A randomised controlled trial of the effects upon asthmatics of eradicating moulds from within their homes.

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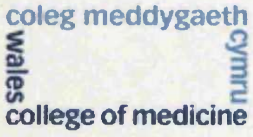

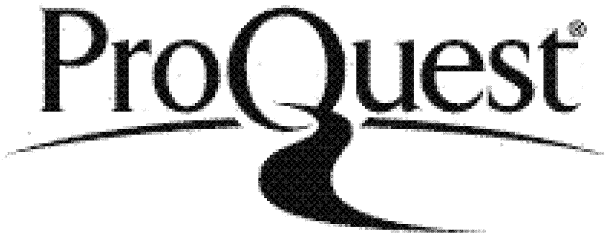

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Abstract

In the UK the prevalence of wheeze in the last twelve months is 30% in 13-14 year olds and the rate of diagnosed asthma for all ages is 14.5%. Approximately 70% of asthmatics are atopic with specific immunoglobin E (IgE) to environmental allergens.

The thesis is based on an Experimental Investigation (Randomised Controlled Trial) of the effect on symptoms and Peak Expiratory Flow Rate (PEFR) of mould eradication in the homes of asthmatic patients. It is also aimed to measure the amounts and types of indoor moulds as well as determining the effects on this of built form, positive input ventilation and other factors.

Methods

Asthmatics were recruited primarily from asthma registers held by General Practitioners in Neath and Port Talbot (NPT), and Cardiff; 177 households were identified as contaminated with mould and 232 asthmatics resided in these. The sample of households also included asthmatics whose homes were registered with NPT Local Authority as being contaminated with mould and some who were randomly sampled from the community.

A baseline visit was made to each home to ascertain both mould and asthma status. At this visit subjects were asked to complete a symptoms questionnaire, PEFR record for two weeks and tested for their skin reactivity to certain moulds and other allergens.

Swab samples of mould were taken from contaminated surfaces and a 24 hour air sample was collected for analysis of ergosterol. Indoor relative humidity and temperature were recorded and details of built form were collected.

If eligible the household was then randomly allocated to intervention or a control group by means of serially numbered sealed envelopes (83 controls and 81 interventions). The intervention consisted of removal of all visible mould from affected surfaces and application of a fungicide as well as installation of Positive Input Ventilation (PIV). Questionnaires and PEFRs were obtained 6 months and 12 months after randomisation, and mould sampling was also repeated at 12 months.

Results

The Intervention group reported greater reduction in medication usage and more improvement in breathing than the control group at 6 months from baseline and this remained at 12 months. Comparison of the questionnaire responses showed a tendency for chest symptoms to improve in the intervention group at 6 months compared to controls and the tendency was present at 12 months.

Certain mould genera were found to predominate and built form was observed to be associated with mould concentration. PIV reduced the indoor humidity and it reduced the likelihood of mould reoccurring in the intervention homes compared to the control homes.

Conclusions

The intervention resulted in a reduction in medication usage and improvement in breathing. Despite the reduction in medication usage in the intervention group there was a small improvement in recent wheeze compared to the control group but this was not statistically significant. For those subjects whose wheeze limited their speech or affected their breathing the improvements observed were larger. There were also improvements in symptoms of rhinitis and rhinoconjunctivitis in the intervention group, especially in those homes where mould remained absent. The prevalence of skin sensitivity observed was high and was approximately 56% in children and 75% in adults.

Built form affected mould contamination; and flats, bungalows and end terraces were found to be at an increased risk from moulds. Positive Input Ventilation had a considerable affect on corrected indoor mixing ratios and reduced this by an average of 20%.

Summary

The rate of diagnosed asthma is 14.5% for all ages and the prevalence of wheeze in the last 12 months is 30% in 13-14 year olds in the UK. This thesis aimed, by means of a randomised controlled trial, to examine the effect on symptoms and Peak Expiratory Flow Rate (PEFR) of mould eradication in the houses of asthmatic patients. Subsidiary aims were to measure mould genera indoors and investigate the effects of built form and ventilation on mould contamination.

Asthmatics were recruited primarily from General Practices and asked to complete a symptom questionnaire and to record PEFR for two weeks. Sensitivity to allergens, including moulds, was measured by skin prick tests. Swab samples of mould were taken from contaminated surfaces and an air sample was collected for analysis of ergosterol. Indoor relative humidity, temperature and details of built form were recorded.

The household was then randomly allocated to either the intervention or the control group. The intervention consisted of removal of all visible mould from affected surfaces and application of a fungicide as well as installation of Positive Input Ventilation (PIV). Questionnaires and PEFRs were obtained 6 months and 12 months after randomisation, and mould sampling was also repeated at 12 months.

