
Effect modification of modelled indoor NO_2 across ECRHS II and III~~	On wheeze	0.14	0.070	0.020	-
	On asthma score	-	-	-	0.095

*Wald test ; ^non-smokers vs current smokers at ECRHS I; ^^ defined as having any specific IgE for HDM, *Cladosporium,* cat or grass allergens above 0.35kU/L at ECRHS I;~ GEE analysis for ECRHS I, II and III; ~~ GEE analysis for ECRHS II and III.

6.4 Discussion

6.4.1 Main findings

In this chapter I have assessed the association of asthma symptoms with the use of gas cooking, in which both symptoms and exposure have been prospectively ascertained in an adult population sample of 17 ECRHS centres over a 20-year period. There was no evidence that gas cooking compared to electric cooking had a long-term association with asthma-related symptoms (wheeze and asthma score). There was some heterogeneity across centres in the association with asthma score and none in the association with wheeze.

Secondly, I have shown some evidence that asthma-related symptoms were associated with indoor NO_2 (modelled). As for the analyses examining the associations of symptoms with the use of gas, there was little variation in these associations across centres when examining the effect of NO_2 on wheeze, but some variation on asthma score. Due to missing information on key predictors of NO_2 the analysis could only be conducted in 9 of the ECRHS centres for the 10-year period between ECRHS II and ECRHS III.

Previous work in the ECRHS has suggested associations of symptoms with use of gas for cooking may be modified by host factors. However I found no evidence of a modifying effect of sex (as previously observed), and limited evidence that effects were modified by atopy and smoking. Unexpectedly, non-atopic individuals were at higher risk of having asthma-related symptoms than atopic individuals when exposed to the same level of indoor NO_2.

People with asthma tended to have more symptoms (as shown by asthma score) when exposed to gas cooking compared to people without asthma - but the effect was not significant, possibly due to the small sample size. Even though modelling NO_2 exposure should provide opportunities to conduct analyses on larger samples, this was not possible due to lower than expected response rates at ECRHS III.

6.4.2 Limitations

The study has some limitations. More than 50% of individuals who participated at ECRHS I did not respond to the invitation to participate at the following surveys. This group of people tended to be different from those who continued to participate at the following surveys; they were younger, more likely to be current or ex-smokers and less educated. A sensitivity analysis that

included an Inverse Probability Weighting to adjust for this response bias suggested that this did not alter the results. More complex, systematic strategies for dealing with non-response have recently been suggested (Sterne *et al.*) and may be of value to consider or future work.

Results showed no variations across centres when examining the exposures (gas cooking and modelled indoor NO_2) effects on wheeze but some when examining the effects on asthma score suggesting that the effect of gas cooking and modelled indoor NO_2 on some asthma-related symptoms vary across centres.

The main findings of this study suggest that there is an association of wheeze and asthma score with modelled indoor NO_2 but not with the use of gas for cooking. There is some difficulty in interpreting this and it is further complicated by the observation that in the subset of centres (n=7) with modelled indoor NO_2 data, the use of gas for cooking **was** also associated with respiratory symptoms. There are some possible interpretations for this:

1. The associations of symptoms with the use of gas are inconsistent between centres (i.e. effects can only be seen in the centres included in the modelled NO_2 work) as analyses on asthma score suggested (i.e. heterogeneity in the analyses on asthma score). However, there was no evidence of heterogeneity in the analysis on wheeze when all 17 centres were included. This may suggest that had we been able to model NO_2 for a larger number of centres no association of wheeze with NO_2 would have been observed.
2. 'Gas cooking' and 'modelled indoor NO_2 are not the same exposure. Gas cooking is more likely to suffer from misclassification, which biases the effect estimate towards the null value. Modelled indoor NO_2 is more precise as it includes other sources of indoor NO_2 (UFGH, gas oven). This suggests that had we been able to model NO_2 for a larger number of centres we would expect different findings and possibly significant (but perhaps not).

As previously discussed the NO_2 model developed in Chapter 5 has some limitations. It tends to under predict and over predict high and low indoor NO_2 concentrations respectively, which biased the estimated health effects.

Finally, these analyses have used self-reported symptoms, and it is possible that all associations would be different if an objective marker of respiratory health had been used.

6.4.3 Recommendations

There is some evidence that exposure to indoor NO_2 is associated with an increased risk of wheeze in adults. Available data was biased towards some selected centres, in which there was also a significant association of symptoms with gas cooking. It is recommended that future research should:

- Consider a much larger single centre study in non-smokers to control for large differences and unknown levels of confounding by centre levels characteristics and smoking.
- Investigate the association between modelled indoor NO_2 and respiratory health by assessing the associations with some objective measures, i.e. lung functions (FEV_1, FEV_1/FVC), FeNO, airway responsiveness
- Consider a more complex, systematic strategy for dealing with complex non-response as suggested by Sterne *et al* (2009).
- Consider some methodologies for evaluating the exposure model in the context of health assessment estimation.

6.5 Summary

This study shows no evidence of an effect of gas cooking on asthma-related symptoms over a 20-year period. There was some evidence that exposure to indoor NO_2 (modelled) was associated with an increased risk of having wheeze in adults over a 10 year period, but analyses were only conducted in some of the centres (and in these centres gas cooking was also associated with wheeze). There was limited evidence that tobacco smoking, asthmatic status and atopy modify these associations.

7. Final discussion and conclusion

7.1 Compendium

In order to examine whether indoor NO_2 exerts an effect on human health at levels associated with gas cooking I began (**Chapter 2**) by systematically reviewing epidemiological studies that measured indoor NO_2 and assessed its association with respiratory health. The literature review identified 50 studies, most of them in children published up to December 2013. Nearly all studies reported positive associations between respiratory outcomes and indoor NO_2 with over half of the studies reporting at least one significant estimate. Studies were very heterogeneous in design, exposure and outcome assessments and in statistical methodology making it difficult to combine the effect estimates. Eight studies that examined the association of 12-month period prevalence of wheeze and indoor NO_2 could be included in a meta-analysis. The combined estimate suggested that a 10µg/m^3 increase of indoor NO_2 is associated with approximately a 6% increase in wheeze. This confirmed a previous meta-analysis (Hasselblad, 1992) conducted more than 30 years ago and which has formed the basis for setting WHO air quality guidelines on annual average exposure to outdoor and indoor NO_2 (40µg/m^3). Visual assessments of funnel plots and formal tests for publication bias of the meta-analysis suggested some evidence of publication bias. A few studies adjusted for other pollutants such as particles, but did not provide strong evidence that any observed health effect was confounded by exposure to other highly correlated indoor or outdoor pollutants. Studies, mainly in children, suggested that asthma severity may increase with increasing exposure. Very few studies were in adults with asthma. This led me to Chapter 3.

In **Chapter 3** I examined the effects of indoor NO_2 on asthma severity in a sub-group of adults with asthma who participated in a multi-centre adult study (the European Community Respiratory Health Survey - ECRHS) between 1999 and 2001. These participants had indoor NO_2 sampled in their kitchen for two weeks. Asthma severity was determined by several measures, such as asthma score, GINA score and bronchial responsiveness after methacholine challenge. There was no evidence that asthma severity was associated with elevated kitchen NO_2 or use of gas for cooking (electric cooking as a baseline), or that effects were modified by sex, atopy, or use of inhaled steroids. There was limited evidence that asthmatics who smoke were at a greater risk of having more severe asthma when exposed to indoor NO_2 than asthmatics who do not smoke. Overall, there was some evidence that wheezing was associated with indoor NO_2 (OR 1.05,

95%CI 1.01 to 1.09 per 10μg/m^3 increase) in the general population sample who participated in ECHRS II and had indoor NO_2 measured (n=1527). The generalizability of these findings was limited by the relatively small number of participants with asthma and indoor NO_2 measurements (n=257). Furthermore, indoor NO_2 had been measured with passive diffusion samplers, which only provide information on average exposure and do not provide any information on exposure to short-term peaks generated during gas cooking/combustion. Clinical studies suggest that short-term high exposure to NO_2 may increase bronchial responsiveness in people with asthma but very few epidemiological studies have been conducted to examine associations of peak exposures with asthma exacerbations.

One major reason for the lack of information on potential respiratory effects of peak exposures has been the lack of proper instrumentation. At the start of my doctoral training a portable, low-cost, real-time monitor (Aeroqual S500) had just entered the market and offered an opportunity to conduct a panel study that could measure and assess whether indoor NO_2 peaks generated from gas combustion are associated with adverse health effects. To assess the feasibility of conducting a panel study using this instrument for exposure assessment I designed and conducted a pilot study (**Chapter 4**). The study aimed to recruit 20 women (75% who cooked with gas) with asthma and follow them for 8 weeks during which indoor NO_2 weekly average as well as NO_2 peaks were monitored and daily changes in respiratory health (i.e. PEF and respiratory symptoms) assessed. The study could not be completed because of poor recruitment. Several strategies to identify potential participants were adopted: an organisation for people with asthma, GP practices, community-based voluntary organisations and staff notice-board at the academic institution where I am based. Only the latter two, led to recruitment of participants who cooked with gas. Furthermore, studies published while this work was ongoing recommended calibration and validation of the monitor independently from the manufacturer. This recommendation was based on observations that the electro-chemical gas sensor is sensitive to temperature and humidity and tends not to perform well when tested outdoors. Laboratory facilities beyond those available to me within the timeframe of my work are required to conduct such calibration. Recommendations have been made on how future large scale studies should be planned and conducted.

Collecting exposure data at individual level has always been a major limitation in any air pollution epidemiological study because of time and related cost. An increasing number of epidemiological studies of outdoor air pollution have used modelled exposure data. So far no large scale epidemiological study of indoor air pollution has attempted the same. In **Chapter 5** I developed a regression model to predict average indoor NO_2 exposure for a sub-group of ECRHS participants in 5 European countries for which information on indoor NO_2 potential predictors was available.

The model predicted that the main determinants of indoor NO_2 are gas hob and gas oven, UFGH, bottled gas, outdoor NO_2, ambient temperature, age of building and country. The model predicted that the presence of a gas stove (hob and oven included) was equivalent to an additional (from the background level) two-week average NO_2 of about 28μg/m^3 of indoor NO_2 (a concentration very close to the estimation used in the Hasselblad meta-analysis over 30 years ago) and that 2μg/m^3 of NO_2 for every 10μg/m^3 of outdoor NO_2 (annual average) was present indoors. The model explained 46% of variability, similar to the explained variability achieved by other published indoor NO_2 models. There was some substantial difference between countries, which could not be explained by the information available. As in any regression model values tended toward the average, leading to an underestimation of the highest exposures. Within its own limitations, I have shown that it is possible, using data available from ECRHS and ESCAPE (which supplied outdoor NO_2 data) to develop a model which includes important variables enabling prediction of indoor NO_2 concentrations. Two-weekly average exposure to indoor NO_2 for the month of October was modelled for a sub-group of participants at ECRHS II and ECRHS III, providing an opportunity to apply modelled exposure in health assessments within the ECRHS cohort, when participants were followed up ten years later.

The ECRHS study is one of the few adult cohorts that have prospectively collected information on symptoms and exposure to gas for cooking over a twenty-year period (from 1990 to 2013). In **Chapter 6**, as data from the third phase (second follow-up) became available I examined whether long-term exposures to gas cooking and modelled indoor NO_2 increase the risk of having asthma-like symptoms in adults using generalised estimating equation modelling. Analysis included 17 ECRHS centres. There was no evidence of an association of wheeze with cooking gas on average across the three ECRHS surveys and no evidence of heterogeneity to suggest that the effect may vary across centres. Analysis of the sub-set of participants of 7 ECRHS centres with modelled indoor NO_2 data showed some evidence that wheeze and asthma score were associated with indoor NO_2 (modelled). Interpretation of these findings is difficult as in this sub-set of centres the use of gas for cooking was also associated with wheeze. We can argue that as there is no heterogeneity in this association of wheeze across the 17 centres, the observation of a ‘gas-cooking effect’ in the 7 centres may have occurred by chance. However, some heterogeneity across centres was observed in the analyses on asthma score. It is also possible that as ‘gas cooking’ and ‘modelled indoor NO_2’ do not strictly describe the same exposure different health effects could be seen with each.

7.2 Does indoor NO_2 cause asthma-related symptoms at levels associated with gas cooking?

7.2.1 The Bradford Hill criteria

In his essay 'The Environment and Disease: Association or Causation" Bradford Hill (Bradford, 1965) listed nine items to evaluate whether an association is causal or not. Bradford stated that *'none of the 9 viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required a sine qua non'* (pg. 295*).* These 'viewpoints' have become known as the Bradford Hill Criteria and have long provided a background framework for the study of simple, direct causation.

In the context of the evidence for a causal association of asthma-related symptoms and exposure to indoor NO_2 which I have gathered in this thesis, I make the following comments:

Strength

'First upon my list I would put the strength of the association.'

Bradford Hill suggested that strong associations were indicative of a causal relationship. Findings from the meta-analysis of existing epidemiological studies and from cross-sectional and longitudinal analyses carried out in a large multi-centre adult cohort (ECRHS) suggest that the risk of having 12-month prevalence of wheeze is associated with indoor NO_2 exposure and that this risk increases as exposure increases. The main findings are listed below:

<u>In children:</u>

- **OR 1.06 (95%1.02, 1.12)** per 10µg/m^3 indoor NO_2 for 12-month prevalence of wheeze - source: my meta-analysis of existing published evidence (Chapter 2)
- **OR 1.05 (95%1.02, 1.08)** per 10µg/m^3 indoor NO_2 for 12-month prevalence of wheeze - source: meta-analysis published by Lin (2013)
- **OR 1.08 (95%1.01, 1.16)** per 10µg/m^3 indoor NO_2 for 'respiratory symptoms' (not wheeze) – source: original meta-analysis published by Hasselblad (1992)

In adults:

- **OR 1.05 (95%1.01, 1.09)** per 10µg/m^3 indoor NO_2 for 12-month prevalence of wheeze in adults: (n=1,527 in 14 ECRHS of 8 European countries) - source: cross-sectional analysis at ECHRS II (Chapter 3)

In adults (long-term exposure):

- **OR 1.10 (95%1.03, 1.18)** per 10µg/m^3 modelled indoor NO_2 for 12-month prevalence of wheeze in adults over a 10-year period: (n=2,376 in 7 ECRHS of 4 European countries) - source: longitudinal analysis across ECRHS II and ECRHS III using a GEE approach (Chapter 6).

Using the most conservative figures I can estimate that as the annual average exposure to indoor NO_2 increases by 10µg/m^3 the risk of having wheeze in children and adults increases by 5%.

The strength of this risk estimate is far lower than those originally considered as indicative of causal associations by Bradford Hill (a 200-fold increase in mortality from scrotal cancer in chimney sweeps exposed to tar and mineral oil and a 20-fold increase in risk of lung cancer in smokers compared to non-smokers). Strong associations are not common in modern epidemiology which studies diseases with multiple risk factors and different causal pathways. Controlling for all the risk factors associated with the disease and the exposure is difficult. On the other hand, strong associations are less likely to be affected by uncontrolled residual confounding.

Consistency

*'Whether chance is the explanation of whether a true hazard has been revealed may sometimes be answered only by a repetition of the circumstances and observation*s'

Overall, findings from studies of indoor NO_2 were inconsistent.

There was some evidence of publication bias in the meta-analyses I have conducted. This restricts my ability to comment on consistency since findings with positive results were more likely to be published. However, in the systematic review it was noted that significant associations were more frequently reported by studies carried out in children and older people

living in US inner cities and children attending schools in China and Korea. They were less frequently reported by studies in children living in Europe. It has been suggested that a possible explanation for the inconsistency of findings is the heterogeneity of study characteristics.

Within ECRHS it was observed that significant associations of wheeze with indoor NO_2 were more likely to be observed in the Spanish, Belgian and UK centres, but these inconsistencies were less than would be expected by chance. However, some heterogeneity across centres was observed when examining the association of asthma score and indoor NO_2.

Observational studies tend to vary in study design, selection of study population, assessment of exposure and outcome and statistical analysis and because of this are difficult to replicate. Residual confounding, in this case smoking, co-pollutants and SES may vary across countries and study population and lead to inconsistent effects.

Specificity

This criterion is generally considered to be one of the weakest. For example smoking is a proven cause of multiple disorders (i.e. lacks specificity). We look at the specificity of an association because if there is no association between indoor NO_2 and other diseases (apart from asthma) then there is clearly a strong argument in favour of causation. However, "*One-to-one relationships are not frequent"*.

Existing evidence suggests that indoor NO_2 is only associated with respiratory health (although studies on outdoor NO_2 have observed some associations with cardiopulmonary mortality). However, asthma lacks specificity as it is associated with a large array of risk factors.

Temporality

'*Which is the cart and which the horse*?'

Longitudinal studies that follow people over a long period of time are better equipped to answer this question than cross-sectional studies. They observe the temporal order of events and answer this question by looking at the onset of a disease. This is particularly relevant with diseases of slow development but not with asthma, whose clinical manifestations are characterised by strong time variability. This variability is a key feature of asthma and makes it difficult to establish whether these changes simply reflect variation in a determined time course or are responses to variations in exposure to environmental triggers for the disease. An increase

in asthma incidence may represent added cases, old cases that had been in remission for a long time or sub-clinical cases brought forward in time by some environmental exposure.

Biological gradient (or dose-response curve)

In general effects of NO_2 on respiratory health are considered with NO_2 examined as a continuous variable, which implies a dose-response. These associations are frequently observed at levels below current WHO guidelines with little evidence of a threshold (WHO 2006; 2013).

