A Randomised Controlled Trial Investigating The Effects Of Nitrogen Dioxide In Classrooms On The Respiratory Health Of Asthmatic Primary School Children.

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Statement

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Abstract

A Randomised Controlled Trial Investigating The Effects Of Nitrogen Dioxide On Asthmatic Children In Primary School Classrooms

(356 words)

The aim of this study was to determine the effects of a randomised controlled trial of unflued gas heater replacement on asthma in children.

18 schools (134 classrooms) using unflued gas heaters in winter were randomly allocated an intervention of heater replacement with either flued gas heaters (4), or electric heating (4), or remained unflued (10). The main eligibility criteria were (i) doctor diagnosed asthma with (ii) no unflued gas sources at home (a priori sample). The sample was extended to asthmatic children with home gas cooking (extended sample). Participants kept a daily diary of symptoms for 12 weeks in order to establish symptom rates in the intervention and control groups. Lung function and bronchial hyper-responsiveness (BHR) tests were performed at the beginning and end of the study period. Indoor NO_2 was monitored in classrooms and homes during the study period.

Mean NO_2 exposure was significantly lower in intervention schools (15.5 ppb SD:6.6) compared to control schools (47.0 ppb SD:26.8). Mean kitchen NO_2 levels were significantly lower in the a priori sample compared to the extended sample (14.3 ppb CI:10.3-18.3 vs 28.7 ppb CI: 24.1-33.3; $p<0.001$).

In the a priori sample there were 45 and 73 children in the intervention and control groups respectively, and 43 in each group in the extended sample.

In the a priori sample, difficulty breathing (RR: 0.32; CI: 0.14-0.69), chest tightness (RR: 0.45; CI:0.25-0.81), and asthma attack (RR: 0.39; CI:0.17-0.93) rates were significantly decreased in the intervention group compared to the control group. In the extended group, symptom rates were not significantly different. Mean %predicted $FEV_1\%$ and BHR were similar between intervention and control groups.

Significantly reduced NO_2 levels in classrooms were accompanied by more than a 50% reduction in some asthmatic symptoms in the intervention a priori group. This was not found in the extended sample, likely due to misclassification of exposure associated with home exposure from gas cooking.

Nitrogen dioxide is associated with increased asthma symptoms in children, and replacement of unflued gas heating in schools should become a public health priority for school authorities. Furthermore, the result may implicate unflued gas appliances in environments other than classrooms.

Chapter 1- Introduction

Over fifty years ago, it was recognised that major air pollution episodes were associated

with adverse health effects. A fog concentrated with industrial pollutants covered the

Meuse valley in Belgium in 1930 and contributed to the death of at least 60 people (1). A

similar episode of trapped industrial smog in a river valley was reported from Pennsylvania

in 1948 (2) where 40% of the population were reported to suffer from acute respiratory

symptoms at the time of the disaster. This was surpassed by the London smog in 1952

were smog had hastened the death of 4000 people (2).

The main pollutants in these early days were smoke and sulphur dioxide (SO_2) arising from

domestic heating and power plants both using combustion of coal for energy production.

Public concern over air pollution subsided once the "Clean Air Act" had been put into

place and the visible smoke had disappeared, but surveillance of health effects revealed

that remaining pollutant levels still posed a threat to the respiratory health of the population

(2).

Over the years ambient guidelines have been established and health related studies have

driven an ongoing revision process of these guidelines, accompanied by downwardly

trends for some pollutants. For example, in 1997 the National Ambient Air Quality

Standards of the Environmental Protection Administration (EPA) in the USA reduced their

standards for ozone (O_3) from 120 ppb to 80 ppb (1). The World Health Organisation

(WHO) lowered NO_2 guidelines from 212 ppb to 110 ppb based on clinical studies (3), and

Australia followed reducing guidelines from 160 ppb to 120 ppb (Environment Protection

and Heritage Council- including National Environmental Pollution Council: Ambient Air Quality: http://www.ephc.gov.au/nepms/air/air_nepm.html)

This interest in improving outdoor air has also resulted in the implementation of regular ambient monitoring stations bringing concentrations of relevant pollutants directly into every household via the internet. These information systems have manifold aims including ongoing surveillance of air pollution levels and related health effects, early warning for susceptible sub-populations, and the ability for the public to monitor air improvement achieved through guideline setting and public awareness (1). Indoor air was not included into this development, although activity pattern research has shown that people spend only a minimum of their time outdoors and 90% indoors (4).

Recognition of indoor air as being different to ambient air in relation to levels of substances shared, but also in relation to substances specific to sources indoors, has only recently been addressed by three major indoor reports in Australia (5) (6) (7). Key issues identified in these reports were the need of more health related indoor studies and the lack of co-ordinated indoor research in Australia.

Internationally, the WHO has also recognised indoor air in their air quality guideline information report in 1999 (8). It was stated that concentrations of many substances with adverse health effects are higher indoors than outdoors because of tighter housing structures and reduced ventilation. Little children spend most of their time indoors and are particularly vulnerable during their years of growth and development. One of the important organs under attack from indoor pollutants is the lung which is in continuous volume expansion from birth, right through the primary school years (9).

Prevalence of wheeze and asthma have increased up to threefold in the last few decades in 'Western affluent' countries (10). In Australia, asthma prevalence was 8.5% in 1988 for all age groups, but increased to 11.3% as reported in the 1995 National Health Survey (11).

Asthma prevalence in the primary school age group (5-14) is particularly high at 18.9%. Concern for this growing problem has initiated large research projects, particularly in Europe, indicating that the indoor environment may play a role in the aetiology of asthma. Comparison of asthma and atopy prevalence in genetically identical populations, which had been separated politically and economically, have shown differences which led to the hypothesis of a possible relationship of asthma and atopy to Western lifestyle (12) (13) (14). Interestingly, in the case of West and East Germany where significant differences in the prevalence of asthma and atopy were found, converging tendencies have been reported only 6 years after the two countries have been re-united (15). Environmental factors are believed to play a role and indoor factors such as changing building characteristics leading towards energy efficient structures have been implicated (16).

This lifestyle related difference in asthma and allergy prevalence has been observed in economical gradients worldwide (17).

The WHO has identified indoor air as one of the key areas where environmental interaction with genetic factors may take place (10). Indoor air pollution in the form of tobacco smoke, bio-mass combustion, formaldehyde and nitrogen dioxide (NO_2) from gas household appliances were all considered to be key pollutants in the indoor environment. The WHO aims to support research that measures the impact of air pollution reduction in children.

NO_2 is one of the ambient criteria pollutants recognised to be of concern for public health. This is the reason for it to be regulated and regularly monitored in the ambient environment, but it can occur in much higher concentrations indoors. Levels of NO_2 concentrations in the vicinity of an operating gas cooker can reach 500 ppb of NO_2 exceeding NO_2 concentrations during major air pollution episodes (18). In a recent locally conducted panel study, mean daily concentrations of NO_2 in households of asthmatics were

measured (19). The results have shown that in households with unflued gas household appliances (gas cookers, unflued heating) 36% exceeded WHO guidelines for ambient NO_2. In the case of unflued gas heating the percentage increased to 80%. Overall, use of gas appliances doubled the daily mean indoor concentration of NO_2 from 14 ppb to 28 ppb in this Australian study.

NO_2 is produced during combustion of gas combining nitrogen and oxygen of the surrounding air. In the last 25 years health effects associated with indoor exposure to NO_2 have been studied widely using all available observational study designs. Study questions raised to this day were related either to current asthma severity in relation to NO_2 (19) (20) or to the initiation of asthma in relation to gas appliances (21) (22). Inconsistencies in the results of some of these studies, associated with design issues, such as misclassification of exposures and outcomes, confounding and bias, have so far precluded a conclusive linking of asthma symptoms to NO_2 at levels occurring in households with gas appliances.

This thesis sets out to improve on former study designs in order to overcome previous sources of non-causal explanations in relation to NO_2 exposure from unflued gas appliances and concurrent asthma severity.

The thesis starts off with a literature review setting the scene for the main study design, followed by a pilot study which was implemented to examine the feasibility of the study and to explore design issues in relation to exposure measurements for the main study. The main section of the thesis comprises methods, results, discussion and conclusion in relation to the main study.

Chapter 2: Literature Review

This chapter summarises the research into the effects of NO_2 on the human respiratory system based on clinical and observational studies. Finally, a conclusion is drawn from the current evidence on this subject, and following from this, the study design for the main study in this thesis is presented.

2.1 Method for literature review

Medline and Embase (1966-2002) were searched using the following terms: asthma, nitrogen dioxide, respiratory disorders, gas cooking/stoves/heating/appliances, and air pollution. Original epidemiological studies, which examined the relationship between NO_2 and respiratory disease and /or asthma, were selected.

In reviewing the relationship between air pollution and respiratory effects, the following criteria of causation, based on Bradford Hill, were evaluated, the presence of dose-response relationships, evidence for a threshold effect, biological plausibility, the specificity and the consistency of the evidence (23). Firstly, clinical studies involving lung function measurements, airways responsiveness, and allergen responsiveness are reviewed. Outdoor studies in the general population and with asthmatics are then discussed, followed by a review of indoor studies.

2.2 Biological plausibility

NO_2, due to its molecular structure, is not as water soluble as SO_2 and O_3 and therefore is transported into the alveolar regions of the respiratory tract where it causes cellular damage related to its oxidative capabilities (24).

The biological plausibility of an adverse effect of NO_2 upon the respiratory tract is suggested by findings from animal studies which have demonstrated NO_2 induced changes in alveolar macrophage function, ciliary movements and reduced antibacterial defences (3) (25). Analysis of lung lavage collected from healthy volunteers exposed to NO_2 in excess of 1000 ppb have also indicated a potential of NO_2 to affect host defence mechanisms in the broncho-alveolar regions. Exposure to NO_2 has shown significant increases in neutrophils in the bronchial fraction (26-28)and reduction of ciliary movements (29). Similar effects had been shown in earlier in vitro studies (30). These studies suggest an inflammatory effect of NO_2 in the small airways.

2.3 Controlled Clinical studies

This chapter briefly reviews human chamber studies.

Randomised controlled trials provide the strongest evidence for adverse acute effects of NO_2. Controlled studies examining short-term health outcomes after exposure to various NO_2 levels have been conducted among the 'non-asthmatic population', asthmatic subjects and patients with chronic obstructive pulmonary disease (COPD). Outcome measures included:

(i) lung function measurements,

(ii) bronchial hyper-responsiveness (BHR) to inhaled broncho-constrictors, and

(iii) specific allergen responsiveness to NO_2.

2.3.1 Effects on lung function

Chamber studies in asthmatics have been conducted exclusively in adults and have shown inconsistent results. Small sample sizes and intra study variations in asthmatic subject selection have been discussed to have contributed to these contradictory results (31). Also, it has been suggested that the large airways may not be the primary site of NO_2 response and that spirometry may therefore be an insensitive test for the effects of NO_2 (31).

In asthmatic subjects, significant reductions in lung function, measured as forced expiratory volume expired in one second (FEV_1), were demonstrated by Bauer at 300 ppb NO_2, but other studies within a similar exposure range could not show similar results (32) (33).

The non-asthmatic population remains unaffected at NO_2 concentrations up to 1000 ppb in clinical studies (34)

2.3.2 Effects on bronchial hyper-responsiveness

Orehek first demonstrated increased airway responsiveness of asthmatics to a non-specific broncho-constrictor at concentrations of 100 ppb of NO_2 (35). However this study had significant methodological limitations including multiple post hoc comparisons. The results of 20 subsequent studies were inconsistent due to small sample sizes and the use of 8 different broncho-constrictors. These studies were reviewed and subjected to a meta-analysis (36). In this meta-analysis the number of positive and negative individual responses for each study were classified according to NO_2 exposure level, and stratification by challenge type, and exercise.

Folinsbee concluded that a significant increase of airway reactivity occurred after resting exposure to NO_2 in asthmatics at levels of 100 to 200 ppb for an hour. This is similar to concentrations occurring in peak hour traffic, in areas of urban air pollution, and in particular indoors when using gas for cooking or heating, and underscores the potential public health importance of NO_2 exposure in community settings (25).

2.3.3 Effects on allergen responsiveness

In the last 10 years interest has shifted towards exploration of a possible interactive effect between NO_2 and allergens. Two recent studies from the United Kingdom combined mite allergen inhalation with NO_2 exposure in order to test whether allergen responsiveness is enhanced by air pollutants. They demonstrated that NO_2 alone (400 ppb) (37), and also concurrently with SO_2 at concentrations of 400 ppb NO_2 and 200 ppb SO_2 (38), enhanced the broncho-constrictor response to inhaled house dust mite allergen in asthmatics. At lower NO_2 levels significant increases in allergen responsiveness were not detected. However, the sample size of 10 asthmatics provided limited statistical power. Later, a similar experiment was repeated with O_3 as the second gaseous pollutant (39). It was demonstrated that NO_2 (400 ppb) alone, O_3 (200ppb) alone, and NO_2 in combination with O_3, (400 and 200 ppb) significantly reduced the allergen dose required to decrease FEV_1 by 20% ($PD_{20}FEV_1$).

Another study observed that NO_2 concentrations of 260 ppb increased the pollen (birch and grass pollen) induced asthmatic response in 18 asthmatic subjects with an allergy to pollen, but no other NO_2 concentrations were studied (40). This experiment was repeated with 16 subjects, but this time NO_2 and allergen doses were applied on 4 subsequent days mimicking a more 'real life' situation (41). Early phase and late phase (4-day mean) FEV_1 was significantly decreased in the NO_2 exposed group.

2.3.4 Summary of clinical studies

Largely based on the meta-analysis by Folinsbee, the WHO has recently reduced hourly outdoor NO_2 guidelines from 212 ppb to 110 ppb (42). This appears to offer a margin of safety for most outdoor situations, but such levels can be readily encountered in the indoor urban setting.

Furthermore, a dose-response relationship and a clear threshold level for exposure have not been found, so there is uncertainty about setting a margin of protection for the public based on such clinical studies.

2.4 Epidemiological studies conducted outdoors

All, but one of the 30 outdoor studies discussed in this review were of cohort design and one study had a case-control design. Outcome measures included hospital admissions, lung function parameters and symptom scores such as daily respiratory symptom diaries. Exposure measurements for NO_2 levels were obtained through static air monitoring stations set up primarily for routine observation purposes. Participant exposure was estimated based on the nearest monitoring site.

2.4.1 General Population studies

Two of the earlier studies used an ecological design, comparing respiratory symptoms incidence between areas of low and high NO_2 exposure (43) (44). These two earlier environmental studies showed that acute upper respiratory symptoms were significantly increased in areas with higher NO_2 levels.

2.4.1.1 Cohort studies

The following cohorts were based on personal observations of symptoms over time in relation to measured NO_2 levels.

In the general population the following cohort studies demonstrated positive associations between respiratory events and increasing levels of NO_2:

- 53% increase in upper respiratory symptoms in schoolchildren (OR:1.53; 95%CI: 1.01-2.31)(mean hourly NO_2 max. 24 h; 26-251 ppb) (45)

- 23 % increase in upper respiratory problems in pre-school children associated with an increase of 10 ppb NO_2 at generally low levels of NO_2 which did not exceed WHO hourly guideline (RR:1.23; 95%CI: 1.03-1.48) (46), and

- 26% increase in sore throats in nurses at levels commonly reported in polluted urban areas such as Los Angeles which regularly exceed current guidelines (OR: 1.26; 95%CI: 1.18-1.35) (47).

2.4.1.2 Studies including lung function measurements in the general population

Two cohort studies among the general population tested lung function in 4300 children (48), and in 423 children and young adults (49), after baseline NO_2 levels were obtained (Schwartz et al,1989: yearly means in different areas NO_2=25-63 ppb) (Frischer et al, 1993: ½ hourly NO_2 means of 10-32 ppb concentrations). Both studies showed a significant decrease in lung function at higher NO_2 levels. Frischer, using regression modelling, predicted a 1.5 ml decrease in forced vital capacity (FVC) with every 0.5 ppb increase of NO_2, while Schwartz demonstrated a significant relationship between NO_2 and an increased risk of having a FVC less than 70% of predicted, additionally a non-linear relationship for NO_2 was identified with a steep decline of FVC commencing at 40 ppb. The only negative cohort was reported by Moseler et al, who compared median weekly NO_2 levels and concurrent lung function measurements in 467 schoolchildren (50). Although negative for the general population of schoolchildren, a subgroup of children with asthma (n=106) did have a significant relationship between outdoor NO_2 levels, use of individual room heaters, and reduction in lung function measurements. The NO_2 levels encountered were low due to mainly vehicular rather than industrial sources (range: 6-27 ppb).

2.4.1.3 Summary of outdoor studies in the general population

The general population studies have consistently demonstrated positive findings, without clear evidence of a dose-response relationship between increasing NO_2 exposure and reduction in lung function parameters. However inference of causation is limited due to potential confounding by other pollutants, occupational exposures and cigarette smoking. Static allocation of exposure levels also may have contributed considerably to misclassification of participants.

Nevertheless, consistently adverse effects were noted at NO_2 levels of the same order of magnitude as the recently introduced hourly WHO outdoor NO_2 guidelines (110 ppb), and also below the current annual US-EPA annual guideline of 54 ppb.

2.4.2 Asthmatic population

Numerous studies focused on the asthmatic population, including children and adults. Outcome measurements comprised lung function, hospital admission for asthma, and daily symptom scores. In most of the studies asthmatics had been diagnosed by a physician.

2.4.2.1 Studies including lung function in the asthmatic population

Although Moseler did not demonstrate any lung function changes in the non-asthmatic group of schoolchildren, the sub-population of 106 asthmatic children had a significant decrease in lung function. This was related to either the presence of an individual room heater, or increased NO_2 concentrations (Forced expiratory volume in 1 second=FEV_1; reduction= 3.5% per 0.5 ppb additional NO_2) (50). A non-linear relationship between NO_2 and lung function indicated a threshold level at 20 ppb.

2.4.2.2 Hospital admissions

Time series analyses conducted in London (51) (52) (53), Belfast (54), Rome (55), Hong Kong (56), Spain (57), Finland (58;59), Sydney (60) and Germany (61), have demonstrated a positive relationship between NO_2 exposure and hospital admissions for asthma at NO_2 levels regularly occurring in domestic indoor and outdoor settings.

In particular:

- a mean of 198 ppb NO_2, and a maximum hourly exposure of 423 ppb NO_2 were associated with a 22% rise in respiratory admissions (51);

- a rise of 15 ppb NO_2 led to a 63% increase in asthma (52);

- Visits to London hospitals for asthma and other respiratory symptoms were observed from 1992-1994 in relation to outdoor air pollution. Asthma visits for children (1 day lag, 8,97 % change, p: <0.001) were associated with daily NO_2 levels (change from 10th-90th percentile) (53).

- a rise of 13 ppb NO_2 was associated with a 4.5% increase in asthma admissions (57);

- periods of high NO_2 compared to low NO_2 (range 0 - 90ppb per hour) were associated with a 20-30% increase in asthma admissions (58;59).

- an increase of 32 ppb NO_2 (mean NO_2 20-30 ppb) was associated with a 27% increase in croup (61).

- In Sydney the risk of childhood asthma admission associated with an increase of NO_2 (1-hourly) from the 10th-90th percentile was 5.3% (60).

- In Rome the risk associated with NO_2 (inter quartile range increase of 11 ppb) for asthma admission in children was 10.7% (1 day lag) (55).

- In Belfast daily childhood admission to hospital was increased by 10 % in relation to increased NO_2 (54).

- Finally, a meta-analysis of data from four European cities, Barcelona, Helsinki, London and Paris, also showed an increased risk of children's admission to hospitals for asthma in associations with an increase in NO_2 levels of 25 ppb (62).

2.4.2.3 Panel studies

Panel studies are designed to follow a group of asthmatics who record daily health outcomes over several months. Health effects such as daily respiratory symptoms and lung function changes have been followed up. Concurrent NO_2 levels were recorded, along with potential confounders which may be associated with the health outcomes such as meteorological data, co-pollutants and activity patterns.

Nine cohort panels studied the relationship between outdoor NO_2 levels and daily symptoms using a diary. Moseholm utilised neural network analytic techniques (to account for collinearity and autocorrelation), and demonstrated an association between peak flow measurements and NO_2 at relatively low NO_2 levels (21 ppb) (63).

In a panel of 60 adult (18-55) asthmatics in Holland, symptoms of asthma and medication use were assessed in relation to daily air pollution over three months in summer. A positive effect was seen mainly for shortness of breath (1.05; CI: 1. 01-1.12) in relation to a NO_2 increase of 5 ppb. When stratified by severity of asthma (airway hyper-responsiveness, steroid use) the relationship to NO_2 was not as expected increased among the severe asthmatics, but levels of pollution were low (64).

In a Swedish panel of 38 asthmatic adults an increase in risk of daily measured severe asthma symptoms (10 weeks) was demonstrated in relation to increasing NO_2 levels. These findings have occurred against a backdrop of very low NO_2 concentration (mean: 15 ppb) (65).

Personally measured weekly exposure (13 weeks) to NO_2 (range: 4-47ppb) was found to be related to increased risk of cough (RR: 1.52 CI: 1-2.31) in a diary study of 163 pre-school children (66).

A greater susceptibility to increases in lower respiratory symptom (wheeze, shortness of breath, asthma attacks) in relation to NO_2 levels was seen in children (n=459) who had been earlier diagnosed with bronchial hyper-responsiveness (BHR) and a high level of total serum IgE (26%) (67). Odds ratios were significant for increments of NO_2 of 20 ppb on the same day (1.20; CI: 1.03-1.39), 1 day lag (1.16; CI:1.01-1.33), 2 day lag (1.18; CI:1.03-1.35) and 5 day lag (1.79; CI:1.39-2.30). In other subgroups, BHR and low IgE, no BHR and no high IgE, no BHR and high IgE, no relationship to outdoor pollution and NO_2 was observed.

Another panel study was based on the hypothesis that asthma is precipitated by an upper respiratory infection initiated by higher NO_2 levels (68). In this study, personally measured (weekly) NO_2, overlapping the time period of an upper respiratory episode was related to an episode of asthma measured by peak flow measurements. Compared with exposures of <4 ppb of NO_2, exposures of >14 ppb of NO_2 were associated with a relative risk of 1.9 (CI:1.1-3.4) for the development of an asthmatic episode within seven days of an infection.

Negative studies:

All three of the negative diary studies were conducted at moderate NO_2 background concentrations and had a limited sample size. :

- An Australian panel of 99 asthmatic primary school children was followed up in two geographically different areas where the NO_2 mean maximum hourly values were 85 vs 35 ppb NO_2 (69). There was no relationship between NO_2 and daily symptoms,

although the prevalence of asthma was significantly higher in the area with higher NO_2 in the baseline cross-sectional study.

- An 8 month diary study in 122 asthmatic children did not show any correlation with either peak flow or symptoms and NO_2 (mean daily NO_2 concentration was 21 ppb NO_2) (70).

- A 2 month diary study in 31 asthmatics also showed no relationship, although subjects were exposed to mean daily NO_2 levels of only 10 ppb (71).

Finally, a case-control study, nested within a birth cohort in Norway tested the hypothesis of relationship between NO_2 levels and bronchial obstructive illness in the first 2 years of life (72). NO_2 sources were almost entirely outdoors (mainly traffic) and personally measured levels were low in comparison to guidelines. The result showed that cases (n=153) were not exposed to higher NO_2 concentrations than matched controls (15.67 vs 15.37 ppb).

2.4.2.4 *Summary of outdoor studies in the asthmatic population*

Studies of asthmatics generally demonstrate a positive relationship between outdoor NO_2 and hospital admissions and lung function, with consistency of findings across several continents. However, this evidence is again weakened by the role of potential confounders, such as other air pollutants, additional indoor NO_2 exposure, un-stated selection criteria for so-called "asthmatic" subjects, and limited sample size in the diary studies. Selection of asthmatic subgroups by severity of their disease may be important in future large population studies. Also, mean outdoor NO_2 levels were generally low, and do not necessarily reflect the more important effect of more prolonged exposures to higher levels of indoor NO_2. Results were also limited due to misclassification of exposure as

most of the exposure measurements of NO_2 were obtained from static observation stations. This may have contributed to inconsistencies in the results experienced in the small panel studies.

Outdoor studies in general did not provide a clear dose-response relationship between NO_2 and outcome measures for either asthmatics or the general population, however, limited data from 2 studies were indicative of a non-linear relationship, with threshold effects at 40 ppb in a "healthy" population (48), and at 20 ppb NO_2 in an asthmatic sample (50).

2.5 Gas appliances exposure studies not measuring NO_2 levels

2.5.1 General population

These studies use information about the presence and absence of unflued household gas appliances, mostly about gas cooking, as proxy indicator (proxy studies) for NO_2 exposure.

A meta-analysis combining 5 earlier proxy studies, and assigning a surrogate value of an additional 15 ppb NO_2 for the presence of a gas stove, resulted in a combined OR of 1.15 (CI: 1.09-1.22) for the relationship between respiratory illness and gas cooking for children (73).

Results of 15 studies of children which investigated the relationship between respiratory symptoms and gas cooking were inconsistent (4 negative and 11 positive), without evidence of change in pulmonary function tests, or dose response relationship (74) (75;76) (77). The evidence of harmful NO_2 effects in the general population is therefore weak, and is likely to be further limited by misclassification of both disease and exposure status, and by confounding variables.

2.5.2 Asthmatic population

Two small diary studies by Lebowitz among asthmatics found decreased peak flow and increased daily symptoms related to use of gas stoves (78) (79).

More recently, 8 major studies (5 cross-sectional and 3 cohort) evaluated respiratory symptoms and the prevalence of asthma in households with gas appliances compared to the prevalence in non-gas households (21) (22) (75) (80) (81) (82) (83) (84).

2.5.2.1 Cross-sectional studies

Five recent cross-sectional studies assessed the relationship between gas cookers and asthma.

- A cross-sectional study by Volkmer et al found an increased prevalence of asthma among pre-school children living in gas cooking households compared to households with electric stove (OR:1.24 CI: 1.07-1.42). Colds (OR:1.14 CI: 1.01-1.29), hay fever (OR:1.13 CI: 1.03-1.33) and wheezing (OR: 1.16 CI: 1.01-1.32) were also significantly higher in gas households (21).

- Gas for cooking was significantly more prevalent in households of physician diagnosed asthmatics in a study of 17, 962 kindergarten and schoolchildren in Canada (OR: 2.0 CI: 1.41-2.68) (82).

- Using gas, oil or wood in stoves was significantly related to asthma in a study of 704 children (OR: 4.79 CI:1.95-11.8) (80).

- Gas appliances were associated with a 3.1 % reduction in FEV_1 and also wheezing in young women (OR: 2.07 CI: 1.41-3.05), and greater use of asthma medication: (OR 2.18 CI: 1.46-5.70) in a study of 500 male and 659 female adults (81). This study was part of the European Community Respiratory Health Survey (ECRHS) in the UK. Lung function reduction was also reported in French centres of the ECRHS (85). The

Belgian subset demonstrated a positive relationship between gas appliances and respiratory symptoms for both genders (86).

- In a survey of 884 randomly selected households in Port Adelaide an increased risk of asthma in males was related to (i) unflued gas heating compared to flued gas heating (OR 4.9 CI: 1.96-12.45) and (ii) unflued gas heating compared non gas heating (OR 3.3 CI:1.40-7.64) after adjustment for age, smoking and geographical area (83).

We performed a meta-analysis to combine the 3 cross-sectional studies among a general population of children (21) (82) (80) using Revman (87). The three studies were identical in their ascertainment of asthma (ever physician diagnosed asthma) and exposure (use of natural gas for cooking or not), as well as using very similar age groups.

For all 3 studies the number of children exposed to gas cookers and information regarding diagnosis of asthma was extracted, and the combined odds ratio of the unadjusted data was 1.20 (95% CI: 1.11-1.30) (Fig.1). Two of these studies also had gathered data on general wheezing (21) (82). The combined unadjusted odds ratio of children who ever wheezed in relation to exposure to gas was 1.12 (95% CI: 1.04-1.20) (Fig.2). Within each of these studies, adjustment for potential confounders, such as household smoking did not effect the odds ratio with respect to gas appliances.

Fig. 1 : Risk of asthma in children in gas cooking households : meta-analysis of 3 cross-sectional questionnaire studies.

n = number of children with asthma
N = total number of children exposed / not exposed to gas cookers

Study	Age Group	Gas cooking : Yes n/N	Gas cooking : No n/N	OR of asthma (95% CI Fixed)	Weight %	OR (95% CI Fixed)
Kuhr	7-16 yrs	9/60	32/644		0.4	3.38(1.53,7.46)
Dekker	4-8 yrs	60/566	574/10253		5.2	2.00(1.51,2.65)
Volkmer	4-5 yrs	1309/5500	1396/6525		94.3	1.15(1.05,1.25)
Total (95% CI)		1378/6126	2002/17422		100.0	1.20(1.11,1.30)

Chi-square 20.28 (df = 2) z = 4.39

.1   .2   5   10
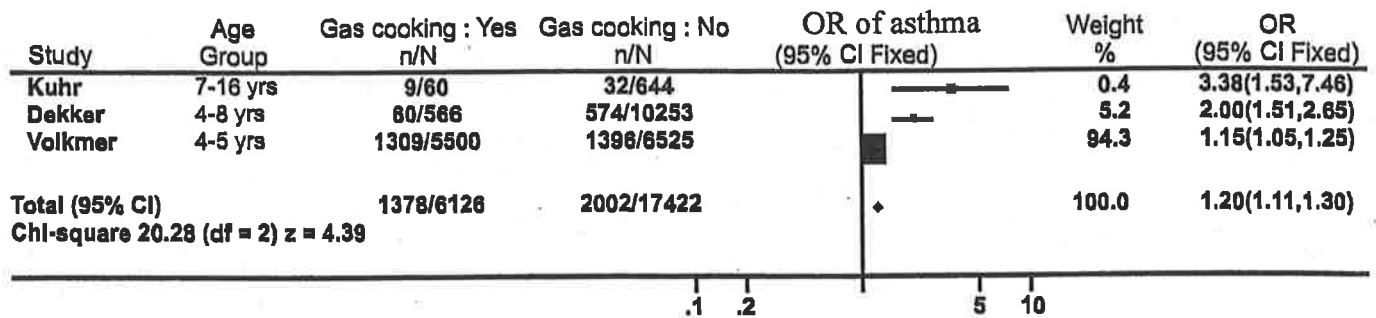
Figure 1: Risk of asthma in children in gas cooking households: meta-analysis of 3 cross-sectional studies

Fig. 2 : Risk of wheezing in children in gas cooking households : meta-analysis of 2 cross-sectional questionnaire studies.

n = number of children with asthma
N = total number of children exposed / not exposed to gas cookers

Study	Age Group	Gas cooking : Yes n/N	Gas cooking : No n/N	OR of wheeze (95% CI Fixed)	Weight %	OR (95% CI Fixed)
Dekker	4-8 yrs	124/566	1488/10253		8.0	1.65(1.34,2.03)
Volkmer	4-5 yrs	2205/5601	2502/6623		92.0	1.07(0.99,1.15)
Total (95% CI)		2329/6167	3990/16876		100.0	1.12(1.04,1.20)

Chi-square 15.17 (df = 1) z = 3.12

.1   .2   5   10
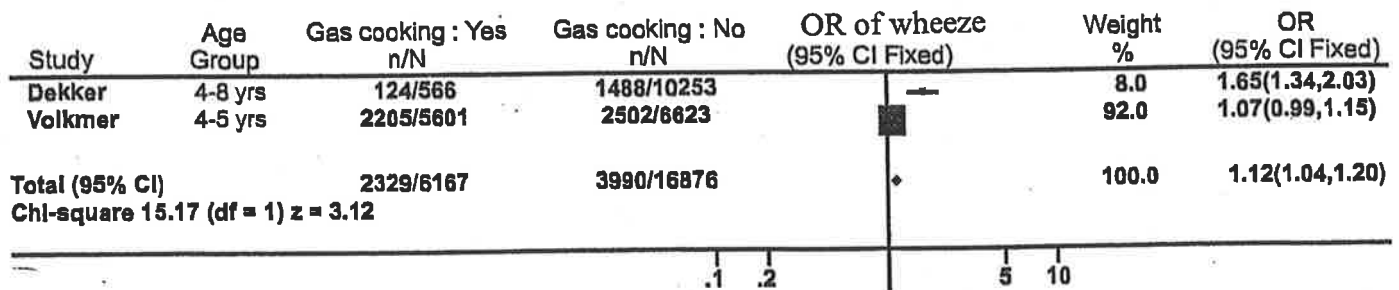
Figure 2: Risk of wheezing in children in gas cooking households: meta-analysis of 2 cross-sectional studies.