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Thesis Aims

The principle aim of this thesis was to test the effect on symptoms and Peak Expiratory Flow Rate of the eradication of mould from the homes of asthmatics, by means of a Randomised Controlled Trial. A subsidiary aim was to measure the distribution of moulds and to determine how built form contributed to this, as well as to examine the effectiveness of Positive Input Ventilation in reducing indoor humidity and preventing reoccurrence of mould growth.

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Rob

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Chapter 1 - Aetiology of Asthma

1.1. Asthma: Prevalence and contributing factors

Asthma is a complex, chronic lung disease of multifactorial aetiology. It is difficult to distinguish it from respiratory infections in infants and from chronic obstructive pulmonary disease (COPD) in older adults. Historically, asthma was the name given to a disorder that occurred in people who episodically breathed with a whistling sound (known as 'wheezing'). No universally accepted definition for asthma exists, which makes it difficult to draw conclusions from epidemiological studies, as they are definition dependent. Von Ehrenstein quotes The World Health Organization (WHO) together with the United States National Heart, Lung and Blood Institute who have developed the following definition:

> "Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells, eosinophils and T-lymphocytes. In susceptible individuals this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough particularly at night and/or in the early morning. These symptoms are usually associated with widespread but variable airflow limitation that is at least partly reversible either spontaneously or with treatment. This inflammation also causes an associated increase in airway responsiveness to a variety of stimuli." (Tamburlini, et al., 2002)

Thus, the principal characteristic of asthma is one of airway hyper responsiveness, an inflammatory reaction to allergens and other stimuli, which can lead to wheezing, shortness of breath, mucus accumulation and airway obstruction. These symptoms are caused by inflammatory mechanisms in the airways that elicit the clinical response associated with an asthma episode. Sensitisation to a heterogeneous group of triggers may provoke an asthma attack. In addition, nocturnal awakening, or sleep disruption may be present in some asthmatics (Johnson et al., 2002). Heredity seems to play a role in asthma, as do environmental factors and allergens, with a combination necessary to initiate development of the condition. A list of the most common factors that can have an effect on asthmatics is shown in table 1.1, overleaf.

Table 1.1. Factors that can have an effect on asthmatics.

Allergens	**Pollutants**	**Other Factors**
Bacteria	Carbon monoxide (CO)	Atopy
Cockroaches	Environmental Tobacco Smoke (ETS)	Exercise
Foods	Nitrous oxides (NO_x)	Genetics
Fungi (moulds)	Ozone (O_3)	Infections
House Dust Mites (HDM)	Particulate Matter (PM)	Stress
Parasites	Sulphur dioxide (SO_2)	
Pets	Volatile Organic Compounds (VOC)	
Pollens		
Vaccines		
Viruses		

Asthma is a common disease of western civilisations. The mortality rate is low but it is a major source of morbidity and hospital admissions, especially amongst children. Extrinsic or atopic asthma usually affects children and peaks before school age. It often goes into remission at adolescence but can reappear at any time in adulthood. Intrinsic asthma usually occurs later in life. Asthma is prevalent in the United Kingdom (UK), New Zealand (NZ), Australia, Western Europe and the United States of America (USA), affecting between five and ten percent of the population (Quiñones et al., 2003). The International Study of Asthma and Allergies in Childhood (ISAAC) was conducted to describe the prevalence and severity of asthma, rhinitis and eczema in children living in different areas and to enable comparisons within and between countries (Asher et al., 1995). It was undertaken in 155 centres in 56 countries around the world and found the prevalence of wheeze (in the past year) amongst 13-14 year olds to be 29-32%. A similar pattern was found in the European Community Respiratory Health Survey (ECRHS) conducted in 48 centres in 22 countries mostly in Western Europe with approximately 140,000 individuals (Janson et al., 2001). A review in 2000 compared the prevalence of wheeze in the participant's past year in the ECRHS survey with those of the International Study of Asthma and Allergies in

Childhood (ISAAC; 317,000 children in 40 countries) and found that in the 17 countries in which both surveys were undertaken that the prevalence patterns showed a good overall agreement from childhood through to adulthood (Pearce et al., 2000). An interim report from The Welsh Health Survey recorded for 2003-2004 that 10% of adults over 16 years of age and 12% of children were currently being treated for asthma (Welsh Health Survey, 2004). Doctor diagnosed asthma was reported in The Health Survey for England at 20.5% in 0-15 year olds and 14.5% for all ages (Primatesta, 2002).