Plausibility

'*Causation is biologically plausible'*

The 'free radical' theory hypothesizes that NO_2 as a free radical has the potential to induce oxidative stress in cells. *In vitro* studies and studies on animals have shown that this can lead to cell injury and airway inflammation. The accumulation of evidence from controlled animal and human toxicology studies is pointing towards the possibility of a causal role for NO_2 (at least in part) at concentrations experienced while cooking with gas or in near traffic environments.

Coherence

'*The cause-and-effect interpretation of our data should not seriously conflict with the generally known facts of the natural history and biology of the disease'*.

Air pollution as a cause of asthma coheres with trends in developed countries of both increasing levels of air pollution and raising levels of asthma prevalence.

Experiment

'*Because of an observed association some preventive action is taken- does it in fact prevent? Is the frequency of the associated events affected*? '

As a sort of semi-experimental approach intervention may reveal ***'the strongest support for the causation hypothesis'*.**

Within this thesis I have not assessed this criterion – which requires the conduct of randomised controlled trials. Others have made such attempts in Australia and New Zealand (Pilotto, 2004; Howden-Chapman 2008; Marks, 2010) and these studies have provided some support for

causation. Briefly, these trials showed that removal of unflued gas heaters (a major source of indoor NO_2) from children's homes or schools found improvements in their respiratory health.

It is not possible to establish whether there is a relationship between exposure to indoor NO_2 and/or other air pollutants generated from gas combustion and respiratory symptoms since intervention studies are designed to answer a different question: '*What is the relationship between a specific intervention and health'* rather than *'What is the relationship between exposure to air pollutants and health outcome*?'. This viewpoint is often under-emphasised by epidemiologists in spite of the fact that a simple intervention (e.g. replacement of UFGH with ducted gas heaters) may improve respiratory symptoms in children. As Galea (2013), the President of the Society for Epidemiological Research, commented ''*there is an overwhelming preoccupation with etiology and causal inference, and little emphasis on intervention*''.

Analogy

In some circumstances it would be fair to make a judgment by analogy.

The most recent reviews on outdoor NO_2 (EPA, 2013) confirm that there is an effect of outdoor NO_2 on respiratory health. This could be reason to infer that there is an effect of indoor NO_2 on respiratory health.

7.2.2 Conclusion

In conclusion, the dose-response effect, the biological plausibility, the health improvement observed following intervention trials and the analogy with outdoor NO_2 suggest that there is some evidence of a causal association of NO_2 and asthma. The lack of evidence to suggest strength of the association, consistency, specificity, temporality and coherence of such association suggest that the observed evidence is not sufficient to imply causality.

More recently some authors have commented that causality cannot in general be proven in human studies due to practical and ethical considerations and can only be induced from demonstrated associations between an exposure and a health outcome (Lucas and McMichael, 2005). Epidemiological studies should instead strive to understand the links between environment and health and give full consideration to epidemiological noise – confounding, bias and chance – to provide support for evidence-based practice.

7.3 Assessment of major issues

7.3.1 Exposure measurement error

One of the major issues emerging from this thesis is that problems related to indoor NO_2 assessment (misclassification, exposure to peaks and NO_2 modelling, errors in quantifying the exposure) can lead to error or bias in the health-effect regression model.

Exposure measurement error is regarded as an unavoidable problem in air pollution epidemiological studies, where ambient exposure is usually based on a few observations (central-site monitoring) and then used to represent individual exposure for health-effect assessment. There are two types of errors:

- Non-differential errors - when error is unbiased with a mean equal to zero. They are sub-divided into two groups (Armstrong, 1998):
    - Random error (also known as misclassification) that is independent of the true exposure and the outcome. This biases the effect measure towards the null value
    - Berkson error - when part of the exposure variability is not captured because exposure is based on a few monitoring sites (particularly relevant to outdoor air pollution studies). This reduces the power of the study.

- Differential errors tend to systematically underestimate or overestimate the true exposure. This causes bias in the effect measure downwards or upwards respectively.

When 'gas cooking' is used as a proxy for NO_2 exposure there is considerable risk of misclassification of exposure. This might explain some of the heterogeneity between studies. It may partly explain why some studies see no effect.

Epidemiological studies on indoor NO_2 are not affected by Berkson type error since indoor NO_2 is measured in each participant's home; for this reason they have more statistical power than traditional outdoor NO_2 studies where exposure is based on central-site monitoring stations. However, a health-effect regression model that uses indoor NO_2 measurements based on passive diffusion samplers is still likely to be affected by misclassification (random error). Measurements are conducted at one or a few points in time and then used to represent average exposure. Average exposure does not consider variations in occupant habits (e.g. time spent indoors) or variations that may occur during the day or year or from year to year.

This is particularly relevant in the context of short-term peaks generated during gas cooking which may cause an effect independent from long-term exposure (WHO, 2013) but the effect cannot be captured when using long-term average exposures. Time-series studies that examined short-term (24-h average and 1-h average) exposure to outdoor NO_2 and emergency hospital admissions for asthma consistently report positive associations (EPA, 2008). This is supported by evidence from controlled exposure studies but the health effects of short-term exposure to indoor NO_2 peaks generated while cooking with gas have rarely been assessed. There is a need to conduct studies that examine the health effects of repeated exposure to NO_2 peaks. New portable, low-cost, real-time monitors that measure NO_2 peaks exist but more work needs to be done to assess their reliability in large-scale field studies.

Finally, the use of modelled exposure in a health-effect regression model may induce a complex form of measurement error leading to biased health effect estimates (Basagana, 2013; Cefalu and Dominici, 2014; Szpiro, 2014). This bias cannot be removed by simply improving the exposure prediction in terms of prediction error (Szpiro, 2011). Simulation studies (Cefalu, 2014) have shown that bias can be influenced by:

- The exposure prediction model
- The type of confounding adjustment used in the health-effects regression model
- The relationship between these two.

In chapter 6 the health effect estimated using modelled NO_2 was larger than the effect estimated using measured NO_2, suggesting a bias directing the effect measure upwards. There are several possible explanations for this. The exposure model tended to overestimate the lowest exposures, which biases the estimate upwards - but at the same time, the model underestimated the higher exposures and this would bias the effect downwards. The exposure model included four predictors that are also associated with the outcome (country, temperature, age of building and outdoor NO_2). One of these predictors was also a modelled exposure (outdoor NO_2). Complex causal pathways with structural equation modelling may overcome some of this.

In conclusion, it is possible to use modelled exposure in a health-effect assessment but current literature treats confounding and exposure prediction as separate statistical issues and great care should be taken when interpreting the results. It has been recommended that exposure assessment should be evaluated in the context of health effect estimation (Sheppard, 2012) and that further methodological consideration should be given to confounding adjustment in the exposure and health-effect models (Cefalu, 2014).

7.3.2 Assessment of asthma

Asthma is a complex chronic disease without a gold standard to define it. In this thesis, assessment of asthma was confined to the assessment of self-reported asthma-related symptoms in the last 12 months. Deficiencies in this approach include recall bias, inability to distinguish different phenotypes and inadequate quantification of the frequency and duration of episodes.

The degree of symptoms' severity was quantified using an asthma score, a composite measure of asthma-related symptoms in the last 12 months on a continuous scale. The associations of asthma score with indoor NO_2 were inconsistent as there was evidence of heterogeneity between ECRHS centres. Misclassification of diseases and residual confounding by smoking may explain some of this inconsistency.

As asthma score is a composite of symptoms it is more likely to be affected by misclassification of disease. Respiratory symptoms can be incorrectly classified. For example, some asthma-related symptoms can be a feature of other diseases, such as COPD, heart failure, gastro-oesophageal reflux and non-specific virus-induced wheeze (Marks, 2005). People with asthma who have been on CST for a prolonged period may not have symptoms at all. Reporting of symptoms may be influenced by other factors such as perception of symptoms and current environmental exposure. Asthma score may incorporate different phenotypes of asthma, such as non-asthmatic symptoms related to smoking (Sunyer, 2007). Analyses were adjusted for smoking status but did not take into account the intensity of smoking or the confounding effect of second-hand smoking.

A much larger single centre study in non-smokers may be able to disentangle the effect of smoking from those associated with air pollution. For example, the Adventist Health Study (AHSMOG), a famous longitudinal study on the long-term exposure to ambient air pollutants included only non-smoking adults thus avoiding any modifying effects of active tobacco exposure (Abbey *et al*, 1993).

Assessment of objective measures (i.e. lung function, BR) could give more information on the association of asthma with indoor NO_2. Such measures have been conducted but at completion of this work were not available for analysis.

7.3.3 Co-pollutants

There has long been a concern that other air pollutants may confound reported associations of respiratory health with both indoor and outdoor NO_2. Indoor gas combustion sources emit particles, formaldehyde and water as well as NO_2. This thesis focused only on the health effects of NO_2 and did not consider the health effect associated with other emissions. Particulate matter is thought to be detrimental to respiratory health, formaldehyde respiratory effects are yet unknown (WHO, 2010) and damp may lead to elevated allergen levels (Sharpe, 2104).

Furthermore, if indoor NO_2 is coming from the outdoor air – it is likely to be part of a complex mixture of traffic-generated air pollutants, which have been found to be associated with respiratory health. Indoors air-borne particles differ from those found outdoors in terms of sources, composition and concentrations. This makes indoor air mixtures different from outdoor air mixture and as a consequence, concerns have been raised about whether the health effect of outdoor NO_2 can be readily extrapolated from studies on indoor NO_2. Results of the meta-analyses showed that some of the largest significant effect estimates were reported by those studies that measured indoor NO_2 inside schools where no gas or other fossil fuels appliances had been reported. This may suggest that:

a. The strength of the association was due to the quality of study. Studies conducted in schools tend to be better controlled than those conducted at home.
b. The strength of the association was due to the stronger confounding health effect of PM generated from outdoor sources.

The observed differences between indoor and outdoor NO_2 support a recent statement from a WHO technical report: '*There is a case for WHO to revise its current guidelines and to consider a short-term guideline based on epidemiological studies and a long-term guideline based on the outdoor, as opposed to the indoor, epidemiology* (pg. 194)*.'* (WHO, 2013)

Two studies (Neas, 1991; Hansel, 2008) included in the systematic review took into account the confounding effect of indoor particles but they did not provide strong evidence that any observed health effect of indoor NO_2 was confounded by exposure to indoor particles.

Some studies prefer to examine the effect of personal exposure to NO_2, usually assessed over 24 hours. Personal NO_2 exposure is measured by wearing passive diffusion samplers for 24 hours or longer; this provides an average exposure. Assessing temporal variations in personal exposure to NO_2 requires knowledge of indoor and outdoor sources, activity patterns and has

proven to be a complex task. Continuous monitoring that can identify exposure to NO_2 peaks has been attempted but remains limited by the available technology.

7.3.4 Public health

In this thesis it has emerged that there are groups of people who are particularly affected by indoor NO_2: those who are exposed to levels above WHO guidelines and those who are particularly vulnerable to NO_2 because of age or respiratory conditions.

People who continue to use unvented gas appliances, cook with poorly maintained gas cookers and/or live near busy roads are still likely to be exposed to levels of NO_2 that exceed WHO guidelines. A recent simulation study (Logue et al, 2014) has estimated that 62% of people who use unvented gas cooking appliances are regularly exposed to levels that exceed 1-hour standards for NO_2 (188µg/m^3). People who live near busy roads are also exposed to higher levels of NO_2 as outdoor NO_2 penetrates indoors. This is of particular concern as outdoor NO_2 levels in UK have been in breach of EU limits for years to the extent that as I am currently writing The Supreme Court of the United Kingdom has ordered the UK government to make plans for tackling the air pollution problem (Harvey, 2015).

Some people may be more susceptible to exposure to NO_2 because they may be affected by lower levels of NO_2 than the general population. They may also experience a greater effect than the general population with the same level of exposure. They include:

- Children, as they breathe more air per kilogram of body weight than adults and their lungs are growing
- Older people, as their ability to compensate for environmental insults tends to decline
- People with asthma or COPD as they are more likely to experience health effects when exposed to short-term high peaks of NO_2 that occur while cooking with gas (or on a busy road).

These groups of people could be specifically targeted for interventions to reduce exposure. Housing interventions such as fitting fans/hoods over the gas stove, which remove combustion fumes to the outside, replacement of unflued gas heaters with flued gas heaters and/or central heating and control of outdoor NO_2 levels within standards are some of the measures that could be taken to minimise the effect of NO_2 exposure on susceptible and vulnerable people.

Although NO_2 related health risks may appear to be small they may well be critical from an overall public health perspective due to the large number of persons in the potential risk groups. Certainly advice to the public should be: to use all appliances as per manufactures instructions, to use them in well ventilated rooms and ensure that they are well maintained.

7.4 Recommendations

- To conduct a panel study of people with asthma of any age to assess the effect of repeated exposure to short-term high concentration of indoor NO_2 on asthma exacerbation, taking into account the confounding/modifying effects of indoor particles. This study would require a prolonged phase of assessment of the reliability of the monitoring device, and would require considerable investment.

- To further investigate the long-term association of respiratory health with indoor NO_2 by assessing the association using objective measures of respiratory health, i.e. lung function, FeNO, airway responsiveness within the ECRHS cohort.

- To conduct a single-centre study on a homogeneous non-smoking population to examine the effect of exposure to indoor NO_2 and indoor particles. This study would avoid the residual confounding and/or modifying effect effects of smoking.

- To develop country-specific and season-specific models for indoor NO_2 with specific reference to how predictor variables may act as confounders/cause of asthma

7.5 Conclusions

Indoor air quality is an important area for research and public health policy. There is some evidence for a link between indoor NO_2 and asthma-related symptoms in children and adults but insufficient to imply causality.

Many people cook with gas and/or live in areas where outdoor NO_2 levels exceed WHO guidelines. Some groups of people may be particularly susceptible to NO_2, namely children, older

people and people with asthma and other chronic respiratory conditions. Although NO_2 related health risks are small they are applied to a substantial proportion of the population.

Interventions such as removal of unflued gas heaters, fitting of fans/hoods over gas stoves, ventilating rooms by opening doors and windows when cooking and heating with gas, and ensuring that long-term and short-term outdoor NO_2 levels are kept to a minimum are measures that may reduce exposure to indoor NO_2.

References

Abbey, D. E., Petersen, F., Mills, P. K. & Beeson, W. L. 1993. Long-term ambient concentrations of total suspended particulates, ozone, and sulfur dioxide and respiratory symptoms in a nonsmoking population. *Arch Environ Health,* 48**,** 33-46.

Accordini, S., Janson, C., Svanes, C. & Jarvis, D. 2012. The role of smoking in allergy and asthma: lessons from the ECRHS. *Current allergy and asthma reports,* 12**,** 185-191.

Algar, O. G., Pichini, S., Basagana, X., Puig, C., Vall, O., Torrent, M., Harris, J., Sunyer, J., Cullinan, P. & Grp, A. 2004. Concentrations and determinants of NO2 in homes of Ashford, UK and Barcelona and Menorca, Spain. *Indoor Air,* 14**,** 298-304.

Allen, S., Britton, J. & Leonardi-Bee, J. 2009. Association between antioxidant vitamins and asthma outcome measures: systematic review and meta-analysis. *Thorax,* 64**,** 610-619.

Amaral, A. F., Ramasamy, A., Castro-Giner, F., Minelli, C., Accordini, S., Sorheim, I. C., Pin, I., Kogevinas, M., Jogi, R., Balding, D. J., Norback, D., Verlato, G., Olivieri, M., Probst-Hensch, N., Janson, C., Zock, J. P., Heinrich, J. & Jarvis, D. L. 2014. Interaction between gas cooking and GSTM1 null genotype in bronchial responsiveness: resu lts from the European Community Respiratory Health Survey. *Thorax,* 69**,** 558-64.

Annesi-Maesano, I., Hulin, M., Lavaud, F., Raherison, C., Kopferschmitt, C., De Blay, F., Charpin, D. A. & Denis, C. 2012. Poor air quality in classrooms related to asthma and rhinitis in primary schoolchildren of the French 6 Cities Study. *Thorax***,** thoraxjnl-2011-200391.

Armstrong, B. G. 1998. Effect of measurement error on epidemiological studies of environmental and occupational exposures. *Occupational and environmental medicine,* 55**,** 651-656.

Asher, M., Keil, U., Anderson, H., Beasley, R., Crane, J., Martinez, F., Mitchell, E., Pearce, N., Sibbald, B. & Stewart, A. 1995. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *European respiratory journal,* 8**,** 483-491.

Atkinson, R. W., Ross Anderson, H., Sunyer, J., Ayres, J. O. N., Baccini, M., Vonk, J. M., Boumghar, A., Forastiere, F., Forsberg, B., Touloumi, G., Schwartz, J. & Katsouyanni, K. 2001. Acute Effects of Particulate Air Pollution on Respiratory Admissions. *American Journal of Respiratory and Critical Care Medicine,* 164**,** 1860-1866.

Ayyagari, V. N., Januszkiewicz, A. & Nath, J. 2007. Effects of nitrogen dioxide on the expression of intercellular adhesion molecule-1, neutrophil adhesion, and cytotoxicity: studies in human bronchial epithelial cells. *Inhalation toxicology,* 19**,** 181-194.