2.5.2.2 Cohort studies

- Daily use of gas for cooking was related to daily recorded asthma symptoms in a cohort panel of 164 adult asthmatics in Denver Colorado studied over a period of 3 months (75) (restrictions in activity: OR: 1.47 CI: 1.0-2.16; severe cough: OR: 1.71 CI: 0.97-3.01; shortness of breath: OR: 1.60 CI: 1.11-2.).

- Prospective incidence of asthma and allergy has been ascertained of 1449 participants of a 1958 birth cohort in Great Britain. Subjects were asked about cooking fuels used in their households when they were 11 years of age and at present. There was no relationship between use of gas and asthma when assessed for age 11 exposure or for use of gas for cooking at presence, but subjects who currently used gas for cooking had a significantly reduced FEV_1 (-70 ml CI: ± 56) which was particularly pronounced in asthmatics (84).

863 children were followed up from birth to (1988/89) until 1995 in Tasmania. Gas heater use during infancy was associated with an increased risk of asthma at follow up in 1995 (relative risk=1.92 CI:1.33-2.76). Gas cooking during infancy was also associated with asthma after follow up for 443 children who had not changed their address since the beginning of the study (RR: 2.17 CI: 1.06-4.43) (22).2.5.3 Summary of indoor gas appliances exposure studies: general population and asthmatic population

In asthmatics there is clearer evidence of a relationship between use of gas appliances and asthma symptoms than it was seen in the general population studies with consistently positive results from five cross-sectional studies and two cohort studies. One hypothesis points to a relationship between asthma and current gas cooking. Recently, the Tasmanian birth cohort linked exposure to unflued gas appliances at birth to asthma in later childhood. This outcome was not reproduced in a British birth cohort, although, in this study knowledge about heater use was only retrospectively collected at age eleven. However,

actual NO_2 levels were not measured in these "proxy" studies, therefore dose-response relationships could not be evaluated and the mainly cross-sectional design provides only weak evidence for causation. Meta-analysis demonstrated an increase of 15 % in respiratory illness in children in households with gas cookers (73), while our meta-analysis demonstrated an overall increase in asthma by 20 % and symptoms of wheezing by 12 %.

2.6 Studies measuring indoor NO_2

Actual indoor measurements of NO_2 have been used in 16 original studies (12 in the general population and four in the asthmatic population). Indoor NO_2 measurements were averaged over either one-week or over 24 hours using static monitors in households and schools or personalised monitors.

2.6.1 General population

Seven of the 12 studies investigating the general population (2 cross-sectional and 5 cohort studies) showed a positive relationship between respiratory problems and higher NO_2 concentrations (93) (88) (90) (73) (89) (91) (20) (92).

2.6.1.1 Cross-sectional studies

Positive cross-sectional study findings in the general population reported on prevalence questionnaires associated with NO_2 measurements averaged over a week.

- An increase of 10 ppb NO_2 (related to the presence of un-vented kitchen water heaters) measured over a week was associated with increased cough (OR: 3.19, P<0.01) and shortness of breath (OR: 1.97, P<0.1) in a study of 630 primary school children in the Netherlands (93).

- An increase of 15 ppb NO_2 was significantly associated with increased respiratory illness in a study of 103 English primary school children(OR: 1.53 CI: 1.04-2.24) who

lived in households with gas cooking (88). This relationship was only positive for NO_2 measured in the bedroom (4-169 ppb NO_2). Kitchen NO_2 levels were not related to respiratory illness (5-317 ppb NO_2).

Two further cross-sectional studies did not demonstrate a relationship between NO_2 levels and respiratory problems in general population subjects.

- Although not statistically significant (OR:1.11 CI 0.83-1.49), Melia reported a higher prevalence of respiratory illness in houses with high NO_2 levels in a study of 179 children in a British studywas highest (NO_2 in bedrooms: 5-160 ppb; NO_2 in living rooms: 9-300 ppb)(94).

- No significant relationship (OR:0.84 CI 0.48-1.47) was found between NO_2 producing water heaters (increase of 20 ppb NO_2) and the respiratory health of 1051 children in the Netherlands. However, only 9.9% of the homes were exposed to NO_2 concentrations above 30 ppb, thus an inadequate range of NO_2 exposures may explain the negative findings (95).

2.6.1.2 Cohort studies

The following cohort studies measured NO_2 once or twice and followed up participants over a period of time in relation to the incidence of their respiratory symptoms.

Positive cohort study findings included:

- An increase of 15 ppb NO_2 was associated with lower respiratory symptoms incidence in a study of 1567 children from 6 different North American communities (89) (OR: 1.40 CI: 1.1-1.7). The effect was stronger in girls than in boys. NO_2 was averaged over one week and measured in winter and summer.

- An increase of 15.4 ppb of NO_2 was associated with increased lower respiratory symptoms experience over a period of 12 weeks in a study of the effects of un-vented kerosene heaters on 121 US children <7 years of age (OR:2.25 CI:1.69-4.79) (90). NO_2 was averaged over a week and measurements ranged from 50 ppb in households with a kerosene heater and a gas stove, to 3 ppb of NO_2 when no gas source was present.

- In a study in Hong Kong a significant dose response relationship was found between the number of respiratory symptoms and mean daily NO_2 levels (19-25 ppb) among mothers in a study of 319 mothers, but not in their 362 children (91). This was considered to be related to the high short-term levels experienced by the mothers using gas cookers.

- Shima studied the respiratory health of 842 schoolchildren over 3 years. Indoor levels of NO_2 were measured over 24 hours on two occasions, with 40 % of the children exposed to concentrations of greater than 40 ppb of NO_2. Girls had consistently a higher incidence of wheeze and asthma over the three years related to NO_2 measurements in their homes (92).

- Pilotto et al measured NO_2 over shorter periods in 41 unflued gas heated classrooms over 9 alternate weeks in Winter, demonstrating a 6 hourly mean NO_2 concentration of 60 ppb (range 7 ppm to 116 ppm), and hourly NO_2 values up to 700 ppb (20). The findings of higher mean NO_2 values can be explained due to shorter measurement periods taking place during actual time of gas usage, and due to most heaters being of the older convection types in the classrooms studied. Pilotto also found that exposure to NO_2 at hourly peak levels of the order of 80 ppb and above, compared with background levels of 20 ppb, was associated with a significant increase in sore throat, colds and absences from school. An increase in cough with phlegm was

marginally significant. Significant dose-response relationships were demonstrated for these four outcomes with increasing levels of NO_2 exposure.

Negative cohort studies included:

- Two prospective studies with negative findings were conducted in Switzerland, again with very low indoor mean concentrations due to a lack of unflued gas appliances in these households (range 6-17 ppb NO_2) (46).

- Samet et al studied a large birth cohort of 1315 infants and found no relationship between incidence of respiratory symptoms among infants and indoor NO_2 levels (96). However, the two-weekly NO_2 measurements may have been too insensitive to give indications about relatively brief NO_2 peaks due to the averaging effect associated with periods of little or no NO_2 production within the residence. Secondly, exposure was generally low in this study. Only 5% of the mean winter bedroom NO_2 concentration were above 40 ppb and 77% of the bedroom concentration were less than 20 ppb. Thirdly, the results may be different for asthmatic infants. This is supported by results in a small subset of 36 asthmatic infants, where an odds ratio of 1.83 (CI 0.81-4.14) was found for the relationship between wheezing and a higher exposure to NO_2 (>40 ppb NO_2 versus 0-20 ppb NO_2), whereas in healthy infants the odds ratio was only 0.91 (CI 0.79-1.06).

2.6.2 Asthmatic population

Indoor NO_2 measurements, specifically in asthmatics, have been performed in two case-control studies and two cohort studies.

2.6.2.1 Case-control studies

Both of the two case-control studies explored whether asthmatics are exposed to higher NO_2 levels in their households and therefore are more prone to develop asthma. Hoek et al measured weekly NO_2 concentrations in homes of 128 children in the Netherlands who were classified to suffer from bronchitis, asthma or frequent coughs and colds and compared these to NO_2 levels (average over one week) in homes of 103 controls, and found no difference in NO_2 exposure (97). Cases were identified through school records, followed up by home interview.

Infante Rivard compared 457 children in Canada with asthma diagnosed by paediatricians and 457 community controls in regard to a number of environmental factors (98). Twenty percent of the children wore a personal NO_2 badge for 24 hours. A dose response relationship between the presence of asthma and increasing NO_2 concentrations was demonstrated (NO_2 mean per 24 h: 17.16 ppb), although surprisingly, an increased odds ratio (OR:2.27 CI 1.42-3.65) was found for electric heating versus gas heating, while the presence of a gas cooker was not significantly different between cases and controls (OR:1.33 CI 0.68-2.58). This may be explained by the very low numbers of gas users among controls (30/457) and cases (23/457).

2.6.2.2 Cohort studies

In an Australian study 53 asthmatic and 95 non-asthmatic children (9-14) were followed up in relation to household measurements of NO_2 and their respiratory health (99). NO_2 levels were extremely low in this study (median 6 ppb). Nitrogen dioxide levels, measured on five occasions over 4 days, were only marginally related to respiratory symptoms, while presence of gas stoves was significantly related to respiratory symptoms which were more common in the asthmatic children (94% vs 48%) (OR: 2.2 CI:1-4.8).

Another Australian study used a panel cohort of 125 asthmatics to evaluate daily differences in household NO_2 concentrations in relation to daily collected asthmatic symptoms (19). A novel approach was the measurement of NO_2 on a personal level, during peak daily use of gas appliances and participants acting as their own control as repeated measurements were conducted over six weeks. Mean NO_2 concentrations were 28.7 ppb (CI: 21-40) in gas households in comparison to 12 ppb (CI: 11-15) in electric households, while in unflued gas heated households mean concentration of NO_2 reached 67 ppb (CI:27-165). While no association between NO_2 and symptoms was evident when all participants were combined, interaction analysis with age categories indicated that children below the age of 14 had significantly more asthma symptoms in relation to daily NO_2 levels. Significant same day relationships were demonstrated for chest tightness (OR 1.29 CI: 1.16-1.43), daytime asthma attack (OR 1.13 CI: 1.02-1.26), night asthma attacks (OR 1.16 CI: 1.03-1.30) and with one day lag for chest tightness (OR 1.29 CI:1.14-1.46), breathlessness on exertion (OR 1.13 CI: 1.0-1.28) and night asthma attacks (OR 1.15 CI: 1.03-1.29).

2.6.3 Summary of indoor studies: general population and asthmatics

There is some evidence that NO_2 is associated with an increase in general respiratory illness among the general population, particularly in children. A meta-analysis combined and reanalysed four of the studies conducted among children, and reported that children may have a 20% higher risk (CI:1.08-1.41) of respiratory symptoms related to an increase of NO_2 by 15 ppb, which is easily achieved by the presence of a gas cooker in a household (73).

But, when assessed individually, cross-sectional indoor studies among the general population have shown ambivalent results. This may be due to the inherent problems in

their study design, mainly in relation to the temporality of exposure and health outcomes. Low concentrations of NO_2 in these studies, and possible misclassification of exposure, may have contributed to the negative results in two of these studies discussed earlier. Cohort studies in the general population have shown consistent positive associations in relation to short term symptoms exacerbations. This may be due to the ability of follow up of symptoms in relation to the respective exposure over time.

The negative findings of the cohort study conducted by Samet may again be explained by low level NO_2 exposures and prolonged measurement periods. Also, this study used a cohort of infants which may not be comparable to the predominant findings from the other studies in school children.

Indoor studies in households of asthmatics have focused on the susceptible population of children. Especially the Canadian case-control study was well executed, cases were based on new asthma cases recruited in the emergency department of a paediatric hospital, and shows dose response evidence for the risk of the incidence of asthma being increased in households with the higher NO_2 exposure. Hoek's case-control study, on the other hand, may have been weakened by less stringent case ascertainment, sample size restrictions and exposure misclassification.

In relation to the effect of NO_2 on short term exacerbations of asthma symptoms, the most conclusive design approach so far was used in the Port Adelaide indoor panel study. Repeated measurements of asthmatic symptoms in children were related to personally measured NO_2. The result shows increasing risk of daily symptoms in relation to daily NO_2 exposure.

Concentrations of NO_2 found in the indoor studies were considerably higher than outdoors, however, health effects have been found in some of the studies at mean NO_2

concentrations as low as 20-30 ppb. Underlying hourly concentrations could amount to at least a doubling of these values according to findings by Pilotto . Thus in studies where respiratory illness was associated with weekly NO_2 exposure of 20-30 ppb, hourly exposures of at least 40-60 ppb NO_2 would have been likely, and such levels are lower than the present WHO guideline (110 ppb).

2.7 Objective lung measurements and indoor NO_2 exposure

In a number of indoor studies lung measurements have been observed in relation to exposure to NO_2 or gas appliances.

As discussed earlier, randomised assigned NO_2 exposure under laboratory conditions did not show any conclusive results in relation to lung function measurements. Recently, in an experimental community study in Singapore where acute responses to single episodes of cooking were studied, short term changes in peak expiratory flow rates (PEFR) were observed in 16 asthmatic women. These changes in PEFR were directly linked to cooking with gas, but also correlated to actual NO_2 concentration levels measured during the cooking process (100).

Long term effects on lung function related to gas appliances in households or to measured indoor NO_2 exposure were explored in a few cross-sectional and cohort studies.

This category comprises results from five large community studies indicating changes of lung function parameters in relation to current gas use, current gas heating, and in relation to gas heating during infancy:

- A large study in the UK found that gas for cooking was associated with a 3.1% reduction in FEV_1 in young women (n=1864) (also wheeze and use of asthma medication) (81).

- Atopic subjects (n=1921) were sensitive to adverse effects of gas cooking. Twenty one percent of subjects with high IgE levels and exposed to gas cooking tested bronchial hyper-responsive (expressed as dose response slope), compared to 14 percent in non-gas cooking households (101).

- Reduction in lung function was detected in girls with high IgE levels exposed to gas cooking (-4.84 % CI:-9.28, -0.19%) (102).

- In an Australian study (Tasmania) of 498 children, children sensitised to house dust mites (HDM) with current gas use, had significantly reduced lung function (-6.2% CI: -10 to -2.4) compared to HDM positive children without gas use (-0.3% CI: -2.5 to 1.8) (103).

- In another recent Australian study (Australian Capital Territory) of 344 children, current gas heating (-2% CI: -3.7% to –0.2%) and personally measured NO_2 levels (per 1 ppb increase in NO_2 –0.12 % CI: 0.23-0.01) were associated with decreased lung function in children (104).

In summary:

Lung function levels and their relationship with NO_2 exposure have been mainly investigated with one off measurements before or after assessment of exposure to either proxy exposure to gas appliances or actual NO_2 measurements. The results suggest an association with both types of exposure appraisal.

The majority of studies addressed current gas use which was positively linked to reduction in objective lung measurements. In particular, this has been observed in atopic women and young girls.

For the first time significant acute lung function changes were observed in asthmatics directly linked to cooking with gas appliances and concurrently measured NO_2 levels .

This is an important finding as it shows an appropriate time relationship between exposure and effect using repeated measurements of exposure and health outcome.

2.8 NO$_2$ exposure measurements - what are the important issues of exposure ascertainment in epidemiological NO$_2$ studies

Chemical formation

NO$_2$ is formed during high temperature combustion processes using oxygen (O$_2$) and nitrogen (N$_2$) gas from the atmosphere. In the first instance nitric oxide (NO) is formed and then quickly oxidised to NO$_2$ (see equation 1) using oxygen or other available oxidating substances (e.g. ground level ozone) (3). While a number of other nitrogen oxygen species (NOx: N2O, N2O3, N2O4) are produced, NO$_2$ is the predominant and more toxic oxide of nitrogen and has therefore been identified as the substance to be regulated under air quality standards (1). Remaining NO, on the other hand, is not toxic and is also produced naturally in the body from the amino acid L-arginine and performs messenger functions in various organ systems (3).

Measurement techniques for NO$_2$ are discussed in detail in chapter 5.2 (see pilot study).

$$(1)\ N_2 + 2O_2 \longrightarrow 2\ NO_2$$

In epidemiological studies exposure measurements of the substances that are thought to bring about health problems are crucial in the process of causal identification. The following paragraphs briefly discuss the main problems that have to be addressed when measuring pollutant exposure and in particular NO$_2$.

Confounding

Confounding is the main problem in outdoor studies, because pollutants co-occurring with NO_2 may be partially or totally responsible for the health effects in question. For example, NO_2 is often correlated with black smoke during vehicle related pollution episodes, and it is therefore often impossible to separate their effects on health from each other.

In the last decade air pollutant monitoring stations have incorporated measurement of all possible co-pollutants within one station, and therefore it has become possible to better adjust for co-pollutants during statistical analysis. However, in case of high correlations of air pollutants, it is still difficult to obtain conclusive results.

Exposure misclassification

Outdoor studies also suffer from imprecise exposure assessment. Exposure measured at central monitoring stations may not reflect the true exposure of individuals. This is especially relevant in studies where acute health effects are related to short term exposure. Misclassification leads to reduced statistical power and may reduce the association towards the null hypothesis.

For example, outdoor measurements do not take into account additional exposure to indoor NO_2 levels from indoor gas combustion sources. A number of studies have shown that indoor levels of NO_2 are much higher than outdoors if unflued gas appliances are present indoors (68) (146).

In locations with low NO_2 levels in the outside environment, personal NO_2 exposure was highly correlated with indoor NO_2 levels, but not with outdoor NO_2 (68). Therefore NO_2 exposures derived from outdoor measurement stations probably seriously misrepresent actual NO_2 exposure of participants.

Indoor exposure measurements and misclassification of NO2

Most measurements of NO_2 in indoor studies were taken over a number of days and NO_2 measurements were averaged to represent a mean hourly NO_2 level. These averaged levels do not give an insight into peak hourly exposure levels experienced during actual usage of unflued gas appliances. In the Port Adelaide cohort panel, indoor NO_2 levels were measured daily during peak use of indoor gas appliances and results indicated that NO_2 levels fluctuated from very low levels (6 ppb NO_2) to very high levels (>100 ppb NO_2) within households (19). Averaging over several days would have therefore misrepresented peak NO_2 levels and their association to acute health outcomes. In general, personal monitoring improved the accuracy of exposure to NO_2. This has been demonstrated in an Australian study of three microenvironments where static measurements of the residential, workplace and outdoor environment did not accurately predict personal exposure (146).

Estimation of the size of the effect

In a study of schoolchildren, Pilotto measured NO_2 over 6 hours in classrooms with background levels of NO_2 (electrical heating) and classrooms with high levels of NO_2 due to unflued gas heating (20). Because of hourly co-measurements of NO_2 it was possible to establish that one hour levels were at least twice as high as the 6-hourly averaged concentrations. When children exposed to background levels of NO_2 were compared to children with high NO_2 levels, significant increases in upper respiratory symptoms were observed. These results suggest that hourly level of 80 ppb of NO_2 or greater are effective in producing respiratory ill health in the general population of children.

In the Port Adelaide study an increased risk of 20-30% was associated with an increment of NO_2 of 43.8 ppb (equivalent to standard deviation of NO_2). It can be argued that this

risk estimate reflects the risk associated with living in a household with unflued gas appliances compared to a household without such appliances, but uncertainty remains as participating households had a variety of gas appliances in their household . Other factors which contribute to this uncertainty are discussed in the next chapter.

An effective design for direct estimation of health effects caused by NO_2 would be an intervention trial. Any differential in health effects experienced after random exchange of sources of NO_2 would be caused by this intervention. The difference in exposure between intervention and control location would be the biological dose responsible for the extra health effects experienced in the intervention group.

2.9 Conclusion from literature review

This literature review has shown strong evidence of an effect of NO_2 on respiratory health, particularly in children, at levels of NO_2 which are below WHO guidelines.

These health effects have been demonstrated mainly in children between 5-13 years of age. While some inconsistent results occurred when NO_2 or presence of gas appliances were linked to respiratory symptoms in the general population of children, consistently positive results of NO_2 have been demonstrated in relation to asthmatic children using epidemiological study designs ranging from cross-sectional to cohort designs. Significant health effects in asthmatic children were observed regardless of whether NO_2 measurements were conducted outdoors or indoors. In comparison to outdoor studies, indoor NO_2 studies have remained rare, despite the advantage of minimisation of misclassification of exposure and reduced confounding by other pollutants. This may be explained by the fact that indoor studies are relatively expensive and labour intensive. Clearly, in the case of NO_2, well designed indoor studies are better able to establish the relationship between health effects and pollutant level.

As has been set out before, outdoor studies may have significant problems with confounding and misclassification of exposure. An additional problem arises with the uncertainty of whether short time NO_2 levels or long term averaged NO_2 levels may better represent the biologically relevant dose. Indoor studies are better equipped to avoid confounding and misclassification due to their individually based approach in health outcomes ascertainment and NO_2 assessment over predestined measurement periods. Due to the high variability of indoor exposure to NO_2 it is possible to compare individuals exposed to high NO_2 levels with individuals in locations with medium to very low background concentrations, or, as has been done in the Port Adelaide panel study, to use daily variability of NO_2 concentrations within

one location to compare participants health outcomes in relation to changing exposures over time (19).

Studies among the general population of children have shown that when symptoms were measured on an incidence basis in relation to measured NO_2 in their households, then it was possible to capture significant increases of symptoms over time (91) (92) (20). For example, in Pilotto's study, daily measurements of NO_2 and symptoms reduced misclassification and gave information about the relationship between peak exposures and acute symptoms (20). Therefore, measurement of symptoms incidence may better reflect the nature of the biological effect of NO_2 than the occurrence of symptoms in general.

But doubts have remained about the impact of NO_2 on the general population, mainly because of Samet's large cohort study of infants which could not confirm an increased incidence of respiratory symptoms in relation to NO_2 in this population (96). It has been discussed earlier that NO_2 levels were generally low in this study and infants may not be as affected as somewhat older children . However, when looking more carefully at a subset of 36 asthmatic infants in this study it was shown that they had a much higher risk of wheezing associated with exposure to >40 ppb of NO_2 than those infants exposed to only <20 ppb of NO_2.

Results from cross-sectional studies using proxy exposure to gas appliances to indicate exposure to NO_2 have also indicated that presence of asthma and asthma symptoms may have a higher prevalence in gas households (21) (80) (82) (83) (86). Subsequent cohort studies confirmed the increased risk of asthma (22) and asthma symptoms in gas cooking and heating households (75).

Of the four studies where NO_2 was determined indoors, two were case-control studies whose aim was to assess whether NO_2 levels were higher in households of asthmatics and were therefore not adequate for assessment of short-term exacerbations of asthma symptoms (97) (98).

Symptom scores were related to actual measurements in the remaining two studies. In Garrett's study symptom's scores were only marginally related to higher levels of NO_2 (99). But exposures had been extremely low in this study and this may have been due to misclassification of peak levels due to exposure averaging over four days.

The last and most recent study in asthmatics tried to overcome previous problems by linking daily personally measured indoor NO_2 levels with daily symptoms of asthma. (19). This study could demonstrate an increase of asthma symptoms of 30% associated with an increase in exposure to NO_2 by 50 ppb.

While this study design was able to minimise recall bias due to repeated follow up of health outcomes in relation to daily changes of NO_2 exposure within households, there were still limitations due to possible confounding by characteristics that may be related to gas appliances. For example, it is possible that the use of gas appliances is related to a number of characteristics that also influence asthma. One of these characteristics may be socio-economic status. Gas appliances may be more prevalent in lower socio-economic settings and asthma may be a disease more common in poor households. Socio-economic status may be independently linked to both, exposure and asthma, and would therefore be a possible confounder. In fact, in two recent studies NO_2 was found to be higher in suburbs linked to low socio-economic status (105) (106). While adjustment for socio-economic status did not change the results in this cohort panel (19), confounding by socio-economic status is an important factor to be considered in the conduct of environmental indoor studies. A similar issue was recently discussed in relation to cooking with open fires and increased respiratory health effects in Guatemala (107) (108). Uncertainty was expressed regarding socio-economic factors confounding the positive outcome of respiratory health in women cooking with flued wood burning stoves (plancha). It was concluded from this and similar other projects that confounding may pose a problem in observational studies of indoor air pollution

and implementation of randomised intervention trials was recommended to overcome this problem.

A randomised controlled trial is also a step forward in relation to NO_2 research, particularly in a situation where it is claimed that there is still uncertainty surrounding the current evidence of NO_2 and health effects. A randomised trial with an appropriate intervention would also be of benefit for the purpose of guideline setting for NO_2, giving policy makers the opportunity to make decisions based on clear evidence about the health benefits gained from such an intervention.

Environmental intervention studies are rarely conducted because they are expensive or more often not possible at all, but the opportunity to conduct such an environmentally based intervention trial existed in Adelaide where the use of unflued gas heaters is still common in classrooms. The advantage presented in this case was the presence of asthmatic children in their respective primary schools where intervention could be organised by the school department without the necessity of the researchers involvement in the intervention process. Also, it was clear that more than one asthmatic child would be available per classroom, making it organisationally and economically more feasible to conduct such a complex study. In addition to providing more conclusive evidence about causal health effects of gas appliances on the acute respiratory health of children, an intervention trial would directly quantify symptom and objective lung function reduction benefits achieved during the intervention period.

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2.10 Aim, hypotheses and objectives of the proposed study

Our aim was therefore to investigate the respiratory health effects associated with randomised replacement of unflued gas heaters in schools, during winter, with electrical or flued gas heaters (depending on economical considerations) on the respiratory health of asthmatic primary school children. The main chemical pollutant emitted during the unflued combustion process of heating with gas is NO_2. Intervention schools were therefore expected to have lower levels of NO_2 exposure than control schools.

As a consequence the three hypotheses under study were:

1. Occurrence of respiratory symptoms in asthmatic primary school children would be significantly lower in schools where unflued gas heaters were replaced with electric or flued gas heaters.

2. At the end of the study period asthmatic children in schools randomised to electrical/flued gas heating would have significantly improved lung function compared to asthmatic children in the control group.

3. At the end of the study period asthmatic children in schools randomised to electrical/flued gas heating would have significantly decreased airways responsiveness after airways challenge with histamine compared to asthmatic children in the control group.

In order to test the above hypotheses the following objectives were pursued:

1. To quantify occurrence of daily asthma symptoms and compare rates of symptoms between children in the intervention and control groups.

   (Hypothesis 1)

2. To measure lung function at baseline and to compare lung function results between the two groups at the end of the study.

   (Hypothesis 2)

3. To measure airways-responsiveness at baseline, and to compare airways-responsiveness between the two groups at the end of the study.

   (Hypothesis 3)

4. To measure indoor NO_2 levels to examine exposure of children in intervention and control classrooms.

(to test effect of intervention on exposure)

Prior to the main study it was necessary to conduct a pilot study to assess levels of NO_2 measured in South Australian primary school classrooms and to assess possible alternatives for heater replacement. This exploratory study will be presented in the next chapter.

Chapter 3 - Pilot study

3.1 Introduction

In 1999 a pilot study was conducted with the main purpose to investigate environmental asthma triggers in primary school classrooms.

A number of studies had previously investigated the classroom environment in relation to house dust mites (Der p I, Der f I), cat allergens (Fel d I) (109) (110) (111) (112) and respirable dust (113). Most of these studies had been conducted in the Northern Hemisphere and therefore could not be necessarily generalised to Australian conditions. Similar studies had not yet been undertaken in Australia.

In a study funded by the Asthma Foundation in South Australia, a survey questionnaire was sent to all Principals of South Australian primary schools asking for information regarding potential sources of environmental asthma triggers in classrooms.

Subsequently, levels of cat allergen, house dust mites and respirable dust, as well as NO_2 levels were quantified in classrooms with unflued and flued gas heaters.

It was within this general framework that specific preparations and investigations for the planned randomised controlled trial were taking place without biasing school personnel, children and their families in relation to the future intervention study.

This chapter outlines the pilot work undertaken specifically in relation to the identification of unflued gas heated schools and the measurement of classroom levels of NO_2 in a subset of schools in South Australia.

3.2 Aim and objectives of the pilot study

Objective one: Environmental survey

The main objective for this pilot study was to identify schools and classrooms heated with unflued or flued gas using an environmental survey.

Objective two: Protocol development for classroom exposure measurements of NO_2

Assessment of NO_2 levels in South Australian schools were to be undertaken using passive diffusion badges (see figure 3)

These monitors had been especially developed for indoor and personal sampling for periods exceeding one hour. NO_2 levels in classrooms with unflued gas heaters and flued gas heaters had not been monitored before in South Australia.

Our objectives were:

1. To compare the mean maximum one hourly NO_2 levels in classrooms with six hourly mean NO_2 classroom levels, which constitute the levels over a school day.

This relationship was investigated in order to be able to infer from six hourly levels in classrooms to hourly peak levels.