1.2. Atopy

The proportion of asthmatics with atopy has been estimated at 70% with the highest prevalence occurring in the UK, New Zealand and Australia (Souhami and Moxham, 2002). Atopy, a genetic predisposition for the development of an immunoglobulin E (IgE) mediated response to common allergens, is the single most predisposing factor for the development of allergic rhinitis, atopic eczema and asthma (Institute of Medicine, 2000). Development of atopic disorders involves the participation of many different cells as well as various genetic and environmental factors that create major problems for patient based studies (Loza et al., 2005). Hopkin describes atopy as, "a T-helper 2 (Th2) cell driven hypersensitivity to innocuous antigens which causes most cases of asthma," (Hopkin, 1997). Allergens can illicit two types of immune response; in non-atopic children the Th2 cell response shifts to a more cytokine one derived from a type Th1 cell response, and in atopic children the Th2 cell stays active into adulthood. Interferon-γ production from Th1 cells inhibits the proliferation of Th2 cells that may otherwise increase, resulting in a predisposition to atopic disorders; this appears to happen within the first 6 months of life (Ball et al., 2000).

> "It is becoming clear that atopic disease is a two-stage process, involving a discrete phase for induction (i.e., immunological memory development favouring allergic sensitisation) which is regulated via interactions between Th1 and Th2 cytokines, followed by a second and more complex effector phase involving the expression in target tissues of sensitised individuals of highly variable levels of Th2-mediated inflammation" (Holt, 2000).

Atopic events during infancy are often associated with food allergy based gastrointestinal problems and atopic dermatitis. These symptoms are useful for identifying potential problems in early childhood, which may lead to asthma or allergic rhinitis (Bergmann et al., 1997). In older children and adults, identification of atopy can quickly be measured using a skin prick test (SPT) for various allergens. A wheal larger than 3mm is usually considered as a positive allergic response. A direct connection between atopy and asthma is as yet undetermined but it has been suggested that there is a potential association between the degree of atopy (number of positive SPT reactions) and asthma (Simpson, et al., 2001).

1.3. Genetics

Asthma and allergy are not inherited as single gene disorders but show a complex pattern of inheritance intermixed with a range of environmental factors (Anderson & Cookson, 1999). The contribution of genetics to asthma expression must be regarded as having some causative relationship. A Danish study observing twins whose asthma incidence was 6.2%, suggests their susceptibility has some genetic component with the remaining variation a result of gene-environment interactions, which may influence the asthma phenotypes (Skadhauge et al., 1999). Problems arise from the variety of asthma phenotypes observed and the definitions, which accompany them. Numerous studies are attempting to identify candidate gene clusters but as this research is in its infancy, the results are often contradictory, possibly from small sample sizes or from different combinations of genes and environmental exposures. Some of the chromosome locations identified are 2q, 5q, 6q, 12q and 13q, which contain loci that consistently affect asthma and atopy with sufficient strength to be detected by genetic linkage (Moffat & Cookson, 1999). A Tucson, Arizona, USA child cohort was used to identify a specific polymorphism in the 5' region of the CD14 gene. They found that polymorphism C-159T was associated with regulating either serum IgE levels or other atopic phenotypes (Baldini et al., 1999). A follow-up study of two cohorts in the north-eastern United States, looked at 5 single nucleotide polymorphisms (SNPs) specific to the CD14 antigen and its polymorphisms, located along the chain 5q22-q32, which affect the development of atopy. They found two SNPs were associated with eczema, suggesting further studies in this gene region (Litonjua et al., 2005).

Although genes are being identified as having links to asthma and atopy the large increase in the prevalence of these diseases over the past few decades cannot be explained by changes in gene frequencies alone. It is probable that pre-existing genetic factors are interacting with an environment, which is rapidly changing, rendering a large proportion of the population susceptible to asthma and atopic diseases (Sengler et al., 2001). These environmental factors may include immunisations, a decline in infectious diseases and helminth challenges, changes in diet, increased pollutants levels indoors and the greater time spent indoors. Genetic variations have developed to help fight infections, which may likely promote allergic inflammation in the absence of sufficient infections. These redundancies in host defence mechanisms may explain the wide variation in chromosomal regions and the increasing number of genetic variants related to atopic conditions (Sengler et al., 2001).

Everyone inhales a vast mix of pollutants on a daily basis but most do not result in allergic reactions, other factors as yet not isolated, accumulated or verified must be at work. Evidence continues to suggest that there is a progression from moderate to severe allergic diseases that are associated with a series of host and environmental interplays during early childhood, these interact to increase the risk of allergic sensitisation within the individual (Holt, 2004).