Barck, C., Sandström, T., Lundahl, J., Halldén, G., Svartengren, M., Strand, V., Rak, S. & Bylin, G. 2002. Ambient level of NO2 augments the inflammatory response to inhaled allergen in asthmatics. *Respiratory Medicine,* 96**,** 907-917.

Basagaña, X., Aguilera, I., Rivera, M., Agis, D., Foraster, M., Marrugat, J., Elosua, R. & Künzli, N. 2013. Measurement error in epidemiologic studies of air pollution based on land-use regression models. *American journal of epidemiology,* 178**,** 1342-1346.

Basu, R. S., Jm. 1999. A review of the epidemiological evidence on health effects of nitrogen dioxide exposure from gas stoves. *Journal of Environmental Medicine***,** 173-187.

Bateson, T. F. & Schwartz, J. 2007. Children's response to air pollutants. *Journal of Toxicology and Environmental Health, Part A,* 71**,** 238-243.

Bauer, M., Utell, M., Morrow, P., Speers, D. & Gibb, F. 1986. Inhalation of 0.30 ppm nitrogen dioxide potentiates exercise-induced bronchospasm in asthmatics. *The American review of respiratory disease,* 134**,** 1203-1208.

Baxter, L. K., Clougherty, J. E., Laden, F. & Levy, J. I. 2007. Predictors of concentrations of nitrogen dioxide, ne particulate matter, and particle constituents inside of lower socioeconomic status urban homes. *Journal of Exposure Science and Environmental Epidemiology,* 17**,** 433-444.

Bayram, H., Sapsford, R. J., Abdelaziz, M. M. & Khair, O. A. 2001. Effect of ozone and nitrogen dioxide on the release of proinflammatory mediators from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients in vitro. *Journal of Allergy and Clinical Immunology,* 107**,** 287-294.

Beelen, R., Hoek, G., Pebesma, E., Vienneau, D., De Hoogh, K. & Briggs, D. 2009. Mapping of background air pollution at a fine spatial scale across the European Union. *Science of the Total Environment,* 407**,** 1852-1867.

Begg, C. B. & Mazumdar, M. 1994. Operating characteristics of a rank correlation test for publication bias. *Biometrics,* 50**,** 1088-101.

Belanger, K., Beckett, W., Triche, E., Bracken, M. B., Holford, T., Ren, P., Mcsharry, J.-E., Gold, D. R., Platts-Mills, T. A. & Leaderer, B. P. 2003. Symptoms of wheeze and persistent cough in the first year of life: associations with indoor allergens, air contaminants, and maternal history of asthma. *American Journal of Epidemiology,* 158**,** 195-202.

Belanger, K., Gent, J. F., Triche, E. W., Bracken, M. B. & Leaderer, B. P. 2006. Association of indoor nitrogen dioxide exposure with respiratory symptoms in children with asthma. *American Journal of Respiratory and Critical Care Medicine,* 173**,** 297-303.

Belanger, K., Holford, T. R., Gent, J. F., Hill, M. E., Kezik, J. M. & Leaderer, B. P. 2013. Household Levels of Nitrogen Dioxide and Pediatric Asthma Severity. *Epidemiology,* 24**,** 320-330.

Berwick, M., Leaderer, B. P., Stolwijk, J. A. & Zagraniski, R. T. 1989. Lower respiratory symptoms in children exposed to nitrogen dioxide from unvented combustion sources. *Environment International,* 15**,** 369-373.

Beuther, D. A., Weiss, S. T. & Sutherland, E. R. 2006. Obesity and asthma. *American Journal of Respiratory and Critical Care Medicine,* 174**,** 112-119.

Bland, J. M. & Altman, D. G. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet,* 1**,** 307-10.

Bland, J. M. & Altman, D. G. 1994. Regression towards the mean. *BMJ : British Medical Journal,* 308**,** 1499-1499.

Bland, M. 2000. *An introduction to medical statistics*, Oxford University Press.

Blomberg, A., Krishna, M. T., Bocchino, V., Biscione, G. L., Shute, J. K., Kelly, F. J., Frew, A. J., Holgate, S. T. & Sandström, T. 1997. The Inflammatory Effects of 2 ppm NO2 on the Airways of Healthy Subjects. *American Journal of Respiratory and Critical Care Medicine,* 156**,** 418-424.

Bouzigon, E., Corda, E., Aschard, H., Dizier, M.-H., Boland, A., Bousquet, J., Chateigner, N., Gormand, F., Just, J. & Le Moual, N. 2008. Effect of 17q21 variants and smoking exposure in early-onset asthma. *New England Journal of Medicine,* 359**,** 1985-1994.

Brauer, M., Henderson, S., Kirkham, T., Lee, K.S., Rich, K. And Teschke, K. 2002. Review of the health risks associated with nitrogen dioxide and sulfur dioxide in indoor air. Technical report. Vancouver: University of British Columbia.

Brauer, M., Spengler, J. D., Lee, K. & Yanagisawa, Y. 1993. Air pollutant exposures inside ice hockey rinks: exposure assessment and reduction strategies. *ASTM SPECIAL TECHNICAL PUBLICATION,* 1212**,** 142-142.

Braun-Fahrlander, C., Ackermann-Liebrich, U., Schwartz, J., Gnehm, H. P., Rutishauser, M. & Wanner, H. U. 1992. Air pollution and respiratory symptoms in preschool children. *American Review of Respiratory Disease,* 145**,** 42-47.

Breusch, T. S. & Pagan, A. R. 1979. A simple test for heteroscedasticity and random coefficient variation. *Econometrica: Journal of the Econometric Society***,** 1287-1294.

Breysse, P. N., Buckley, T. J., Williams, D. A., Beck, C. M., Jo, S.-J., Merriman, B., Kanchanaraksa, S., Swartz, L. J., Callahan, K. A. & Butz, A. M. 2005. Indoor exposures to air pollutants and allergens in the homes of asthmatic children in inner-city Baltimore. *Environmental Research,* 98**,** 167-176.

Breysse, P. N., Diette, G. B., Matsui, E. C., Butz, A. M., Hansel, N. N. & Mccormack, M. C. 2010. Indoor Air Pollution and Asthma in Children. *Proceedings of the American Thoracic Society,* 7**,** 102-106.

Breysse, P., Diette, G., Curtin-Brosnan, J., Aloe, C., Matsui, E. & Lu, K. 2012. Overweight/obesity Modifies The Effect Of Indoor Nitrogen Dioxide On Asthma Symptoms In Urban Children. *Am J Respir Crit Care Med,* 185**,** A4066.

Briggs, D. J., Collins, S., Elliott, P., Fischer, P., Kingham, S., Lebret, E., Pryl, K., Vanreeuwijk, H., Smallbone, K. & Vanderveen, A. 1997. Mapping urban air pollution using GIS: a regression-based approach. *International Journal of Geographical Information Science,* 11**,** 699-718.

Brunekreef, B., Houthuijs, D., Dijkstra, L. & Boleij, J. S. 1990. Indoor nitrogen dioxide exposure and children's pulmonary function. *Journal of the Air & Waste Management Association,* 40**,** 1252-6.

Burney, P., Chinn, S., Luczynska, C., Jarvis, D., Vermeire, P., Bousquet, J., Nowak, D., Prichard, J., Demarco, R. & Rijcken, B. 1996. Variations in the prevalence of respiratory

symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survey (ECRHS). *European respiratory journal,* 9**,** 687-695.

Burney, P., Papacosta, A., Withey, C., Colley, J. & Holland, W. 1991. Hospital admission rates and the prevalence of asthma symptoms in 20 local authority districts. *Thorax,* 46**,** 574-579.

Bylin, G., Lindvall, T., Rehn, T. & Sundin, B. 1985. Effects of short-term exposure to ambient nitrogen dioxide concentrations on human bronchial reactivity and lung function. *European journal of respiratory diseases,* 66**,** 205-217.

Carlsten, C., Brauer, M., Dimich-Ward, H., Dybuncio, A., Becker, A. B. & Chan-Yeung, M. 2011. Combined exposure to dog and indoor pollution: incident asthma in a high-risk birth cohort. *European Respiratory Journal,* 37**,** 324-30.

Cazzoletti, L., Marcon, A., Corsico, A., Janson, C., Jarvis, D., Pin, I., Accordini, S., Bugiani, M., Cerveri, I., Gislason, D., Gulsvik, A., De Marco, R., Therapy & Health Economics Group of the European Community Respiratory Health, S. 2010. Asthma severity according to Global Initiative for Asthma and its determinants: an international study. *International Archives of Allergy & Immunology,* 151**,** 70-9.

Cefalu, M. & Dominici, F. 2014. Does exposure prediction bias health effect estimation? The relationship between confounding adjustment and exposure prediction. *Epidemiology,* 25**,** 583.

Chauhan, A. 1999. Gas cooking appliances and indoor pollution. *Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology,* 29**,** 1009.

Chinn, S., Burney, P., Jarvis, D. & Luczynska, C. 1997. Variation in bronchial responsiveness in the European Community Respiratory Health Survey (ECRHS). *European Respiratory Journal,* 10**,** 2495-501.

Ciuk, J., Volkmer, R. E. & Edwards, J. W. 2001. Domestic nitrogen oxide exposure, urinary nitrate, and asthma prevalence in preschool children. *Archives of Environmental Health,* 56**,** 433-438.

Coggon, D., Barker, D., Inskip, H. & Wield, G. 1993. Housing in early life and later mortality. *Journal of Epidemiology and Community Health,* 47**,** 345-348.

Comstock, G. W., Meyer, M. B., Helsing, K. & Tockman, M. S. 1981. Respiratory effects of household exposures to tobacco smoke and gas cooking. *Am Rev Respir Dis,* 124**,** 143-148.

Cook, D. G. & Strachan, D. P. 1997. Health effects of passive smoking. 3. Parental smoking and prevalence of respiratory symptoms and asthma in school age children. *Thorax,* 52**,** 1081-1094.

Cook, R. D. 1977. Detection of influential observation in linear regression. *Technometrics***,** 15-18.

Cyrys, J., Heinrich, J., Richter, K., Wolke, G. & Wichmann, H. E. 2000. Sources and concentrations of indoor nitrogen dioxide in Hamburg (west Germany) and Erfurt (east Germany). *Science of the Total Environment,* 250**,** 51-62.

Delgado-Saborit, J. M. 2012. Use of real-time sensors to characterise human exposures to combustion related pollutants. *Journal of Environmental Monitoring,* 14**,** 1824-1837.

Dennekamp, M., Howarth, S., Dick, C. A., Cherrie, J. W., Donaldson, K. & Seaton, A. 2001. Ultrafine particles and nitrogen oxides generated by gas and electric cooking. *Occupational & Environmental Medicine,* 58**,** 511-6.

Dersimonian, R. & Laird, N. 1986. Meta-analysis in clinical trials. *Control Clin Trials,* 7**,** 177-88.

Devalia, J. L., Rusznak, C., Herdman, M. J., Trigg, C. J., Tarraf, H. & Davies, R. J. 1994. Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation. *Lancet,* 344**,** 1668-71.

Dietert, R. R., Etzel, R. A., Chen, D., Halonen, M., Holladay, S. D., Jarabek, A. M., Landreth, K., Peden, D. B., Pinkerton, K. & Smialowicz, R. J. 2000. Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary. *Environmental health perspectives,* 108**,** 483.

Diette, G. B., Hansel, N. N., Buckley, T. J., Curtin-Brosnan, J., Eggleston, P. A., Matsui, E. C., Mccormack, M. C., Williams, D. L. & Breysse, P. N. 2007. Home indoor pollutant exposures among inner-city children with and without asthma. *Environmental Health Perspectives,* 115**,** 1665-1669.

Dijkstra, L., Houthuijs, D., Brunekreef, B., Akkerman, I. & Boleij, J. S. 1990. Respiratory health effects of the indoor environment in a population of Dutch children. *American Review of Respiratory Disease,* 142**,** 1172-8.

Dockery, D. W., Spengler, J. D., Reed, M. P. & Ware, J. 1981. Relationships among personal, indoor and outdoor NO 2 measurements. *Environment international,* 5**,** 101-107.

Douwes, J., Gibson, P., Pekkanen, J. & Pearce, N. 2002. Non-eosinophilic asthma: importance and possible mechanisms. *Thorax,* 57**,** 643-648.

Ebrahim, S. & Smith, G. 2013. Commentary: Should we always deliberately be non-representative? *International Journal of Epidemiology,* 42**,** 1022-1026.

Egger, M., Davey Smith, G., Schneider, M. & Minder, C. 1997. Bias in meta-analysis detected by a simple, graphical test. *BMJ (Clinical research ed),* 315**,** 629-34.

Egger, M., Schneider, M. & Davey Smith, G. 1998. Spurious precision? Meta-analysis of observational studies. *BMJ (Clinical research ed),* 316**,** 140-4.

Eisner, M. D. 2002. Environmental tobacco smoke exposure and pulmonary function among adults in NHANES III: impact on the general population and adults with current asthma. *Environ Health Perspect,* 110**,** 765-70.

Eisner, M. D. & Blanc, P. D. 2003. Gas stove use and respiratory health among adults with asthma in NHANES III. *Occupational & Environmental Medicine,* 60**,** 759-64.

Eisner, M. D., Yelin, E. H., Katz, P. P., Earnest, G. & Blanc, P. D. 2002. Exposure to indoor combustion and adult asthma outcomes: environmental tobacco smoke, gas stoves, and woodsmoke. *Thorax,* 57**,** 973-8.

Emenius, G., Pershagen, G., Berglind, N., Kwon, H. J., Lewne, M., Nordvall, S. L. & Wickman, M. 2003. NO2, as a marker of air pollution, and recurrent wheezing in children: a nested case-control study within the BAMSE birth cohort. *Occupational & Environmental Medicine,* 60**,** 876-81.

Environmental Protection Agency 2008. Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (Final Report, 2008). *EPA/600/R-08/071.* Washington, DC, United States: Environmental Protection Agency.

Erdei, E., Bobvos, J., Brozik, M., Paldy, A., Farkas, I., Vaskovi, E. & Rudnai, P. 2003. Indoor air pollutants and immune biomarkers among Hungarian asthmatic children. *Archives of Environmental Health,* 58**,** 337-347.

Escape. 2010. *Exposure assessment manual* [Online]. Utrecht University - The Netherlands. Available: http://www.escapeproject.eu/manuals/ESCAPE_Exposure-manualv9.pdf [Accessed 12/11/2013.

Esplugues, A. 2013. Indoor and outdoor concentrations and determinants of NO2 in a cohort of 1-year-old children in Valencia, Spain (vol 20, pg 213, 2010). *Indoor Air,* 23**,** 264-264.

Esplugues, A., Ballester, F., Estarlich, M., Llop, S., Fuentes-Leonarte, V., Mantilla, E., Vioque, J. & Iniguez, C. 2011. Outdoor, but not indoor, nitrogen dioxide exposure is associated with persistent cough during the first year of life. *Science of the Total Environment,* 409**,** 4667-4673.

Ezratty, V., Guillossou, G., Neukirch, C., Dehoux, M., Koscielny, S., Bonay, M., Cabanes, P.-A., Samet, J. M., Mure, P. & Ropert, L. 2014. Repeated nitrogen dioxide exposures and eosinophilic airway inflammation in asthmatics: a randomized crossover study. *Environmental health perspectives,* 122**,** 850.

Farrow, A., Greenwood, R., Preece, S. & Golding, J. 1997. Nitrogen dioxide, the oxides of nitrogen, and infants' health symptoms. *Archives of Environmental Health,* 52**,** 189-194.

Fecht, D., Fischer, P., Fortunato, L., Hoek, G., De Hoogh, K., Marra, M., Kruize, H., Vienneau, D., Beelen, R. & Hansell, A. 2015. Associations between air pollution and socioeconomic characteristics, ethnicity and age profile of neighbourhoods in England and the Netherlands. *Environmental Pollution,* 198**,** 201-210.

Fine, G. F., Cavanagh, L. M., Afonja, A. & Binions, R. 2010. Metal oxide semi-conductor gas sensors in environmental monitoring. *Sensors,* 10**,** 5469-5502.

Fischer, P., Brunekreef, B., Biersteker, K., Boleij, J. S. M., Van Der Lende, R., Schouten, J. & Quanjer, P. H. 1989. Effects of indoor exposure to nitrogen dioxide on pulmonary function of women living in urban and rural areas. *Environment International,* 15**,** 375-381.

Fischer, P., Remijn, B., Brunekreef, B., Van Der Lende, R., Schouten, J. & Quanjer, P. 1985. Indoor air pollution and its effect on pulmonary function of adult non-smoking women: II.

Associations between nitrogen dioxide and pulmonary function. *International Journal of Epidemiology,* 14**,** 221-6.

Flamant-Hulin, M., Caillaud, D., Sacco, P., Penard-Morand, C. & Annesi-Maesano, I. 2010. Air pollution and increased levels of fractional exhaled nitric oxide in children with no history of airway damage. *Journal of Toxicology and Environmental Health - Part A: Current Issues,* 73**,** 272-283.

Fletcher, C., Gilson, J., Hugh-Jones, P. & Scadding, J. 1959. Terminology, definitions, and classification of chronic pulmonary emphysema and related conditions. A report of the conclusions of a Ciba guest symposium. *Thorax,* 14**,** 286-299.

Florey, C. V., Melia, R. J., Chinn, S., Goldstein, B. D., Brooks, A. G., John, H. H., Craighead, I. B. & Webster, X. 1979. The relation between respiratory illness in primary schoolchildren and the use of gas for cooking--III. Nitrogen dioxide, respiratory illness and lung infection. *International Journal of Epidemiology,* 8**,** 347-53.