2. To compare badges situated parallel to each other (6-hourly) and in different parts of a classroom (6 hourly and 1 hourly badges) to investigate the spatial variation and to determine reliability of passive badges.

3. To compare NO_2 levels to WHO guidelines

4. To conduct continuous measurements of NO_2 in a number of classrooms for 24 hours by method of chemiluminescence to determine reliability and validity of NO_2 exposure measurements with passive badges.

This method is routinely used by the Environmental Protection Authority (EPA-SA) in South Australia to measure outdoor NO_2 and is considered to be the "gold standard" for NO_2 measurements (114). Continuous NO_2 monitoring of classrooms with unflued and flued gas heaters was undertaken to provide information about the comparability of one hourly and six hourly NO_2 levels measured by passive badge method with NO_2 levels recorded with the EPA method.

3.3 Methods used in pilot study

3.3.1 Environmental survey

In collaboration with the manager of the Health Care Unit (Education Department SA) an information letter and questionnaire were sent out to all primary school principals in South Australia, and information was sought at classroom level about the type of heating used in each of these classrooms, together with the number of students occupying each of these classrooms.

In relation to the main study this survey was helpful in that it quantified, among other things, unflued gas heaters in South Australian primary schools. This information was necessary to make an informed decision about the feasibility of the intervention study. Other questions about school pets, carpets, moulds etc. were included to ensure gas heating was not singled out as the centre of attention, but that information does not form part of this thesis.

3.3.2 Passive diffusion badge monitoring

Passive sampling badges were manufactured and analysed in the environmental and occupational hygiene laboratory of the Department of Public Health at the University of Adelaide (Thebarton Campus) under the supervision of Dr. Dino Pisaniello who has extensive experience in the measurement of NO_2 indoors.

3.3.2.1 Chemical analysis

This badge type sampling unit is based on the Australian standard (AS 2365.1.2-1990) for determination of NO_2 (115).

Each badge consisted of a 50mm petri-dish containing a tri-ethanol-amine impregnated cellulose filter covered by a porous diffusion barrier (figure 3). These elements were kept in place with a stainless steel clip. After exposure the diffusion process of NO_2 was stopped by placement of a tight fitting lid over the petri-dish.

The principal chemical concept is based on the formation of an azo-dye after reaction of tri-ethanol-amine with NO_2. The intensity of the azo-dye is then measured spectro-photometrically (read at 540 nm) and compared to a calibration graph. In a previous study, precision and detection limit for the passive monitors had been tested by exposure of badges to a controlled NO_2 atmosphere. This experiment showed agreement within 10% compared to a chemiluminescense analyser at various concentrations and a detection limit of 38 ppb for a one hourly exposure, and 6 ppb for a six hourly exposure (personal communication with Dr. Pisaniello).

To determine the concentration of NO_2 on badges:

1. NO_2 absorbed on the badge filters was determined by spectrophotometric method

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2. The absorbance measured was corrected by the absorbance determined on a blank or control badge (absorbance of exposure monitor – absorbance of blank).

3. Blanks were kept parallel to each exposure monitor in a sealed container during classroom monitoring.

4. After completion of measurements, exposure monitors were covered with a lid and reunited with their respective control badges in a sealed plastic bag.

5. Badge concentration was determined from the calibration graph according to the formula:

Equation 1:   badge concentration = slope × corrected absorbance + constant

Equation 2: $c = k \times$ badge concentration/ exposure time

Where c= concentration of NO_2 in μg and k= calibration constant

6. transformation from micrograms to parts per billion (ppb)

7. exclusion of measurements below detection limit.
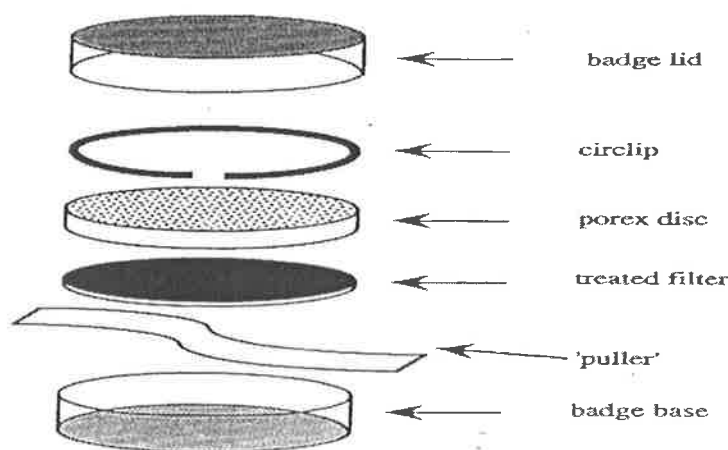
Figure 3: constituents of a passive diffusion badge monitor

3.3.2.1 Sampling protocol

Sampling of NO_2 was undertaken in 8 schools (29 classrooms) with unflued gas heating and in two schools (13 classrooms) with flued gas heating. Each of the classrooms was monitored one day during normal school time in July 1999. Two one hourly monitors were placed horizontally and at breathing height into the classroom, one at the front (on shelves) and one at the back, (bookcases) approximately 30 cm away from the wall. These badges were replaced on an hourly basis six times until the end of the school day (9 am-3 pm). This experimental arrangement was used to explore possible spatial differentials in NO_2 levels across the room. Another two badges were placed next to each other in a horizontal position and at breathing height for six hours to ascertain repeatability. Another six hourly badge was placed on the opposite side of the room for observation of spatial differences over six hours.

For each classroom, control badges were kept in sealed plastic bags for correction of unwanted NO_2 that may have entered the bags during transportation and storage after exposure assessment. A scavenger badge (NO_2 reactive filter, but no lid) was also placed into the sealed bag to react with any unwanted NO_2.

For monitoring outdoor levels of NO_2, two parallel passive badges were placed horizontally next to each other on ledges within the school yard, at least 5m away from open windows of classrooms for 6 hours during school time.

3.3.3 Chemiluminescense monitoring

Robert Mitchell, manager of the air monitoring laboratory of the Environmental Protection

Authority (EPA) of South Australia organised this monitoring.

Standardised chemiluminescense based NO_2 equipment, normally used for routine outdoor

monitoring by the EPA (Australian Standard 3580.5.1.) was installed into 6 different

classrooms in the afternoon and monitoring took place until after school on the next day

(approximately 24 hours) (116). Passive badges were used parallel (1-hourly and 6

hourly) to the continuous monitoring of NO_2 for measurement of agreement between these

two methods (see picture 1, page 55).

The principle behind continual monitoring of NO_2 is the chemiluminescent reaction of

nitric oxide with ozone present in the continuous air sample. NO_2 is then converted to

nitric oxide (NO) which in turn reacts with ozone. During this reaction photons in the

600-3000 nm range are emitted, and the amount of light generated is proportional to the

concentration of nitric oxide. NO_2 is then indirectly determined by comparison with the

signal of an air sample that has not been converted (117).

3.3.4 Statistical analysis

For comparison of the passive versus the continuous method of sampling NO_2, and for

assessment of repeatability of NO_2 levels by passive monitoring, the statistical method of

assessing agreement between two methods was used (118). This method is based on the

calculation and visualization of the limits of agreement between two methods. Limits of

agreement (LA) are derived by calculation of the mean difference between the two

methods, and by estimation of the 95% limits of agreement by the following formula:

LA= d ± 1.96 SD   with d= mean difference; SD= standard deviation of the mean

difference and LA = upper and lower limits of agreement.

These upper and lower limits of agreement indicate the possible difference between two

pairs of measurements and predict that 95% of the measurements made with these two

methods will be closer together than these limits.

Graphically it is depicted by plotting the differences against the mean of the values which

have been measured with the two methods. This same approach was also used for

assessment of repeatability of measurements when the same method (passive method) was

used.
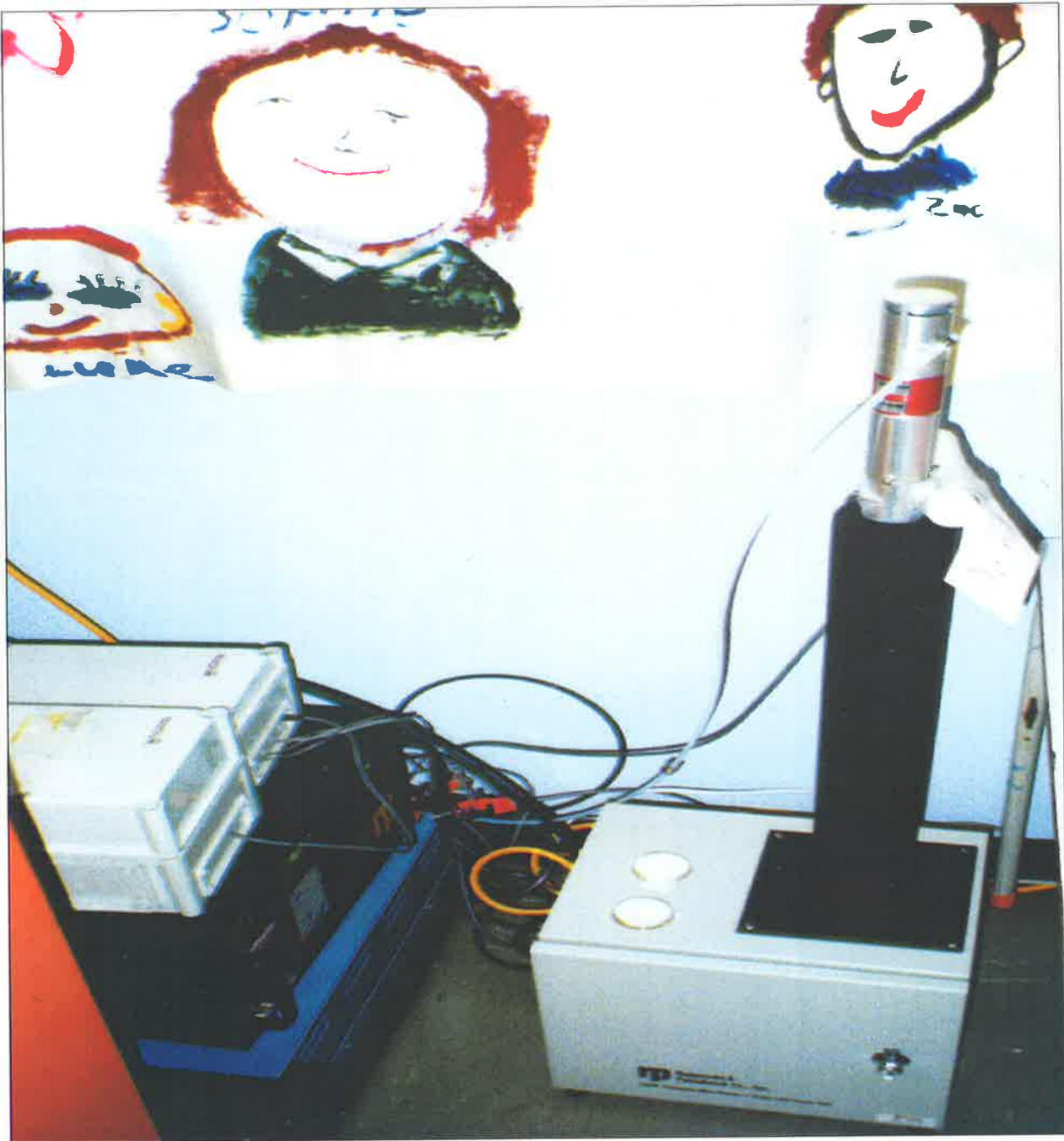
picture 1: Agreement of two sampling techniques. Concurrent NO$_2$ sampling with chemiluminescense (EPA/SA) and passive sampling method

3.4 Results

3.4.1 Objective one: Environmental Survey

Completed questionnaires were received from 400 out of 564 (71%) South Australian primary schools. Of those not responding were at least 14 schools which had been closed down since the last update of the distribution list.

Of 214 schools with gas heating, 78 schools with 374 classrooms stated that their heaters were not flued to the outside (table 1), 19 of which were situated in the metropolitan area of Adelaide. In total, this involved 7708 children in unflued gas heated classrooms, while 17 241 children were exposed to flued gas heating.

Table 1: Results from the asthma trigger survey in South Australian primary schools

Question	Results are based on 400 schools	
	Number of classrooms	Number of children in classrooms
Reverse cycle	2889	54401
Classrooms with unflued heating	374	7708
Flued heating	889	17 241
No heating	10	162

3.4.2 Objective two: NO$_2$ methodology

3.4.2.1 Descriptive NO$_2$ exposure

Exposure measurements were taken in two flued gas heated schools (13 classrooms) and in eight unflued schools (29 classrooms). Results from 600 exposure measurements are displayed in table 2. Of the 150 monitors which were used for one hourly NO$_2$ levels in flued heated classrooms none were above the detection limit (38 ppb), while of the 329 monitors used in unflued gas heated classrooms 68% were above that limit. On the other hand, 36 out of 37 six hourly monitors were above the limit of detection (6 ppb) in the flued heated classrooms, as well as all six hourly monitors in the unflued heated schools. Mean NO$_2$ concentration of six hourly badges in unflued heated classrooms was significantly higher than of those in flued gas heated classroom (14.1 ppb vs 82.1 ppb of NO$_2$). Mean NO$_2$ level of all one hourly monitors in unflued heated classrooms was 102.8 ppb.

Mean of the outdoor levels was 8 ppb of NO$_2$. All of the schools were situated away from major arterial roads.

The two types of unflued heaters prevalent in South Australian classroom were either free standing or wall mounted (pictured on page 70 in methods section). The mean NO$_2$ one hourly concentration gathered from freestanding heaters (NO$_2$: 89.5; ppb SD: 58.5; n=155) was significantly (p<0.05) lower (t-test of log converted NO$_2$ values) than the mean concentration collected from wall mounted gas heaters (132.8 ppb SD: 139.9; n=69). The mean difference of the six hourly averaged NO$_2$ levels between the freestanding heaters and the wall mounted heaters was not significant (freestanding: NO$_2$: 71 ppb; SD: 37.4 n=60 vs wall: 110.2 ppb; SD: 160.7 n=24).

None of the one or six hourly monitors in the flued heated classrooms exceeded WHO guidelines, while 26% of the one hourly and 14% of the six hourly monitors were in excess of 110 ppb of NO_2 in the unflued gas heated schools. Of the 29 classrooms with unflued gas heating, 14 had measurements that at least once exceeded WHO guidelines.

Table 2: Descriptive NO_2 results from 1 hourly and 6 hourly exposure measurements in flued and unflued gas heated classrooms in 10 metropolitan primary schools.

	Flued gas heaters	Unflued gas heaters
Number of schools	2	8
Number of classrooms	13	29
Number of badges	1 hour: 150 6 hours: 37	1hour: 329 6 hours: 87
Number of badges > limit of detection	1 hour = 0% 6 hours = 97.3%	1 hour = 68% 6 hours = 100%
Mean 1 hour (number) standard deviation range	No observations over detection limit of 38 ppb	102.8ppb (n=224) 93.5 38-754
Mean 6 hours (number) standard deviation range	14.1 ppb (36) * 3.6 8 - 20	82.1 ppb (84) * 92.0 12 - 826
Mean outside 6-hourly (number) standard deviation range	8 ppb (10) 6.4 3 – 19	

*P<0.001

3.4.2.2 Comparison of one hourly maximum NO_2 levels with six hourly maximum NO_2 classroom levels.

In a previous school study the mean of the ratios of maximum one-hourly (peak) to six-hourly averages was established for classrooms which had one hourly levels in excess of 80 ppb of NO_2 (20). The ratio indicated a doubling of NO_2 exposure for peak levels of NO_2 measured over one hour when compared to an averaged six hourly exposure.

In this study, the mean of the ratio calculated at classroom level for maximum one hourly levels (> 80 ppb NO_2) (max.1 hour mean: 229.9 SD 185) over six hourly levels (max 6 hour mean: 140.6 SD 172) repeated this earlier result, showing a mean ratio of 2.2 (SD 1.6). Comparison of the mean of all maximum one hourly classroom levels with the mean of all maximum six hourly levels, only excluding badges below detection, equally resulted in a two to one ratio (181.5 ppb : 85 ppb).

3.4.2.3 Agreement between and repeatability of NO_2 measurements

One hourly in different locations:

One hourly concurrent passive monitors situated in different locations within classrooms showed agreement only at the lower end of NO_2 levels (figure 4). Paired monitors indicating exposures to NO_2 of greater than 100 ppb did not show very good agreement. The limits of agreement ranged from –86 ppb to + 90 ppb and several differences between measurements exceeded the 95% limits of agreement. This result indicated possible large NO_2 differentials within classrooms, particularly at the higher end of the exposure scale.
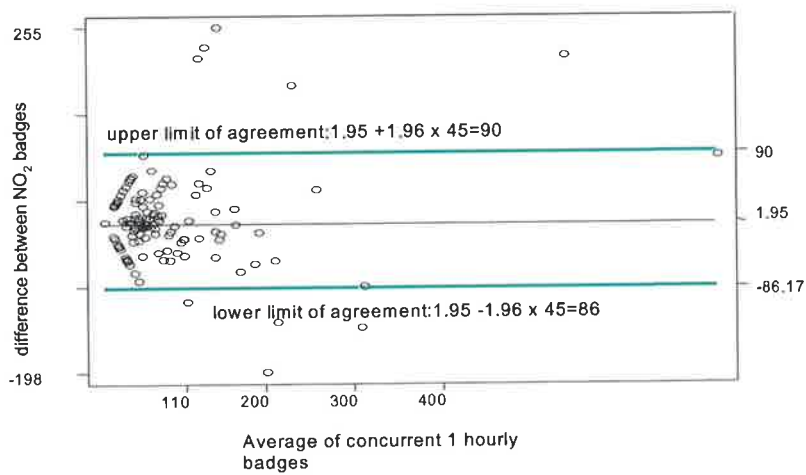
Figure 4: one hourly passive NO₂ monitors situated in different locations within the classroom: difference between the concurrent monitors versus average of the pair.
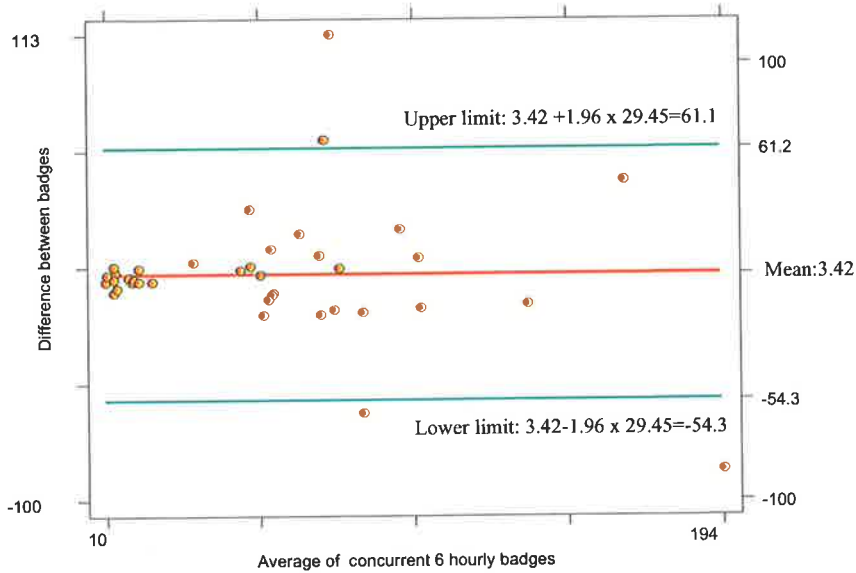
Figure 5: six hourly passive NO₂ monitors situated in different locations within the classroom: difference between the concurrent monitors versus average of the pair

Six hourly in different locations

The limits of agreement indicate possible differences in NO_2 values ranging from 54 ppb to 61 ppb of NO_2, with several measurements outside the 95% limit (figure 5). Again, agreement was good at the lower end of the NO_2 levels and variability between the monitors increased above 50 ppb of NO_2, although the excursions to either side were not as wide as in the one hour pairs of badges.
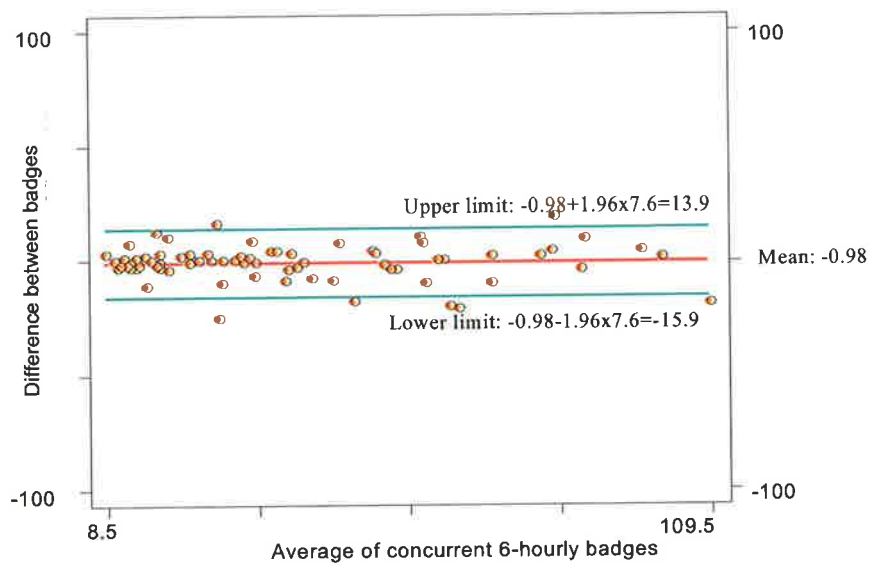
Figure 6: six hourly passive NO_2 monitors situated next to each other: difference between the concurrent monitors versus average of the pair

Six hourly located next to each other

Unlike with the exposure badges located at a distance from each other, badges lying next to each other showed very good agreement (figure 6). In relation to this experiment it can be expected that 95% of the differences between the monitor pairs would be less than the range of 14 ppb –16 ppb defined by the limits of agreement.

One hourly and six hourly continuous sampling compared with the passive diffusion method

For comparison of the two different methods for measuring NO_2, 29 one hourly observation pairs (not directly next to each other) were available. Figure 7 indicates that lines of agreement are much closer together compared to those depicted in figure two displaying one hourly results for comparison of passive badges. Differences for most of the sampling pairs ranged from –38 ppb (passive badges exceeding epa monitor) to + 34 ppb of NO_2 (epa method exceeding passive method).

Only 5 pairs of NO_2 measurements (5 classrooms) were available for testing the agreement between continuous and passive badges over six hours. Measurement pairs showed relatively similar NO_2 levels and any differences were likely to be due to distance in location (EPA versus passive diffusion method: 87-73; 63-68; 53-74; 84-80; 22-25 NO_2 in ppb).
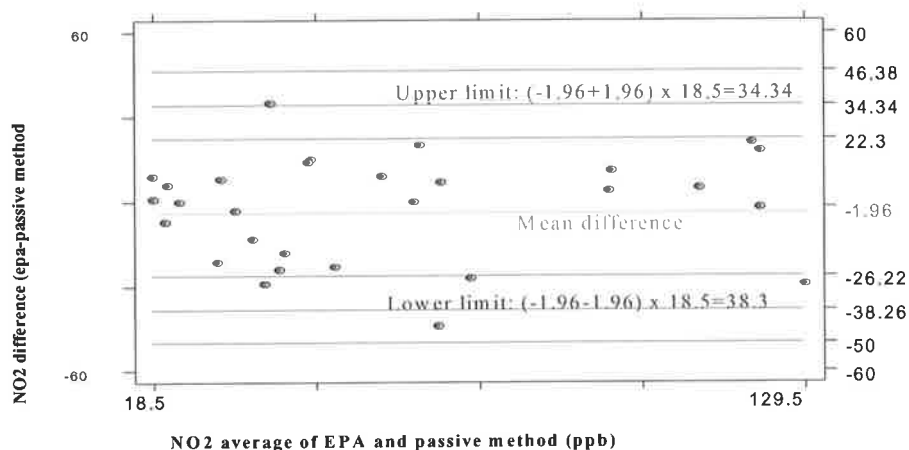
Figure 7: One hourly NO2 exposure in ppb in classrooms: difference between continuous method and passive method versus average of the concurrent measurements.

Discussion

The school survey provided important information in relation to the feasibility of conducting an intervention study. It registered a large number of schools with unflued gas heating in South Australia. Unflued gas heated schools were more prevalent in the country area. For the main study 19 schools of this type were available in the metropolitan area, including approximately 4000 children exposed to unflued gas heated classrooms.

Air sampling in a selection of these schools showed six hourly and one hourly high levels of NO_2. These levels were generally higher than those measured in studies investigating the health effect of NO_2 in the general and asthmatic population.

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One hourly samples of NO_2 were taken during the pilot study to investigate peak exposure levels in relation to levels averaged over the school day. As most of the heating was occurring during the first lessons in the morning, the six hourly averaged exposure level represented a more diluted picture of possible classroom levels during the day, as heating usually stopped around lunchtime or even earlier. But, as the one to two ratio of maximum one hourly versus maximum six hourly NO_2 levels was confirmed during the pilot study, it was reasonable to decide on an easily implemented six-hourly measurement regime for the main study, with the benefit of still being able to infer peak levels. Lack of agreement, especially at higher NO_2 exposure levels, of six hourly and one hourly NO_2 monitors situated in different parts of the room, led to two assumptions. Either, that the badges lacked repeatability or that a NO_2 gradient was present in the classrooms leading to differential levels of NO_2. Tests with six hourly proximal monitors strongly pointed towards the second possibility as agreement between the NO_2 levels was good, with 95% of the differences between the monitors being between acceptable limits of -16 ppb and $+14$ ppb of NO_2.

Additionally, passive sampling averaged over six hours resulted in very similar NO_2 levels to those achieved by the gold standard method. Therefore, monitors placed into the classrooms for six hours using passive diffusion badges would accurately reflect the classroom level of NO_2.

In response to the findings of differential NO_2 levels within unflued gas heated classrooms, it was decided to distribute three six hourly NO_2 monitors per classroom during the main study and hence calculate a mean representative classroom level of NO_2 on a daily basis.

Results from NO_2 levels in flued heated classrooms during the pilot study were very similar to exposure levels in electrically heated classrooms. For example, in Pilotto's school study the average level in electrically heated classrooms was 10 ppb (range of 8-23 ppb) of NO_2 (20) compared to 14 ppb (range of 8-20 ppb) of NO_2 for the flued gas heated classrooms in this study. Also, there was little

variability of NO_2 levels in flued gas heated classrooms and it was determined therefore that two monitors would suffice for representative NO_2 exposure in those classrooms where heaters were to be replaced by flued gas heaters or electric heaters. Overall, this pilot study concluded that flued gas heaters and electrical heaters were equally suited as intervention heaters.

Chapter 4 - Methodology for main study

This chapter describes the methods used for the randomised controlled asthma prevention trial. Ethics approval was sought and approval given by The Queen Elizabeth Hospital Ethics Committee and by the Research Council of the Department of Education Training and Employment (DETE) in South Australia. A letter of approval from the Catholic and Independent school board was also received.

4.1 School selection

A metropolitan survey of primary schools in Adelaide identified a total of 19 schools that used unflued gas heating in school classrooms during winter, making them eligible to participate in this study.

Prior to recruitment of schools (January to February 2000) a letter was sent to the principals to seek their support and involvement in the study. Subsequently a meeting was arranged to discuss the study and the necessary responsibilities of involvement for the schools. Principals were asked to maintain confidentiality about the link between replacement of heaters and this study, which was identified as a study of the indoor environment of schools and asthma. This was to blind school staff, the research participants and their families to the intervention. Consent for the study was finally achieved for 18 schools.

4.2 Randomisation

Practical consideration in relation to the randomisation process resulted in the use of cluster randomisation of schools.

For random allocation of schools into intervention and control groups the random number generator in Stata 7.0 was used (119). To be able to reproduce the results of the randomisation process a seed (**1989**) was preset. A new variable was created expressing random values between 0 and 1 for each school (table 3). Schools with a value greater than 0.5 were categorized to be intervention schools, while those with numbers smaller than 0.5 were to be control schools.

Table 3: Randomisation allocation

Identity number of school	Group allocation 0=control 1=inter-vention	Type of intervention: Flued gas =FG Electric heating = EH	Random number
15	0		0.4880412
18	0		0.045022
10	0		0.083379
6	0		0.4923418
3	0		0.4779098
5	0		0.0207296
11	0		0.073205
1	0		0.1336867
13	0		0.3977869
9	0		0.3311375
2	1	FG	0.6510205
14	1	EH	0.9393665
16	1	EH	0.8849291
17	1	EH	0.9259387
7	1	FG	0.5739803
12	1	FG	0.5026757
8	1	EH	0.9686185
4	1	FG	0.5778779

4.3 Intervention

Installation of heaters was organised by the education department such as to have the replacement appear as part of the normal school maintenance program. The costs for the heaters were to a large extent covered by NHMRC funds allocated for this study and supplemented by the school authority.

Picture two (page 70) shows examples of old heaters and a new (flued gas) replacement heater in classrooms of participating schools.

During the pilot study extensive measurements in classrooms showed that NO_2 levels in classrooms with flued gas heaters were very similar to classrooms with electrical heaters (see chapter 3: pilot study). Therefore, the Education Department was able to use either of the two heaters as replacement for unflued heaters as their budget for the extra costs allowed.

picture 2: Examples of unflued gas heaters and an intervention heater

Freestanding unflued gas wall heater

Flued gas replacement heater

Wall mounted unflued gas heater

4.4 Recruitment and maintenance of the study

All materials distributed for recruitment purposes are gathered chronologically in the attachments section.

4.4.1 Personnel

A research assistant was engaged for a period of 6 months to assist with recruitment and maintenance of the study.

Additionally, school support staff assisted in a paid capacity (one off payment of $100.00) and facilitated access to children on site.

Their tasks were to:

- Ensure that all students in unflued gas heated classrooms in the selected schools were identified and that all of these students would receive recruitment packages (eligibility questionnaire and information letter).

- Collect parent completed eligibility questionnaires.

- Promote the study in classrooms with low participation rate and report back to the investigators.

- Distribute consent forms and information sheets to eligible asthmatic children and follow up

- Place and collect 6-hourly NO_2 exposure badges in predetermined positions within classrooms

4.4.2 Asthma survey

Figure 8 shows the stepwise progression of the recruitment process.

In the first instance, an information letter (appendix I) was distributed to all children listed by the school support officers. Attached to this letter was a short questionnaire (appendix I) to be completed by the parents or carers in relation to location of their child within the respective school (classroom, teacher), home address, parents names, information regarding home appliances, and whether the children had been ever diagnosed with asthma by a doctor. Children whose questionnaires were not returned to the school support staff within a week received two more reminders together with an information package to take home.