1.4. Hygiene Hypothesis

The hygiene hypothesis (the suggestion that keeping the body relatively free from biological intrusions increases the risk of developing atopic disorders) has arisen from attempts to explain the differences in allergy occurrence related to socioeconomic and geographical factors. One of the earliest reports from 1976 by Gerrard and colleagues involved an allergy study of Métis Indians in northern Canada. They found that asthma, eczema and urticaria were less common in the Métis Indians than the white population and suggested that atopic disease in the white community was the consequence of relative freedom from viruses, bacteria and helminths found amongst the Métis (Gerrard et al., 1976). The presence of atopic disorders is generally low in developing countries yet in western urbanized countries there has been an alarming rise in the prevalence of allergy and asthma over the past four decades (Björkstén et

al., 1998). There are a number of reasons suggested; one is that a reduction in the combined incidence of childhood infections and contact with bacterial antigens has contributed to the higher risk of allergy; this is often referred to as the "hygiene hypothesis". Another is that large helminth infestations now mostly limited to developing countries, particularly Africa, stimulate strong Th2 cytokine responses with high IgE concentrations. What is intriguing and as yet the answer is unknown, is why these subjects do not develop an allergic reaction. This occurs without a shift to the non-allergic Th1 response, thus requiring some modification of the hygiene hypothesis (van den Biggelaar et al., 2000). A third possibility involves the introduction of vaccines, which may replace the Th1 stimulus an individual would receive from the disease with a Th2 vaccine response thus depriving the immune system of development (Rook & Stanford, 1998).

1.5. Allergens

An allergy is an inappropriate response of the body's immune system reacting to contact with a foreign substance or antigen. Substances producing a reaction are known as allergens and whether inhaled, ingested or through contact, they may cause an IgE-mediated response in susceptible individuals. Inhaled allergens are thought to play a key role in asthma exacerbations based on conclusions from studies in diverse climates in which measured allergens were associated with sensitised asthmatics. A review on behalf of the European Community Respiratory Health Survey II (ECRHS), which included ~140,000 individuals from 22 countries, concluded that a sensitisation response to indoor allergens from mites and cats produced the strongest bronchial responsiveness but that asthma often goes under-treated (Janson et al., 2001). Another study questions whether there is a difference between children with just allergy and those with allergy and wheezing and how important is this distinction (Platts-Mills et al., 2000b). There is further evidence showing that asthmatics with sensitisation to at least one allergen have a more severe form of asthma (Langley et al., 2003). Mould allergen sensitisation has been suggested as a major risk factor for severe asthma (Zureik et al., 2002). This sensitisation appears to be most prevalent in early childhood and reaches a maximum as the child approaches 8 years of age, and then declines (Nolles et al., 2001). It is usually determined by a positive skin prick test

(SPT) to an allergen or from a blood sample analysis, commonly referred to as a RAST (Radio Allergo Sorbent Test), looking for a specific IgE response. Allergies can develop at any age but are common in children, especially those who have atopic parents.

Allergens can be divided between indoor and outdoor sources although invariably there will be some migration, these may include cat, dog and horse dander, moulds, house dust mite excrement, pollens, cockroach detritus, vaccines and food proteins. Other asthma triggers that do not produce an IgE response but may irritate the lungs include; chemical irritants (including perfumes), environmental tobacco smoke (ETS) and diesel particulates. Lifestyle, standard of living and the season of birth may also play a role with atopy in this complex system (Sears et al., 1996). People spend approximately 90 percent of their time indoors so it is prudent to investigate those allergens affecting asthmatics in indoor environments (Robinson & Thomas, 1991).

Allergen avoidance measures in the domestic environment focussed on a reduction in allergen-driven sensitisation should begin in infancy, especially if the parents are atopic (Wahn et al., 1997). Allergen removal from the source has a beneficial effect in reducing the persistence of asthma symptoms in both adults and children (Nelson, 2000).