Folinsbee, L. J. 1992. Does nitrogen dioxide exposure increase airways responsiveness? *Toxicol Ind Health,* 8**,** 273-83.

Franklin, P., Runnion, T., Farrar, D. & Dingle, P. 2006. Comparison of peak and average nitrogen dioxide concentrations inside homes. *Atmospheric Environment,* 40**,** 7449-7454.

Franklin, P. J., Loveday, J. & Cook, A. 2012. Unflued gas heaters and respiratory symptoms in older people with asthma. *Thorax,* 67**,** 315-20.

Fuentes-Leonarte, V., Tenias, J. M. & Ballester, F. 2009. Levels of pollutants in indoor air and respiratory health in preschool children: a systematic review. *Pediatr Pulmonol,* 44**,** 231-43.

Galea, S. 2013. An argument for a consequentialist epidemiology. *American journal of epidemiology***,** kwt172.

Galea, S. & Tracy, M. 2007. Participation rates in epidemiologic studies. *Annals of epidemiology,* 17**,** 643-653.

Garn, H., Siese, A., Stumpf, S., Barth, P. J., Müller, B. & Gemsa, D. 2003. Shift Toward an Alternatively Activated Macrophage Response in Lungs of NO2-Exposed Rats. *American Journal of Respiratory Cell and Molecular Biology,* 28**,** 386-396.

Garrett, M. H., Hooper, M. A., Hooper, B. M. & Abramson, M. J. 1998. Respiratory symptoms in children and indoor exposure to nitrogen dioxide and gas stoves. *American Journal of Respiratory & Critical Care Medicine,* 158**,** 891-5.

Gent, J. F., Kezik, J. M., Hill, M. E., Tsai, E., Li, D. W. & Leaderer, B. P. 2012. Household mold and dust allergens: Exposure, sensitization and childhood asthma morbidity. *Environmental Research,* 118**,** 86-93.

Gilbert, N. L., Gauvin, D., Guay, M., Héroux, M.-È., Dupuis, G., Legris, M., Chan, C. C., Dietz, R. N. & Lévesque, B. 2006. Housing characteristics and indoor concentrations of nitrogen dioxide and formaldehyde in Quebec City, Canada. *Environmental Research,* 102**,** 1-8.

Gillespie-Bennett, J., Pierse, N., Wickens, K., Crane, J., Howden-Chapman, P., Housing, H. & Health Study Research, T. 2011. The respiratory health effects of nitrogen dioxide in children with asthma. *European Respiratory Journal,* 38**,** 303-9.

Gillespie-Bennett, J., Pierse, N., Wickens, K., Crane, J., Nicholls, S., Shields, D., Boulic, M., Viggers, H., Baker, M., Woodward, A. & Howden-Chapman, P. 2008. Sources of nitrogen dioxide (NO2) in New Zealand homes: Findings from a community randomized controlled trial of heater substitutions. *Indoor Air,* 18**,** 521-528.

Gilliland, F. D., Mcconnell, R., Peters, J. & Gong Jr, H. 1999. A theoretical basis for investigating ambient air pollution and children's respiratory health. *Environmental health perspectives,* 107**,** 403.

Gilmour, M. I., Park, P. & Selgrade, M. K. 1996. Increased immune and inflammatory responses to dust mite antigen in rats exposed to 5 ppm NO2. *Toxicological Sciences,* 31**,** 65-70.

Global Initiative for Asthma 2002. Global stratey for asthma management and prevention, 2002.

Goldstein, B. D. & Melia, R. 1981. Indoor nitrogen oxides. *Bulletin of the New York Academy of Medicine,* 57**,** 873.

Goldstein, I. F., Lieber, K., Andrews, L. R., Kazembe, F., Foutrakis, G., Huang, P. & Hayes, C. 1988. Acute respiratory effects of short-term exposures to nitrogen dioxide. *Archives of environmental health,* 43**,** 138-42.

Goodman, J. E., Chandalia, J. K., Thakali, S. & Seeley, M. 2009. Meta-analysis of nitrogen dioxide exposure and airway hyper-responsiveness in asthmatics. *Critical reviews in toxicology,* 39**,** 719-42.

Goodman, S. N. & Berlin, J. A. 1994. The Use of Predicted Confidence Intervals When Planning Experiments and the Misuse of Power When Interpreting Results. *Annals of Internal Medicine,* 121**,** 200-206.

Graham, J. A., Grant, L., Folinsbee, L., Kotchmar, D., Garner, J. & Organization, W. H. 1997. Nitrogen oxides.

Groves Rm, C. R., Couper Mp. 1992. Understanding the decision to participate in a survey. *Public Opin Q.,* 56**,** 347-367.

Gul, H., Gaga, E. O., Dogeroglu, T., Ozden, O., Ayvaz, O., Ozel, S. & Gungor, G. 2011. Respiratory health symptoms among students exposed to different levels of air pollution in a Turkish city. *Int J Environ Res Public Health,* 8**,** 1110-25.

Halliwell, B., Hu, M.-L., Louie, S., Duvall, T. R., Tarkington, B. K., Motchnik, P. & Cross, C. E. 1992. Interaction of nitrogen dioxide with human plasma antioxidant depletion and oxidative damage. *FEBS letters,* 313**,** 62-66.

Hansel, N. N., Breysse, P. N., Mccormack, M. C., Matsui, E. C., Curtin-Brosnan, J., Williams, D. L., Moore, J. L., Cuhran, J. L. & Diette, G. B. 2008. A longitudinal study of indoor nitrogen dioxide levels and respiratory symptoms in inner-city children with asthma. *Environmental Health Perspectives,* 116**,** 1428-1432.

Hansel, N. N., Mccormack, M. C., Belli, A. J., Matsui, E. C., Peng, R. D., Aloe, C., Paulin, L., Williams, D. L., Diette, G. B. & Breysse, P. N. 2013. In-home air pollution is linked to respiratory morbidity in former smokers with chronic obstructive pulmonary disease. *American Journal of Respiratory & Critical Care Medicine,* 187**,** 1085-90.

Harvey, F. 2015. Supreme court orders UK to draw up air pollution cleanup plan. *The Guardian*. http://www.theguardian.com/environment/2015/apr/29/supreme-court-orders-uk-to-draw-up-air-pollution-cleanup-plan

Hasselblad, V., Eddy, D. M. & Kotchmar, D. J. 1992. Synthesis of environmental evidence: nitrogen dioxide epidemiology studies. *J Air Waste Manage Assoc,* 42**,** 662-71.

Hasselblad, V., Stead, A. G. & Galke, W. 1980. Analysis of Coarsely Grouped Data from the Lognormal-Distribution. *Journal of the American Statistical Association,* 75**,** 771-778.

Health Effects Institute 2010. Traffic-related air pollution: a critical review of the literature on emissions, exposure, and health effects. Boston,: Health Effects Institute.

Hernán, M. A. & Robins, J. M. 2006. Estimating causal effects from epidemiological data. *Journal of epidemiology and community health,* 60**,** 578-586.

Heroux, M. E., Clark, N., Van Ryswyk, K., Mallick, R., Gilbert, N. L., Harrison, I., Rispler, K., Wang, D., Anastassopoulos, A., Guay, M., Macneill, M. & Wheeler, A. J. 2010. Predictors of Indoor Air Concentrations in Smoking and Non-Smoking Residences. *International Journal of Environmental Research and Public Health,* 7**,** 3080-3099.

Hesterberg, T. W., Bunn, W. B., Mcclellan, R. O., Hamade, A. K., Long, C. M. & Valberg, P. A. 2009. Critical review of the human data on short-term nitrogen dioxide (NO2) exposures: evidence for NO2 no-effect levels. *Critical reviews in toxicology,* 39**,** 743-81.

Higgins, J. P., Thompson, S. G., Deeks, J. J. & Altman, D. G. 2003. Measuring inconsistency in meta-analyses. *BMJ,* 327**,** 557-60.

Hill, A. B. 1965. The Environment and Disease: Association or Causation? *Proceedings of the Royal Society of Medicine,* 58**,** 295-300.

Hoek, G., Beelen, R., De Hoogh, K., Vienneau, D., Gulliver, J., Fischer, P. & Briggs, D. 2008. A review of land-use regression models to assess spatial variation of outdoor air pollution. *Atmospheric Environment,* 42**,** 7561-7578.

Hoek, G., Brunekreef, B., Meijer, R., Scholten, A. & Boleij, J. 1984. Indoor nitrogen dioxide pollution and respiratory symptoms of schoolchildren. *International Archives of Occupational & Environmental Health,* 55**,** 79-86.

Hoenig, J. M. & Heisey, D. M. 2001. The Abuse of Power. *The American Statistician,* 55**,** 19-24.

Howden-Chapman, P., Pierse, N., Nicholls, S., Gillespie-Bennett, J., Viggers, H., Cunningham, M., Phipps, R., Boulic, M., Fjallstrom, P., Free, S., Chapman, R., Lloyd, B., Wickens, K., Shields, D., Baker, M., Cunningham, C., Woodward, A., Bullen, C. & Crane, J. 2008. Effects of improved home heating on asthma in community dwelling children: randomised controlled trial. *BMJ,* 337**,** a1411.

Hulin, M., Annesi-Maesano, I. & Caillaud, D. 2011. Indoor air quality at school and allergy and asthma among schoolchildren. Differences between rural and urban areas. Qualite de l'air interieur dans les ecoles et asthme et allergies parmi les ecoliers en Auvergne. Differences entre le milieu rural et le milieu urbain. *Revue Francaise d'Allergologie,* 51**,** 419-424.

Janson, C., Anto, J., Burney, P., Chinn, S., De Marco, R., Heinrich, J., Jarvis, D., Kuenzli, N., Leynaert, B., Luczynska, C., Neukirch, F., Svanes, C., Sunyer, J., Wjst, M. & Surve, E. C. R. H. 2001. The European Community Respiratory Health Survey: what are the main results so far? *European Respiratory Journal,* 18**,** 598-611.

Jarvis, D., Chinn, S., Luczynska, C. & Burney, P. 1996. Association of respiratory symptoms and lung function in young adults with use of domestic gas appliances. *Lancet,* 347**,** 426-31.

Jarvis, D., Chinn, S., Sterne, J., Luczynska, C. & Burney, P. 1998. The association of respiratory symptoms and lung function with the use of gas for cooking. European Community Respiratory Health Survey. *The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology,* 11**,** 651-8.

Jarvis, D. L., Leaderer, B. P., Chinn, S. & Burney, P. G. 2005. Indoor nitrous acid and respiratory symptoms and lung function in adults. *Thorax,* 60**,** 474-479.

Jerrett, M., Arain, A., Kanaroglou, P., Beckerman, B., Potoglou, D., Sahsuvaroglu, T., Morrison, J. & Giovis, C. 2005. A review and evaluation of intraurban air pollution exposure models. *Journal of Exposure Science and Environmental Epidemiology,* 15**,** 185-204.

Katsouyanni, K., Touloumi, G., Samoli, E., Gryparis, A., Le Tertre, A., Monopolis, Y., Rossi, G., Zmirou, D., Ballester, F., Boumghar, A., Anderson, H. R., Wojtyniak, B., Paldy, A., Braunstein, R., Pekkanen, J., Schindler, C. & Schwartz, J. 2001. Confounding and Effect Modification in the Short-Term Effects of Ambient Particles on Total Mortality: Results from 29 European Cities within the APHEA2 Project. *Epidemiology,* 12**,** 521-531.

Kattan, M., Gergen, P. J., Eggleston, P., Visness, C. M. & Mitchell, H. E. 2007. Health effects of indoor nitrogen dioxide and passive smoking on urban asthmatic children. *Journal of Allergy and Clinical Immunology,* 120**,** 618-624.

Keller, M. D., Lanese, R. R., Mitchell, R. I. & Cote, R. W. 1979a. Respiratory illness in households using gas and electricity for cooking: I. Survey of incidence. *Environmental research,* 19**,** 495-503.

Keller, M. D., Lanese, R. R., Mitchell, R. I. & Cote, R. W. 1979b. Respiratory illness in households using gas and electricity for cooking: II. Symptoms and objective findings. *Environmental research,* 19**,** 504-515.

Kelly, F. & Tetley, T. 1997. Nitrogen dioxide depletes uric acid and ascorbic acid but not glutathione from lung lining fluid. *Biochem. J,* 325**,** 95-99.

Kelly, F. J. 2003. Oxidative stress: its role in air pollution and adverse health effects. *Occupational and environmental medicine,* 60**,** 612-6.

Kim, J. L., Elfman, L., Wieslander, G., Ferm, M., Toren, K. & Norback, D. 2011. Respiratory Health among Korean Pupils in Relation to Home, School and Outdoor Environment. *Journal of Korean Medical Science,* 26**,** 166-173.

Koenig, J. Q., Covert, D. S., Marshall, S. G., Van Belle, G. & Pierson, W. E. 1987. The Effects of Ozone and Nitrogen Dioxide on Pulmonary Function.

Kotzias D Et Al. 2005. The INDEX project. Critical appraisal of the setting and implementation of indoor exposure limits in the EU. Ispra.

Lai, H. K., Bayer-Oglesby, L., Colvile, R., Gotschi, T., Jantunen, M. J., Kunzli, N., Kulinskaya, E., Schweizer, C. & Nieuwenhuijsen, M. J. 2006. Determinants of indoor air concentrations of PM2.5, black smoke and NO2 in six European cities (EXPOLIS study). *Atmospheric Environment,* 40**,** 1299-1313.

Lash, T. L. 2015. Truth and Consequences. *Epidemiology,* 26**,** 141-142.

Lawson, S. J., Galbally, I. E., Powell, J. C., Keywood, M. D., Molloy, S. B., Cheng, M. & Selleck, P. W. 2011. The effect of proximity to major roads on indoor air quality in typical Australian dwellings. *Atmospheric Environment,* 45**,** 2252-2259.

Leaderer, B. P., Zagraniski, R. T., Berwick, M. & Stolwijk, J. a. J. 1986. Assessment of Exposure to Indoor Air Contaminants from Combustion Sources - Methodology and Application. *American Journal of Epidemiology,* 124**,** 275-289.

Lee, K., Levy, J. I., Yanagisawa, Y., Spengler, J. D. & Billick, I. H. 1998. The Boston residential nitrogen dioxide characterization study: Classification and prediction of indoor NO2 exposure. *Journal of the Air & Waste Management Association,* 48**,** 736-742.

Lee, K., Xue, J., Geyh, A., Ozkaynak, H., Leaderer, B., Weschler, C. & Spengler, J. 2002. Nitrous acid, nitrogen dioxide, and ozone concentrations in residential environments. *Environmental Health Perspectives,* 110**,** 145-149.

Levy, J. I. 1998. Impact of residential nitrogen dioxide exposure on personal exposure: An international study. *Journal of the Air & Waste Management Association,* 48**,** 553-560.

Levy, J. I., Clougherty, J. E., Baxter, L. K., Houseman, E. A. & Paciorek, C. J. 2010. Evaluating heterogeneity in indoor and outdoor air pollution using land-use regression and constrained factor analysis. *Res Rep Health Eff Inst***,** 5-80; discussion 81-91.

Leynaert, B., Sunyer, J., Garcia-Esteban, R., Svanes, C., Jarvis, D., Cerveri, I., Dratva, J., Gislason, T., Heinrich, J. & Janson, C. 2012. Gender differences in prevalence, diagnosis and incidence of allergic and non-allergic asthma: a population-based cohort. *Thorax***,** thoraxjnl-2011-201249.

Lin, C., Gillespie, J., Schuder, M., Duberstein, W., Beverland, I. & Heal, M. 2015. Evaluation and calibration of Aeroqual series 500 portable gas sensors for accurate measurement of ambient ozone and nitrogen dioxide. *Atmospheric Environment,* 100**,** 111-116.

Lin, M., Chen, Y., Burnett, R., Villeneuve, P. & Krewski, D. 2003. Effect of short-term exposure to gaseous pollution on asthma hospitalisation in children: a bi-directional case-crossover analysis. *Journal of epidemiology and community health,* 57**,** 50-55.

Lin, W., Brunekreef, B. & Gehring, U. 2013. Meta-analysis of the effects of indoor nitrogen dioxide and gas cooking on asthma and wheeze in children. *International Journal of Epidemiology,* 42**,** 1724-1737.

Loo, C., Foty, R. G., Wheeler, A. J., Miller, J. D., Evans, G., Stieb, D. M. & Dell, S. D. 2010. Do questions reflecting indoor air pollutant exposure from a questionnaire predict direct measure of exposure in owner-occupied houses? *International journal of environmental research and public health,* 7**,** 3270-3297.

Lucas, R. M. & Mcmichael, A. J. 2005. Association or causation: evaluating links between" environment and disease". *Bulletin of the World Health Organization,* 83**,** 792-795.

Magnus, P., Nafstad, P., Oie, L., Carlsen, K. C., Becher, G., Kongerud, J., Carlsen, K. H., Samuelsen, S. O., Botten, G. & Bakketeig, L. S. 1998. Exposure to nitrogen dioxide and

the occurrence of bronchial obstruction in children below 2 years. *International Journal of Epidemiology,* 27**,** 995-9.

Marks, G. B. 2005. Identifying asthma in population studies: from single entity to a multi-component approach. *European Respiratory Journal,* 26**,** 3-5.