4.4.3 Eligibility and consent

The second information letter (appendix II) was sent to parents of children who fulfilled the two main **eligibility criteria:**

(1) Doctor diagnosed asthma and (2) no unflued gas appliances in the household of the children. (gas cooking or unflued gas heating). This group is referred from here on as the a priori sample.

Once all eligibility questionnaires had been received it had become clear that only a third of the asthmatic population of children in Adelaide were free of unflued gas at home. It was then decided to modify the eligibility criteria for participation for a second group of children who had gas cookers in their homes. Children with unflued gas heating at home

were not eligible because of the exceptionally high contribution to NO_2 levels from this sort of heating. This group is referred to from here on as the extended sample. Measurement of household background levels of NO_2 in these children in comparison to the exposure in the group of children based on the a priori study hypothesis would then determine further the status of this extended sample in relation to the overall results of the study.

The letter invited parents and children to take part in an environmental assessment of the classroom environment in relation to their children's asthma. It included detailed description of the outcome assessments in which the children were to take part. These were (i) keeping of a daily diary, (ii) objective lung measurements and (iii) giving a sample of saliva for assessment of environmental tobacco smoke exposure. It also detailed any possible adverse events from lung measurements.

Each child's lung performance result was to be tabulated in a format to be interpreted readily by their general practitioner. This document was made available to the parents of all participants. Parents were also informed in general terms about environmental monitoring of classrooms as part of the study.

Although it was preferred that children took part in all elements of the study, parents were able to opt for the diary part only, as some parents had concerns about any form of physical testing.

Parents or caregivers were then asked to sign the attached consent forms (appendix II) and return them to the investigators. The children received their own consent forms addressing special concerns they may have had.

Finally, after consent forms were received, parents were issued with the first 4-weekly diary sheet together with instructions, a fridge magnet and pencil (appendix VI). For children who had consent to take part in spirometry, invitation letters for their baseline lung tests were issued at the same time.

Parents of eligible asthmatic children who did not respond in the first instance were recruited during a telephone information campaign.

Finally, a quality control survey was to be conducted after the parents had received the personal results for their children. This telephone survey using 10 randomly selected intervention and 10 control households had the purpose (i) to find out whether the study had been conducted to the satisfaction of the parents, (ii) whether the results from the personal measurements had been useful for the participating families, and (iii) whether the participants had noticed any change in relation to the air quality of the classrooms during the study period. The latter question was asked to ensure that participants did not relate heater replacement with the asthma study.

Figure 8: Flow diagram of recruitment material and information material

```
┌──────────────────────────────────┐
│  1.  identification of schools    │
│  information to principal         │
│  and enumeration of all students  │
│  in unflued heated classrooms     │
└──────────────────────────────────┘
                  │
                  ▼
    ┌──────────────────────────────┐
    │  2.  all students            │
    │  information sheet (1) and    │
    │  eligibility questionnaire    │
    └──────────────────────────────┘
                  │
                  ▼
    ┌──────────────────────────────┐
    │  3. students with asthma and │
    │  no gas at home:             │
    │  information sheet (2) and    │
    │  consent forms for RCT        │
    └──────────────────────────────┘

    ┌──────────────────────────────┐
    │  4. for all children with    │
    │  consent to RCT:             │
    │  Diary                        │
    │  severity questionnaire       │
    │  invitation to lung tests     │
    │  home NO  measurements        │
    └──────────────────────────────┘
```

4.5 Demographic and clinical factors

Baseline information about participants was gathered at the beginning and at the end of the study period to identify whether the randomisation process had succeeded and to assess possible imbalances between the groups.

Particularly in this case of cluster randomisation, baseline data may suffer from bias since the units of randomisation were schools rather than individual children within the schools.

As the main aim from randomisation was to obtain groups that are similar regarding factors related to the outcome, it was important to verify through baseline comparison whether imbalances had occurred and whether any statistical adjustment had to be made during outcome analysis (120).

For example, if low birth weight were to be unequally distributed between the two groups, then this in turn could lead to an imbalance of children with airways disease, which could have an effect on outcome variables such as symptoms. In this case the results would reflect the health status of the participants that caused the imbalance rather than the health effect caused by the intervention. Depending on which side the imbalance would occur, it could lead to the acceptance of the null-hypothesis (type II error) even though it is not true or it could lead to an erroneous relationship (type I error).

Significance testing of baseline characteristics as basis for inclusion into covariate adjustment has been discussed as inappropriate for randomised trials (120). As described above, only characteristics related to the outcome could potentially distort the results and in this case significant and non-significant baseline characteristics could have an effect on the outcome. In relation to this trial baseline variables were all selected on the basis that they may be important in relation to symptoms outcomes and therefore all were included for adjustment during analysis.

The following variables were gathered for baseline purposes: age, birth weight, chest illnesses before the age of two, presence of hay fever in the last 12 months, asthma status of the biological parents, ethnic background and information about exposure to environmental tobacco exposure.

During a telephone interview at the beginning of the study, parents were also asked about the severity of their children's asthma and the use of medication in the previous 12 months (see Appendix IV).

4.6 Study overview

Figure 9 gives an overview over all elements of the study period:

Recruitment of schools started in the last two weeks of the summer school holidays in January 2000. Recruitment of eligible children took place from the beginning of first term until mid May. Lung function tests and bronchial hyper-responsiveness measurements were conducted in May, while asthma symptom collection started in June, extending over 12 weeks until the 20th of August 2000. Every two weeks the parents and carers were contacted and interviewed about their children's symptoms over the previous two weeks. Diaries were also kept during the two weeks of school holidays. Lung function and hyper-responsiveness testing was repeated after the diary part of the study was concluded, which also constituted the end of winter and the end of the heating season.

Heaters were replaced over the first four weeks of term two and were finally in place by end of May.

Exposure to NO2 was monitored repeatedly during the study period in all classrooms and in the children's home.
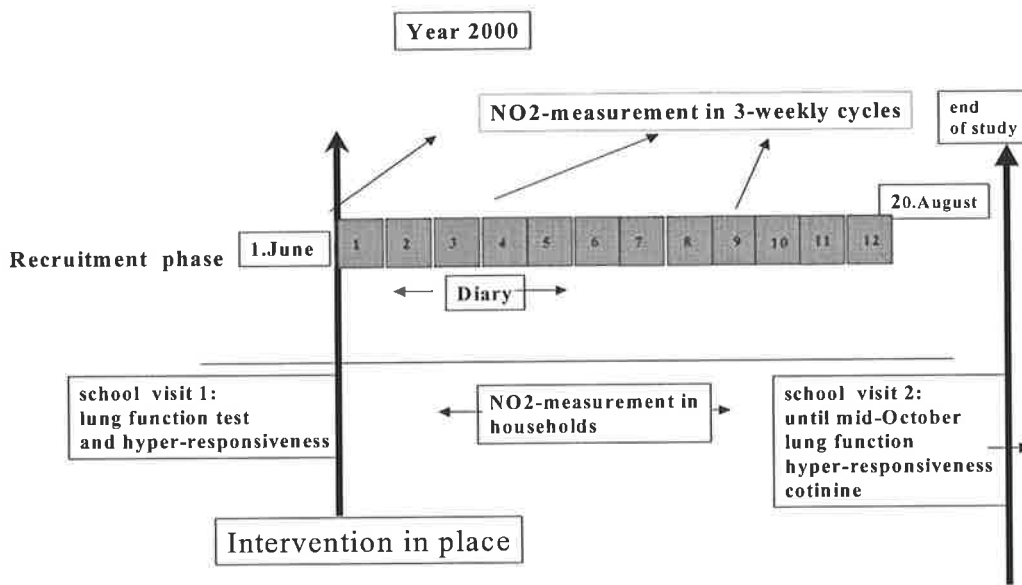
Figure 9: Diagram of study design and time flow

4.7 Exposure measurements

4.7.1 Nitrogen dioxide assessment in classrooms

Nitrogen dioxide was measured using the Australian standard (AS 2365.1.2.-1990) for the sampling of indoor air (115) as set out previously (see pilot study, page 50).

NO_2 was measured in three-weekly cycles in all schools. Each week all classrooms of 6 different schools were monitored for a period of 3 consecutive days for 6 hours from 9am to 3pm. After all schools were monitored initially (three weeks), another two cycles followed extending the exposure measurement period over 9 weeks during school heating, not including two weeks of school holidays.

Monitors were put into place and collected by the school support staff of the respective schools. The school support staff had been trained in the procedure of how to handle and where to place monitors prior to the exposure period. At the beginning of each school's exposure assessment, the school support staff received three sealed plastic bags for each classroom containing the following set of badges:

- Control classrooms

3 exposure badges labelled with: school ID classroom ID, date, round (1-3)

1 scavenger badge without lid, marked with a cross and a label indicating that it should be left unsealed in the sealed bag

1 control badge labelled: Do not open. Type: control classroom and school ID, date, round
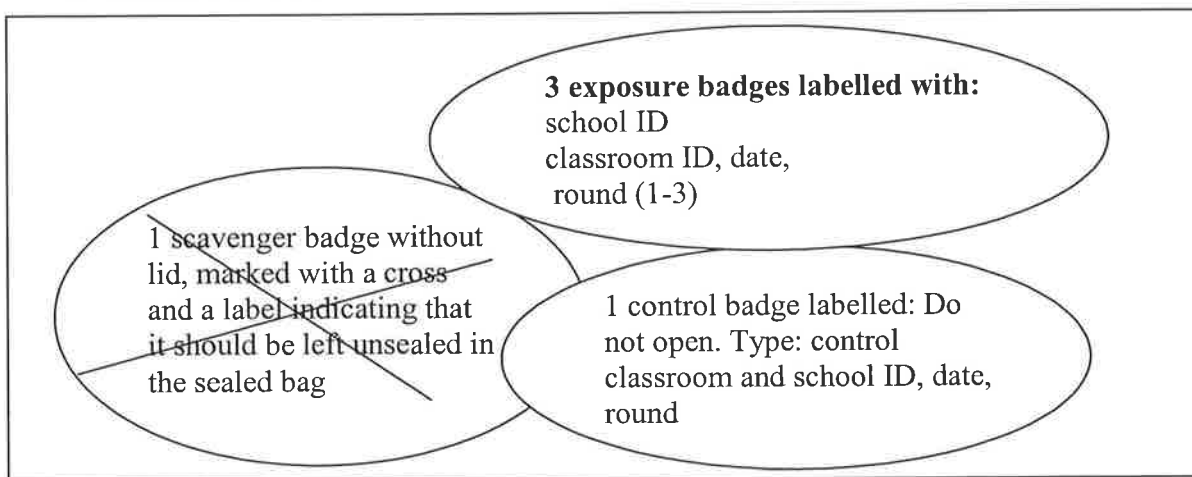
- Intervention classrooms received the same package, but instead of three exposure badges, only two were placed into every classroom. This was because during the pilot study no spatial differences in NO₂ levels were observed within electrically or flued gas heated classrooms.

At the end of each school day school support staff collected exposure badges from each classroom and packed them with their lids closed into their respective sealed bags. Over night the bags were kept in a closed plastic container in a fridge on the school premises. At the end of the exposure week containers were collected and brought to the processing laboratory.

4.7.2 Nitrogen dioxide exposure assessment in children's homes

Personal and kitchen NO_2 measurements were taken on three consecutive days. Children had an exposure badge pinned on to their clothes from the time they came home from school until bedtime. Parallel to the personal exposure two other badges were placed on the kitchen bench side by side. Parents recorded beginning and end of the time of exposure and this time was used for calculation of the time averaged level of NO_2. At the end of the three days children brought the monitors back to their respective school. Parents were instructed on the use of the exposure badges during a home visit and were given additional information sheets (see appendix: VII). Measurement of indoor NO_2 levels at home was explained as part of an environmental indoor study which also included collection of dust (house dust mite exposure) from the living room and the children's beds for another research project which is still ongoing.

4.7.3 Environmental tobacco smoke

Exposure of children to environmental tobacco smoke was measured by detection of saliva cotinine, a metabolite of nicotine which is the most sensitive and specific biochemical marker for tobacco smoke exposure for use in epidemiological studies.

Cotinine levels were detected using a microplate assay (STC Diagnostics: Micro-Plate EIA-kit) equipped with a solid phase enzyme reagent. The method was adapted using standards of 0, 10, 50 and 100 ng/ml of nicotine. The lower limit of detection was determined to be 5 ng/ml (personal communication with Elaine Whitham, Head of Toxicology/Special biochemistry, Chemical Pathology at the Adelaide Women's and Children's Hospital)

Saliva was gathered during lung tests, at the end of the study, from all students. Approximately 2-5 ml of saliva was collected into test tubes and immediately placed on ice, transferred to a freezer until analysis in the laboratory.

4.8 Outcome Measurements

4.8.1 Symptom diaries

To find out about daily asthma symptoms in the intervention and control groups a diary was maintained by the children with the help of their parents. The diary was based on a symptoms questionnaire previously used to elicit daily asthma symptoms in a panel of asthmatics in relation to household NO_2 (19). Daytime and night time occurrence of symptoms such as wheeze, difficulty breathing, chest tightness, cough and asthma attacks

were noted. Daily records of non-attendance at school and health care visits due to asthma were kept, as well as records of asthma medication.

Diary data were collected fortnightly over a 12-week period during a telephone interview with parents of participating children. In order to increase participation, to improve recall and to decrease observer bias, an independent market research company conducted the interviews. Interviewers used a pre-formulated script which set out in details how to approach parents during the fortnightly interview. At the beginning of the study they were trained in using the script and regular check ups of their interviewing technique were conducted by their supervisor.

4.8.2 Lung measurements

4.8.2.1 Personnel

Spirometry was conducted by two experienced lung technicians who had been previously involved in respiratory testing at The Queen Elizabeth Hospital and The Women's and Children's Hospital over several years.

For medical assistance during histamine testing, physicians were present at all times. Prior to the testing period, the physicians had completed an update of their basic life support skills.

All personnel were also blinded to whether the children were situated in a control or intervention school.

4.8.2.2 Information For Parents

Parents of the participating children were informed one week prior to spirometry and histamine testing, explaining the procedure and asking them to cease asthma medication according to the following instructions (Appendix V):

"Your child may be on medication which could get in the way of proper test results, therefore we ask you to stop any of the following medication according to the following time plan:"

5 days before test: antihistamines such as Teldane, Claratyne

3 days before test: antihistamines such as Zadine, Piriton Polaramine

24 hours before test: Theophyllines, such as Theodur, Neulin, Austyn, Elixophylline, Brondecon

24 hours before test: long acting bronchodilators such as Serevent, Foradile, Singulair, Salmeterol

night before test: Intal or Tilade

4 hours before test: bronchodilators such as Respolin, Bricanyl, Atrovent, Berotec, Alupent

4.8.2.3 Methods Of Measurement

Definitions of spirometry outcomes:

- Forced Expiratory Volume$_1$: indicating volume expired in 1 second (FEV_1) during complete expiration.

- Predicted FEV_1: FEV_1 expected for specific age, gender and height

- %predicted FEV_1: Percent of predicted FEV_1 which was calculated as

$$\frac{FEV_1 \text{ of child}}{\text{Predicted } FEV_1} \times 100$$

- Forced vital capacity (FVC): Volume measurement on complete forced expiration after full inspiration. (%predicted FVC as for %predicted FEV_1)

- Peak expiratory flow: Maximal expiratory flow rate achieved very early in the process of the FVC process (PEF). (%predicted PEF as for %predicted FEV_1)

Spirometry and broncho-dilation test:

Spirometry was performed in accordance with the protocol set out by the American Thoracic Society (ATS statements: standardisation of spirometry; www.//thoracic.org/statements/spirometry1-30.pdf).

For computerised adjustment of spirometry calculations, each child's gender, height and age were entered into a direct reading spirometer (Toshiba Satellite 2590CDT and Canon Notejet 11CX with Jaeger Toennis Master Scope, software version 4.34).

Forced expiratory curves were measured with the child standing using a nose clip. Measurements were repeated until two curves were obtained with reproducible FEV_1 and FVC to within 100ml. With some of the younger children the procedure had to be abandoned as no reproducible measurements were achieved.

Calibration of each spirometer was checked daily using a 2 l syringe. Barometric pressure and ambient temperature were checked twice daily and data adjusted to it accordingly.

Reversibility of airways:

Children were also subject to a broncho-dilation test.

Salbutamol (5mg/2.5 ml) was administered with a nebuliser for 10 minutes. Lung function was then measured again. An increase of 15% or greater was regarded to be significant, indicating reversibility of airways.

Response was measured as the increase in FEV_1 as a percentage of the initial value of FEV_1 which is referred to as reversibility :

$$\frac{\text{Post } FEV_1 - \text{pre } FEV_1}{\text{Pre } FEV_1} \times 100 = \% \text{ reversibility}$$

Bronchial hyper- responsiveness:

The method used in this study is based on the rapid method for measurement of bronchial responsiveness by Yan et al which has been successfully used in population studies (121). This method has since been evaluated and tested in children during several recent studies in Australia (122) (123) (124). Based upon the experience gathered during these studies a measurement protocol for children has been developed (125). This protocol has been shown to be reliable and safe in its use in children and has therefore been adopted for this study.

Two summary outcome measures of BHR derive from this method, firstly the dose of histamine that causes a 20% decrease in FEV_1 (PD20FEV_1), and secondly the percent fall in FEV_1 at last dose divided by the total dose administered which corresponds to the dose response slope (DRS).

PD20FEV$_1$

The use of responsiveness to a provocative dose of histamine in a population is restricted

by the concentration that can be given safely to an asthmatic child during field-work. For

this study the cut off point for histamine concentration was a cumulative dose of 3.8micro

mol (μmol) according to Woolcock (125).

From this follows that PD20FEV$_1$ can only be calculated for subjects who experience a

fall of 20% in FEV$_1$ within the boundaries of the administered doses.

Dose response

In many studies the information from those whose FEV$_1$ did not fall by 20% or more is

summarised categorically "as not responsive". To make use of all the gathered data and to

formulate a summary measure of severity of response for all participants the dose response

slope (DRS) was calculated (126) (127). The DRS was computed as the ratio of the total

percentage fall in FEV$_1$ from the saline value of FEV$_1$ to the FEV$_1$ at total cumulative

dose, divided by the total cumulative dose.

Procedure

Increasing doses of histamine were delivered into the child's airways by use of Devilbiss

hand-held glass nebulisers. By squeezing of the Devilbiss bulb a histamine aerosol is

produced, which in turn is inhaled by the child.

Before the application of histamine, baseline lung function was assessed, followed by one

dose of saline, which acted as a control for subsequent histamine inhalations.

Table 4 shows the application regime for histamine in this study:

Table 4: dosage regime for histamine challenge

Dose No	0	1	2	3	4	5	6	7	8
bulb	1	2	2	3	3	4	4	4	5
Hist conc. mg/ml	saline	3.13	3.13	6.25	6.25	25	25	25	50
No. of inhalations	1	1	1	1	2	1	2	4	4
Cumulative dose (μmol)	--	0.035	0.069	0.129	0.251	0.467	0.899	1.762	3.78

After inhalation of each dose the procedure was as follows:

After a 3 second holding period (ensured by a nose clip), the children were asked to close their mouths and to breathe slowly through their nose. A stopwatch was set for 50 seconds after which the child performed a lung function test. This regime continued until FEV_1 had dropped greater than 20% or until all the above doses were administered, the highest dose being 3.78 μmol of histamine.

Children with an initial FEV_1 less than 80% predicted did not proceed with histamine testing.

After the histamine challenge test was completed, children were given salbutamol (5mg/2.5 ml) with the help of a nebuliser in order to restore lung function back to normal and to overcome any adverse effects of histamine.

Spirometry testing

Histamine challenge with Devilbiss bulb

Picture 3: Objective lung measurements

4.9 Statistical analyses

For data entry Epi-info 6 was used. In case of lung measurements, spirometry results were re-entered using the Epi-info re-entry module (128). For descriptive analysis, bivariate and multivariate analysis Stata 7.0 was used (119).

Estimation of significance of differences between the two groups was calculated with the cluster option in Stata. This command takes into account clustering by schools by producing robust variances and standard errors of outcome estimates in situations where observations were not independent.

4.9.1 Adjustment for clustering

As discussed previously, participants in this study were not randomised as individuals, but as groups of asthmatics clustered into schools. With this study design it was necessary to take into account a possible cluster effect of the school. For example, if the children in the cluster had something in common which may in turn be related to the outcomes measured, then this could lead to false interpretations of results. Taking into account cluster effect during analysis can lead to potential loss of power and therefore to a need for an increased sample size (129). A way to overcome and to accommodate this dependency of data is to estimate a potential design effect due to clustering with the help of an intra-cluster correlation coefficient and thus adjust the originally calculated sample size for non clustered randomisation (130).

For this study a correlation coefficient was calculated based on a previous school cohort of 40 asthmatics who were part of a general population cohort of 598 children distributed into 8 schools (20). The intra-class and intra-school correlation coefficients of this

asthmatic sub-population were zero for wheeze, and were 0.01 for non-specific asthma symptoms such as cough with phlegm and dry cough. This information indicated that adjustment of the sample size was not needed in the case of recording specific asthma symptoms.

Nevertheless, a sample size based on the intra cluster correlation coefficient calculated from Pilotto's study of 0.01 for non-asthmatic symptoms was computed so as to allow for possible correlation effects.

The design effect was based on a formula which had been used in community trials by several authors previously (130) (131) (132) (133).

$$D = 1 + (m-1) \times rt$$
$$Nc = D \times Nn$$

D=design effect, m=the average cluster size, rt=intra class correlation Nc=D x Nn factor, Nc=sample size corrected, Nn=normal sample size.

4.9.2 Non clustered (Nn) sample size calculation for asthma symptom rates

Win EpiScope was used to calculate the non-clustered sample size (134).

The sample size calculation was approached in two different ways:

Firstly, the sample size was determined by assuming a 50% reduction in mean symptom rates based on a standard deviation of 80%, with 95% confidence and 80% power in a two tailed test which resulted in a sample size of 86 participants (43 per group). This would allow the detection of a clinically significant reduction in symptoms from an average of

four to two asthma symptom days over a period of 100 days. This period of 100 days is approximate to the winter heating period in Adelaide.

A possible design effect was calculated and adjustment was made to the sample size:

$$D=1+(18-1)\ 0.01= 1.17$$
$$Nc=1.17 \times 86 =101$$

Sample size: 101

Secondly, the mean symptom rate for wheeze from Pilotto's study was used to estimate a sample size. In this study the mean symptom rate for wheeze among asthmatic children over winter was calculated to be 0.048 (sd: 0.07). Based on this data it was estimated that **136** participants per group would be needed to determine a 50% reduction in symptom occurrence during the study period, with 95% confidence and 80% power in a two-tailed test. This would allow detection of a clinically significant reduction in symptoms in an average child over winter from 4.8 to 2.4 days. Due to the large standard deviation derived from this study the calculated sample size increased considerable. This can be explained by the small number of asthmatics (40) included in the overall number of primary school children in this study.

A possible design effect was calculated and adjustment was made to the sample size:

$$D=1+(18-1)0.01= 1.1$$
$$Nc=1.17 \times 272=318$$

Sample size: 318

The true sample size needed was thought to lie in between 101 and 318 participants and the aim was to include as many eligible children as possible.

4.9.3 Sample size calculation for objective lung measurements

The estimated sample sizes for objective lung measurements were smaller. For example, the sample size required to detect a 10% shift in PD20 based on a mean PD20 of 0.9 (sd: 0.1) with 95% confidence and 80% power was 22 children per group.

Additionally, the sample size was increased by a factor of 1.7 as only 60% (1:0.6) of self reported asthmatics were expected to be responsive. Also, similar to the symptoms diary, possible cluster effects had to be taken into account and the sample size was therefore increased to an upper limit, using the design effect previously calculated (D=1.17):

responsiveness factor: 22 x 1.7=37

design factor: Nc=1.7 x 37= 43 per group and therefore requiring a total of 86 subjects.

In relation to lung function the study had a 80% power to detect a difference of 3.5% in %predFEV$_1$ (SD:10) expressed as a percentage of the predicted value.

4.9.4 Estimation of source population and asthmatic children

Eligible schools were predetermined by the two surveys undertaken prior to the main study (see pilot study, page 48). After completion of the pilot study (winter 1999) there were in excess of 36 primary schools which had unflued gas heating within the metropolitan area and nearby. But, 6 months later, by the time these eligible schools were

enrolled to the randomised controlled trial, some of them had already switched to electricity and were not eligible any more, leaving only 19 eligible primary schools. The projection at the outset was that we would expect to find a prevalence of at least 19% of asthmatic children among the 5-13 age group of primary school children (135). Therefore, approximately 190 asthmatics were expected per 1000 primary school students. It was estimated, based on a previous survey in Port Adelaide, that roughly 50% of the households would have unflued gas household appliances and therefore 95 children per 1000 primary school children would fulfil the eligibility criteria. Based on the above projection, 19 primary schools were sufficient to achieve the projected sample size.

4.9.5 Analysis of lung function results

Mean FEV_1, FVC, PEF and %predicted FEV_1, FVC and PEF were calculated for intervention and control groups at baseline and at the end of the study period. Only children with successful spirometry at baseline and at the end of the study were included. To estimate the significance of any differences in spirometry outcomes between the 2 groups at the end of the study period, regression analysis was performed. The main focus was the difference in %predicted FEV_1 between the two groups. Baseline data outcome variables (FEV_1, FVC, PEF and %predicted thereof) were included into the regression analysis as co-variates to allow for adjustment for pre-study lung function in accordance to Frison et al (136).

To determine the significance of differences in proportions of reversibility between the exposed and the intervention groups, chi square analysis was used.

4.9.6 Analysis of bronchial hyper-responsiveness

4.9.6.1 Calculation of PD20FEV₁

To derive PD20FEV$_1$ (dose of histamine which causes a 20% fall in FEV$_1$), linear interpolation between the fall in FEV$_1$ at the log of the second highest cumulative concentration of histamine and fall in FEV$_1$ at the log of the highest cumulative concentration was undertaken to calculate the cumulative concentration at the point where FEV$_1$ dropped by 20 percent. **_Interpolation formula (119):_**

$$\ln(PD20FEV_1) = ((\ln \text{cumdoseb}) - \ln \text{cumdoseb2})/ (\text{fallb-fallb2})) * (20-\text{fallb2}) + \ln(\text{cumdoseb2})$$

Definitions:

- %Fall between FEV$_1$%pred post saline and FEV$_1$%pred at second highest histamine concentration = **fallb2**

- %Fall between FEV$_1$%pred postsaline and FEV$_1$%pred at highest histamine concentration = **fallb**

- cumulative concentration of histamine at fallb2 =**cumdoseb2**

- cumulative concentration of histamine at fallb =**cumdoseb**

For example:

$$\frac{(\ln 3.78 - \ln 1.76)}{(44.13 - 13.17)} \times (20 - 13.17) + \ln 1.76 = 0.73 = \exp(0.73) = 2.08 = PD20FEV_1$$

From the above follows that the lower the value of $PD20FEV_1$ the more severe is the degree of airways responsiveness.

Analysis of variance was used to compare mean values of $PD20FEV_1$ between the two groups. $PD20FEV_1$ prior to the study was included as co-variate and cluster adjustment was made for schools. $PD20FEV_1$ values were converted to base10 logarithm prior to analyses, and geometric mean values were reported, because of the log normal distribution of $PD20FEV_1$.

Severity of bronchial hyper-responsiveness was established by categorizing of $PD20FEV_1$ into grades of severity. These grades have been shown in previous studies to be reproducible and highly sensitive to identify 100% of current symptomatic asthmatics (125) (137):

Severe BHR: $PD20 \leq 0.1$ µmol

Moderate BHR: $PD20 = 0.11-0.8$ µmol

Mild BHR: $PD20 = 0.81-3.2$µmol

Slight BHR: $PD20 = 3.21-7.8$ µmol

(in this study the maximum concentration was 3.78 µmol)

4.9.6.2 Calculation of DRS

The following steps show how the DRS (%fall in FEV_1 at the highest cumulative dose of histamine divided by the total cumulative dose of histamine given) was computed

1. % fall= ((FEV_1%pred post saline - FEV_1%pred at highest cumulative dose)/FEV_1%pred post saline) x 100

As some children increased their FEV_1 during histamine exposure and therefore would receive a zero or negative DRS, a constant of 3 was added to all DRS values.

2. DRS= (%fall / highest cumulative dose)+ 3

3. DRS were converted to base 10 logarithm prior to further analysis in order to reach an approximately normal distribution for the purpose of further regression analysis.

4. geometric means of DRS were calculated.

Example: FEV_1%pred post saline = 108; FEV_1%pred at highest cumulative dose of histamine=86

Highest cumulative dose of histamine administered = 1.762 μmol

Step 1 % fall= (108-86/108) x 100= 20.4

Step 2 DRS=(20.4 / 1.762) + 3 = 14.6

From the above follows that the higher the value of the DRS the more severe is the degree of airways abnormality.

Analysis of variance was used to compare DRS between the two groups. DRS values measured at baseline were included as co-variates and cluster adjustment was made for schools. DRS values were converted to base10 logarithm prior to analyses, and geometric mean values were reported because of the log normal distribution of DRS.

4.9.7 Statistical analyses of diaries

Symptom diary data were used for the following outcomes:

Asthma symptom rates were calculated for (1) a priori group of children, (2) extended sample of children and (3) for the combined group of children.

Symptom incidence rates were calculated as ratio of the sum of all days where symptoms were present over the number of days children were participating in the study.

Example: participation (exposure): 84 days; wheeze on 8 days: wheeze rate =0.095

The relative risk (RR) was then calculated as the main outcome by dividing the mean incidence rates of asthma symptoms in the intervention group by the mean incidence rates in the control group.

Example: 0.065/0.095=0.68

In case of a RR smaller than one this would indicate a decrease of symptom occurrence in the intervention group compared to the control group. In the event of a RR of one, or near to one this would mean no difference between the groups, and finally, in the event of the RR being greater than one, this would indicate an increase of symptoms in the intervention group.

To compare rates between the two groups and for calculation of the 95% confidence intervals and p-values, negative binomial regression analysis was used, adjusting for clustering by schools (119).

This model describes recurrent events where the standard deviation of the mean rate is much larger than the mean, representing the fact that symptoms occur more frequently in some participants than in others (138). In this case of overdispersion of event data, negative binomial regression analysis is superior to a poisson based analysis, and it also allows for variable follow up times for participants.

Chapter 5 - Results from randomised controlled trial

5.1 Randomisation Outcome

5.1.1 Participation

A flowchart for cluster randomised studies developed by Elbourne has been adapted to show the flow of participants through all stages of the study (139). It was designed to extend the "consort flow diagram" from individual participants to cluster level and to be able to illustrate comparability of clusters after randomisation.