Marks, G. B., Ezz, W., Aust, N., Toelle, B. G., Xuan, W., Belousova, E., Cosgrove, C., Jalaludin, B. & Smith, W. T. 2010. Respiratory health effects of exposure to low-NOx unflued gas heaters in the classroom: a double-blind, cluster-randomized, crossover study. *Environ Health Perspect,* 118**,** 1476-82.

Martins, P. C., Valente, J., Papoila, A. L., Caires, I., Araujo-Martins, J., Mata, P., Lopes, M., Torres, S., Rosado-Pinto, J., Borrego, C., Annesi-Maesano, I. & Neuparth, N. 2012. Airways changes related to air pollution exposure in wheezing children. *Eur Respir J,* 39**,** 246-53.

Matsui, E. C., Hansel, N. N., Aloe, C., Schiltz, A. M., Peng, R. D., Rabinovitch, N., Ong, M. J., Williams, D. a. L., Breysse, P. N. & Diette, G. B. 2013. Indoor pollutant exposures modify the effect of airborne endotoxin on asthma in urban children. *American journal of respiratory and critical care medicine,* 188**,** 1210-1215.

Mead, M., Popoola, O., Stewart, G., Landshoff, P., Calleja, M., Hayes, M., Baldovi, J., Mcleod, M., Hodgson, T., Dicks, J., Lewis, A., Cohen, J., Baron, R., Saffell, J. & Jones, R. 2013. The use of electrochemical sensors for monitoring urban air quality in low-cost, high-density networks. *Atmospheric Environment,* 70**,** 186-203.

Melia, R., Florey, C., Altman, D. & Swan, A. 1977. Association between gas cooking and respiratory disease in children. *British medical journal,* 2**,** 149.

Melia, R. J., Florey, C. D., Chinn, S., Morris, R. W., Goldstein, B. D., John, H. H. & Clark, D. 1985. Investigations into the relations between respiratory illness in children, gas cooking and nitrogen dioxide in the U.K. *Tokai Journal of Experimental & Clinical Medicine,* 10**,** 375-8.

Melia, R. J. W., Du Ve Florey, C. & Morris, R. W. 1982. Childhood respiratory illness and the home environment. II. Association between respiratory illness and nitrogen dioxide, temperature and relative humidity. *International Journal of Epidemiology,* 11**,** 164-169.

Melia, R. J. W. F., C. V.; Chinn, S., Et Al. 1980. The relation between indoor air pollution from nitrogen dioxide and respiratory illness in primary schoolchildren. *Clinical Respiratory Physiology,* 16.

Mi, Y. H., Norback, D., Tao, J., Mi, Y. L. & Ferm, M. 2006. Current asthma and respiratory symptoms among pupils in Shanghai, China: influence of building ventilation, nitrogen dioxide, ozone, and formaldehyde in classrooms. *Indoor Air,* 16**,** 454-64.

Minelli, C., Wei, I., Sagoo, G., Jarvis, D., Shaheen, S. & Burney, P. 2011. Interactive effects of antioxidant genes and air pollution on respiratory function and airway disease: a HuGE review. *American Journal of Epidemiology,* 173**,** 603-20.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G. & Grp, P. 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Bmj-British Medical Journal,* 339.

Monn, C., Brandli, O., Schindler, C., Ackermann-Liebrich, U., Leuenberger, P. & Team, S. 1998. Personal exposure to nitrogen dioxide in Switzerland. *Science of the Total Environment,* 215**,** 243-251.

Monn, C., Carabias, V., Junker, M., Waeber, R., Karrer, M. & Wanner, H. U. 1997. Small-scale spatial variability of particulate matter <10 mu m (PM10) and nitrogen dioxide. *Atmospheric Environment,* 31**,** 2243-2247.

Moran, S. E., Strachan, D. P., Johnston, I. D. & Anderson, H. R. 1999. Effects of exposure to gas cooking in childhood and adulthood on respiratory symptoms, allergic sensitization and lung function in young British adults. *Clinical & Experimental Allergy,* 29**,** 1033-41.

Moskowitz, R. L., Lyons, H. A. & Cottle, H. R. 1964. Silo filler's disease: clinical, physiologic and pathologic study of a patient. *The American Journal of Medicine,* 36**,** 457-462.

Mukala, K., Alm, S., Tiittanen, P., Salonen, R. O., Jantunen, M. & Pekkanen, J. 2000. Nitrogen dioxide exposure assessment and cough among preschool children. *Archives of Environmental Health,* 55**,** 431-8.

Neas, L. M., Dockery, D. W., Ware, J. H., Spengler, J. D., Speizer, F. E. & Ferris, B. G., Jr. 1991. Association of indoor nitrogen dioxide with respiratory symptoms and pulmonary function in children. *American Journal of Epidemiology,* 134**,** 204-19.

Neas, L. M. W., J. H.; Dockery, D. W., Et Al. Association of Indoor Nitrogen Dioxide Levels with Respiratory Symptoms and Pulmonary Function in Children. Indoor air '90: Precedings of the 5th International Conference on Indoor Air Quality and Climate, Volume 1, Human Health, Comfort and Performance, 1990 Ottawa, ON, Canada. International Conference on Indoor Air Quality and Climate, Inc.,, 381-386.

Ng, T. P., Seet, C. S., Tan, W. C. & Foo, S. C. 2001. Nitrogen dioxide exposure from domestic gas cooking and airway response in asthmatic women. *Thorax,* 56**,** 596-601.

Nitschke, M. 1999. Respiratory health effects of nitrogen dioxide exposure and current guidelines. *International Journal of Environmental Health Research,* 9**,** 39-53.

Nitschke, M., Pilotto, L. S., Attewell, R. G., Smith, B. J., Pisaniello, D., Martin, J., Ruffin, R. E. & Hiller, J. E. 2006. A cohort study of indoor nitrogen dioxide and house dust mite exposure in asthmatic children. *Journal of Occupational and Environmental Medicine,* 48**,** 462-469.

Noy, D., Brunekreef, B., Boleij, J. S., Houthuijs, D. & De Koning, R. 1990. The assessment of personal exposure to nitrogen dioxide in epidemiological studies. *Atmospheric Environment. Part A. General Topics,* 24**,** 2903-2909.

Ogawa. Pompano Beach FL USA: OgawaUSA. Available: http://ogawausa.com/ [Accessed 05/06/2013.

Orehek, J., Massari, J., Gayrard, P., Grimaud, C. & Charpin, J. 1976. Effect of short-term, low-level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. *Journal of clinical investigation,* 57**,** 301.

Osman, L. M., Douglas, J. G., Garden, C., Reglitz, K., Lyon, J., Gordon, S. & Ayres, J. G. 2007. Indoor air quality in homes of patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine,* 176**,** 465-472.

Ostro, B. D., Lipsett, M. J., Mann, J. K., Wiener, M. B. & Selner, J. 1994. Indoor air pollution and asthma. Results from a panel study. *American journal of respiratory and critical care medicine,* 149**,** 1400-6.

Palmes, E. D. 1981. Development and application of a diffusional sampler for NO2. *Environment International,* 5**,** 97-100.

Paulin, L., Diette, G., Scott, M., Mccormack, M., Matsui, E., Curtin-Brosnan, J., Williams, D., Kidd-Taylor, A., Shea, M. & Breysse, P. 2014. Home interventions are effective at decreasing indoor nitrogen dioxide concentrations. *Indoor air,* 24**,** 416-424.

Pearce, N. & Douwes, J. 2013. Lifestyle changes and childhood asthma. *Indian journal of pediatrics,* 80 Suppl 1**,** S95-99.

Persinger, R. L., Poynter, M. E., Ckless, K. & Janssen-Heininger, Y. M. 2002. Molecular mechanisms of nitrogen dioxide induced epithelial injury in the lung. *Molecular and cellular biochemistry,* 234**,** 71-80.

Pilotto, L. S., Douglas, R. M., Attewell, R. G. & Wilson, S. R. 1997. Respiratory effects associated with indoor nitrogen dioxide exposure in children. *International Journal of Epidemiology,* 26**,** 788-796.

Pilotto, L. S., Nitschke, M., Smith, B. J., Pisaniello, D., Ruffin, R. E., Mcelroy, H. J., Martin, J. & Hiller, J. E. 2004. Randomized controlled trial of unflued gas heater replacement on respiratory health of asthmatic schoolchildren. *Int J Epidemiol,* 33**,** 208-14.

Platts-Mills, T. A. 1992. Dust mite allergens and asthma: report of a second international workshop. *Journal of Allergy and Clinical Immunology,* 89**,** 1046-1060.

Ponsonby, A. L., Glasgow, N., Gatenby, P., Mullins, R., Mcdonald, T., Hurwitz, M., Pradith, B. & Attewell, R. 2001. The relationship between low level nitrogen dioxide exposure and child lung function after cold air challenge. *Clinical & Experimental Allergy,* 31**,** 1205-1212.

Popoola, O. a. M. 2012. *Studies of urban air quality using electrochemical based sensor instruments.* University of Cambridge.

Quackenboss, J. J., Krzyzanowski, M. & Lebowitz, M. D. 1991. Exposure assessment approaches to evaluate respiratory health effects of particulate matter and nitrogen dioxide. *Journal of Exposure Analysis and Environmental Epidemiology,* 1**,** 83-107.

Quackenboss, J. J., Spengler, J. D., Kanarek, M. S., Letz, R. & Duffy, C. P. 1986. Personal exposure to nitrogen dioxide: relationship to indoor/outdoor air quality and activity patterns. *Environmental science & technology,* 20**,** 775-783.

Raaschou-Nielsen, O., Hermansen, M. N., Loland, L., Buchvald, F., Pipper, C. B., Sorensen, M., Loft, S. & Bisgaard, H. 2010. Long-term exposure to indoor air pollution and wheezing symptoms in infants. *Indoor Air,* 20**,** 159-67.

Rabe, K. F., Adachi, M., Lai, C. K., Soriano, J. B., Vermeire, P. A., Weiss, K. B. & Weiss, S. T. 2004. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. *Journal of Allergy and Clinical Immunology,* 114**,** 40-47.

Radiello. 2012. Fondazione Salvatore Maugeri IRCCS. Available: http://www.radiello.com/ [Accessed 05/06/2013 2013].

Rage, E., Siroux, V., Kunzli, N., Pin, I., Kauffmann, F., Epidemiological Study on The, G. & Environment Of, A. 2009. Air pollution and asthma severity in adults. *Occupational & Environmental Medicine,* 66**,** 182-8.

Rona, R. J. 2000. Asthma and poverty. *Thorax,* 55**,** 239-244.

Roorda-Knape, M. C., Janssen, N. A., De Hartog, J. J., Van Vliet, P. H., Harssema, H. & Brunekreef, B. 1998. Air pollution from traffic in city districts near major motorways. *Atmospheric Environment,* 32**,** 1921-1930.

Ross, D. 1996. Continuous and passive monitoring of nitrogen dioxide in UK homes. *Environmental Technology,* 17**,** 147-155.

Rothman, K. J. 1976. Causes. *American Journal of Epidemiology,* 104**,** 587-592.

Ryan, P. B., Soczek, M. L., Spengler, J. D. & Billick, I. H. 1988. The Boston residential NO2 characterization study: I. Preliminary evaluation of the survey methodology. *JAPCA,* 38**,** 22-27.

Saffell, J. R. & Dawson, D. H. 2004. Electrochemical gas sensor. Google Patents.

Saffell, J. R., Smith, R. & Dawson, D. H. 2010. Electrochemical sensor. Google Patents.

Sakai, K., Norback, D., Mi, Y. H., Shibata, E., Kamijima, M., Yamada, T. & Takeuchi, Y. 2004. A comparison of indoor air pollutants in Japan and Sweden: formaldehyde, nitrogen dioxide, and chlorinated volatile organic compounds. *Environmental Research,* 94**,** 75-85.

Samet, J. & Utell, M. 1990. The risk of nitrogen dioxide: what have we learned from epidemiological and clinical studies? *Toxicology and industrial health,* 6**,** 247-262.

Samet, J. M., Lambert, W. E., Skipper, B. J., Cushing, A. H., Hunt, W. C., Young, S. A., Mclaren, L. C., Schwab, M. & Spengler, J. D. 1993. Nitrogen dioxide and respiratory illnesses in infants. *American Review of Respiratory Disease,* 148**,** 1258-1265.

Samet, J. M., Marbury, M. C. & Spengler, J. D. 1987. Health effects and sources of indoor air pollution. Part I. *American Review of Respiratory Disease,* 136**,** 1486-1508.

Sanderson, S., Tatt, I. D. & Higgins, J. P. 2007. Tools for assessing quality and susceptibility to bias in observational studies in epidemiology: a systematic review and annotated bibliography. *International Journal of Epidemiology,* 36**,** 666-76.

Sarnat, J. A., Schwartz, J., Catalano, P. J. & Suh, H. H. 2001. Gaseous pollutants in particulate matter epidemiology: confounders or surrogates? *Environmental health perspectives,* 109**,** 1053.

Sarnat, S. E., Raysoni, A. U., Li, W.-W., Holguin, F., Johnson, B. A., Luevano, S. F., Garcia, J. H. & Sarnat, J. A. 2011. Air Pollution and Acute Respiratory Response in a Panel of Asthmatic Children along the U. S.-Mexico Border. *Environmental health perspectives,* 120**,** 437-444.

Schweizer, C., Edwards, R. D., Bayer-Oglesby, L., Gauderman, W. J., Ilacqua, V., Jantunen, M. J., Lai, H. K., Nieuwenhuijsen, M. & Kunzli, N. 2007. Indoor time-microenvironment-activity patterns in seven regions of Europe. *Journal of Exposure Science and Environmental Epidemiology,* 17**,** 170-181.

Scott, M., Diette, G., Mccormack, M., Matsui, E., Curtin-Brosnan, J., Williams, D., Breysse, P., Hansel, N. & Paulin, L. 2013. Home Interventions Are Effective At Decreasing Indoor Nitrogen Dioxide Concentrations. *Am J Respir Crit Care Med,* 187**,** A3507.

Seltenrich, N. 2014. Take care in the kitchen: Avoiding cooking-related pollutants. *Environmental Health Perspectives,* 122**,** A155-A159.

Sexton, K. 2005. Comparison of recruitment, retention, and compliance results for three children's exposure monitoring studies. *Journal of Exposure Science and Environmental Epidemiology,* 15**,** 350-356.

Sexton, K., Letz, R. & Spengler, J. D. 1983. Estimating Human Exposure to Nitrogen-Dioxide - an Indoor Outdoor Modeling Approach. *Environmental Research,* 32**,** 151-166.

Sharpe, R., Thornton, C. & Osborne, N. 2014. Modifiable factors governing indoor fungal diversity and risk of asthma. *Clinical & Experimental Allergy,* 44**,** 631-641.

Sheppard, L., Burnett, R. T., Szpiro, A. A., Kim, S.-Y., Jerrett, M., Pope Iii, C. A. & Brunekreef, B. 2012. Confounding and exposure measurement error in air pollution epidemiology. *Air Quality, Atmosphere & Health,* 5**,** 203-216.

Shima, M. & Adachi, M. 2000. Effect of outdoor and indoor nitrogen dioxide on respiratory symptoms in schoolchildren. *International Journal of Epidemiology,* 29**,** 862-70.

Shy, C. M., Creason, J. P., Pearlman, M. E., Mcclain, K. E., Benson, F. B. & Young, M. M. 1970. The Chattanooga School Children Study: Effects of Community Exposure to Nitrogen Dioxide: 1. Methods, Description of Pollutant Exposure, and Results of Ventilatory Function Testing. *Journal of the Air Pollution Control Association,* 20**,** 539-545.

Shy, C. M., Goldsmith, J. R., Hackney, J. D., Lebowitz, M. D. & Menzel, D. B. 1978. Health effects of air pollution. American Thoracic Society, New York. Scientific Assembly on Environmental and Occupational Health.

Sign. 2014. *SIGN 141 British guideline on the management of asthma* [Online]. Edinburgh: Scottish Intercollegiate Guidelines Network. Available: http://www.sign.ac.uk/index.html [Accessed 11/02/2015 2015].

Simoni, M., Carrozzi, L., Baldacci, S., Scognamiglio, A., Di Pede, F., Sapigni, T. & Viegi, G. 2002. The Po River Delta (North Italy) indoor epidemiological study: Effects of pollutant exposure on acute respiratory symptoms and respiratory function in adults. *Archives of Environmental Health,* 57**,** 130-136.

Simoni, M., Scognamiglio, A., Carrozzi, L., Baldacci, S., Angino, A., Pistelli, F., Di Pede, F. & Viegi, G. 2004. Indoor exposures and acute respiratory effects in two general population samples from a rural and an urban area in Italy. *Journal of Exposure Analysis and Environmental Epidemiology,* 14**,** S144-S152.

Smedje, G., Norback, D. & Edling, C. 1997. Asthma among secondary schoolchildren in relation to the school environment. *Clinical and Experimental Allergy,* 27**,** 1270-1278.

Smith, B. J., Nitschke, M., Pilotto, L. S., Ruffin, R. E., Pisaniello, D. L. & Willson, K. J. 2000. Health effects of daily indoor nitrogen dioxide exposure in people with asthma. *European Respiratory Journal,* 16**,** 879-85.

Sorensen, M., Loft, S., Andersen, H. V., Nielsen, O. R., Skovgaard, L. T., Knudsen, L. E., Ivan, V. N. B. & Hertel, O. 2005. Personal exposure to PM2.5, black smoke and NO2 in Copenhagen: relationship to bedroom and outdoor concentrations covering seasonal variation. *Journal of Exposure Analysis and Environmental Epidemiology,* 15**,** 413-422.

Spengler, J., Schwab, M., Mcdermott, A., Lambert, W. & Samet, J. 1996. Nitrogen dioxide and respiratory illness in children. Part IV: Effects of housing and meteorologic factors on indoor nitrogen dioxide concentrations. *Research report (Health Effects Institute)***,** 1-29; discussion 31-6.

Spicer, C. W., Kenny, D. V., Ward, G. F. & Billick, I. H. 1993. Transformations, lifetimes, and sources of NO2, HONO, and HNO3 in indoor environments. *Air Waste,* 43**,** 1479-85.

Sterne, J., Sutton, A., Ioannidis, J., Terrin, N., Jones, D., Lau, J., Carpenter, J., Rucker, G., Harbord, R., Schmid, C., Tetzlaff, J., Deeks, J., Peters, J., Macaskill, P., Schwarzer, G., Duval, S., Altman, D., Moher, D. & Higgins, J. 2011. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ (Online),* 343.

Sterne, J. A., Gavaghan, D. & Egger, M. 2000. Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. *J Clin Epidemiol,* 53**,** 1119-29.

Sterne, J. A., White, I. R., Carlin, J. B., Spratt, M., Royston, P., Kenward, M. G., Wood, A. M. & Carpenter, J. R. 2009. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *Bmj,* 338**,** b2393.

Stewart, A. W., Mitchell, E. A., Pearce, N., Strachan, D. P. & Weiland, S. K. 2001. The relationship of per capita gross national product to the prevalence of symptoms of asthma and other atopic diseases in children (ISAAC). *International journal of epidemiology,* 30**,** 173-179.

Strand, V., Rak, S., Svartengren, M. & Bylin, G. 1997. Nitrogen dioxide exposure enhances asthmatic reaction to inhaled allergen in subjects with asthma. *American journal of respiratory and critical care medicine,* 155**,** 881-7.

Stroup, D. F., Berlin, J. A., Morton, S. C., Olkin, I., Williamson, G. D., Rennie, D., Moher, D., Becker, B. J., Sipe, T. A., Thacker, S. B. & Grp, M. 2000. Meta-analysis of observational studies in epidemiology - A proposal for reporting. *Jama-Journal of the American Medical Association,* 283**,** 2008-2012.

Sunyer, J., Pekkanen, J., Garcia-Esteban, R., Svanes, C., Kunzli, N., Janson, C., De Marco, R., Anto, J. & Burney, P. 2007. Asthma score: Predictive ability and risk factors. *Allergy: European Journal of Allergy and Clinical Immunology,* 62**,** 142-148.

Sunyer, J., Puig, C., Torrent, M., Garcia-Algar, O., Calico, I., Munoz-Ortiz, L., Barnes, M., Cullinan, P. & Asthma Multicentre Infants Cohort, S. 2004. Nitrogen dioxide is not associated with respiratory infection during the first year of life. *International Journal of Epidemiology,* 33**,** 116-20.

Sutton, M. A., Howard, C. M., Erisman, J. W., Billen, G., Bleeker, A., Grennfelt, P., Van Grinsven, H. & Grizzetti, B. 2011. *The European nitrogen assessment: sources, effects and policy perspectives*, Cambridge University Press.

Swedish Environmental Research Institute Ivl. 2013. [Online]. Available: http://www.ivl.se/english/startpage.4.4a08c3cb1291c3aa80e80001374.html [Accessed 12/12/2014 2014].

Szpiro, A. A., Paciorek, C. J. & Sheppard, L. 2011. Does more accurate exposure prediction necessarily improve health effect estimates? *Epidemiology (Cambridge, Mass.),* 22**,** 680.

Szpiro, A. A., Sheppard, L., Adar, S. D. & Kaufman, J. D. 2014. Estimating acute air pollution health effects from cohort study data. *Biometrics,* 70**,** 164-174.

Tavernier, G., Fletcher, G., Francis, H., Oldham, L., Fletcher, A., Blacklock, G., Stewart, L., Gee, I., Watson, A. & Frank, T. 2005. Endotoxin exposure in asthmatic children and matched healthy controls: results of IPEADAM study. *Indoor air,* 15**,** 25-32.

Triche, E. W., Belanger, K., Bracken, M. B., Beckett, W. S., Holford, T. R., Gent, J. F., Mcsharry, J. E. & Leaderer, B. P. 2005. Indoor heating sources and respiratory symptoms in nonsmoking women. *Epidemiology,* 16**,** 377-384.

Tsujino, I., Kawakami, Y. & Kaneko, A. 2005. Comparative simulation of gas transport in airway models of rat, dog, and human. *Inhalation toxicology,* 17**,** 475-485.

Tunnicliffe, W. S., Burge, P. S. & Ayres, J. G. 1994. Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. *Lancet,* 344**,** 1733-6.

Valero, N., Aguilera, I., Llop, S., Esplugues, A., De Nazelle, A., Ballester, F. & Sunyer, J. 2009. Concentrations and determinants of outdoor, indoor and personal nitrogen dioxide in pregnant women from two Spanish birth cohorts. *Environment International,* 35**,** 1196-1201.

Van Strien, R. T., Gent, J. F., Belanger, K., Triche, E., Bracken, M. B. & Leaderer, B. P. 2004. Exposure to NO2 and nitrous acid and respiratory symptoms in the first year of life. *Epidemiology (Cambridge, Mass.),* 15**,** 471-478.

Venables, K. M. & Chan-Yeung, M. 1997. Occupational asthma. *The Lancet,* 349**,** 1465-1469.

Venn, A., Cooper, M., Antoniak, M., Laughlin, C., Britton, J. & Lewis, S. 2003. Effects of volatile organic compounds, damp, and other environmental exposures in the home on wheezing illness in children. *Thorax,* 58**,** 955-960.

Vos, T., Flaxman, A. D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., Shibuya, K., Salomon, J. A., Abdalla, S. & Aboyans, V. 2013. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet,* 380**,** 2163-2196.

Wang, M., Beelen, R., Eeftens, M., Meliefste, K., Hoek, G. & Brunekreef, B. 2012. Systematic evaluation of land use regression models for NO2. *Environmental science & technology,* 46**,** 4481-4489.

Wells Ga, S. B., O'connell D, Petersen J, Welch V, Losos M, Tugwell P. *The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses.* [Online]. Department of Epidemiology and Community Medicine, University of Ottawa, Canada. Available: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp [Accessed September 2014 2014].

Williams, D. E., Salmond, J., Yung, Y. F., Akaji, J., Wright, B., Wilson, J., Henshaw, G. S., Wells, D. B., Ding, G. & Wagner, J. Development of low-cost ozone and nitrogen dioxide measurement instruments suitable for use in an air quality monitoring network. Sensors, 2009 IEEE, 2009. IEEE, 1099-1104.

Wilson, A., Colome, S., Baker, P. & Becker, E. 1986. Residential indoor air quality characterization study of nitrogen dioxide. *Los Angeles, CA: Southern California Gas Company*.

World Health Organization 2006. Air quality guidelines: global update 2005: particulate matter, ozone, nitrogen dioxide, and sulfur dioxide. World Health Organization.

World Health Organization 2010. WHO guidelines for indoor air quality : selected pollutants. *In:* EUROPE, R. O. F. (ed.). Copenhagen: WHO Regional Office for Europe.

World Health Organization 2013. Review of Evidence on Health Aspects of Air Pollution–REVIHAAP Project. Technical Report, Regional Office for Europe, Copenhagen, Denmark.

World Health Organization. Regional Office for Europe. 2013. *"Review of evidence on health aspects of air pollution - REVIHAAP Project" First results,* Copenhagen, WHO Regional Office for Europe.

Yang, W., Lee, K. & Chung, M. 2004. Characterization of indoor air quality using multiple measurements of nitrogen dioxide. *Indoor air,* 14**,** 105-111.

Yeatts, K. B., El-Sadig, M., Leith, D., Kalsbeek, W., Al-Maskari, F., Couper, D., Funk, W. E., Zoubeidi, T., Chan, R. L., Trent, C. B., Davidson, C. A., Boundy, M. G., Kassab, M. M., Hasan, M. Y., Rusyn, I., Gibson, J. M. & Olshan, A. F. 2012. Indoor air pollutants and health in the United Arab Emirates. *Environ Health Perspect,* 120**,** 687-94.

Yu, C. H., Morandi, M. T. & Weisel, C. P. 2008. Passive dosimeters for nitrogen dioxide in personal/indoor air sampling: a review. *Journal of Exposure Science and Environmental Epidemiology,* 18**,** 441-451.

Zeger, S. L. & Liang, K. Y. 1986. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics,* 42**,** 121-30.

Zhang, G., Spickett, J., Rumchev, K., Lee, A. H. & Stick, S. 2004. Snoring in primary school children and domestic environment: a Perth school based study. *Respiratory Research,* 5**,** 19.

Zhang, Q., Gangupomu, R. H., Ramirez, D. & Zhu, Y. 2010. Measurement of ultrafine particles and other air pollutants emitted by cooking activities. *International journal of environmental research and public health,* 7**,** 1744-1759.

Zhang, X., Zhao, Z., Nordquist, T. & Norback, D. 2011. The prevalence and incidence of sick building syndrome in Chinese pupils in relation to the school environment: a two-year follow-up study. *Indoor Air,* 21**,** 462-71.

Zhao, Z. H., Zhang, Z., Wang, Z. H., Ferm, M., Liang, Y. L. & Norback, D. 2008. Asthmatic symptoms among pupils in relation to winter indoor and outdoor air pollution in schools in Taiyuan, China. *Environmental Health Perspectives,* 116**,** 90-97.

Zota, A., Adarnkiewicz, G., Levy, J. I. & Spengler, J. D. 2005. Ventilation in public housing: implications for indoor nitrogen dioxide concentrations. *Indoor Air,* 15**,** 393-401.

Appendix Chapter 2 – Extraction forms for the systematic review

INDOOR NITROGEN DIOXIDE
LEVEL 1 Extraction Form

EndNote ID First author: Title: Year of publication **1. Reviewed in Hasselblad?** y/n **2. Study design** Case –control Cross-sectional Intervention Birth cohort Cohort (not birth cohort) High risk cohort **3. Name of study (if applicable)** **4. Country** **5. Cities** **6. Enter details of location if necessary** (e.g. urban, rural, etc) **7. Number participants :** **8. Other details about participants (if applicable)** **9. Period and follow-up when study was carried out**	**Reviewed (date)** **Data entered (date)** Journal/Vol(no): **10. Outcomes definitions (if applicable)** **11. Adjusted for** List confounders **12. Enter details of gas appliance (type and percentage)** **13. Any estimate for gas appliance? y/n** **14. Any comments**

INDOOR NITROGEN
LEVEL 2 Extraction Form

EndoNote ID: **Enter the table/tables' number from which the following information has been extracted:**

		Access_id=	Access_id=	Access_id=	Access_id=	Access_id=
Outcome	Outcome as reported[1]					
	How is reported?[2]					
	Period[3]					
	Measure					
	Prevalence %					
	Combined effect?					
Participants	Age (yr)					
	Sex					
	Symptomatic - enter details[4]					
	Family history of atopy[5]					
	Stratified by					
Exposure mearsumrent	Exposure					
	Range[6]					
	Instrument					
	Which rooms?[7]					
	How long for?[8]					
	How often?[9]					
	When?[10]					
Analysis	[11] Statistical method[12]					
	Type of estimate					
	Increment[13] and Unit					
	Effect estimate					
	95% LCI, 95% UCI (or SE)					

[1] E.g.Wheeze, persistent wheeze, asthma, evening PEFR, number of days with wheeze, etc
[2] E.g. Doctor dx, questionnaire, diary, measurement, etc.
[3] E.g. During first year of life, during the year while indoor NO2 measurements were done, month before NO2 measurement, etc
[4] Enter whether asthmatic, atopic, etc.
[5] Enter details, e.g. mother, sibling, etc
[6] Enter min/max/medium (with units) or as reported in the paper
[7] Enter in which room/rooms the measuring instrument was located
[8] Enter how long each reading was carried out (e.g. 1 wk, 2 wk)
[9] Enter how often the measurements were taken (e.g. twice, 6 times)
[10] Enter when the measurements were carried out (e.g. over a year, summer and winter, etc.)
[11] Enter whether it is RR, OR, difference, IRR (Incidence Risk Rate), etc
[12] Enter the statistical method used, enter details if Poisson regression was used (e.g. GAM Poisson regression, GEE Poisson regression with smoothing spline, etc)
[13] Enter the increment the effect has been calculated for (e.g. 10 ppb, 20 ppb, high versus low, 21-40ug/m3 vs 41-60 ug/m3, etc)

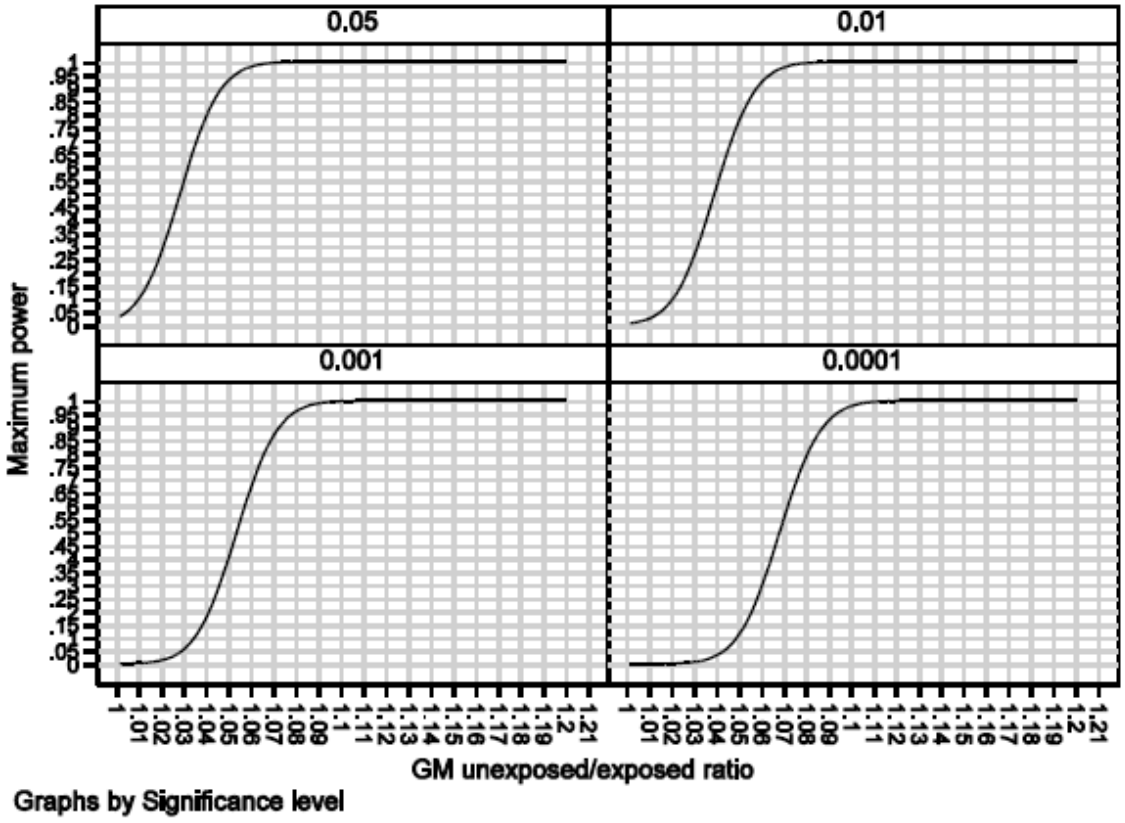
Appendix Chapter 4 – Power calculation

Power calculations for proposed repeated-measures study

Roger Newson, 5 Dec 2012

"Please find attached a version of the power calculation, based on the conservative coefficient of variation of 0.34 from the Franklin paper and a conservative zero intraclass correlation, with 4 unexposed measurements and 2 exposed measurements for each of 20 subjects. Under these conservative assumptions, 20 subjects should give us the power to detect an unexposed/exposed GM ratio of 1.1, corresponding to a 10 percent increase from exposed to unexposed.

Figure S1. Power by geometric mean unexposed/exposed ratio for 20 subjects assuming 2 exposed and 6 unexposed measurements each, a CV of .34 and an intraclass log correlation of 0



Appendix Chapter 4 – Main questionnaire

WINAP - INTERVIEW-LED QUESTIONNAIRE v3 02/05/13

I AM GOING TO ASK YOU SOME QUESTIONS. AT FIRST THESE WILL BE MOSTLY ABOUT YOUR ASTHMA, THEN YOUR KITCHEN AND YOURSELF. WHEREVER POSSIBLE, I WOULD LIKE YOU TO ANSWER 'YES' OR 'NO'.