Baseline data, including severity of asthma information will be reported, firstly, to characterise the children included, secondly, to demonstrate the balance achieved between the two groups after the randomisation process, and thirdly to discuss necessity of adjustment for baseline characteristics during analyses.

The randomisation diagrams (figures 10 and 11) show the flow of participants through the stages of the recruitment process.

Nineteen schools with unflued gas heating in 143 classrooms in the metropolitan area were identified. Eighteen of the principals readily agreed for their schools to take part in the study. After randomisation of schools there where 8 schools (56 classrooms) in the intervention group and 10 (76 classrooms) in the control group. Cluster size by school was similar with an average of 197 children in the intervention schools and 178 in the control schools (figure 10).

Of the total number of students available, 66.2% (945) completed the eligibility questionnaire in the intervention group and 62% (1224) in the control group. Response to this questionnaire was comparable at the cluster level (mean cluster size: 118 vs 122

children). The prevalence of doctor diagnosed asthma was only slightly higher in the control group (intervention: 23.9%; control: 26.8%) (figure 10).

After application of a priori eligibility criteria, there were only 7.8% (74) of asthmatic children with home electric cooking (and no other source of unflued gas) in the intervention group and 10% (125) in the control group (figure 11). Within this group of children, the a priori group with no background home exposure to NO2, final consent was received for 45 children in the intervention group and 73 in the control group. Due to the unexpected low number of children with no exposure to unflued gas at home and in order to potentially increase sample size, a random selection of children from gas cooking households (but without unflued gas heating) was also made. These children are followed through the flow diagram in two separate groups (figure 11). Out of 356 asthmatic children with gas cooking at home 155 were randomly selected and asked to take part in the study. Consent was received for 43 children from the intervention sample and 43 from the control sample.

As children did not take part in all elements of the study, participation of children, both individually and combined, in the various parts of the study are presented in figure 11. With all children combined, mean cluster size after consent comprised 13 children in the intervention group, and 12 in the control school.

Overall, the flow chart indicates an equal participation in the study by intervention and control group on the individual and on the cluster level.

For statistical analyses, the participants under study therefore comprised three groups of children:

(i)   *The "a priori sample"* (children with no gas appliances at home)

(ii)   *The "extended sample"* (children with gas cooking at home)

(iii)   *The combined sample (a priori sample plus extended sample)*

Figure 10: Flow diagram of randomisation of schools and participation of students in the eligibility questionnaire.
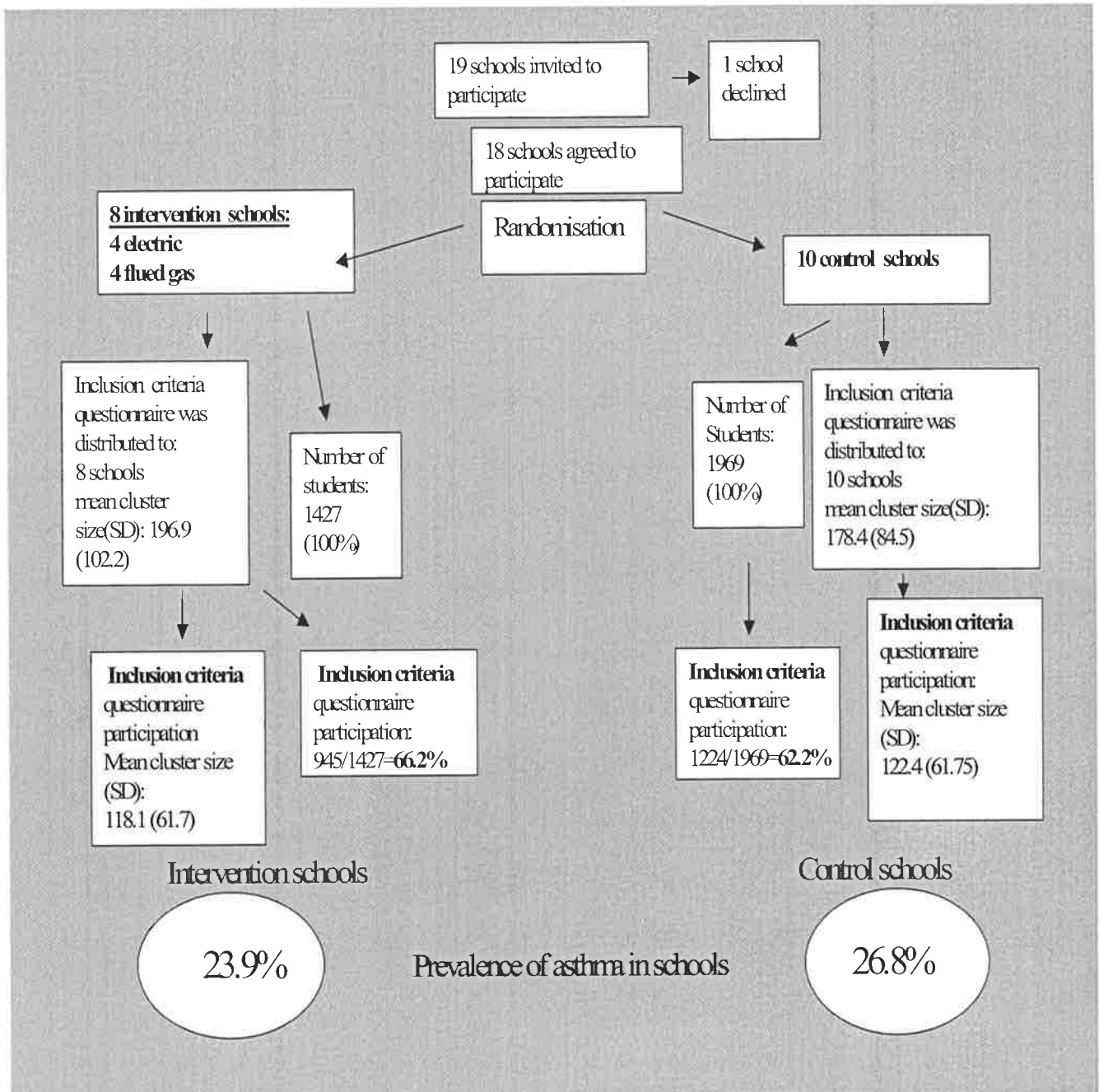
Figure 11: Flow of eligible asthmatics through the study protocol

Intervention group 226

Doctor diagnosed asthma

Control group 329

Eligible cluster size for combined children:
Mean (SD) cluster size in 8 schools: 17.6 (7.5)

Eligible children with electric appliances (a priori sample): 74

Eligible children with gas cooking (extended sample): 152

152/945 random selection of 73/152

Eligible children with gas cooking (extended sample): 204

Random selection: 82/204

Eligible children with electric appliances (a priori sample): 125

Eligible cluster size for combined children: Mean (SD) cluster size in 10 schools: 20.4 (9.7)

Consent from parents in the a priori sample: 45/74=60%
Participation in:
1.severity of asthma: 100%
2.baseline quest.: 100%
3.diary: 100%
4.lung function: 84%
5.BHR: 60%

Consent from parents in the extended sample: 43/73=59%
Participation in:
1.severity of asthma: 84%
2.baseline quest.: 100%
3.diary: 93%
4.lung function: 63%
5.BHR: 16%

Consent from parents in extended sample: 43/82=52%
Participation in:
1.severity of asthma: 84%
2. baseline quest: 100%
3.diary: 100%
4.lung function: 72%
5.BHR: 28%

Consent of parents in a priori group: 73/125=58%
Participation in:
1.severity of asthma: 65/73=89%
2.baseline quest: 73/73=100%
3.diary: 65/73=89.0%
4.lung function: 52/73=71%
5.BHR: 37/73=51%

Combined children:
Consent of parents from 7 schools was received. 1 school had no participant.
mean cluster size (SD): 12.6 (6.4)
1.Severity baseline: 11.7 (6.1)
2.Baseline: 12.3 (6.5)
3.Diary: 12.5 (6.5)
4.lung function: 9.3 (6.6)
5.BHR: 5.0 (2.9)

Combined children:
Consent of parents from 10 schools was received.
Mean (SD) cluster size: 11.6 (5.1)
particular in: mean (SD)
1.severity baseline: 10.1 (4.7)
2.baseline: 11 (4.8)
3.diary: 11.6 (5.1)
4.lung function: 9.2 (3.5)
5.BHR: 5.0 (2.8)

5.1.2 Distribution of baseline variables

For the a priori children randomisation produced very similar groups in relation to demographic variables such as age, gender and ethnicity and prognostic variables such as birth weight and smoking of carers (table 5). Small baseline imbalances (>5%) were present for hay fever, chest illness before the age of two and education of main carer. In particular, hay fever in the last 12 months was more prevalent in the control group and having finished high school was a more prevalent feature for carers in the intervention group.

In the extended group of children there were imbalances between intervention and control group for all baseline variables apart from birth weight.

No major imbalances of characterising variables were seen in the combined group of children. None of the children had any detectable levels of cotinine in their saliva sample. The laboratory was initially surprised that none of the samples tested positive. Methods and results were therefore re-checked, but no error was found. A low smoking prevalence (9% intervention - vs 11% control group) and information from smoking parents that smoking only occurred outdoors may explain the result.

5.1.3 Baseline severity of asthma

Table 6 sets out the results of the baseline asthma severity questionnaire (Appendix IV).

In the a priori sample and the extended sample there were slight differences in relation to when the last asthma medication had been taken. When the first two categories were combined (in the last 4 weeks and in the last 12 months) an equal majority of intervention and control children in the a priori sample (84% versus 84%) fitted into this combined medication category. While in the extended sample there were slightly more children in the control group who had taken asthma medication in the last 12 months (82% versus 89%).

For the combined children the distribution of their asthma medication usage was very similar.

When current status of asthma severity was established in form of symptoms and medication combined, most of the children fell into the category of "some asthma and occasional medication". The percentage for this category was similar for intervention and control groups regardless of their home cooking category.

Table 5: Characterisation of children by demographic and prognostic variables (mean age, otherwise %), by home exposure to NO_2 and by intervention and control group.

Questions Variables	A priori sample		Extended sample		Combined sample	
	Intervention N=45	Control N=73	Intervention N=43	Control N-=43	Intervention N=88	Control N=116
Age, SD, range	8.4 (2.2) 5-12	8.7 (2.3) 5-12	8.2 (2.4) 5-12	7.9 (2.3) 5-12	8.3 (2.3) 5-12	8.4 (2.3) 5-12
Gender female %	51	49	42	58	47	53
Australian born % (non-aboriginal)	80	79	77	84	82	79
Birth weight <2500 g %	9	7	12	9	9	9
Chest illness before the age of two %	42	37	37	51	40	42
Hay fever in last 12 months %	29	36	30	35	30	35
High school completed: main carer %	71	60	54	67	63	63
Smoking main carer %	9	11	16	9	13	10
Smoking second carer %	13	14	26	14	19	14
Cotinine	No detectable levels in any of the children					

Table 6: Distribution of severity of asthma in relation to use of asthma medication and symptoms (%), by category of home exposure to NO$_2$ and by intervention and control group.

	A priori sample		Extended sample		Combined sample	
	Intervention N= 45	Control N=65	Intervention N=37	Control N=36	Intervention N=82	Control N=101
Last medication?	%(n)	%(n)	%(n)	%(n)	% (n)	%(n)
Taken in the last 4 weeks	48	55	61	47	54	53
Taken in last 12 months	36	29	21	42	29	33
Taken since in reception	9	9	13	11	11	10
Taken when under two years of age	2	5	0	0	1	3
Never taken	5	2	5	0	5	1
Current symptoms						
no trouble with asthma	11	17	24	14	17	16
some asthma, no medication	9	6	8	3	9	5
some asthma, occasional medication	64	57	55	53	60	55
asthma and routine medication	5	3	8	8	6	5
asthma, routine medication + additional asthma medication when necessary	11	17	5	22	9	19

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5.1.4 Discussion of baseline characteristics

The percentage distribution of baseline variables between intervention and control groups were similar for the a priori sample and the extended samples. In the extended group small differences were observed for all of the variables. This result reflects the effect of a decreased sampling error with increasing size of the trial group.

Altmann recently declared in the revised "CONSORT" statement that the choice of variables for adjustment during the main analysis should be declared as to whether they were chosen due to predetermination at the protocol stage or due to baseline imbalances at the analysis stage (140). In this study, baseline variables had been predetermined at protocol stage and therefore, to allow for possible effects of these characteristic variables on the result, all the variables were included in an analysis of covariance in case of symptoms comparison.

On the other hand, results of lung function (FEV_1, FVC, PEF) and bronchial hyper-responsiveness testing (PD20, DRS) were adjusted for by inclusion of the relevant baseline (beginning of the study) data as co-variates.

5.2 Results from Nitrogen dioxide measurements

This chapter presents detailed results from atmospheric NO_2 measurements taken in classrooms, outdoors and in children's homes.

For classroom and home measurements, NO_2 data is presented by categories of children in relation to their home background exposure to unflued gas cooking or electric cooking, and by intervention and control status. Classroom measurements were gathered to demonstrate differences in NO_2 levels in children's classrooms after heater exchange. Outdoor exposure to NO_2 is presented at school level by intervention and control status. It was measured in order to detect possible imbalances in outdoor NO_2 between intervention and control schools. Home exposure to NO_2 was measured for the purpose of investigating potential misclassification of exposure status.

5.2.1 NO_2 concentration in classroom

5.2.1.1 All NO_2 measurement badges

After randomisation of schools monitors for NO_2 were organised for the 57 intervention and 77 control classrooms. As described previously in the methods chapter, NO_2 was measured over 9 weeks during the study period. Each week, six schools were monitored for three days rotating over three cycles, thus recording NO_2 levels on 9 days in each classroom during the study period.

This also included classrooms infrequently used by students for music, art and computing. The box and whisker graph presented in figure 12 includes the exposure measurements of all 979 intervention and 1539 control badges.
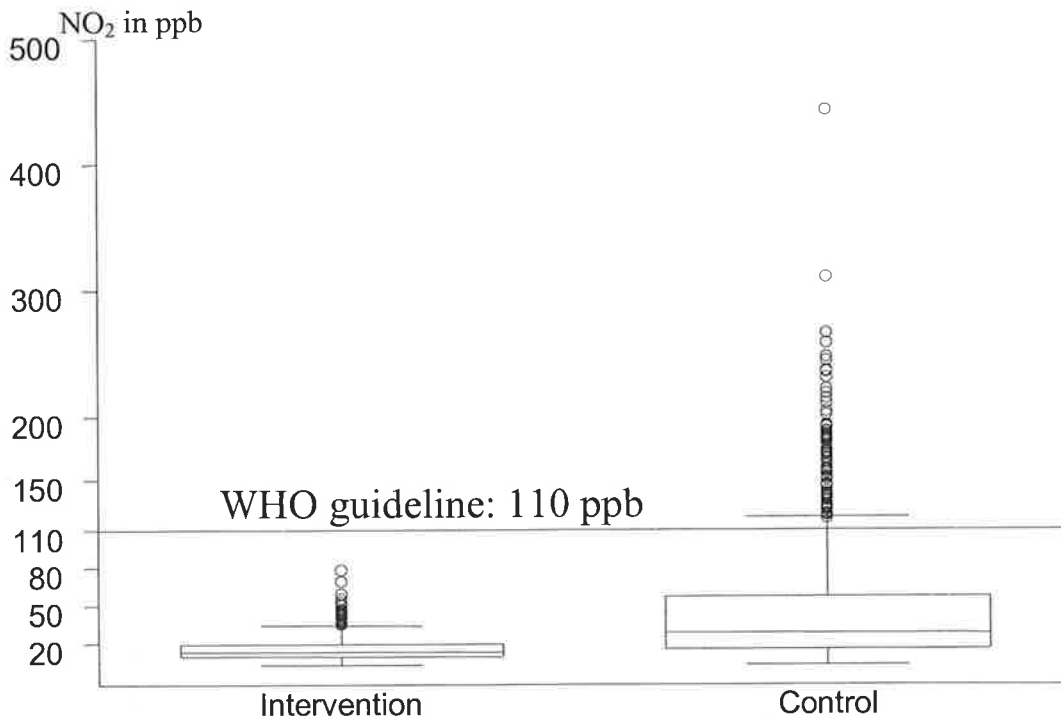
Figure 12: Distribution of time averaged NO$_2$ levels in ppb (all measurement badges) after exchange of unflued gas heaters in intervention classrooms with electric or flued gas heaters in 134 classrooms, by intervention and control schools.

Overall, there were 6.4 % NO$_2$ measurements below the six-hourly detection level of 6 ppb in the control group and 1.8% in the intervention group. Because of the imprecise nature at NO$_2$ levels below detection limit, a NO$_2$ level of half the detection limit (3 ppb) was allocated. The overall mean concentration of NO$_2$ for all badges in the intervention group was 15.2 ppb (SD: 9.3; 95%CI: 14.6-15.8 range: 3-78) and 45.1 ppb (SD: 44.0; 95% CI: 42.9-47.3 range: 3-442). Classrooms in intervention schools had significantly lower levels of NO$_2$ compared to classrooms in control schools (P<0.001) (t-test of log transformed NO$_2$).

A number of classrooms had been included in the pilot study a year prior to the intervention. When 6-hourly classroom levels of NO_2 taken during the pilot study were compared to levels measured in the intervention classrooms after the intervention, a mean reduction of 68 ppb of NO_2 was observed.

5.2.1.2. Mean NO_2 levels at classroom level for participating children

This section describes levels of NO_2 for intervention and control groups based on mean classroom levels, excluding classrooms without participating children. Mean classroom levels were established as follows:

(1) Daily six-hourly mean classroom levels of NO_2 were calculated by averaging the exposure levels of two badges per classroom in intervention schools, and three badges in control schools. This resulted in up to 9 daily mean NO_2 observations per classroom during the study period.

(2) These daily six-hourly measurements were subsequently averaged to result in an overall mean classroom level for each of the classrooms.

(3) These mean classroom observations were then averaged for (i) the a priori group, (ii) the extended group and (iii) the combined group by intervention and control categories.

Results for mean NO_2 measurements for intervention and control group classrooms are set out in table 7 and are also shown in form of a box and whisker plots in figure 13. Mean NO_2 classroom levels were based on 755 measurements in 34 intervention classrooms and 1001 measurements in 53 control classrooms.

Mean levels of NO_2 for the total of 87 classroom were calculated using 6 measurements in 10 classrooms, 8 measurements in 14 classrooms and 9 measurements in 63 classrooms. Some

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Table 7: Mean NO$_2$ levels, categorised by exposure to household cooking of participating children, based on averaged classroom, outdoors and home measurements with passive diffusion monitors.

	A priory sample		Extended sample		Combined sample	
	Intervention N=45	Control N=73	Intervention N=43	Control N=43	Intervention N=88	Control N=116
Number of children with asthma						
Number of school classrooms	29	44	21	33	34	53
Number of measurements	384	632	371	369	755	1001
Mean exposure (SD) Range	15.5 (6.6) 6.8-38.1	47.0 (26.8) 11.6-115.6	13.6 (4.3) 6.8-23.7	51.3 (32.5) 10.7-117.3	14.6 (5.7) 6.8-38.1	48.6 (29.0) 10.7-117.3
Geometric mean	14.0 (12.5-15.7)	39.5 (34.8-44.7)	14.1 (12.6-15.7)	45.14 (37.1-54.9)	14.04 (12.9-15.2)	41.45 (37.3-46.1)
%children ≥WHO	0	27 (37.0%)	0	18 (42.0%)	0	45 (38.8%)
% of classrooms ≥ WHO guidelines	0	20/45=44.4%	0	15/33=45.5%	0	24/53=45%
Outdoor NO$_2$ mean (SD), range	Intervention N=38 10.4 (4.9) 3-21			Control N=56 12.3 (9.5) 3-58		

classrooms had less than 9 measurements because of pupil free days and sport days occurring on allocated measurement days.

For the a priori children, the mean classroom level of NO_2 in intervention schools was significantly lower than in the control classrooms (15.5 ppb 95% CI: 13.5-17.5 vs 47.0 ppb 95% CI:40.7-53.2 p<0.001). This was also true for the extended group of children (13.6 ppb 95% CI:12.3 -14.9 vs 51.3 ppb 95%CI: 41.3-61.3) and when the children were combined (14.6 ppb 95% CI:13.4-15.8 vs 48.6 CI:43.2-53.9).

Mean classroom concentrations ranged from 6 ppb to up to 117 ppb of NO_2.

Comparison of the NO_2 data gathered in this study with data compiled during the pilot study suggests that control classrooms with low NO_2 levels may reflect classrooms that were infrequently heated, or that heaters may have been switched on for short periods of time only. Whereas, higher NO_2 concentrations in intervention classrooms may reflect either high ambient NO_2 concentrations or initial adjustment problems with newly installed flued gas heaters.

5.2.1.3 Classroom measurements of NO_2 compared to WHO guidelines

The WHO guideline for NO_2 is currently set at 110 ppb for a time period of one hour (3). In this study NO_2 was time averaged over a 6 hour period equating to one school day. Considering all NO_2 measurements, including in classrooms with no participating children, 130 (5%) of badges exceeded WHO guidelines. WHO guidelines were exceeded (one or more times) in relation to 42% of the children and in 45% of classroom (Table 7).

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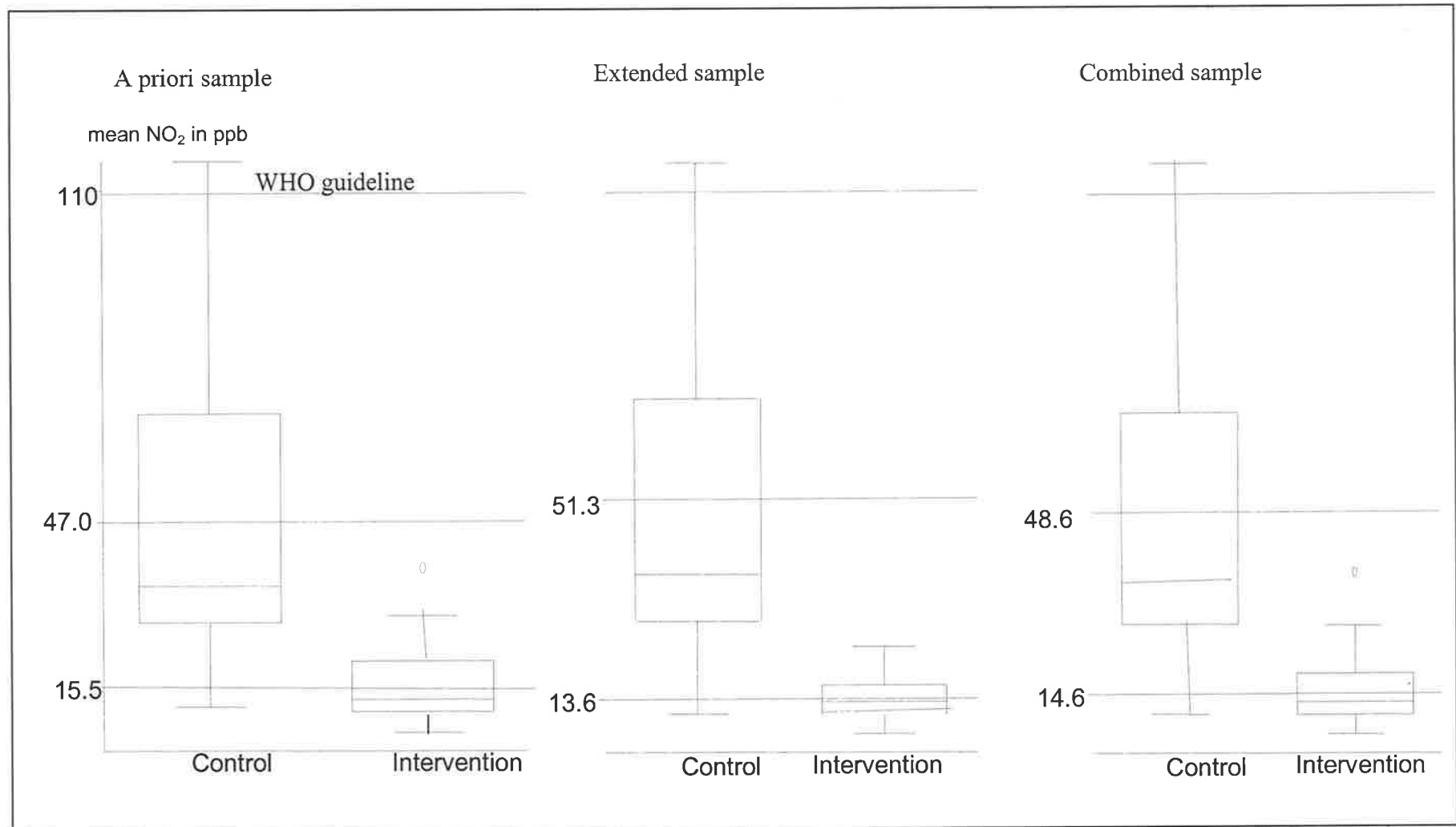
Figure 13: Comparison of children's mean NO₂ classroom levels (based on a maximum of 9 daily averaged measurements per classroom during 12 weeks study period), by category of home exposure to NO₂ and by intervention and control group.

5.2.2 Outdoor levels of NO_2

During the first three weeks of NO_2 sampling at schools, 94 outdoor measurements of NO_2 were obtained from the school area adjacent to the classrooms. Mean outdoors levels of NO_2 are summarised in table 7.

Mean outdoor NO_2 levels and the range of levels gathered in the intervention and control schools were very similar, and were consistent with the relative low levels measured outdoors during a previous environmental study in Adelaide (83).

5.2.3 NO_2 results from children's homes

Home measurements of NO_2 were taken in 180 out of 196 households over a period of three consecutive week days (table 8). Overall, mean number of kitchen measurements taken per household were 5.5 (SD: 1.6) (maximum 6: 2 badges per kitchen/per day over 3 days) and 2.6 (SD: 0.9) (maximum 3: 1 per day) for personal measurements on participants.

A mean kitchen concentration was calculated for every participating household and similarly, a mean personal level was established using the measurements of NO_2 taken over three days. Average time of daily exposure for kitchen badges was 4.3 hours (SD: 1.8) and 4.0 (SD: 1.5) hours for personal exposure. Within all three categories of children the concentrations measured personally and in kitchens were very similar for intervention and control children. Personal and kitchen distributions of NO_2 measurements are shown in figures 14 and 15 for households with and without gas cooking, and for combined households.

Gas cooking at home doubled the mean NO_2 level when measured by passive monitors in the kitchen, and when monitors were personally worn by the children (table 8). When comparing

personal and kitchen exposure to NO_2 of children (intervention and control) from homes with only electrical appliances (a priori sample) with levels of NO_2 measured in households of children with gas cooking (extended sample), a significantly lower NO_2 concentration was observed for the a priori children in relation to mean kitchen (14.3 ppb CI: 10.3-18.3 vs 28.7 ppb CI: 24.1-33.3; $p<0.001$) and mean personal NO_2 exposure (12.9 ppb CI: 10.4-15.4 vs 24.6 ppb CI: 20.8-28.3; $p<0.001$).

Personally measured mean NO_2 levels correlated well with mean kitchen NO_2 levels ($p<0.001$, R=0.75, R-squared = 0.6). Only two of the home measurements were above WHO guidelines and both of them occurred in the category of children with unflued gas exposure at home. There were also a number of higher exposures of NO_2 in the a priori children which may be explained by either high outdoor exposure through car exhaust or, in the case of personal exposure, by children going to places where they were exposed to NO_2. It is also possible that there was some misclassification of children regarding their background NO_2 sources, but generally households were checked for unflued gas sources during the home visit.

Table 8: Home exposure to time averaged NO$_2$ in ppb, by intervention and control group and by type of household appliances.