DATE: ____/_____/_____ **ID:**

ABOUT YOUR ASTHMA

		NO	YES
1.	Have you had wheezing or whistling in your chest at any time in the last year?	☐	☐
2.	Have you woken up with a feeling of tightness in your chest at any time in the last year?	☐	☐
3.	Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last year?	☐	☐
4.	Have you had an attack of shortness of breath that came on following strenuous activity at any time in the last year?	☐	☐
5.	Have you been woken by an attack of shortness of breath at any time in the last year?	☐	☐

6. What medication do you take for your asthma?

NAME	DOSE (unit)	x TIMES/ DAY

		NO	YES
7.	Have you visited a hospital department because of breathing problems in the last year?	☐	☐
8.	Do you have any nasal allergies, including hay fever?	☐	☐
9.	Do you have eczema or any kind of skin allergy?	☐	☐
10.	When you are near animals or trees or grass or flowers or in a dusty part of the house do you ever start of cough, wheeze, become short of breath, get a runny or stuffy nose, get itchy or watery eyes?	☐	☐

WINAP - INTERVIEW-LED QUESTIONNAIRE v3 02/05/13

ABOUT YOUR HOME

11. When was your present home built? (YEARS)
If not sure, please write in your best guess.

12. Is the building in which you live? (NO / YES)
   a. a one family house detached from any other house?
   b. a one family house attached to one or more houses?
   c. a building for two or three families?
   d. a building for three or more families?
   e. other _____

13. How many rooms does your home have? (NUMBER)
(excluding kitchen, bathroom, toilet, laundry)

14. How many people live in your home? (NUMBER)

15. Which type of cooking stove do you use? (NO / YES)
   a. electric hob/electric oven
   b. gas hob/gas oven
   c. gas hob/electric oven
   d. other

If **YES** in 15.b or 15.c
15.1. Does your cooker have constantly lit pilot light? (NO / YES)

15.2. Does your cooker have a gas grill? (NO / YES)

15.2.1. If **YES**, is it at eye level? (NO / YES)

15.3 When was the last time your gas cooker was serviced by a gas engineer? (NO / YES)
   a. In the last year?
   b. In the last 2 years (but not the last year)?
   c. In the last 5 years (but not the last two years)?
   d. Never
   e. Don't know

16. How old is your cooker? (YEARS)
If not sure, please write in your best guess.

17. How many hobs has your cooker got? (NUMBER)

18. How wide is your cooker? (NO / YES)
   a. less than 50 cm
   b. 50-55 cm
   c. 56-60 cm
   d. larger than 60 cm

WINAP - INTERVIEW-LED QUESTIONNAIRE v3 02/05/13

		NO	YES
19.	Are you the person who does most of the cooking at home?	☐	☐
		NUMBER	
20.	How many people do you regularly cook for?	☐☐	
		NUMBER	
21.	On how many days a week do you normally cook at home?	☐☐	
22.	On average how long do you spend cooking on those days?	NO	YES
	a. less than 30 minutes	☐	☐
	b. between 30 min- 1 hour	☐	☐
	c. more than a hour	☐	☐
23.	Does your kitchen have	NO	YES
	a. a window to the outside?	☐	☐
	b. a door that open to the outside?	☐	☐
	If **YES** for 23.a. or 23.b.		
	23.1. Over the last month when you were cooking did you have them open	NO	YES
	a. most of the time?	☐	☐
	b. some of the time?	☐	☐
	c. never?	☐	☐
24.	Do you have an extractor fan over the cooker?	NO ☐	YES ☐
25.	When cooking do you used the fan	NO	YES
	a. all of the time?	☐	☐
	b. some of the time?	☐	☐
	c. none of the time?	☐	☐
	d. Not applicable *(I don't have a fan)*	☐	☐
26.	Does the fan take the fumes outside the house?	NO ☐	YES ☐ DK ☐
27.	Does your home have a central heating?	NO ☐	YES ☐
28.	Which appliances do you use for heating and hot water?	NO	YES
	a. gas-fired boiler (located inside home)	☐	☐
	b. gas-fired boiler (located outside home)	☐	☐
	c. electric heater	☐	☐
	d. open gas fire	☐	☐
	e. portable gas fire	☐	☐
	f. other _____	☐	☐
29.	Within the last year have you had wet or damp spots on surfaces in your kitchen (e.g. wall, ceiling, carpet)?	NO ☐	YES ☐

WINAP - INTERVIEW-LED QUESTIONNAIRE v3 02/05/13

		NO	YES
30.	Have you seen or smelt any mould or mildew in your kitchen (excluding food) in the last year?	☐	☐
31.	Do you keep a cat or a dog or a bird pet in the house?	☐	☐

ABOUT YOURSELF

		YEARS		
32.	At which age were you diagnosed with asthma?	☐☐		
		NO		YES
33.	Have you ever smoked?	☐		☐
	33.1. How old were you when you gave up smoking?	☐☐		
		NO		YES
34.	Do people in your household smoke inside the home?	☐		☐
		NO	YES	DK
	34.1. If **YES,** Do they ever smoke in the kitchen?	☐	☐	☐

		NO	YES
35.	What best describe your current status?		
	1. full-time house person	☐	☐
	2. house duties with job (less than 8 hours per week)	☐	☐
	3. house duties with job (9-20 hours week)	☐	☐
	4. house duties with job (more than 20 hours per week)	☐	☐
	5. unemployed	☐	☐

36. If **YES** for 35.2 or 35.3 or 35.4
    36.1. What is this job? _____
    If **YES** for 35.1 or 35.5
    36.2. What was your last job? _____

Date: _____/_____/_____ **Interviewer:** _____

Appendix Chapter 4 – PIS Form

Imperial College London

Respiratory Epidemiology and Public Health
Imperial College London

Emmanuel Kaye Building, Manresa Road
National Heart & Lung Institute
London SW3 6LR
Tel: +44 (0)207 352 8121 ext 3510
Fax: +44 (0) 207 351 8322
Secretary (Hilary Barton) 0207 352 8121 x 3506

www.imperial.ac.uk/nhli/respiratory/popgenetics/reph/

Participant Information Sheet for Screening Questionnaire

The Women and Indoor Air Pollution Study – WINAP

(A Pilot Study)

Dear Madam

We invite you to fill in a screening questionnaire for the Women and Indoor Air Pollution Study (WINAP). You have recently contacted us showing an interest in participating in our study and we are now sending you a questionnaire enclosed with this pack. Before you decide to fill it in, it is important for you to understand why we are sending the screening questionnaire and why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish.

If you decide to fill in the questionnaire, please send the completed questionnaire back to us using the enclosed pre-addressed envelope.

What is the purpose of the screening and the study?
The purpose of the screening questionnaire is to see whether you have all the criteria that make you a suitable participant in this research study.

The purpose of the study is to collect information from 20 women with mild/moderate asthma who regularly cook at home. We want to see if their asthma worsens when they are exposed to some air pollutants present in kitchens. This is a pilot study. It means that only a small number of participants are recruited because we want to see whether the study design works before carrying out a larger study with more participants. This study is part of a student research project.

What does the study involve?
The whole study will last one year, but you will be involved in the study for eight weeks only. You will be provided with small air monitors, weekly diaries, a peak flow meter and pre-stamped Jiff bags. The air monitors will be positioned on a shelf in the kitchen and replaced by the participant herself every week. You will measure your own peak flow every morning and evening and record the readings in the diary. In this diary you will also score their respiratory symptoms, reliever usage and the time you cook every morning and evening for 8 weeks. You will be in contact with research staff by phone every week. At the end of every week you will send the air monitor to the research department by post along with the completed weekly diary.

Patient Information Sheet – Screening questionnaire: WINAP	Version 3 020513

Why have I been sent the questionnaire?
You have been sent the questionnaire because you have shown an interest in participating in the study.

Do I have to fill in the questionnaire?
It is up to you to decide to fill in the questionnaire and which questions to answer or not. We will be able to decide whether you can join in the study only from the information you will give us in the questionnaire.

If you are eligible, we are going to send you further information that will help you make a decision whether to take part or not in the study. If you decide to take part you are still free to withdraw at any time and without giving a reason. If anything is unclear, please contact us (see details below).

.

Will my screening questionnaire be kept confidential?
Yes. All the information about your screening questionnaire will be kept strictly confidential according to the Data Protection Act 1998. All the personal information will be securely stored for 3 years after which it will be destroyed. No information that identifies you will be transmitted outside Imperial College. Only authorised researchers will have access to identifiable data. Neither your name nor any other identifiable information will be kept with the results of the tests or will appear in any publication or presentation related to this study.

Who is organising and funding the research?
This study is funded using internal departmental funding within the Department of Respiratory Epidemiology and Public Health Group.

Who has reviewed the study?
This study was given a favourable ethical opinion by **xxx REC (insert reference number)**

Contacts for Further Information:
If you need any help or have any questions, do not hesitate to contact the Research Team:

Ms Graziella Favarato
Respiratory Epidemiology and Public Health Group
Imperial College London
National Heart & Lung Institute
Emmanuel Kaye Building
Manresa Road
London SW3 6LR
Tel: 02073528121 ext. 3508
Mobile: 07703889083
E-mail: g.favarato11@imperial.ac.uk

Thank you for considering taking time to read this sheet and filling in the questionnaire!

Appendix Chapter 4 – Informed consent form

Imperial College London

Respiratory Epidemiology and Public Health
Imperial College London

Emmanuel Kaye Building, Manresa Road
National Heart & Lung Institute
London SW3 6LR
Tel: +44 (0)207 352 8121 ext 3510
Fax: +44 (0) 207 351 8322
Secretary (Hilary Barton) 0207 352 8121 x 3506

www.imperial.ac.uk/nhli/respiratory/popgenetics/reph/

Study Protocol Number: xxx

INFORMED CONSENT FORM

Title of Study: Short-term respiratory health effects and indoor nitrogen dioxide exposure in a panel of asthmatic women – The Women and Indoor Air Pollution Study - WINAP

Name of Principal Investigator: Prof Debbie Jarvis

Please ask participant to tick each box below to indicate consent to EACH statement.
Please cross out any sections that the participant does NOT consent to.

	Please initial box
1. I confirm that I have read and understand the information sheet dated xx/xx/xxx version xxxx for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.....	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.....	☐
3. I agree to take part in the above study.....	☐

Name of /Participant	Signature	Date
Name of Person taking consent	Signature	Date

When completed, 1 copy for participant, 1 copy for Principal Investigator, 1(original) for researcher file

Appendix Chapter 4 – Letter from GP to patient informing about pilot study

Imperial College London

NHS headed paper

GP headed paper /NHS headed paper

Date xxxxxxx

Dear Madam,

Can you help researchers at Imperial College London to understand how cooking / indoor air pollution affects asthma?

Researchers at Imperial College London are conducting a study to find out if women with asthma get worse asthma symptoms when they come into contact with certain air pollutants present in kitchens – for example fumes from cooking. I am writing to you because these researchers would like to recruit participants for their study from this practice – and you may be eligible.

The enclosed leaflet describes the study. The study takes 8 weeks, and during this time you would need to keep a symptoms and peak flow diary. Small air pollution monitors would be placed in your kitchen. Research workers will visit your home three times over the 8 week period, at a time convenient to you.

Before making any decision please read the information enclosed with this letter. If you like to know more and talk with one of the researchers you can call Graziella Favarato on 020 759 47943 or email her at G.Favarato11@imperial.ac.uk .

Yours faithfully,

Dr xxxxxx

Appendix Chapter 4 – Ethical approval

Imperial College London

Imperial College Research Ethics Committee

Imperial College London
Room 5L10D, 5th Floor, Lab Block
Charing Cross Hospital
Fulham Palace Road
London
W6 8RF

Tel: +44 (0)203 331 0208 Fax: +44 (0) 203 311 0203

researchethicscommittee@imperial.ac.uk

9 May 2013

Debbie Jarvis
Professor of Public Health
Emmanuel Kays Building
Manresa Road
National Heart and Lung Institute
Imperial College
London SW3 6LR

Dear Professor Jarvis,

Study Title: Short term respiratory health effects and indoor nitrogen dioxide exposure in a panel of asthmatic women: The Women And Indoor Air Pollution Study (a pilot study)

ICREC reference: ICREC_13_2_10

The above study was approved by your HoD on 4th March 2013 and by the Joint Research Office on the 8th May 2013.

Under the Imperial College Research Ethics Committee process, a study that has been reviewed by the Joint Research Office and Head of Division/Department (or Principal), where no significant ethical issues have been identified in the protocol or ethics application, can be approved without requiring it to go to full committee.

Documents
The documents reviewed were:

- ICREC Application form
- Research Proposal and Protocol Version 4 2/05/13
- Recruitment Advertisement Version 2 3/04/13
- Participant Information Sheet Version 2 3/04/13
- Participant Information Sheet (screening questionnaire) Version 3 2/05/13
- Participant Consent Form Version 2 3/04/13
- Interview Led Questionnaire Version 3 2/05/13
- Screening Questionnaire Version 2 2/03/2013
- WINAP Weekly Symptom Diary Version 1 18/2/13
- Asthma UK Blurb
- Sponsorship and Insurance registration form

Yours sincerely,

Gary Roper,
Head of Regulatory Compliance,
Imperial College London

West London Primary Care Research Consortium

NHS Brent CCG

Wembley Centre for Health & Care
116 Chaplin Road
Wembley
Middlesex
HA0 4UZ

Tel: 020 8795 6730/5

Email: ricky.banarsee@brentpct.nhs.uk

Prof Debbie Jarvis
Emmanuel Kaye Building
National Heart and Lung Institute
Manresa Rd
London
SW3 6LR

28 November 2013

Dear Debbie

Project Title:	Short-term respiratory health effects and indoor nitrogen dioxide exposure in a panel of asthmatic women: The Women and Indoor Air Pollution Study (pilot study)
REC	13/SC/0560
Portfolio No	Not applicable IRAS 124799 (PIC)

The West London Primary Care Consortium (WLPC) is the lead Research Governance (RG) office for the North West London CCGs and GP practices/pharmacists/dentists.

NHS RG assurance for the above research has been given on the basis described in the application form and supporting documentation approved by an NHS Research Ethics Committee (REC) subject to the conditions listed below and overleaf. Assurance is given on the understanding that the study is conducted in accordance with the Research Governance Framework and NHS Trust policies and procedures. Assurance is only granted for the activities for which a favourable opinion has been given by the REC.

Please note that the ultimate decision as to whether to take part in this PIC activity lies with the Primary Care Independent Contractor. The study team must get written agreement from each GP site confirming their decision to take part in this study.

This assurance, on behalf of the CCGs in North West London, covers our GP practices/services in Hammersmith & Fulham. Please give a copy of this letter to each participating site.

If you require any further information or advice, do not hesitate to contact Sylvia Westrup our Research Governance & Management Manager (s.westrup@imperial.ac.uk)

With kind regards

Ricky Banarsee
Director WLPC/Applied Research Unit
Sent via email
Chief Investigator: d.jarvis@imperial.ac.uk
PhD Student; g.favarato11@imperial.ac.uk
Sponsor: c.buicke@imperial.ac.uk
NWL CSO: shilpi.mehra@nhs.net

Appendix Chapter 4 – Comments from Facebook

Asthma UK Facebook (alive on 16/07/13)

Can you help us to understand how cooking/indoor air pollution affects asthma? Researchers at Imperial College London are collecting information from women with asthma to find out if their asthma symptoms get worse when they come into contact with certain air pollutants in kitchens – for example fumes from cooking. If you're a woman with mild to moderate asthma and you cook regularly at home, you could participate in the study and help us to understand asthma symptoms in women. Due to the limited resources of the researchers, they're currently recruiting only women who live in London. To find out more about the study and to participate, please contact the researcher Graziella Favarato by emailing: G.Favarato11@imperial.ac.uk Thank you for your help!

22 people like it

Asthma UK reply after comment (written with GF and DJ consent)

Thanks for your comments everyone – you've raised some really interesting issues and the researchers have taken them on board. They wanted to let you know a bit about the background of the study. This is a pilot project which will gather initial information to feed into a larger project in the future. The reason they are looking at the effects of indoor pollution / cooking on women is because there is some evidence to suggest that women are affected more than men and have more severe symptoms. We don't know why this happens, and this project might explain the differences between the effects on men and women. This study may be taken forward to a larger study which could involve a wider group of people, including men and people with severe asthma. We're really grateful for everyone's feedback so far, and we hope that some of you might be interested in taking part in this project.

Comments

1. I wonder why only women? Will try research men after for comparison?
2. I agree. A little bit sexist, this. Men cook too. The best chefs in the world are men.
3. I have bad reactions to flash bathroom spray
4. My son is affected by change in air cooking does it ,any aerosols, barbecues ect also when were in hospital and we walked near a coffee shop that set him off so its not just females.
5. Airfreshners, most cleaning products, anything with a floral fragrance especially roses, anything smoking ie the oven/rings if something has spilled, even hovering will trigger an attack..... I think I need a cleaner ha ha ha.
6. I work in catering and steam sets me off.
7. Def deodorants and bleach affect me and some foods especially flour related bread and cakes !
8. Bleach, oven cleaner, some air freshners, heavy perfume. Sometimes steam can aggravate it. (Although I suppose steam should help not harm - not sure).
   a. Steam aggravates my asthma too
   b. Thanks Isabel
9. Cooking hot spicy food affects me badly. Chilies, jalapenos sometimes even ground spices
10. I get bad when its hot or cold weather it seems fine when it's just in between the both when my boys spray things it starts it off or bed time kills me when I lay flat I need loads of pillows but I am bad I am on a nebuliser at home feel sometimes like I am suffocating
11. Spray on deodorants and hair spray affect me. Also air fresheners
12. most air fresheners make me really bad, as do joss sticks and scented candles, also bleach and anything rose scented
13. Some commercial air freshners & strong perfumes affect me and I am not Asthmatic, but occasionally get bronchitis

14. A lot of our customers have told us they're suffering more and more indoors - we suppose as air pollution gets worse outdoors it's creeping in more and more! We've sold out of air purifiers, expecting more stock soon, as we've got over 50 customers waiting, we've shared the post and are hoping one of our followers may be able to help!