	A priori group		Extended group		Combined group	
Number of children with asthma	Intervention N=45	Control N=73	Intervention N=43	Control N=43	Intervention N=88	Control N=116
Number of personal measurements	44 (97%)	64 (88%)	40 (93%)	39 (91%)	84 (95 %)	103 (89%)
Number of kitchen measurements	42 (93%)	62 (85%)	40 (93%)	38 (88%)	82 (93%)	100 (86%)
Mean personal NO$_2$ (ppb) (SD) range	12.8(12.2) 3-42	12.9 (13.9) 3-79	25.1 (18.6) 3-28	23.9 (15.4) 3-69	18.7 (16.7) 3-42	17.1 (15.4) 3-79
Number of personal measurements ≥WHO	0	0	1	1	1	1
Mean kitchen levels of NO$_2$ (ppb) (SD) range	13.8 (19.3) 3-36	14.6 (21.5) 3-38	27.8 (22.1) 3-104	29.6 (18.8) 6-84	20.6 (21.7) 3-104	20.3 (20.7) 3-84
Number of kitchen measurements ≥WHO	0	0	0	0	0	0

Figure 14: Distribution of daily time averaged mean NO₂ levels measured personally (maximum 3 exposure badges) on children in their homes, by category of household appliances and by intervention and control group.
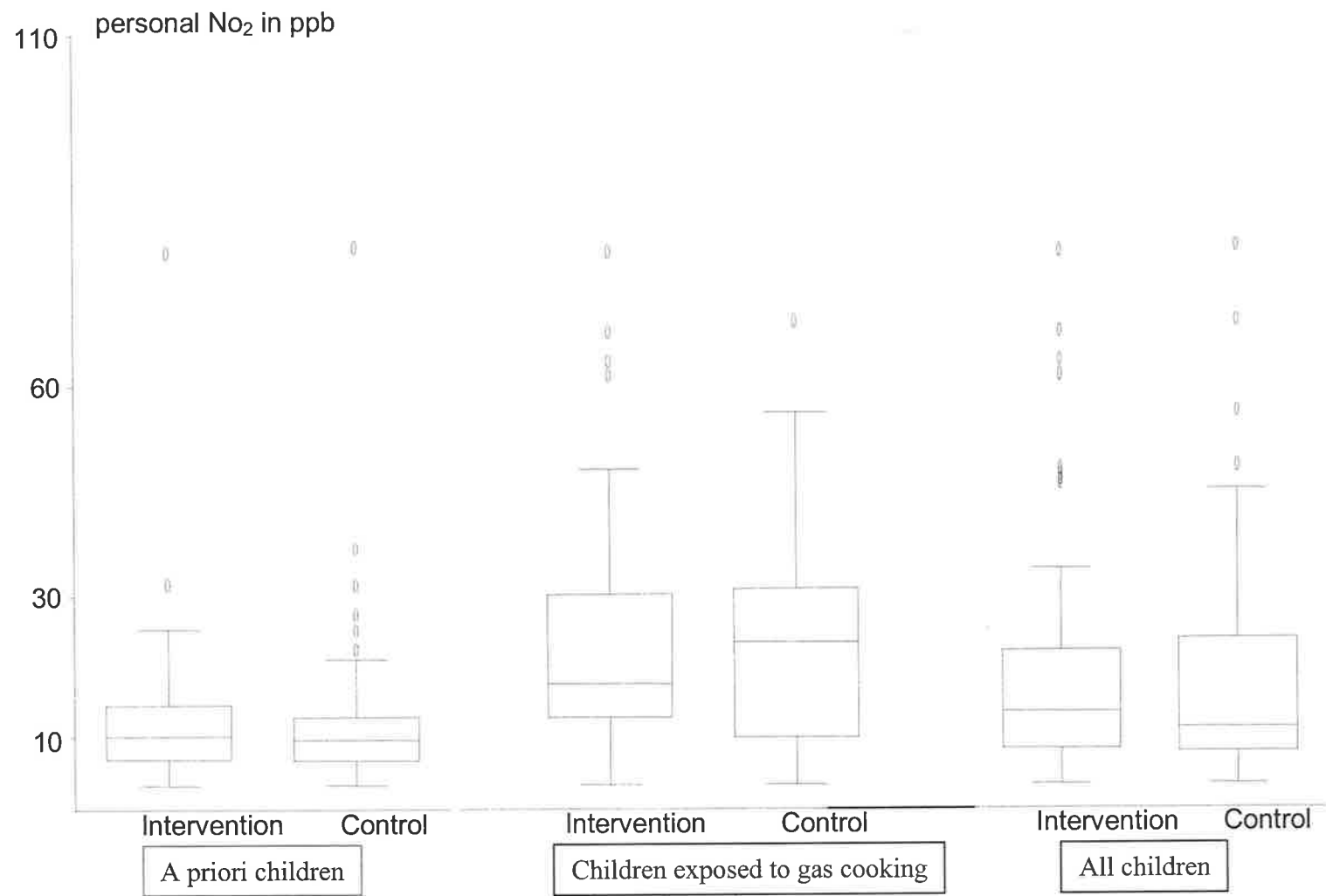
Figure 15: Distribution of daily time averaged mean NO$_2$ levels measured in the kitchen (maximum 6 exposure badges) on children in their homes, by category of household appliances and by intervention and control group.

mean kitchen NO$_2$ in ppb
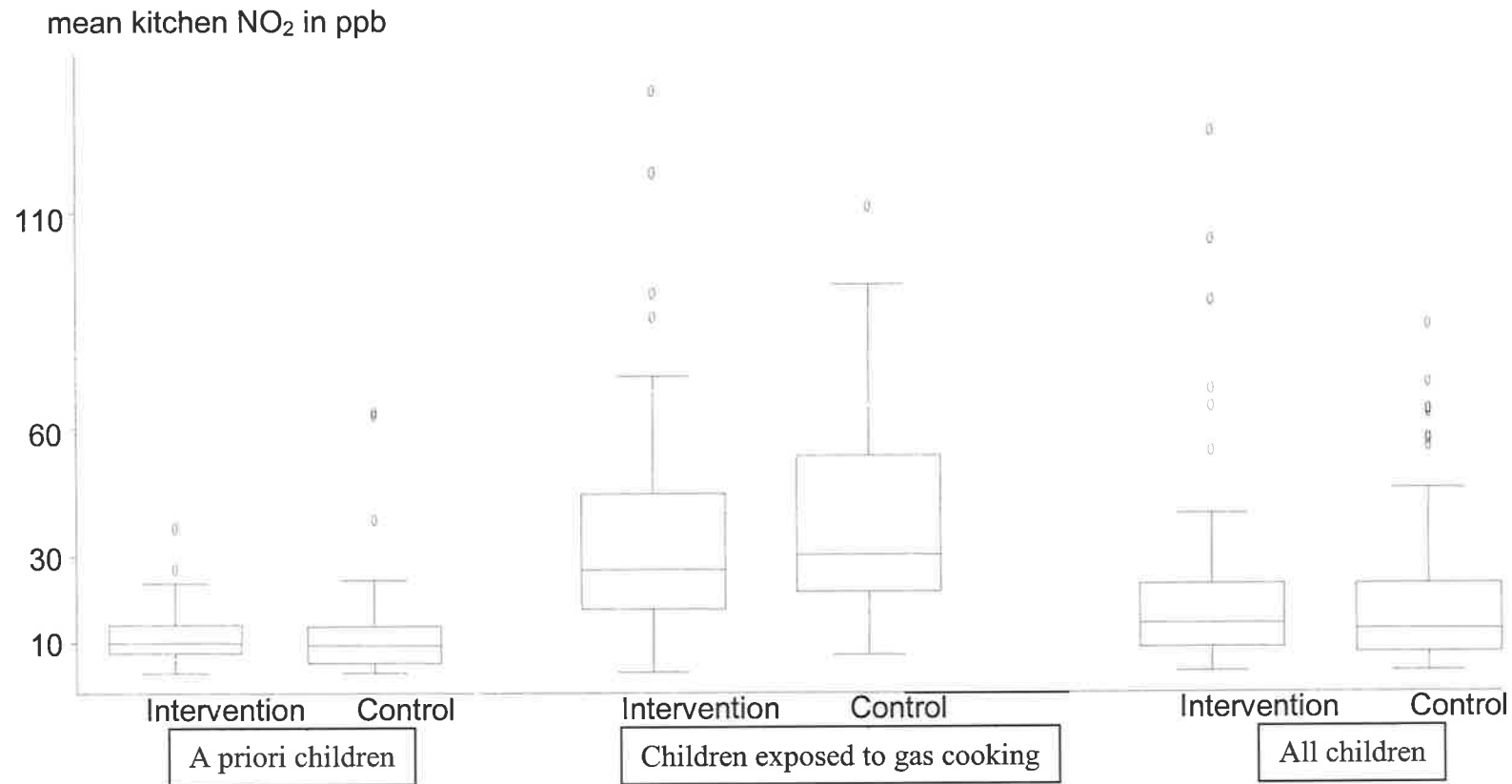
5.2.4 Conclusion and implications from NO$_2$ measurements

Exploration of classroom levels of NO$_2$ after exchange of heaters indicated that intervention was successful. The overall mean 6 hourly classroom concentration in intervention schools was significantly lower than the mean classroom concentration in control schools. There was no difference in school exposure levels in regard to children from the a priori sample compared to children from the extended group.

On the other hand, a significantly higher personal and kitchen exposure to NO$_2$ was measured in households of children with gas cooking compared to those with only electrical appliances for cooking. Mean personal and kitchen concentrations of intervention and control children in this category were similar.

This difference in home background exposure to NO$_2$ has implications for the analysis of outcome variables. Combination of children from both categories of home background exposure to NO$_2$ could lead to substantial misclassification of exposure status between the intervention and the control groups. This misclassification of children's exposure status could be equated in its effect on outcome variables to that of random misclassification. Presence of children exposed to NO$_2$ at home in the intervention group may dilute a real effect of a true association and this could lead to a possible wrong acceptance of the null-hypothesis.

Therefore analysis of outcomes in the following chapters will be reported separately for (1) children in households with electric cooking (a priori sample), (2) children in households with gas cooking (extended sample), and (3) children from both types of households together (combined sample).

5.3 Results for objective lung measurements

Lung measurements were conducted at the beginning and at the end of the study period to explore the beneficial effects or otherwise of heater exchange in relation to objective lung measurements in the intervention children at the end of the study period. Outcome measurements discussed in this chapter refer to lung function results and bronchial hyper responsiveness testing.

5.3.1 Participation in lung measurements

Table 9 shows participation of intervention and control children in spirometry and bronchial hyper-responsiveness testing grouped by their background exposure status, (1) a priori children, (2) extended children, (3) and the combined group.

All children were encouraged to take part in lung testing. The only restriction applied to children younger than 7 years of age who were not invited to take part in bronchial hyper-responsiveness testing due to their likely inability to satisfactorily undertake the complex breathing requirements.

The main reasons for non compliance were:

- children being sick on days of testing

- parents not consenting to any testing

- younger children not being able to conform with breathing techniques

- children having considerable airways problems on the days of testing. Cut off point for hyper-responsiveness testing was a % predictedFEV$_1 \leq 80\%$.

In relation to spirometry and hyper-responsiveness testing, results were based on only those children who took part in baseline and end of study measurements. This was necessary because regression analysis of lung performance outcomes required adjustment by baseline results.

Table 9: Participation in lung testing by categories of home exposure and by intervention and control group

	A priori sample		Extended sample		Combined sample	
	Intervention: 45	Control: 73	Intervention: 43	Control: 43	Intervention:88	Control:116
FEV$_1$ Baseline and end of study	38 (83%)	52 (71%)	27 (63%)	31 (72%)	65 (74%)	83 (71.6%)
Baseline only	5	5	7	3	12	8
End of study only	2	6	3	6	5	12
Reasons:						
No permission	-	3	2	1	2	4
Not at school	-	2	2	-	2	5
Too sick	-	3		-		3
Technique (Were not able to apply technique correctly)	-	2	2	2	3	4
BHR Baseline and end of study	27 (60%)	37 (51%)	7 (16%)	12 (28%)	34 (39.7%)	49 (42%)
Baseline only	1	3	1	2	2	5
End of study only	1	1	4		5	1
Reasons:						
No permission	1	2	2	1	3	3
Not at school		2	2		2	2
Too sick	2	1	1	1	3	2
<7	12	18	15	18	27	36
technique	1	7		5	1	12
other		2	11	4	11	6

5.3.2 Spirometry results

5.3.2.1 A priori sample

Table 10 displays the baseline and end of study spirometry results for children from the a priori group. Results for all lung performance indicators prior to the heating period shows that both groups were very similar at baseline.

When lung function tests were repeated at the end of the study period all lung performance indicators were increased. This highlights that the lungs of the children had matured during the three months study period. The increase was similar for both intervention and control group, and thus no difference was observed between the groups. Specifically, in relation to the main outcome variable, %predictedFEV$_1$, children of both groups were similar with a %predictedFEV$_1$ for the intervention group of 107.5% and 107.3% for the control group (β-coefficient: 1.2; 95% CI: -2.4, 4.9; p=0.5). Equally, the difference between %predictedPEF was not significant (88.9 % vs 85.0 %; (β-coefficient: 2.0; 95% CI: -5.4, 9.4; p=0.6).

5.3.2.2 Extended sample

Spirometry results for the extended group of children are shown in table 11. baseline spirometry results show a small difference in lung performance between the two groups. Predicted FEV$_1$ in the control group is 10 ml less than in the intervention group which may be explained by the slightly lower mean age of the control children (7.9 years) compared to the intervention children (8.2 years) in this category. At the end of the study intervention children had a higher % predicted FEV$_1$ than the control children when baseline % predicted FEV$_1$ was taken into account, but the difference was not significant (β-coefficient: 4.4; 95% CI: -6.2, 15.1; p=0.4). There also was a difference in % predicted

PEF between the groups, with the intervention children having a higher peak flow, but the difference fell short of significance (β-coefficient: 11.2; 95%CI: -0.8, 23.3; p=0.065).

5.3.2.3 combined sample

Table 12 combines the spirometry results from all participating children. For the main outcome variables, %predicted FEV_1 and %predicted PEF, there were overall no differences between the two groups at the end of the heating season. When comparing intervention and control group by regression analysis, a slightly higher %predicted FEV_1 was found in the intervention group (β-coefficient: 2.3; 95%CI: -2.1, 6.7; p=0.3), as well as a higher %predicted PEF (β-coefficient: 5.6; 95%CI: -0.9, 12.2;p=0.08), both of the differences were not significant.

Table 10: Baseline and end of the study spirometry measurements (standard deviation): based on a priori children. Lung parameters as defined in methods chapter.

Spirometry outcomes	Intervention N:38 baseline	end of study	Control N: 52 baseline	end of study
Predicted FEV_1 (litres)	1.9 (0.5)	2.0 (0.5)	1.9 (0.6)	2.0 (0.6)
FEV_1 (litres)	2.0 (0.6)	2.1 (0.6)	2.0 (0.65)	2.1 (0.7)
%predicted FEV_1	102.3 (12.1)	107.5(15.2)	103.4(13.3)	107.3 (17.0)
FVC (litres)	2.3 (0.8)	2.5 (0.9)	2.28 (0.75)	2.52 (0.8)
%predicted FVC	100.9 (12.8)	106.9	101.6 ((18.7)	108.9 (14.6)
PEF	4.0 (1.3)	4.4 (1.5)	3.8 (1.4)	4.3 (1.5)
%predictedPEF	81.8 (15.1)	88.9 (20.5)	79.6 (14.8)	85.0 (18.1)

Table 11: baseline and end of study spirometry measurements (standard deviation): based on the extended group of children.

Spirometry outcomes	Intervention N= 27		Control N=31	
	baseline	end of study	baseline	end of study
Predicted FEV$_1$ (litres)	1.8 (0.6)	1.8 (0.6)	1.7 (0.4)	1.7 (0.4)
FEV$_1$ (litres)	1.9 (0.6)	2.1 (0.6)	1.7 (0.4)	1.8 (0.5)
%predicted FEV$_1$	109.6 (11.9)	118.7 (12.7)	103.7 (13.2)	107.7 (14.5)
FVC (litres)	2.2 (0.7)	2.5 (0.8)	1.9 (0.5)	1.8 (0.5)
%predictedFVC	102.2 (22.9)	111.3 (24.4)	99.4 (22.3)	108.2 (12.6)
PEF (litres)	3.7 (1.1)	4.2 (1.2)	3.3 (1.1)	3.5 (1.4)
%predicted PEF	86.7 (10.8)	97.3 (21.7)	79.6 (15.1)	80.4 (18.3)

Table 12: baseline and end of study spirometry measurements (standard deviation): combining all children.

Spirometry outcomes	Intervention N= 88		Control N=116	
	baseline	end of study	baseline	end of study
Predicted FEV$_1$ (litres)	1.9 (0.6)	1.9 (0.6)	1.8 (0.5)	1.9 (0.5)
FEV$_1$ (litres)	2.0 (0.6)	2.12 (0.6)	1.9 (0.6)	2.0 (0.6)
%predicted FEV$_1$	105.4 (12.5)	112.2 (15.2)	103.6 (13.2)	108.3 (18.3)
FVC (litres)	2.2 (0.7)	2.5 (0.8)	2.1 (0.7)	2.4 (0.8)
%predicted FVC	101.4 (17.4)	108.8 (18.2)	100.7 (20.0)	108.6 (13.8)
PEF (litres)	3.9 (1.2)	4.3 (1.4)	3.7 (1.3)	4.0 (1.5)
%predicted PEF	83.8 (13.6)	92.5 (21.3)	79.6 (14.9)	83.3 (18.2)

5.3.2.4 Reversibility

There were fewer children continuing with reversibility testing than initially participated in lung function testing. Numbers of children participating in the reversibility test and results are displayed in table 13. Reversibility testing at baseline indicated that there were overall only 10 (7.3%) children with lung function improvement of ≥15% after inhalation of salbutamol and the proportion of children with reversible airways was similar for both groups. At the end of the study period reversibility of the a priori children in the control group had increased from one to five, while in the intervention group it was reduced from four to three children. This difference in proportions between the two groups was not significant (OR: 0.45; CI: 0.1-1.9; p=0.3).

The number of children with reversible airways in the extended group remained the same during baseline and at the end of the study in the control group, and was reduced to zero in the intervention group.

At the end of the study, there were less children in the combined group with reversible airways in the intervention group compared to the control group. This overall difference in proportion was not significant (OR: 0.33; CI: 0.1-1.3; p=0.1).

Table 13: Reversibility of lung function after salbutamol inhalation

	A priori group		Extended group		Combined group	
	Intervention N=37	Control N=46	Intervention N=25	Control N=29	Intervention N=62	Control N=75
aseline	4 (11.0%)	1 (2.2%)	2 (8.0%)	3 (10.3%)	6 (9.7%)	4 (5.3%)
nd of tudy	3 (8.1%)	5 (10.9%)	0	3 (10.3%)	3 (4.8%)	8 (10.7%)

5.3.3 Bronchial hyper-responsiveness results

Table 14 shows the results for hyper-responsiveness testing.

5.3.3.1 A priori sample

In the intervention group, 37 % of children were responsive to histamine ($\geq$20% fall of

FEV_1) during baseline investigations and 35% in the control group. At the end of the study

responsiveness in the intervention group rose to 40.7% and 43.2 % in the control group.

This difference in proportions was not significant when expressed as an odds ratio (OR:

0.8; 95%CI: 0.3, 2.3; P=0.7).

In regards to airway responsiveness to histamine, expressed as $PD20FEV_1$ and DRS the

results showed a similar response for the two groups at baseline (1.1 µmol vs 1.3 µmol).

At the end of the study period the geometric mean $PD20FEV_1$ for this group of children

remained almost identical to the original baseline value and DRS values increased

(increased response) to the same geometric mean value for both groups. Linear regression

analysis in relation to end of study outcomes for $PD20FEV_1$ (β-coefficient: -0.2; 95%CI:-

0.5, 0.1; p=0.2) and DRS (β-coefficient: -0.3; 95%CI:-1.3, 0.7; p=0.7), adjusted for pre

study outcomes, indicated no significant difference between intervention and control

group.

5.3.3.2 Extended group of children and the combined group of children

Hyper-responsiveness results for children in the extended group were based on only 19

subjects. Baseline data indicated greater responsiveness to histamine in the intervention

group compared to the control group when expressed as $PD20FEV_1$ and DRS (table: 14).

This difference was maintained at the end of the study period, but linear regression

analysis for end of study results of $PD20FEV_1$ (β-coefficient:-0.3, 95% CI:-1.3-0.7; p=0.5)

and DRS (β-coefficient: -0.03, 95% CI: -0.4-0.33; p=0.9) indicated that these differences between intervention and control group were not significant.

When combining all children (table 14), the risk of intervention children being responsive to histamine was very slightly reduced, but this reduction was not significant (OR: 0.8; 95%CI: 0.3, 1.9; p=0.6). There was no significant difference in dose response values, expressed as $PD20FEV_1$ (β-coefficient: -0.2, 95% CI:-0.5, 0.1; p=0.2) and DRS (β-coefficient -0.03, 95%CI:-0.1, 0.09; p=0.6) between the intervention and control group.

5.3.3.3 Severity of BHR

Categorisation of $PD20FEV_1$ into categories of severity as defined in the methods chapter is also set out in table 14. Categories of severity are similarly distributed between the two groups with slightly more children in the intervention group with moderate asthma compared to the control group with slightly more children in the mild asthma category.

5.3.4 Summary of objective lung measurements

Of the children who underwent lung testing at the beginning and at the end of the study there was no difference in either lung function parameters or bronchial reactivity at baseline.

At the end of the study %predictedFEV_1 had risen in both groups, but no relevant difference was evident between the groups. This overall improvement in lung function may be explained by the lapse of time between the baseline and post study measurement (on average 3 months). During this period of time children's lung function may have increased due to natural lung growth, while their age, a parameter adjusted for during lung function measurements, may have only changed for some of the children during the study

period.   Another explanation may be that the children had improved their blowing technique.

Proportion of children responding to histamine was similar at baseline and remained so after the study period. Linear regression analysis in relation to post study responses to DRS and PD20 indicated no differences between intervention and control children.

Table 14: Responsiveness to histamine (BHR). Number of participants (%) being responsive ($\geq 20\%$ fall of FEV_1), geometric means (95% confidence interval) of $PD20FEV_1$ and DRS (as defined in methods chapter) and severity of bronchial hyper-responsiveness.

Outcome variables	A priori group		Extended group of children		Combined children	
	Intervention (N=27)	Control (N=37)	Intervention (N=7)	Control (N=12)	Intervention (N=34)	Control (N=49)
baseline % BHR positive	10 (37.0%)	13 (35.1%)	3 (42.9%)	4 (33.3%)	13 (38.2%)	17 (34.7%)
baseline PD20 (µmol)	1.1 (0.6-2.1)	1.3 (0.8-2.0)	0.8 (0.08-8.7)	1.9 (0.6-6.1)	1.0 (0.6-1.8)	1.4 (0.1-2.0)
end of study % BHR positive	11 (40.7%)	16 (43.2%)	3 (42.9%)	5 (41.7%)	14 (41.2%)	21 (42.8%)
end of study PD20 (µmol)	1.07 (0.6-1.7)	1.3(1.0-1.6)	1.0 (0.1-8.8)	1.7 (0.7-3.9)	1.1 (0.7-1.6)	1.4 (1.1-1.7)
baseline DRS	7.9 (5.3-11.6)	7.1 (5.3-9.4)	9.7 (3.7-25.2)	6.4 (3.7-10.9)	8.2 (5.8-11.6)	6.9 (5.4-8.8)
end of study DRS	8.8 (6.1-12.7)	8.8 (6.9-11.2)	9.9 (4.2-23.6)	8.2 (5.4-12.4)	9.1 (6.6-12.5)	8.6 (7.1-10.6)
baseline severity of BHR						
Slight	1	3			1	3
mild	5	7	2	3	7	10
moderate	4	2	1	1	5	3
severe		1				1
End of study severity of bhr						
slight				2		2
mild	7	14	2	3	9	17
moderate	4	2	1		5	2
severe						

5.4 Results from symptom diaries

This chapter explores the effect of heater exchange on asthma symptoms during the study period.

To test this hypothesis, logistic regression was used to compare the proportion of children who experienced each symptom in the intervention and control groups. Secondly, for each child the symptom rate for each symptom was calculated as the days of symptom presence as a proportion of observation days recorded in the diary. Relative risks for each symptom were then calculated to compare mean rates between the intervention and the control group using negative binomial regression.

As diary questions also referred to actions taken by the children in relation to asthma, for example, taking of asthma medication, the term asthma symptoms will be extended to include these actions in the following chapters.

5.4.1 Participation in symptom diaries

In the a priori group, all 45 children who had enrolled in the intervention group participated in the diary part of the study, compared to 69 out of 73 children in the control group (table 15). For the children in the extended group, 40 out of 43 took part in the intervention group and all of the 43 enrolled children in the control group. When combining all 197 children, the number of days where symptoms were collected was identical for both groups, with 87% of children participating during all of the 84 days of the study period, 9% during 70 days and 3% on at least 28 days. The distribution for participation in the diary part of the study for children in the a priori group and those with gas cooking at home is shown in table 15.

Table 15: Participation N (%) in asthma diaries (days) by category of home exposure and by intervention (I) and control group (C) children.

Diary days	A priori group		Extended group		Combined group	
	I N=45	C N=69	I N=40	C N=43	I N=85	C N=112
84 days	38 (84)	60 (87)	36 (90)	37 (86)	74 (87)	97 (87)
70 days	5 (11)	7 (10)	3(7.5)	3 (7)	8 (9)	10 (9)
≥ 28 days	2 (5)	2 (3)	1 (2.5)	3 (7)	3 (4)	5 (4)

5.4.2 Distribution of symptom presence/absence during the study period

5.4.2.1 A priori sample

There was a tendency of the odds ratio (OR) to be below one in the intervention children for difficulty with breathing (day and night), chest tightness during the day, difficulty breathing after exercise, asthma attack during day and night, visit to health care facilities due to asthma and taking asthma medication (relieving and preventing) (table 16), but these risk reductions were not statistically significant. Intervention children had a higher proportion of ever coughing at night, and risk of missing school due to problems with asthma was more than doubled in the intervention group. Proportions of wheeze (day and night), chest tightness (night time) and cough during the day were very similar between the two groups. Adjustment for baseline variables did not change the odds ratios and confidence intervals greatly, however missed school due to asthma was no longer significantly different between intervention and control children.

Table 16: Proportion (%) of children with at least one asthma symptom day for each symptom by intervention and control group for the a priori group during the study period (84 days), and estimates of odds ratio (OR) and associated 95% confidence intervals (CI) with and without adjustment for confounding.

A priori group Symptoms/Activities	Intervention (%) proportion	Control	Intervention versus control group			
			Unadjusted N=114		Adjusted N=112	
	N=45	N=69	OR	95% CI	OR	95% CI
Wheeze during the day	46.7	44.9	1.07	0.48-2.38	1.08	0.49-2.42
Wheeze during the night	33.3	34.8	0.94	0.41-2.16	0.91	0.41-2.04
Difficulty breathing during the day	40.0	44.9	0.82	0.41-1.64	0.92	0.43-1.96
Difficulty breathing during the night	22.2	27.5	0.75	0.3-1.87	0.74	0.31-1.74
Chest tightness during the day	37.8	43.5	0.79	0.40-1.55	0.79	0.41-1.57
Chest tightness during the night	28.9	27.5	1.07	0.4-2.8	1.09	0.38-3.12
Cough during the day	84.4	81.2	1.26	0.61-2.59	1.49	0.68-3.27
Cough during the night	82.2	75.4	1.51	0.53-4.28	1.68	0.61-4.60
Difficulty breathing after exercise	44.0	50.7	0.65	0.22-1.89	0.64	0.20-1.98
Asthma attacks during the day	28.9	34.8	0.76	0.32-1.79	0.74	0.31-1.76
Asthma attacks during the night	20.0	30.4	0.57	0.28-1.18	0.55	0.28-1.10
Missed school due to asthma	51.1	28.9	**2.56**	**1.08-6.04***	2.45	0.98-6.08
Visit to health care facilities due to asthma	28.9	33.3	0.8	0.36-1.83	0.74	0.33-1.67
Taking relieving medication	64.5	72.5	0.69	0.25-1.89	0.72	0.26-1.96
Taking preventive medication	33.4	42.0	0.69	0.31-1.51	0.71	0.32-1.61
Any asthma medication	69.1	78.8	0.60	0.23-1.57	0.64	0.25-1.66

*P<0.05

5.4.2.2 Extended sample

Presence of asthma symptoms for this group is shown in table 17.

Similar to the a priori group, the odds ratios of experiencing asthma symptoms were below one in the intervention group for the majority of symptoms, with the exception of wheeze, chest tightness during the night and asthma attacks during the day time, but none were statistically significant. Contrary to the a priori children the percentage of children ever missing school due to asthma during the study was almost identical for the two groups. Adjustment for all baseline characteristics did not change this result significantly.

5.4.2.3 Combined sample

When combining all children, the results obtained prior to the amalgamation of the two categories did not change. Overall, no significant differences in symptoms occurrence were apparent between the intervention and control groups, and adjustment for all potential confounders did not change these results significantly (table 18).

Table 17: Proportion (%) of children with at least one asthma symptom day for each symptom by intervention and control group for the extended group of children during the study period (84 days), and estimates of odds ratio (OR) and associated 95% confidence intervals (CI) with and without adjustment for confounding.

Extended sample Symptoms/Activities	Intervention (%) proportion N=40	Control N=43	Unadjusted N=83 OR	95% CI	Adjusted N=80 OR	95% CI
Wheeze during the day	40.0	46.5	0.77	0.38-1.54	0.91	0.41-2.06
Wheeze during the night	40.0	34.9	1.24	0.68-2.27	1.61	0.84-3.08
Difficulty breathing during the day	32.5	41.9	0.67	0.28-1.59	0.75	0.27-2.08
Difficulty breathing during the night	32.5	30.2	1.11	0.58-2.12	1.18	0.6-2.29
Chest tightness during the day	35.0	46.5	0.62	0.30-1.27	0.66	0.30-1.42
Chest tightness during the night	47.5	32.6	1.87	0.85-4.12	2.37	0.93-6.06
Cough during the day	82.5	90.1	0.48	0.12-1.87	0.57	0.12-2.66
Cough during the night	80.0	81.4	0.91	0.41-2.03	1.09	0.48-2.53
Difficulty breathing after exercise	52.5	48.8	1.16	0.55-2.41	1.19	0.46-3.11
Asthma attacks during the day	32.5	27.9	1.24	0.41-3.79	1.35	0.45-4.08
Asthma attacks during the night	30.0	37.2	0.72	0.289-1.81	0.79	0.33-1.94
Missed school due to asthma	32.5	34.9	0.89	0.51-1.58	0.91	0.47-1.75
Visit to health care facilities due to asthma	32.5	39.5	0.74	0.40-1.35	0.68	0.33-1.41
Taking relieving medication	65.0	76.7	0.56	0.22-1.43	0.48	0.18-1.27
Taking preventive medication	47.5	53.5	0.79	0.38-1.62	0.76	0.34-1.71
Any asthma medication	77.8	85.4	0.6	0.23-1.55	0.57	0.18-1.77

Table 18: Proportion (%) of children with at least one asthma symptom day for each symptom by intervention and control group for combined children during the study period (84 days), and estimates of odds ratio (OR) and associated 95% confidence intervals (CI) with and without adjustment for confounding.

Combined children Symptoms/Activities	Intervention (%) proportion N=85	Control N=112	Intervention versus control group Unadjusted N=197 OR	95% CI	Adjusted N=192 OR	95% CI
Wheeze during the day	43.5	45.5	0.92	0.53-1.59	0.98	0.56-1.70
Wheeze during the night	36.5	34.8	1.07	0.69-1.66	1.13	0.76-1.69
Difficulty breathing during the day	36.5	43.8	0.74	0.43-1.27	0.81	0.44-1.48
Difficulty breathing during the night	27.1	28.6	0.93	0.51-1.67	0.94	0.55-1.62
Chest tightness during the day	36.5	44.6	0.71	0.41-1.24	0.72	0.41-1.27
Chest tightness during the night	37.7	29.5	1.44	0.75-2.78	1.53	0.77-3.04
Cough during the day	83.5	84.8	0.91	0.54-1.51	1.11	0.61-2.01
Cough during the night	81.2	77.7	1.24	0.61-2.51	1.35	0.65-2.81
Difficulty breathing after exercise	45.9	50.0	0.85	0.40-1.77	0.84	0.38-1.85
Asthma attacks during the day	30.6	32.1	0.93	0.46-1.89	0.89	0.43-1.86
Asthma attacks during the night	24.7	33.0	0.66	0.38-1.16	0.66	0.37-1.19
Missed school due to asthma	42.4	31.3	1.62	0.93-2.79	1.56	0.88-2.78
Visit to health care facilities due to asthma	30.6	35.7	0.79	0.43-1.46	0.75	0.39-1.44
Taking relieving medication	64.7	74.1	0.64	0.31-1.32	0.61	0.29-1.27
Taking preventive medication	40	46.4	0.77	0.43-1.38	0.78	0.42-1.44
Any asthma medication	73.1	81.3	0.62	0.33-1.18	0.66	0.34-1.28

5.4.3 Asthma symptom rates

5.4.3.1 A priori sample

Symptom rates, relative risks and confidence intervals for the a priori children are set out in table 19.