15. Deodorants, hairspray, several perfumes and laundry detergents affect me. Plug-in air fresheners are another bad one. I don't think general cooking smells really aggregate me, except if I burn something. I'm well up north as well though so can't take part in this study either

16. **Everytime I cook on the hob, my asthma starts and I cannot stop coughing. I am in Somerset so can't take part in the survey**.

17. such a shame I don't live in london I cook everyday as I'm a chef and sometimes do find it hard especially in heat and when I'm too hot in bed I need loads of pillows to feel like I'm sat up otherwise I feel like someone is strangling me because my chest and back tighten so bad x

18. Thats a shame I m also in Yorkshire and would have been interested in getting involved

19. for brittle asthmatic 17yr old daughter every thing others have said and its 24 /7 all yr round what ever the weather very often do i see her with out a nebuliser mask on i feel for you all

20. welll I live in yorkshire but I have asthma and copd thats effected by diffrent food products and other things

21. doesn't effect me at all

22. air freshners effect me too and so do most cleaning products

23. Cooking in late autumn to spring sets me and my daughter's asthma off regular .opening a window /back door doesnt help as it seems to happen more when the air is more damp which then makes it much worse

24. Only cooking that bothers me is bbq, we don't do that ourselves but have to keep doors & windows shut when other people are having them. Luckily I don't burn things (usually!) So no smoke in kitchen

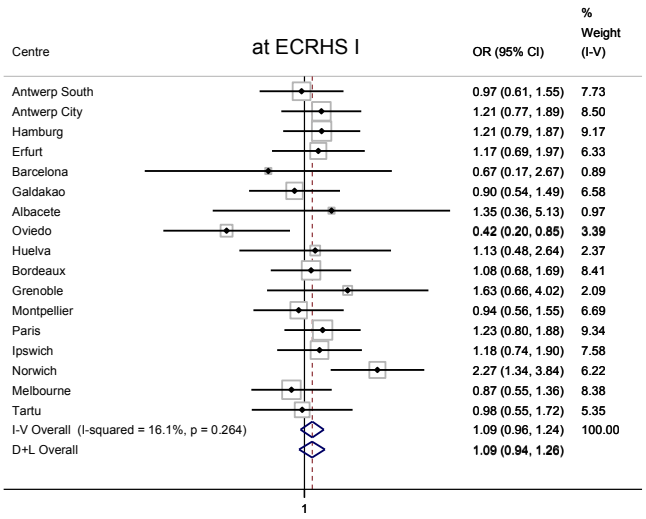
25. I'm pretty sure that men are affected by this and not just women. I have to be very careful what cleaning products I use.

26. What do you class as moderate asthma lol

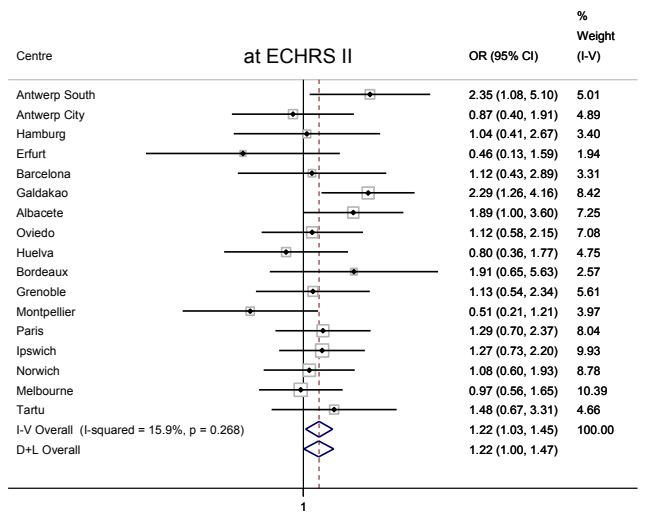
27. Also not in London but would be interested. I find chilli in particular and dry frying/toasting spices sets me off.

This lady emailed me on the same day as the advert appeared on facebook (she does not say where she lives exactly – only north england)
Hi Graziella, I noticed on Facebook that you are looking for female participants to help with your study on how cooking smells affect asthma. Unfortunately, I do not live in London but I am very interested in your study. I am female and have moderate asthma which is affected by cooking smells. I cook most days of the week but I have to adapt what I cook and how I cook a meal. For example, I can't cook anything that contains chilli especially if it is fried or baked. Grilling food can also be a problem at times and I always keep an inhaler in the kitchen just in case of having an attack. In general, I tend to slow cook food to reduce the chance of having an asthma attack. I am an undergraduate, mature student in my final year of study and I would like to help your study in any way that I can. Regards, xxx.

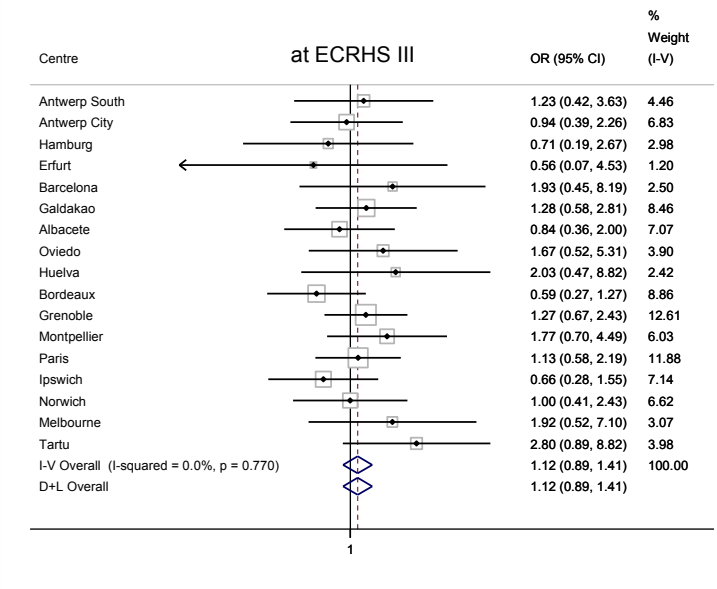
Appendix Chapter 6 – Forest plots of the associations of wheeze and gas by centre and survey



Forest plot showing the odds ratio of having wheeze when cooking mostly with gas rather than electricity at <u>ECRHS I</u> by centre adjusted for sex, age, smoking status and age left education in all cases

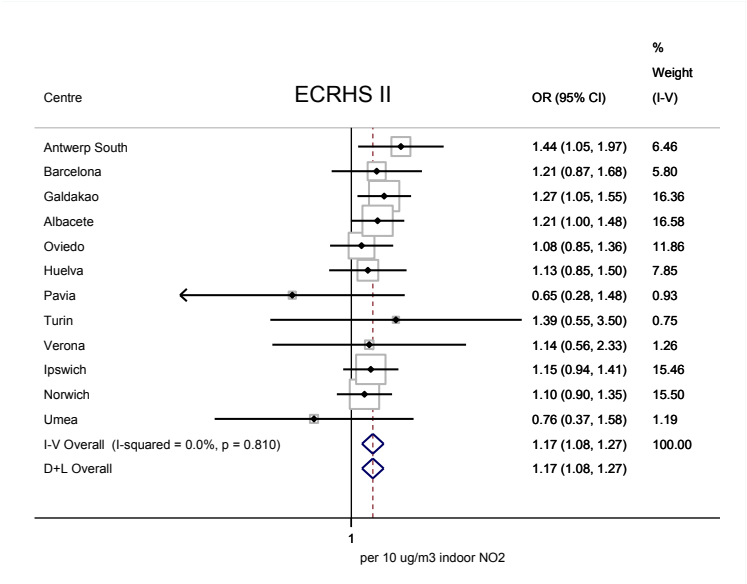


Forest plot showing the odds ratio of having wheeze when cooking mostly with gas rather than electricity at <u>ECRHS II</u> by centre adjusted for sex, age, smoking status and age left education in all cases

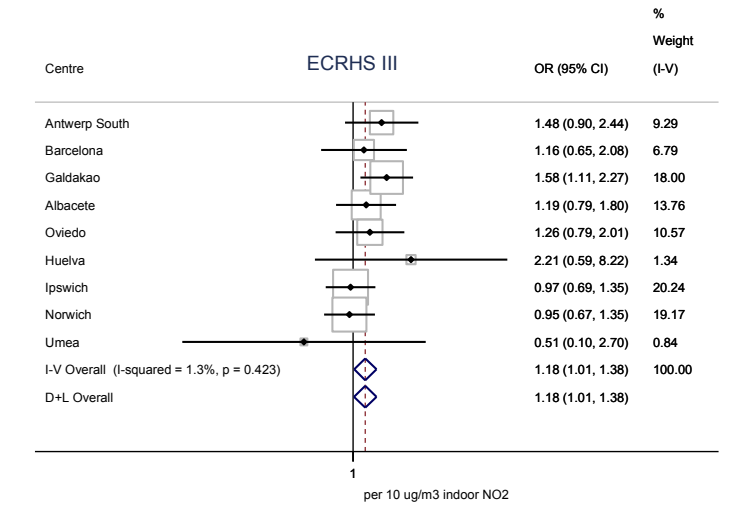


Forest plot showing the odds ratio of having wheeze when cooking mostly with gas rather than electricity at <u>ECRHS III</u> by centre adjusted for sex, age, smoking status and age left education in all cases

Appendix Chapter 6 – Forest plots of the associations of wheeze and modelled indoor NO_2 by centre and survey

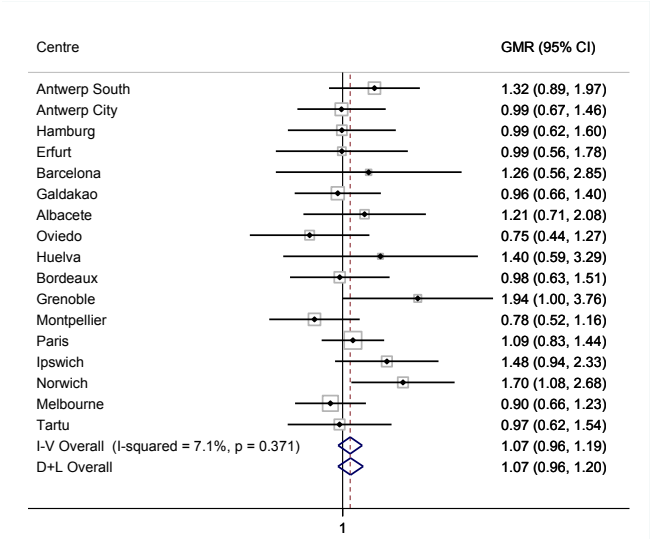


Forest plot showing odds ratio of having wheeze per 10 µg/m^3 increase of <u>modelled</u> indoor NO_2 in the month of October at <u>ECRHS II</u> by centre adjusted for sex, age, smoking status and age left education with random effect by centre in all cases

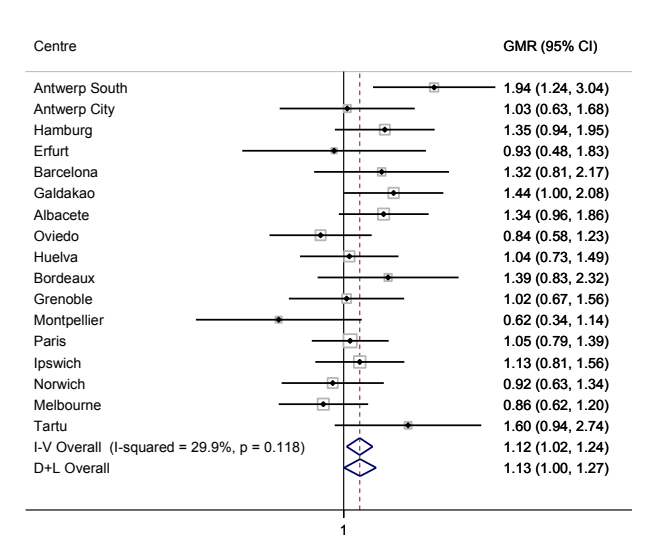


Forest plot showing the odds ratio of having wheeze per 10 µg/m^3 increase of <u>modelled</u> indoor NO_2 in the month of October at <u>ECRHS III</u> by centre adjusted for sex, age, smoking status and age left education with random effect by centre in all cases

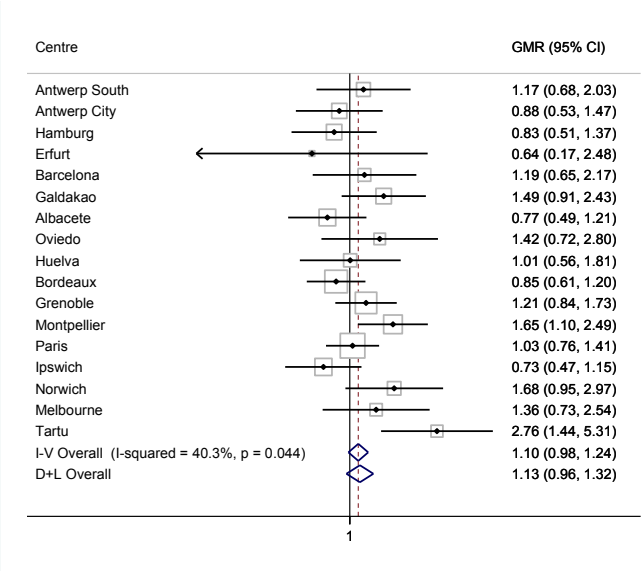
Appendix Chapter 6 – Forest plots of the associations of asthma score and gas by centre and survey



Forest plot showing the geometric means ratio of having a higher asthma score when cooking mostly with gas rather than electricity at <u>ECRHS I</u> by centre adjusted for sex, age, smoking status and age left education in all cases

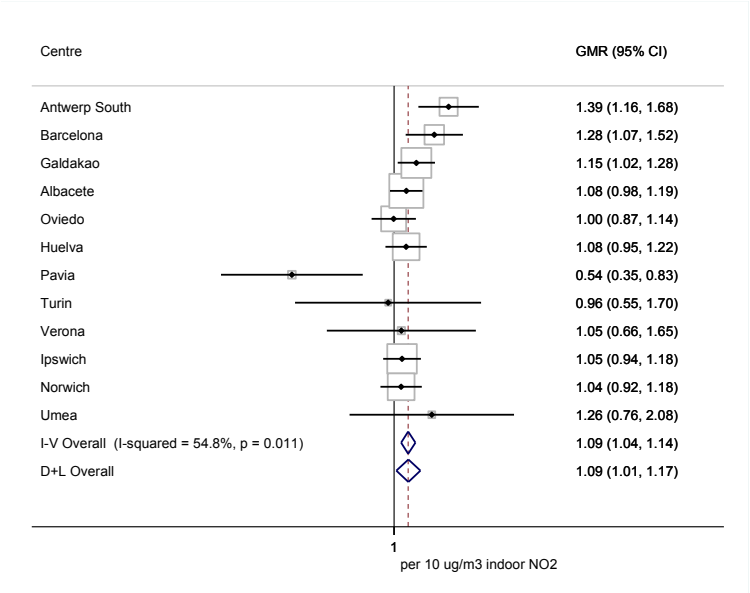


Forest plot showing the geometric means ratio of having a higher asthma score when cooking mostly with gas rather than electricity at <u>ECRHS II</u> by centre adjusted for sex, age, smoking status and age left education in all cases

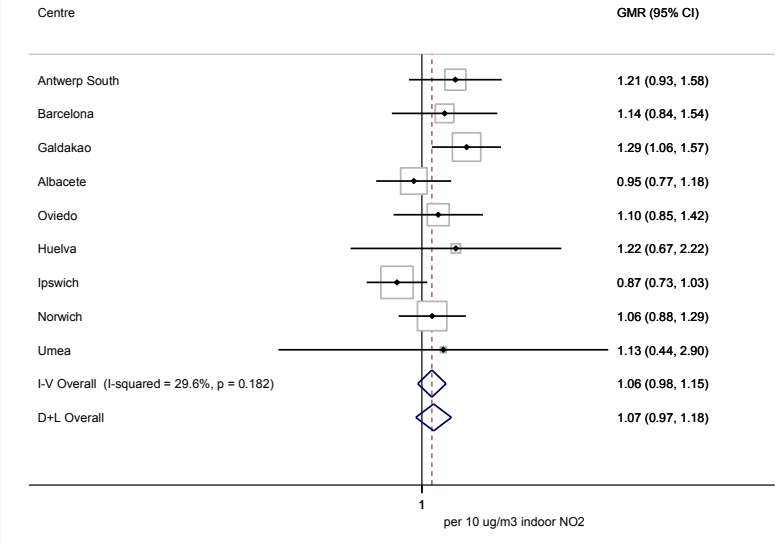


Forest plot showing the geometric means ratio of having a higher asthma score when cooking mostly with gas rather than electricity at <u>ECRHS III</u> by centre adjusted for sex, age, smoking status and age left education in all cases

Appendix Chapter 6 – Forest plots of the associations of asthma score and modelled indoor NO_2 by centre and survey



Forest plot showing the geometric means ratio of having a higher asthma score per 10 µg/m^3 increase of <u>modelled</u> indoor NO_2 in the month of October at <u>ECRHS II</u> by centre adjusted for sex, age, smoking status and age left education with random effect by centre in all cases



Forest plot showing the geometric means ratio of having a higher asthma score per 10 µg/m^3 increase of <u>modelled</u> indoor NO_2 in the month of October at <u>ECRHS III</u> by centre adjusted for sex, age, smoking status and age left education with random effect by centre in all cases