Unadjusted rates

Wheeze (day and night), cough (day and night) and missing school due to asthma were not different between intervention and control group. Difficulty breathing during the day (p=0.045) and night (p=0.004), chest tightness during the day (p=0.008) and asthma attacks during the day (p=0.034) showed a significant reduction in the intervention group compared to the control group. Of border line significance were asthma attacks during the night (p=0.067) and visits to health care facilities due to asthma (0.065), both of which showing a protective effect associated with heater intervention. Also reduced in the intervention group, albeit not statistically significant, were the rates for chest tightness during the night, difficulty breathing after exercise and the use of asthma medication (relieving and preventive medication).

Adjusted rates

When adjustment was made for potential confounding baseline variables, the difference in the rates between intervention and control group for difficulty breathing during the day lost significance (p=0.075) and chest tightness and asthma attacks during the night achieved significance, (p=0.02), but the results were essentially maintained. Adjustment for all of the potential confounders added chest tightness during the night to the symptoms which were significantly decreased (p=0.035) in the intervention group.

Table 19: Mean rates (SD), relative risks (RR), 95% confidence intervals for symptoms/activities for a priori children over 12 weeks. Unadjusted and adjusted for baseline characteristics.

Symptoms/ Activities: A priori children	Mean rate Intervention N=45	Mean rate Control N=69	RR 95% CI unadjusted	RR 95% CI adjusted
Wheeze during the day	0.049 (0.15)	0.051 (0.1)	0.95 0.45-2.01	0.65 0.29-1.43
Wheeze during the night	0.022 (0.06)	0.023 (0.05)	0.94 0.36-2.50	0.48 0.17-1.34
Difficulty breathing during the day	0.022 (0.04)	0.054 (0.1)	**0.41* 0.07-0.98**	0.47 0.20-1.06
Difficulty breathing during the night	0.0083(0.02)	0.026 (0.07)	**0.32** 0.14-0.69**	**0.31** 0.16-0.59**
Chest tightness during the day	0.023 (0.04)	0.051 (0.09)	**0.45** 0.25-0.81**	**0.44** 0.26-0.76**
Chest tightness during the night	0.015 (0.03)	0.025 (0.06)	0.59 0.28-1.29	**0.48* 0.25-0.94**
Cough during the day	0.18 (0.2)	0.14 (0.1)	1.27 0.81-2.00	1.21 0.69-2.08
Cough during the night	0.11 (0.2)	0.12 (0.1)	0.92 0.49-1.73	0.81 0.45-1.43
Difficulty breathing after exercise	0.038 (0.07)	0.064 (0.1)	0.59 0.31-1.13	0.54 0.21-1.38
Asthma attacks during the day	0.011 (0.02)	0.027 (0.05)	**0.39* 0.17-0.93**	**0.38* 0.17-0.88**
Asthma attacks during the night	0.007 (0.02)	0.018 (0.04)	0.38 0.13-1.07	**0.26** 0.10-0.65**
Missed school due to asthma	0.016 (0.02)	0.012 (0.03)	1.34 0.68-2.60	1.55 0.87-2.77
Visit to health care facilities due to asthma	0.0046 (0.001)	0.0075 (0.01)	0.6 0.35-1.03	0.60 0.35-1.06
Taking any asthma medication	0.27 (0.4)	0.35 (0.4)	0.77 0.49-1.21	0.71 0.41-1.22
Taking any reliever	0.14 (0.2)	0.22 (0.29)	0.62 0.31-1.25	0.59 0.34-1.03
Taking any preventer	0.26 (0.4)	0.29 (0.4)	0.87 0.53-1.44	0.88 0.31-2.5

*p<0.05, **p<0.01

5.4.3.2 Extended sample

Unadjusted results

For children with potential background exposure to NO_2 at home through gas cookers, the results from the unadjusted analyses were somewhat different from the a priori sample (table 20). While a small reduction was still evident for some of the symptoms, none of these reductions were significant. This is also evident through the large confidence intervals surrounding the point estimates of the relative risks.

Adjusted analysis

After adjustment for all baseline variables it was clear that some confounding was present. For example, for difficulty breathing at night and day, imbalances at baseline must have contributed to the unadjusted elevated risk ratio in the intervention group. Adjustment for all possible confounders resulted in a significant reduction of difficulty breathing at night in the intervention group (p=0.047). Adjustment also further reduced the relative risk for asthma attacks at night, such that it became significantly reduced in the intervention group (p=0.033).

5.4.3.3 Combined sample

Unadjusted results

As seen in the results before, the relative risk for wheeze, cough and absence from school was close to one indicating similar symptom rates for both groups (table 21). Only chest tightness during the day showed a significantly reduced rate (p=0.018) in the intervention group. Asthma attacks during the night (p=0.06) and visits to health care facilities due to asthma (p=0.06) approached significance. Point estimates of the relative risks for the other symptoms showed a reduction of symptoms in the intervention group, but all of the 95% confidence intervals included one.

Adjusted analysis

In case of the combined sample, adjustment for all pre-determined confounders changed the results significantly for (i) difficulty breathing at night (p=0.002), (ii) asthma attacks during the night (p=0.005), (iii) taking preventative (p=0.013), relieving (p=0.014) and any (p=0.009) asthma medication. In case of difficulty breathing during the day (p=0.077), asthma attack during the day (p=0.089) and visit to health care facilities due to asthma, (p=0.057) border line reduction was reached in the intervention group. This left only the relative risk of "coughing" and "missing school due to asthma" similar between the two groups.

Table 20: Mean rates (SD), relative risks (RR), 95% confidence intervals for symptoms/activities for the extended group of children over 12 weeks, unadjusted and adjusted for baseline characteristics.

Symptoms/ Activity: Extended group	Mean rate Intervention N=40	Mean rate Control N=43	RR 95% CI unadjusted	RR 95% CI adjusted
Wheeze during the day	0.040 (0.1)	0.041 (0.075)	0.99 0.48-2.03	0.92 0.59-1.44
Wheeze during the night	0.019 (0.038)	0.027 (0.067)	0.71 0.33-1.5	1.09 0.61-1.95
Difficulty breathing during the day	0.047 (0.16)	0.044 (0.087)	1.05 0.28-3.95	0.60 0.27-1.33
Difficulty breathing during the night	0.035 (0.15)	0.024 (0.054)	1.48 0.29-7.53	**0.49* 0.24-0.99**
Chest tightness during the day	0.023 (0.05)	0.033 (0.062)	0.69 0.32-1.5	0.66 0.31-1.37
Chest tightness during the night	0.022 (0.039)	0.026 (0.052)	0.85 0.41-1.75	1.07 0.56-2.06
Cough during the day	0.19 (0.22)	0.20 (0.23)	0.97 0.54-1.74	0.99 0.63-1.57
Cough during the night	0.15 (0.20)	0.15 (0.18)	1.01 0.59-1.70	0.94 0.54-1.62
Difficulty breathing after exercise	0.049 (0.15)	0.043 (0.096)	1.17 0.37-3.7	1.04 0.41-2.63
Asthma attacks during the day	0.029 (0.07)	0.023 (0.045)	1.25 0.52-3.04	1.42 0.63-3.2
Asthma attacks during the night	0.014 (0.03)	0.024 (0.042)	0.59 0.27-1.33	**0.45* 0.21-0.94**
Missed school due to asthma	0.010 (0.02)	0.015 (0.028)	0.71 0.34-1.49	0.56 0.29-1.06
Visit to health care facilities due to asthma	0.005 (0.009)	0.008 (0.013)	0.66 0.33-1.31	0.66 0.34-1.29
Taking any asthma medication	0.32 (0.37)	0.42 (0.39)	0.76 0.43-1.34	0.65 0.39-1.09
Taking any reliever	0.31 (0.41)	0.25 (0.32)	0.81 0.48-1.37	0.66 0.35-1.27
Taking any preventer	0.31 (0.41)	0.41 (0.47)	0.68 0.33-1.39	0.61 0.31-1.18

*p<0.05, **p<0.01

Table 21: Mean rates (SD), relative risks (RR), 95% confidence intervals for symptoms/activities over 12 weeks study period for combined children with gas cooking at home, unadjusted and adjusted for baseline characteristics.

Symptoms/ Activities: Combined children	Mean rate Intervention N=85	Mean rate Control N=112	RR 95% CI unadjusted	RR 95% CI adjusted
Wheeze during the day	0.05 (0.13)	0.047 (0.094)	0.95 0.57-1.58	0.81 0.51-1.29
Wheeze during the night	0.021 (0.048)	0.024 (0.059)	0.84 0.46-1.54	0.84 0.47-1.50
Difficulty breathing during the day	0.034 (0.11)	0.051 (0.11)	0.67 0.26-1.69	0.59 0.34-1.05
Difficulty breathing during the night	0.021 (0.11)	0.025 (0.064)	0.78 0.21-3.3	**0.39** 0.21-0.70
Chest tightness during the day	0.023 (0.046)	0.044 (0.087)	**0.51* 0.3-0.89**	**0.50* 0.28-0.88**
Chest tightness during the night	0.018 (0.036)	0.025 (0.058)	0.72 0.4-1.3	0.69 0.42-1.16
Cough during the day	0.18 (0.22)	0.16 (0.18)	1.14 0.81-1.6	1.12 0.79-1.58
Cough during the night	0.13 (0.18)	0.13 (0.15)	0.99 0.64-1.5	0.89 0.59-1.37
Difficulty breathing after exercise	0.044 (0.12)	0.056 (0.12)	0.79 0.37-1.67	0.73 0.42-1.26
Asthma attacks during the day	0.019 (0.05)	0.025 (0.05)	0.76 0.44-1.32	0.68 0.43-1.06
Asthma attacks during the night	0.01 (0.027)	0.020 (0.04)	0.51 0.25-1.02	**0.34** 0.16-0.72
Missed school due to asthma	0.01 (0.021)	0.013 (0.028)	1.03 0.67-1.59	1.05 0.70-1.58
Visit to health care facilities due to asthma	0.005 (0.009)	0.0078 (0.01)	0.63 0.39-1.02	0.61 0.36-1.01
Taking any asthma medication	0.29 (0.37)	0.37 (0.38)	0.78 0.53-1.15	**0.63** 0.44-0.89
Taking any reliever	0.17 (0.27)	0.23 (0.30)	0.69 0.42-1.17	**0.60* 0.40-0.90**
Taking any preventer	0.28 (0.40)	0.34 (0.44)	0.78 0.49-1.19	**0.56* 0.36-0.88**

*p<0.05, **p<0.01

5.4.4 Summary of symptom result

For some of the symptoms, the risk of occurrence during the study period was slightly, but not significantly reduced among the intervention children when compared to the control group. This was regardless of home exposure to unflued gas.

A much clearer picture emerged when symptoms were analysed according to the frequency of their daily occurrence. Intervention children showed significant reductions in clinically important asthma symptoms when analysis was restricted to the population of children with no unflued gas exposure at home. On the other hand, this benefit was not as clearly evident in children exposed to unflued gas cooking at home.

Therefore, when both home exposure categories were combined it seemed initially in the unadjusted analysis that misclassification of children regarding their exposure status to unflued gas sources in their homes in the intervention group may have diluted some of the symptom results towards the null hypothesis. Adjustment for baseline characteristics on the other hand strengthened the direction and the precision of the overall result away from the null hypothesis supporting the unadjusted result rather than contradicting it.

The size of risk reduction in terms of actual number of days is set out in table 22.

To obtain this, the mean rate of symptoms in the intervention group was subtracted from the mean rate in the control group. This rate difference was then multiplied by 100 in order to equate the risk reduction to a period of 100 days.

Example for difficulty breathing during the day (see table 19 for rates):

$$0.054-0.022=0.032 \times 100=3.2 \text{ days}$$

In the intervention group, difficulty breathing during the day was reduced on average from occurring from five to two days, difficulty breathing during the night from three to one day

and chest tightness during the day from five to two days, while asthma attacks during the day were reduced by one day for the average child during the study period.

While there were reductions in days for other symptoms, these were not significant. On average, a four day increase in cough during the day and on average one more day of absence from school due to asthma was experienced by the intervention group, but these increases were not significant.

Table 22: Size of risk in the a priori group of children, expressed as (i) mean change of symptom rates (difference between mean symptom rate in the intervention group and the control group) and (ii) days of reduction over a period of 100 days (multiplication of symptom rate by the factor of 100).

Symptom activities a priori group	Mean change in intervention group rates	Mean reduction over a period of 100 days
Wheeze during the day	-0.002	<1
Wheeze during the night	-0.001	<1
Difficulty breathing during the day	**-0.03**	**3**
Difficulty breathing during the night	**-0.02**	**2**
Chest tightness during the day	**-0.03**	**3**
Chest tightness during the night	-0.01	1
Cough during the day	+0.04	+ 4
Cough during the night	-0.01	<1
Difficulty breathing after exercise	-0.03	2
Asthma attacks during the day	**-0.02**	2
Asthma attacks during the night	-0.01	1
Missed school due to asthma	+0.004	+ 1
Visit to health care facilities due to asthma	-0.003	<1
Taking relieving medication	-0.08	7
Taking preventive medication	-0.08	7
Any asthma medication	-0.03	3

Chapter 6 – Discussion and Conclusion

6.1 Introduction

Studies into the relationship between NO_2 and respiratory health effects in children, especially in asthmatic children, have been conducted over more than 20 years. They included studies ranging from cross-sectional design (21) to case-control (98) and to cohort design (67) (19), exploring daily outdoor or indoor effects of NO_2 in asthmatics. World wide, guideline setting agencies were cautious in the interpretation of results from observational studies and repeatedly issued the verdict of inconclusive evidence in relation to NO_2 effects on health (141). Doubts were raised as some NO_2 studies did not show any health effects, and there was the possibility of bias, confounding and exposure misclassification in observational studies (142). Existing experimental studies were small in size and confined to laboratory conditions, limiting extrapolation to the asthmatic population at large.

Controlled intervention studies are powerful research designs aimed to overcome these limits. However, in relation to environmental issues this design is not used frequently, often because they are too costly or most of all, because it is impossible to intervene and exchange a pollutant source.

This is the first reported community based study to implement a randomised controlled trial for the purpose of studying the respiratory health effects of NO_2 on asthmatic children.

Eighteen out of nineteen primary schools in metropolitan Adelaide, all originally heated with unflued gas, were randomly assigned to either receive intervention in form of

electrical or flued gas heaters or to retain the original unflued gas heaters in their classrooms.

While asthma prevalence was high in the participating schools, asthmatic children who were eligible in relation to the a priori eligibility criteria, living in households without gas appliances (a priori children), were scarce and thus reduced initially eligible asthmatics from in the intervention schools to 7.8% (74/945) and in the control schools to 10.2% (125/1224). Under a modified eligibility criteria, where gas cooking at home was allowed, an extended group of asthmatic children also took part.

6.2 Randomisation

Cluster randomisation by school was successful. Intervention and control groups had similar characteristics and little difference in asthma severity at entry into the study. This was true for both the a priori sample and the combined sample, although small differences in characteristics were seen in the extended group due to smaller sample size. These imbalances could have occurred by using schools as the unit of randomisation rather than classrooms or individuals. Randomisation by school was chosen for practical considerations. Exchange of heaters in some classrooms and not others would have led to schools still retaining sources of NO_2 exposure which would have led to potential misclassification as children are not entirely confined to one classroom within a school.

Confounding by socio-economic differences was avoided by participation of all but one school with unflued gas heating. After randomisation there was no obvious clustering of schools in either of the groups in relation to socio-economic standing of the schools and

their associated community. When socio-economic status was measured in the form of parental education, only a small difference was found in favour of the intervention group.

6.3 Intervention

Intervention results were monitored closely by repeated measurements of NO_2 over the winter period. NO_2 levels ranged 3-78 ppb and 3-442 ppb in the intervention and control classrooms respectively. The mean levels recorded in intervention classrooms were significantly less than in control classrooms. Overall, heater replacement was successful in reducing NO_2 exposure in the intervention group.

On the other hand monitoring of household measurements of NO_2 revealed significant differences in mean personal and kitchen concentrations between households with and without gas cooking. It was decided to analyse data from a priori children (no home exposure to gas) and the extended group (gas cooking at home) separately to examine the effects in the a priori group which would be unlikely to involve misclassification of exposure due to home NO_2 levels.

6.4 Lung function results

Following heater replacement, there was no difference in lung function (FEV_1, FVC, PEF) and bronchial responsiveness (DRS, PD_{20}) between the two groups. This absence of difference in lung function is consistent with the majority of chamber results among adult asthmatics (143) (34). A significant decrease in lung function was seen in a cohort of 106 asthmatic children in relation to outdoor NO_2 exposure (50). A number of studies in the general population of children, with and without asthma, also found small changes in objective lung measurements in relation to outdoor NO_2 (50) (49) (48). These effects may be explained by ecological differences in outdoor air pollution studies, or even socio-economic gradients, rather than by short term effects of NO_2, as most of these observational studies were conducted over a number of communities.

Several indoor studies in the general population reported lung function reductions (FEV_1) in women (81), and in girls (102) in relation to the presence of gas cooking. Ponsonby, in an Australian study, observed airways obstruction (FEV_1/FVC ratio as indicator) in children of both genders exposed to current home gas appliances (104). All these studies explored the long-term effect of home gas appliances, and a possible link to the development of asthma. One study reported immediate responses to gas cooking in the form of a fall in peak expiratory flow (PEF) in asthmatic women who had measured their peak flow rates before and after cooking (100). The effect of cooking may be explained not only by NO_2 exposure, but other substances emanating from the process of food preparation, such as a high concentration of particles smaller than 10 micrometer (PM10).

In the present study exposure to unflued gas heating was restricted to a short winter period and to school hours and this exposure pattern may not have had any persistent

effect on the children's lung performance. Also, tests were applied infrequently, only before and after the study, and therefore were not able to detect any acute disturbances on a day to day basis. It appears that children recover quickly from intermittent airways obstructions. This argument may be assisted by the results of a large clinical study of asthmatic children on inhaled cortico-steroids, where no improvement in lung function was detected compared to placebo (144).

Lung function parameters may not be useful indicators for the behaviour of the large airways. As NO_2 is thought to react with the epithelium of the small airways, doubts have been raised about the appropriateness of lung function tests for detection of pollutant related changes in asthmatics (143). Another possibility is that lung function changes may only be observed in conjunction with additional exposure to relatively high exposures to allergens in sensitised subjects as had been shown in clinical studies (37). Periodic exposure to unflued gas heating may not be captured by lung function measurements in children because of the reversibility of children's airways. This study was not designed to evaluate acute lung function changes in relation to peak daily exposures to NO_2.

6.5 Bronchial responsiveness

In this study, responsiveness to histamine was measured at baseline and after the study period to detect differences in bronchial reactivity due to exposure to unflued gas heating, and therefore to test the hypothesis of a longer-term effect of NO_2 on respiratory responsiveness. However, bronchial responsiveness, measured as mean PD20 and mean DRS did not result in any difference between intervention and control group at the end of the study.

Long term effects of NO_2 on responsiveness to non specific broncho-constrictors have so far only been examined in one study. A higher prevalence of responsiveness (measured as PD20) to methacholine was observed in a general population (n=1921) of adults exposed to gas cooking (101). This difference was significant in the atopic sub population only. In the present study we observed a small increase in the percentage of children who tested positive in the control group, but results were not significant. All other studies which had examined bronchial hyper-responsiveness in relation to NO_2 exposure had been experimental in design and observed daily changes in bronchial responsiveness in relation to NO_2, with (38) (39) (40) (41) (145), and without allergen challenge (36). In this study, daily changes may have occurred, but were not measured in this study.

6.6 Symptoms results

Initial analyses examined results for children with no domestic exposure to gas (a priori children). Symptom rates were established for 45 a priori children in the intervention sample and 69 children in the control sample.

Asthma symptoms measured during the 12 weeks study period, such as difficulty breathing during day and night, chest tightness during the day, and asthma attacks, were significantly reduced following heater replacement in the intervention group, but not cough and wheeze. These symptom reduction findings are consistent with those reported in a previous study conducted in Adelaide that used the same symptoms/activities diary as was used in this study (19). This asthma panel study demonstrated that NO_2 levels and symptom occurrences were related on a same day, and one day lag basis in children under the age of 14.

These findings are confirmed by positive findings from previous studies. A case-control study in children explored the presence of asthma in relation to measured NO_2 indoors and demonstrated that asthma is more prevalent in households with high NO_2 levels (98) and several cross-sectional studies were able to relate asthma in children to the presence of gas cooking (21) (22) (75) (80) (81) (82) (92). Predominantly these studies had positive findings in the age group of children between 5-13 years of age.

Findings of increased asthma symptoms in the present study are also supported by numerous outdoor time series studies showing increased hospital admissions for asthma related to increased NO_2 levels outdoors (51) (57) (60). Several diary based panel studies relating symptoms to outside NO_2 exposure were inconclusive, but were based on relatively low NO_2 exposure (69) (71). On the other hand, a recent panel study of hyper-

responsive primary school children with high IgE serum had significantly increased

lower respiratory symptoms (difficulty breathing, asthma attacks) in relation to outdoor

NO_2 (67).

Mean symptom rate reduction in the current study was in the order of over 50 percent for

the a priori sample. Differences in mean rates were in the range of 0.01-0.03 equating to

symptom/activity reduction for an average child of between one and three days over the

period of 100 days, covering most of the winter period in South Australia.

The asthma symptoms reductions in question are of clinical significance. The reduction

relates to a large reduction in symptom burden as students are still exposed to unflued gas

appliances in the country schools of South Australia, in Canberra and in New South

Wales.

Symptom reduction was also found in the extended group of children, indicating that

NO_2 exposure at school in addition to the NO_2 exposure from cooking at home may have

contributed to this finding. On the other hand, symptom reduction in this group was very

small, extending at the most to one day. This was probably due to children in the

intervention group who were exposed to NO_2 in their homes. This may indicate some

misclassification of exposure manifested by weakening the symptom result when the two

groups of children (a priori and extended children) were combined.

The rate for cough was very similar between intervention and control group. Coughing is

mainly explained by events in the upper airways and therefore may have been not

affected by pollutant exposure.

Differences between the rates of absenteeism from school were small, despite the fact

that children in the intervention group had a higher prevalence of missing school due to

asthma.

6.7 Bias

This study was a double blind randomised controlled trial. Parents and children did not know that the change of heaters was associated with the asthma in the school environment study. This was confirmed by random telephone interview of 10 intervention and 10 control households after the study. None of the interviewees recollected anything different in the classroom of their children during the study period. One parent remembered an exchange of heaters, but thought that the heaters had been installed after this study. This confirms that no measurement bias of symptom rates or the reverse were likely to have occurred in this study.

Observation bias was minimised by the fact that research assistants who gathered data during the telephone interview and conducted lung function tests were not informed about the status of the schools in relation to heater exchange. Data was gathered prospectively after intervention with small time intervals between telephone calls. This temporal relationship has also contributed positively to the precision and completeness of the diary data.

6.8 Limitations of the study

Intervention was achieved with electrical and flued gas replacement heaters. Ideally, the use of only one type of heater would have been preferable for health impact assessment, but instalment of electrical heaters for all schools would have been too expensive as some schools would have required extensive electrical rewiring. In any events the intervention schools had significantly reduced NO_2 levels which was the desired outcome.

Sample size was restricted in this study due to strict eligibility criteria. While it was sufficient to demonstrate significant reductions in relation to some symptoms, relative

risks for some of the other reported symptoms and activities (chest tightness at night, asthma attack at night, difficulty breathing after exercise, visit to doctor's and use of asthma medication) were reduced in the intervention group, but the reduction did not reach significance.

The results of this study did not provide any evidence of an effect from unflued gas heaters on lung function (FEV_1, FVC, PEF) and bronchial hyper-responsiveness, however, acute daily short term changes could have occurred instead which were not measured. Ideally, daily objective lung measurements would have been preferable, but were too expensive to implement.

6.9 Extending the findings to other sources of NO_2

While this study only explores asthmatic symptoms in children related to unflued gas heating, the effects found are clearly related to the significant decrease in NO_2 achieved by heater intervention. There is only one other indoor study of asthmatics which had unflued gas heaters included as indoor exposure source (19). But even in this study the larger percentage of NO_2 sources was from gas cooking. Other indoor studies exclusively reported health effects arising in relation to gas cooking. It is possible that NO_2 emitted from gas cooking has the same effect on asthmatics as demonstrated in this study from unflued gas heating.

Personal indoor and outdoor NO_2 measurements were gathered in an international study extending over 568 participants from 18 cities in 15 countries using the same research protocol to study the contribution of different sources to personal exposure (146). While personal exposure was found to be varied, mean time averaged NO_2 concentration measured over 48 hours ranged from 11 ppb to 51.5 ppb. Indoor NO_2 sources were the

main contributors to personal exposure. Kerosene heaters, gas cooking and gas space heaters, all were associated with a significantly increased indoor/outdoor ratios when compared to ratios without the presence of these gas combustion sources. Indoor NO_2 levels are therefore an international concern. For example, gas dependent cities were shown to use gas for cooking in 75% of households, a number similar to what was found in this study in metropolitan Adelaide.

A report published by the National Environmental Health Forum in Australia (established by directors from Environmental Health Departments from each State and the Commonwealth) estimated that unflued gas space heating is used in 34% of households in Sydney, 5% in Melbourne, 4% in Brisbane and 5% in Adelaide (147) and classrooms with unflued gas heaters were estimated to be 80 000 in NSW.

Sales for unflued gas heaters are booming in the United States, according to a report concerned about the associated risks which are not discussed in the public domain (148). There, vent free gas heaters come in all sizes and imitation gas log fires can be bought cheaply off the shelves for display in disused fire places (148). Unflued gas heaters in Australia are also not just remains of redundant technology. Advertising for newly designed gas heaters without a flue to the outside is wide spread, with the marketing pitch towards ease of installation and modesty in price.

One control school for example, had just recently updated their old heaters with modern unflued gas heaters. NO_2 levels measured in this school were high, with mean NO_2 levels of 32 ppb and daily averaged levels of up to 171 ppb.

6.10 Conclusion

The findings from this randomised controlled study clearly indicate that a number of key asthma symptoms in children were significantly decreased in children whose original unflued classroom heater was either exchanged by a flued gas heater or an electrical heater.

During the trial, NO_2, the main by-product of gas combustion and biologically implicated in negatively effecting host defence mechanisms (25) in the respiratory system, had been significantly reduced in intervention classrooms compared to control classrooms.

This reduction in exposure to NO_2 was not associated with a decrease in lung function or measured hyper-responsiveness when these lung performance indicators were measured after the three months study period.

Consistency of the results with similar studies and the strength of the intervention design add considerable weight to the findings of this study.

In a recent paper it was argued that a revision of NO_2 guidelines should be considered in the light of results from NO_2 studies, particularly those where NO_2 was measured in relation to health outcomes (149) (see also Appendix VIII).

In the current study mean 6-hourly NO_2 concentration in control schools was 45ppb of NO_2. As described in the pilot study chapter, the ratio of six hours averaged NO_2 concentration in school rooms to the mean one hourly concentration of NO_2 is one to two, implying hourly peak levels of approximately 90 ppb of NO_2. Associated with these peak levels are on average one to three days of more asthma symptoms over a winter period compared to background outdoor exposure of approximately 15 ppb averaged over the same measurement period (6 hours). These increased NO_2 levels

concurrent with unflued gas heating were not unusually high. Most of the classrooms in Pilotto's school study in New South Wales experienced mean school concentration levels between 50-130 ppb during the winter months (20).

Studies which evaluated respiratory health effects in indoor studies in the general population of children reported increases in respiratory symptoms at weekly averaged NO_2 concentrations in the range of 10-30 ppb, which would be associated with peak exposures in the vicinity of 80 ppb. Studies where gas cooking was used as proxy measure for NO_2 exposure have reported 20% increase in asthma, 12 % increased risk of wheezing and 15% of increased respiratory symptoms. Gas cooking approximately doubles the NO_2 exposure in households (19) and therefore averaged daily exposures of 30 ppb (background of 15 ppb) can be expected with peak hourly excursions of 60 ppb and over, depending on the length of cooking and use of ventilation.

As yet, only ambient guidelines have been set in Australia, initially by NHMRC (160 ppb) and subsequently by the newly formed National Environmental Protection Council in (NEPC) 1998. This latter NO_2 goal of 120 ppb per hour is rarely surpassed when measured outdoors, but is often exceeded on a daily level in Australian households and schools during gas heating and cooking.

In view of the above evidence on health effects of NO_2, and particularly in relation to the new evidence from the intervention trial, it is recommended that current guidelines for NO_2 should be lowered. This guideline should be of the order of 80 ppb of NO_2 or less in order to provide sufficient safety margins for the susceptible subpopulation of asthmatic children. This new guideline should be valid for outdoor and indoor purposes as it would be artificial to compartmentalise air exposure, especially as outdoor and indoor studies are in good agreement about the size of the health effects of NO_2.

Australian data estimates that more than 60% of the metropolitan households use gas cookers and up to 34% of Sydney households use unflued gas space heaters (147). This may mean that more than half of the asthmatic children (>20% of the primary school age population) are exposed to concentrations of NO_2 which potentially increases their risk of a variety of asthma symptoms. While there are no figures available for the unflued gas heating prevalence in Australia, an increase in sales is to be expected in Victoria where new regulations may allow households to acquire this form of heating for the first time.

This intervention study has used flued gas heaters together with electrical heaters to lower NO_2 concentrations, therefore gas can be successfully used as source of energy without adding an extra burden of asthma triggers in form of NO_2.

Reduction of indoor exposure of NO2 could be achieved effectively through replacement of unvented gas heaters with vented gas heaters and by an increase in ventilation around gas stoves.

Future studies

This study has quantified the health benefit associated with an exchange of unflued gas heaters with electrical or flued gas heaters. Similar intervention studies should identify the risk associated with gas cooking or should study the health benefits achieved with simple modifications such as lowering peak NO_2 levels by using range hoods, extraction fans or by low NOx unflued gas heaters.

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Appendices

Appendices
I-VIII

Appendix I

Eligibility questionnaire and information sheet

Respiratory Health in the School Environment
Questionnaire

Dear Parent/Caregiver, if you wish your child to be considered for this study, please complete the following questionnaire and return it by the end of this week to your schools support officer.

If you have more than one of your children involved in the study, please complete a separate questionnaire for each child.

❖ Please write the answers **in print** into the boxes provided
❖ or tick the right answer

1. First name of child:.................................

2. Surname of child.................................

3. What is this child's age (in years)? ☐☐

4. What is this child's date of birth? ☐☐ ☐☐ ☐☐
   day   month   year

5. What is this child's gender? ☐ female   ☐ male

6. Name of this child's school

....

7. Year level of this child: ☐   or Reception ☐

8. Number (or name) of this child's classroom ☐

9. Main parent/caregiver of this child

First name:.................................

Surname:.................................

10. Address of main parent/caregiver of this child:

Number/street.................................

Suburb/postcode.................................

11. Has your child ever been diagnosed with asthma by a doctor?

Yes ☐   No ☐

At this point, we would like to ask you some questions about your household appliances.

What kind of appliances you have at home may affect the selection of the children into the second part of the study.

12. Please tick, if you use any of the following heater(s) at home:

Wood or coal fired heater(s)......................... ☐

Oil columned heater(s) .... ☐

Electric heater(s)......................... ☐

Gas/LPG heater(s) .... ☐

Other......................... ☐

If other describe.................................

Please, turn the page....

if you have ticked the gas fuelled heater(s) box then continue with question 13, otherwise continue with question 14

13. Is your gas heater based on

unflued gas heater: .... ☐
(unflued: no ventilation duct leading vertically or horizontally outside)

flued gas heater: .... ☐
(ventilation duct to the outside present)

not sure if flued or unflued:..................... ☐

14. Please tick, if you use any of the following cooking appliances:

Electric hotplate................................ ☐

Electric oven.................................... ☐

Gas hotplates................................... ☐

Gas oven....................................... ☐

15. Do you keep any pets in your household?

Yes ☐   No ☐

16. In your household, do you have any mould or mildew on any surfaces?

Yes ☐   No ☐

17. Does your child spend 1 day or more per week in another household

Yes ☐   No ☐

If you answered yes to question 17, please repeat the questions about the household appliances for this household

Please tick, if any of the following heater(s) are used:
/

Wood or coal fired heater(s)...................... ☐

Oil columned heater(s) .... ☐

Electric heater(s)............................... ☐

Gas/LPG heater(s) .... ☐

Other.. ☐

If other describe...............................

If you have ticked gas fuelled heater(s), is the gas heater based on

unflued gas heater: .... ☐
(unflued: no ventilation duct leading vertically or horizontally outside)

flued gas heater: .... ☐
(ventilation duct to the outside present)

not sure if flued or unflued:...................... ☐

Please tick, if any of the following cooking appliances are used:

Electric hotplate............................... ☐

Electric oven.................................. ☐

Gas hotplates................................. ☐

Gas oven..................................... ☐

● ● ●
End of questionnaire
Thank you for help

The
Queen Elizabeth
Hospital

Date

Respiratory Health in the School Environment

Asthma affects:
1 in 4 primary aged children
1 in 7 teenagers
1 in 10 adults

- Asthma is becoming more common and more severe in children
- 15 Australians die from asthma each week
- Asthma is a major cause of childhood admissions to hospital
- Asthma is the most common cause of school absenteeism amongst Australian children

Our research team headed by Dr. Louis Pilotto (The Queen Elizabeth Hospital) has been awarded a research grant by the National Health and Medical Research Council to study the effect of air quality in classrooms on the respiratory health of children in schools in metropolitan Adelaide. The Department of Education, Training & Employment, the Catholic Education Centre, the Independent School Board and the principals of participating primary schools have endorsed our research and we will be working closely together with them throughout the study.

What is the study about?
We don't really know why some people have sensitive airways, but it is clear that certain things can trigger asthma symptoms such as wheezing and coughing. Some of the asthma triggers are especially found indoors such as dust in general, mites, moulds, animals and other pollutants coming from a variety of indoor sources. These triggers may also affect non-asthmatic children causing sore throat, colds and increase the absenteeism of children from school.
The respiratory health of children is a significant health issue for schools, where children spend a considerable amount of time during their day, however there is relatively little known about the indoor school environment. With this study we want to explore potential triggers in classrooms and relate them to children situated in the classrooms.

How does the study work?
Initially we want to find out about the number of children with and without asthma. In the second part of the study we will then measure environmental triggers in classrooms and at the same time ask a selected number of asthmatic and non-asthmatic children to participate. In brief, participation would involve the keeping of a diary of your child's breathing problems, if any, over about 3 months. If your child has asthma, a doctor and a respiratory technician will also visit your school for some breathing and allergy tests which are otherwise routinely performed at hospitals such as the Women's and Children' Hospital on children with asthma. In the end we will draw conclusions between what we have measured in the classrooms, the diary and the results of the medical tests.

What happens during the first part of the study?
If you are willing for your child to be considered for this study, (and we really hope you will agree as we need your help) you are invited to participate by completing the attached questionnaire. Based on this information we will then select children to participate in the next part of the study for which we will provide further information.

What happens to the results of the study
We will provide you with the findings of the study and with the personal breathing and allergy results for those children who will take part in the medical consultations. Also, if the results suggest adverse health effects, short and long term changes will be implemented to the indoor school environment. If you participate in this questionnaire we will make sure that your child's name and information is kept confidential and will not appear in any published material.

What if I have a question about the study?
If you have questions about this research, you can call the following members of the research team: Monika Nitschke (research officer): 82226896, Dr. Louis Pilotto (principal investigator and senior consultant): 8222 7534. Thank you for your help and please do not forget to return the completed questionnaire to your child's school.

Yours sincerely

Dr. Louis Pilotto
Principal Investigator

Appendix II

Information sheet for main study and consent forms
for children and adults

INFORMATION SHEET FOR PARENTS/CAREGIVERS AND CHILDREN

"Asthma Childhood Environment Study (ACES)"

Dear Parents/Caregivers and Children

We are seeking your help and invite you to participate in the second part of this study which aims to reduce the problem of asthma in children. This form describes the purpose, procedures, benefits, risks and discomforts connected with this study. Even if you choose to participate, you have the right to withdraw from the study at any time.

What is the study about?

From the respiratory health questionnaire which you completed recently, we understand that your child has asthma. We are now writing to ask for your help in a study of trigger factors which might worsen asthma in the school environment. Environmental factors such as allergies to animals and dust mites may be triggers of asthma. The study is conducted by The Queen Elizabeth Hospital.

How does the study work?

If you agree to participate in this study,

your child would

attend two sessions where your child 's asthma will be assessed with breathing tests, performed at your child's school. These tests will take place at the beginning (May) and at the end of the study (September). These tests will tell us how the lungs of the children are working. The breathing tests are very easy to do and are not painful or distressing.

what happens during the breathing tests

- First, we will ask your child to do a breathing test which will take about 15 minutes. They involve blowing into a machine which will measure your child's airflow. This will be done before and after Ventolin to measure if there is any change in the breathing tubes.
- In some children we will perform a second test which will take about 30-40 minutes. This further breathing test is routinely done in our hospitals to see if your child's breathing tubes are "twitchy" or asthmatic. They will breathe slowly a small amount of histamine. In people who have asthma, histamine causes a small reduction in blowing capacity.
- During this consultation we will also perform a check on your child's allergy status by doing a skin test. This will take about 10 minutes. With this test we scratch the skin on the inside of the forearm lightly with substances that we have found many people are allergic to. We will test for cat, house dust mite and rye grass.
- We will also take a small sample of saliva from your child in order to test for exposure to environmental tobacco smoke.
- A research team member will make an appointment with you to visit you at your home during the study period and will ask to take a dust sample from your home. This is to investigate whether there are any substances in the dust of your house to which your child may be allergic to.

you as the carer of the child would

- Keep a diary of asthma symptoms together with your child for a period of 16 weeks. This will only take a minute per day.
- In order to communicate the information of the daily diary effectively to the research team, you would be contacted by phone 8 times during the period of the study (once every two weeks). An interviewer will ask you questions about your child's symptoms over the last 2 weeks which you will be able to answer with the help of the diary you and your child have kept on a daily basis. We will ask you simple questions such as whether your child had difficulty breathing today, or whether he/she had any wheezing.

A timetable is attached for your convenience.

What are the risks and / or discomforts in the study?

Breathing tests:
After application of histamine mild irritation of the throat with hoarseness may occur in a few subjects. In case of chest tightness or wheezing the application of Ventolin, a common asthma puffer, will rapidly reverse these effects. Histamine is a natural substance which is routinely used as a test for asthma in Australia.

Skin test:
If allergic, a small hive, similar to a mosquito bite, will appear on the skin which can be relieved by an anti irritant cream. A severe life threatening reaction (anaphylaxis) as a complication of this test is extremely rare, but appropriate medication will be present.
An experienced medical officer will be in attendance during this consultation.

What will I get out of the study?

You and your child will be informed of the results of the breathing and skin allergen tests which may be of assistance to your usual doctor. You also will be informed personally about the results of the overall study which aims to reduce potential asthma trigger factors from the school environment.

What happens to the results?

Your individual results will be communicated to you and, if you wish to your doctor. The overall results of the study will be presented at scientific meetings and published in relevant journals. We will keep your information confidential and your personal details will be destroyed at the end of the study. Your name will not appear at any stage in connection with the results.

What happens if I say no?

Your participation will be highly appreciated. But, you are free to withdraw from this study at any time. If you do not wish to participate or if you withdraw from the study this will not affect your future treatment at the Queen Elizabeth Hospital.

What if I have a question about the study?

If you have questions about the research, you can call the following members of the research team: Monika Nitschke (research officer): 8 222 68 96, Dr. Louis Pilotto (principal investigator and senior consultant): 8 222 75 34.

This study has been approved by the North Western Adelaide Health Service Ethics of Human Research Committee. Should you wish to speak to a person not directly involved, in particular in relation to matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make a confidential complaint, you may contact the Executive Officer of this Committee Mr Paul Miller on 08 8222 6841.

Thank you for your help. Please, if you wish your child(ren) to be considered for this study, return the completed consent form as soon as possible using the attached reply paid envelope.

Yours sincerely

Dr. Louis Pilotto
Senior Consultant

Student Consent Form

Dear Student

You and your parent/caregiver have received today an information sheet about a project called:
"Asthma Childhood Environment Study (ACES)"
You may remember that we asked your parents about the health of your lungs a couple of weeks ago. Now that we know that you have asthma we want to know if you would help us in a further project. In this project we will explore the environment in your classroom and determine whether there is anything which may be bad for your asthma.
Before you say you will be in this study by signing at the bottom of the page, we want to give you some information.

❖ You and your parents will pay attention to your asthma a little bit closer from May to mid August by keeping a very short diary. This will take about a minute a day. Every two weeks we will then ring up your parents/caregivers who will tell us about your health with the help of this diary.

❖ A doctor and a nurse will come to your school and check your breathing twice. Once at the beginning of the project (May) and once at the end (August or September). This may take between 15-45 minutes during school hours and it involves breathing into a plastic tube. Some children will be asked to also inhale a spray and then blow into a tube. It is very easy and the only thing that may happen is that your throat may get a bit croaky. For the unlikely event that you feel ill a doctor is there to help you instantly.

During the second visit:

❖ The same lung tests will be done again, but the nurse will also do a skin test for allergies.

❖ You will also give a saliva sample which will test whether you have been exposed to smoke.

We are doing this project to improve your school environment and your asthma, so please help us by taking part and by signing below.

I, ...(first name and surname of student, please print)

Consent to take part in the Asthma Childhood Environment Study

Signed...Date...............................

School...Class...............................

North Western Adelaide Health Service
The Queen Elizabeth Hospital

Parents/Caregivers Consent Form

❖ I, the undersigned...

❖ hereby consent to my child's involvement in the research project titled:

"Asthma Childhood Environment Study (ACES)"

❖ I have read the information sheet, and I understand the reasons for this study.

❖ I also understand the details of the project as set out on the information sheet, including:

> ➢ **A short phone interview every 2 weeks (8 times) during the study period**
> ➢ **The assessment of my child's asthma during school hours, including a simple breathing test with and without Ventolin and another breathing test with histamine.**
> ➢ **A skin test for assessment of possible allergies**
> ➢ **A short house visit for collecting dust samples**
> ➢ **A saliva sample which tests exposure to environmental tobacco smoke**
>
> *Please, consult attached time table*

❖ I have also been informed about the possible risks and discomforts

❖ I understand that I will receive the results of the breathing and skin tests of my child(ren) at the end of the study.

❖ I understand that while information gained in the study may be published, my child will not be identified and all individual information will remain confidential. The final result of the study findings will be sent to you.

❖ I understand that my child can withdraw from the study at any stage, and that if I do withdraw my child from the project it will not affect any treatment at this hospital in the future.

Signed..Date...............................

Name printed...

Address (printed)...

Phone number: [ ]

Relationship to child..

Name of child...

Telephone interview

Please indicate a convenient time for our telephone interviewers to contact you for the first asthma diary. The interviewers may ring between:
Monday, 15. May— Thursday 18.May

__You may tick more than 1 box__

◯ In the morning (9am-12 am)   phone number   [   ]

◯ In the afternoon (after 12 am – 5pm)   phone number   [   ]

◯ In the evening (after 6 pm – 9 pm)   phone number   [   ]

Your interviewer will ask you about a convenient interview time for the 2nd diary

Appendix III

Baseline characteristics questionnaire

Asthma Childhood Environment Study

Clinical Epidemiology and Health Outcomes Unit
Telephone: (08) 8222 6896 Facsimile: (08) 8222 6121

Children's final study questionnaire

Please post the completed questionnaire back to us as soon as possible. Use the

PERSONAL DATA OF PARTICIPATING CHILD

1. What is this child's ethnic background?

Australian   (Aboriginal)   ☐

Australian (Non-Aboriginal)   ☐

Other   ☐
↓
*Please, specify:*_____

Medical History of this child

The questions in this section apply to the past medical history of your child. In general, we are asking you to recall events that may have occurred several years ago. If you cannot remember the time periods in question, please indicate that by ticking the "Don't know" box.

2. What did this child weigh when he or she was born ?

under **1,500** grams (under 3 lbs 5 ozs)   ☐

2.200-2,500 grams (3 lbs 5 oz – 5 lbs 8 ozs)   ☐

Over **2, 500** grams (over 5 lbs 8 oz)   ☐

Don't know   ☐

3. Was this child seen by a doctor or other health practitioner for a severe chest illness *BEFORE* the age of 2 years?

No ☐   Yes ☐   Don't know ☐
↓   ↓   ↓
go to 4   *go to 3.1*   *go to 4*

3.1 Did the child have more than one such illness?
No ☐   Yes ☐

3.2 What was the diagnosis? *(please, mark all that apply)*

Pneumonia ☐   Croup ☐

Bronchiolitis ☐   Asthma ☐

Bronchitis ☐   Don't know ☐

Other ☐
↓
*Please, Specify:*_____

3.3 Was the child kept in the hospital overnight for any such illness?

No ☐   Yes ☐

4. Was your child seen by a doctor or other health practitioner for a severe chest illness AFTER the age of 2 years?

No ☐   Yes ☐   Don't know ☐
↓   ↓   ↓
go to 5   *go to 4.1*   *go to 5*

4.1 Did the child have more than 1 such illness?

No ☐   Yes ☐

4.2 What was the diagnosis *(mark all that apply)*

Pneumonia ☐   Croup ☐

Bronchiolitis ☐   Asthma ☐

Bronchitis ☐   Don't know ☐

Other ☐
↓
*Please Specify:*_____

4.3 Was the child kept in the hospital overnight for any such illness?

No ☐   Yes ☐

5. Has your child ever had hay fever?

No ☐   Yes ☐   Don't know ☐

6. Has a doctor or other health practitioner ever said that your child had allergies?

No ☐   Yes ☐   Don't know ☐
↓   ↓   ↓
go to 7   *go to 6.1*   *go to 7*

6.1 To which of the following is she or he allergic? *(Mark all that apply)*

- Things that are eaten or ingested, for example, food or medicine. ☐
- Things that are breathed in or inhaled, for example, dust, pollens, moulds, animal fur or dander, smoke. ☐
- Things which come in contact with the skin, for example, wool. ☐
- Other ☐

Please Specify:_____

---

7. during the past 12 months, did your child have hay fever?

No ☐   Yes ☐   Don't know ☐
↓   ↓   ↓
go to 8 **go to 7.1**   **go to 8**

7.1 Was the child seen by a doctor or other health practitioner for hay fever?

No ☐   Yes ☐

8. During the past 12 months, did your child have any chest illness?

No ☐   Yes ☐   Don't know ☐
↓   ↓   ↓
go to 9.1 **go to 8.1**   **go to 9.1**

8.1 Did your child have more than one such illness?

No ☐   Yes ☐

8.2 Were your child's activities restricted for three days or more because of any such illness?

No ☐   Yes ☐

8.3 Was your child seen by a doctor or other health practitioner for any such illness?

No ☐   Yes ☐

8.4 What was the diagnosis? **(Mark all that apply)**

Pneumonia ☐   Croup ☐

Bronchiolitis ☐   Asthma ☐

Bronchitis ☐   Don't know ☐

Other ☐
↓
*Please Specify:*_____

8.5 Did your child take antibiotics for any such illness?

No ☐   Yes ☐

8.6 Was your child kept overnight in the hospital for any such illness?

No ☐   Yes ☐

9.1 Has a doctor ever said the *BIOLOGICAL* father of this child had chronic bronchitis, emphysema, or chronic obstructive lung disease?

No ☐   Yes ☐   Don't know ☐

9.2 Has a doctor ever said the *biological* father of this child had asthma?

No ☐   Yes ☐   Don't know ☐

10.1 Has a doctor ever said the *biological* mother of this child had chronic bronchitis, emphysema, or chronic obstructive lung disease?

No ☐   Yes ☐   Don't know ☐

10.2 Has a doctor ever said the *biological* mother of this child had asthma?

No ☐   Yes ☐   Don't know ☐

Current household members

11. Counting yourself, how many people live in this child's home?

1 ☐   2 ☐   3 ☐   4 ☐   5 ☐

6 ☐   7 ☐   8 or more ☐

12. Is English your primary language?

No ☐   Yes ☐

13. What is the highest grade or educational level you have completed?

- Did not complete primary school ☐
- Completed primary school ☐
- Did not complete highest year of secondary school ☐
- Completed highest year of secondary school ☐

14. Since leaving school, have you completed a trade certificate, degree or any other educational qualification?

No ☐   Yes ☐

15. Do you currently smoke cigarettes?

No ☐ → *go to 16*   Yes ☐ → *go to 15.1*

15.1 About how many cigarettes do you smoke on average per day *inside your home*?

- Fewer than 10 ☐
- 10-14 ☐
- 15-24 ☐
- 25-34 ☐
- 35-44 ☐
- 45 or more ☐

16. Do you currently smoke pipes or cigars?
(Mark all that apply)

Pipes ☐   Cigars ☐   Neither ☐

17. Is there another primary adult *(for example your spouse or your partner)* living in your household?

No ☐ → *go to 18*   Yes ☐ → *go to 17.1*

17.1 What is the highest grade or educational level completed by this other adult?

- Did not complete primary school ☐
- Completed primary school ☐
- Did not complete highest year of secondary school ☐
- Completed highest year of secondary school ☐

17.2 Since leaving school, has this other adult completed a trade certificate, degree or any other educational qualification?

No ☐   Yes ☐

17.3 Does he or she currently smoke cigarettes?

No ☐ → *go to 17.5*   Yes ☐ → *go to 17.4*

17.4 About how many cigarettes does he or she smoke on average per day *inside your home*?
- Fewer than 10
- 10-14
- 15-24
- 25-34
- 35-44
- 45 or more

17.5 Does he or she currently smoke pipes or cigars? (*Mark all that apply*)

Pipes ☐   Cigars ☐   Neither ☐

18. Not counting yourself and your spouse or partner, does anyone smoke cigarettes within your home (*as opposed to smoking only outside your home*) **? Include regular visitors, for instance a grandparent or babysitter.**

No ☐ ➤ the end   Yes ☐ ➤ *go to 18.1*

18.1 Counting *only* these other smokers, about how many cigarettes are smoked *per day* inside your home?
- Fewer than 10
- 10-14
- 15-24
- 25-34
- 35-44
- 45 or more

- The End -
Thank you for completing the questionnaire

Appendix IV

Severity of asthma questions

Asthma Severity Questions (Telephone interview):

1. When was the last time your child took medication for asthma?

1.1. Within the past week

1.2. Within the past month (but not in the past week)

1.3. Within the past twelve months (but not in the past month)

1.4. Since starting reception (but not in the past twelve month)

1.5. Under age 2

1.6. Never took medication specifically for asthma

2. Which best describes the child's current level of symptoms? Please, read out all possible answers first

In the past 12 months...

2.1. The child has not be troubled by asthma

2.2. The child has had some asthma, but did not take any medication for it

2.3. The child has had some asthma, requiring medication only for occasional episodes

2.4. The child has had asthma, requiring medication on a routine basis, but did not have any episodes while on medication.

2.5. The child has had asthma, requiring medication on a routine basis and also had one or more episodes requiring additional treatment.

Appendix V

objective lung function information letters

Asthma Childhood Environment Study

Lung test

As explained in the information sheet we want to find out how much your child's asthma affects your child's breathing by using a simple breathing test. In this test an expert will show your child how to blow into a machine called a spirometer. This involves taking a full breath in and blowing it out very hard. This test is carried out before and after inhaling Ventolin, a common asthma puffer (bronchodilator), to see whether the lung works better after using the puffer.

Invitation to skin tests

We will also perform a check on your child's allergy status by doing a skin test. This will take about 20 minutes. With this test we scratch the skin on the inside of the forearm lightly with substances that we have found many people are allergic to. We will test for cat, dog, house dust mites, grasses and funghi. If allergic, a small hive, similar to a mosquito bite, will appear on the skin which can be relieved by an anti irritant cream. A severe life threatening reaction (anaphylaxis) as a complication of this test is extremely rare, but appropriate medication will be present.

An experienced medical officer will be in attendance during this consultation.

Preparation for your child's lung test and skin test:

Your child may be on medication which could get in the way of proper test results, therefore we ask you to stop any of the following medication according to the following time plan:

5 days before test: antihistamines such as Teldane, Claratyne

3 days before test: antihistamines such as Zadine, Piriton Polaramine

24 hours before test: Theophyllines, such as Theodur, Neulin, Austyn, Elixophylline, Brondecon

24 hours before test: long acting bronchodilators such as Serevent, Foradile, Singulair, Salmeterol

night before test: Intal or Tilade

4 hours before test: bronchodilators such as Respolin, Bricanyl, Atrovent, Berotec, Alupent

---

If your child requires any of the above medication urgently to stop an acute attack of asthma then do not stop the medication. We will ask your child about medication taken prior to commencing the testing and may organise another time to test in case your child needed to continue any of the above medication longer than requested. If you have any further concerns, please do not hesitate to ring Monika Nitschke (research officer) on 8222 68 96 (work), or 81320881 (home), or 0410 698 426 (mobile) and she can arrange for one of the medical doctors involved in the study to address your concerns.

Yours sincerely

Dr. Louis Pilotto
Senior Consultant

Asthma Childhood Environment Study

Clinical Epidemiology and Health Outcomes Unit
Telephone: (08) 8222 6896 **Facsimile:** (08) 8222 6121

Invitation to lung tests

As explained in the information sheet, we want to find out how much your child's asthma affects your child's breathing by using a simple breathing test. In this test an expert will show your child how to blow into a machine called a spirometer. This involves taking a full breath in and blowing it out very hard.

This test is carried out before and after inhaling Ventolin, a common asthma puffer (bronchodilator), to see whether the lung works better after using the puffer.

Also, your child is to perform a second breathing test that takes longer (30-40min) and gives a more precise picture of your child's asthma. The expert will use a substance called histamine to see whether your child's breathing tubes are "twitchy", which tells us how mild or severe your child's asthma is. Histamine is naturally occurring in the human body and is routinely used as a test for asthma in Australia in children and adults. After using histamine, mild irritation of the throat may occur in a few cases. In cases of chest tightness and/or wheezing the application of Ventolin will rapidly reverse the effects. An experienced medical officer will be at the school when the testing takes place.

Invitation to skin tests

During this consultation we will also perform a check on your child's allergy status by doing a skin test. This will take about 20 minutes. With this test we scratch the skin on the inside of the forearm lightly with substances that we have found many people are allergic to. We will test for cat, dog, house dust mites, grasses and funghi.

If allergic, a small hive, similar to a mosquito bite, will appear on the skin which can be relieved by an anti irritant cream. A severe life threatening reaction (anaphylaxis) as a complication of this test is extremely rare, but appropriate medication will be present.

An experienced medical officer will be in attendance during this consultation.

Should you wish to speak with one of the doctor's in charge of the study prior to your child's test, please ring Monika Nitschke on 8 222 68 96 or 0410698 426 and she will arrange for one of the doctor's to return your call.

Preparation for your child's lung function (breathing), histamine inhalation test and skin test:

Your child may be on medication which could get in the way of proper test results, therefore we ask you to stop any of the following medication according to the following time plan:

5 days before test: antihistamines such as Teldane, Claratyne
3 days before test: antihistamines such as Zadine, Piriton Polaramine
24 hours before test: Theophyllines, such as Theodur, Neulin, Austyn, Elixophylline, Brondecon
24 hours before test: long acting bronchodilators such as Serevent, Foradile, Singulair, Salmeterol
night before test: Intal or Tilade
4 hours before test: bronchodilators such as Respolin, Bricanyl, Atrovent, Berotec, Alupent

...please, turn the page

If your child requires any of the above medication urgently to stop an acute attack of asthma then do not stop the medication.. We will ask your child about medication taken prior to commencing the testing and may organise another time to test in case your child needed to continue any of the above medication longer than requested.

Your sincerely

Dr. Louis Pilotto
Senior Consultant

/

Appendix VI

Diary

This is the last diary
Please, use the magnet to keep your diary on the fridge!
Return your diary after your last telephone interview using the reply paid envelope

We ask you to mark this diary on a daily basis. It may be necessary to get some help from your parents/care givers. This diary will help your parents/care givers during the telephone interview with us.

Instructions and examples:

➢ Shade the circle if you have a symptom, leave it unmarked if you have no symptom.

➢ Information about your day time symptoms are best recorded in the evening and night time symptoms in the morning (*for example: Monday's daytime symptoms count from the time you get up until you go to bed. Monday's night time symptoms count from when you go to bed until Tuesday morning when you get up*).

Thank you for your help

Study weeks:13-16 (24.July-20.August)

Day of the week	24.7 M	25.7 T	26.7 W	27.7 T	28.7 F	29.7 S	30.7 S	31.7 M	1.8 T	2.8 W	3.8 T	4.8 F	5.8 S	6.8 S	7.8 M	8.8 T	9.8 W	10.8 T	11.8 F	12.8 S	13.8 S	14.8 M	15.8 T	16.8 W	17.8 T	18.8 F	19.8 S	20.8 S

Symptoms during the day?   week 13 / week 14 / week 15 / week 16

	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S
Chest wheezing	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Breathlessness	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Chest tightness	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Cough	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

Symptoms during the night?

	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S
Chest wheezing	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Breathlessness	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Chest tightness	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Cough	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

Activity today?

	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S
Any physical exercise today?	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
If yes, mark if you had difficulties with your breathing afterwards	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

Day of the week	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S

Did you have any asthma attacks today? (Definition: "Any asthma episode involving breathlessness and/or wheezing and/or chest tightness and/or coughing that interrupts ongoing activities or requires some procedures, such as resting or using of a nebuliser to resume normal and comfortable breathing."

	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S
During the day?	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
During the night?	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S
Missed school today? (exclude weekends and holidays)	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Visit to a health care facility today because of your asthma? (GP, hospital, outpatient hospital, asthma clinic)	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

Asthma Medication: Write down the name of your medication in the following free spaces of the first column and note underneath your regular dose of this medicine (eg. Ventolin 3 puffs). In the daily columns note how often you have taken this medication at that dose (eg. 3 x).
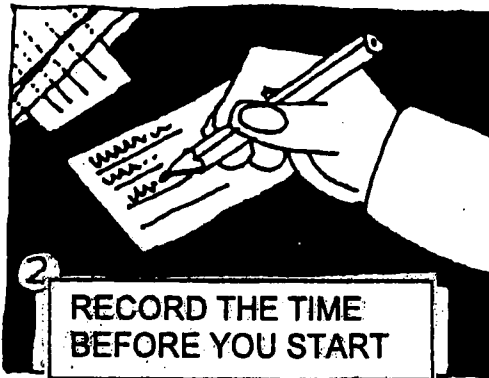
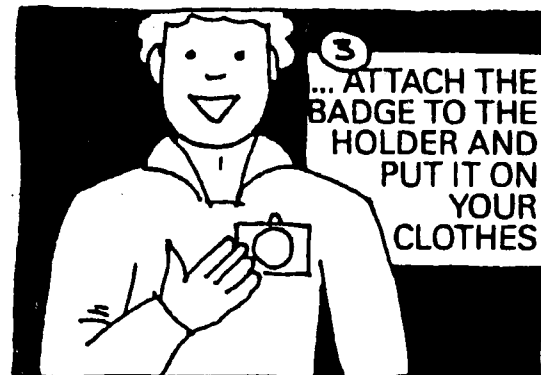| 1. medication ……………………… Regular dose:…………….																												
2. medication ……………………… Regular dose:…………….																												
3. medication ……………………… Regular dose:…………….																												
4. medication ……………………… Regular dose:………….																												

Appendix VII

Information: exposure sampling in homes of participants

1. UNSEAL PLASTIC BAG ...

2. RECORD THE TIME BEFORE YOU START

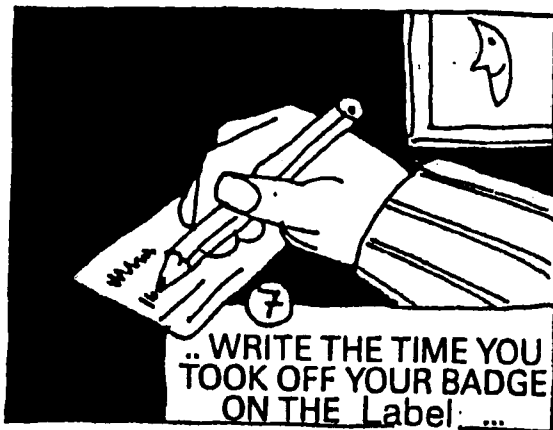3. ... ATTACH THE BADGE TO THE HOLDER AND PUT IT ON YOUR CLOTHES

4. ... REMOVE THE BADGE LID ...

5. START WEARING THE BADGE FROM WHEN YOU COME HOME FROM SCHOOL UNTIL BEDTIME

6. TAKE OFF THE BADGE AND REPLACE THE LID ...

7. .. WRITE THE TIME YOU TOOK OFF YOUR BADGE ON THE Label ...

8. .. REPLACE IN PLASTIC BAG AND SEAL BAG ...

➢ **Open** the other 2 badges at the same time as the personal badge, but position them in the kitchen as discussed.
➢ **Close** the lid of the 2 kitchen monitors at the same time as your personal badge.
➢ **Place** all 3 of your daily badges into their bag before you go to bed.
➢ **Store** the badges in the fridge until you bring them back to school.
➢ **Don't mix** badges from different days
➢ **Always keep** the "*blank*" and the "*badge without lid*" in the sealed bag and in the fridge.
➢ **PLEASE, DO NOT FORGET TO BRING THE BADGES BACK TO SCHOOL**

Appendix VIII

Publication: earlier NO$_2$ review