1.5.1. House Dust Mites (HDM)

House dust mite allergen, Der p1, is the single most studied indoor antigen (Saglani and McKenzie 2002). HDM allergen is a strong inducer of allergic responses in children (Lau et al., 1989) and it may have a primary role in the causation of childhood asthma (Mahmic et al., 1998, Wickens et al., 2004). Increased exposure to HDM and other indoor allergens may be a factor contributing to increased morbidity and mortality relating to asthma (Sporik et al., 1990). Dozens of studies from the early seventies and eighties summarised in a review show that between 48% and 85% of asthmatics worldwide are allergic to mite allergen compared with 5% to 30% of the general population; the table of results did not stipulate the actual percentages found in each study (Platts-Mills et al., 1989). The major source of allergen from dust mites is from gastrointestinal proteins attached to their excrement (Sporik et al., 1990, Tovey et al., 1981). House dust mites live off dead human skin fragments, and have the highest

concentration in mattresses (median 188 mites/g dust) and pillows but are also found in carpets and upholstered furniture (Sidenius et al., 2002). A study found there are two relevant species that occur in homes, *Dermatophagoides pteronyssinus* (primary allergen Der p1), first isolated by Voorhorst in 1967, and *farinae* (chief allergen Der f1)(Voorhorst et al., 1967). Dust mites are incapable of ingesting water, they absorb moisture through hairs covering their bodies and are thus dependent on moist microclimates to live and breed. They prefer temperatures of ~25°C and relative humidity of ≥73% (Arlian, 1992); this explains the dust mites' dominance in bedding where their food source and environment is the most suitable. It was found that increased exposure to HDM was associated with higher risk of sensitisation but was protective if the subject had non-atopic parents (Cole Johnson et al., 2004). Housing conditions in general have improved with energy and heat conservation in the past few decades. These changes include the installation of insulating windows, high efficiency boilers and furnaces and the closing of draughty chimneys that have also benefited the house dust mite. A study running from April 1975 to August 1977 showed that climate and humidity within the Stockholm area are unfavourable to the development HDM in indoor dust (Turos, 1979). A study looking at mechanical ventilation and HDM found that homes with <0.5 air changes per hour (ACH) had significantly greater quantities of HDM allergen (>2μg/g) in dust and with water vapour values exceeding 7g/kg in air ($p=0.01$; comparing homes to those with ≥0.5 ACH). There has been a dramatic change in the dust mite population whose occurrence was rare in the late 1970s and at the end of the century is quite evident (Emenius et al., 1998). It was suggested in 1996 that HDM allergens are in important cause of childhood asthma and that a reduction in exposure would benefit public health in terms of asthma (Peat et al., 1996). The USA book review, Clearing the Air (Institute of Medicine, 2000) is the first publication to conclude that house dust mite exposure has a causal relationship with asthma.

1.5.2. Fungi

Fungi are more commonly known as mushrooms, moulds, mildews and rusts. Those found indoors are usually moulds and produce a characteristic musty smell. The major illnesses associated with mould include allergic bronchopulmonary mycoses, hypersensitivity pneumonitis, asthma, rhinitis and allergic sinusitis. Allergic bronchopulmonary aspergillosis is a pulmonary disease characterised by immunologic response to antigens of *Aspergillus fumigatus,* usually in patients with a history of asthma and elevated total serum IgE (Chiu & Fink, 2002). Hypersensitivity pneumonitis (or extrinsic allergic alveolitis) and inhalation fever (toxic pneumonitis) may be caused by very high exposure to moulds ($5x10^3$ and 10^6 CFU^{∇}/m^3, respectively) (Kolstad et al., 2002). Allergy to the genera *Alternaria* is a risk factor for death in asthmatic patients (O'Hollaren et al., 1991). Other disorders attributed to moulds include sick building syndrome and pulmonary haemorrhage related to *Stachybotrys atra* (Etzel et al., 1998).

Microbial agents including fungi play a major role in exacerbating asthma symptoms but their role as a possible causative agent in asthma is as yet unclear (Institute of Medicine, 2000). A time-series study in Cincinnati, Ohio, USA found no associations between hospital visits for asthma attacks and fungal spore counts or ozone levels (Lierl & Hornung, 2003). Much of the data indicating a possible link between asthma and fungi is qualitative, resulting from observational epidemiological surveys where the participant and/or the researcher may introduce bias. Difficulty is aggravated because there are no accurate, quantitative measures of mould exposure or definition of asthma, which hinders efforts to clearly make a link between fungi and asthma development or its exacerbation. The quantity and variety of moulds that grow indoors is defined by the environment and sources of spores. Indoor mould growth is typically affected by the humidity, ventilation rate, surface and air temperature, food substrate (source), wind strength and direction, season, and the presence or absence of pets, carpets, and houseplants. Moulds are usually identified through culture (growth) of the fungal spores collected on swabs or directly onto culture plates containing a suitable agar-based substrate, and then via visual identification of these growths using

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$^{\nabla}$ CFU – Colony forming unit

microscopy. There are numerous types of media available and some may include compounds added to improve growth of a particular fungi or suppress competing organisms (i.e. bacteria). Surrogates of mould load used to help quantify fungi are: ergosterol, mycotoxins, β-(1-3) D-glucan and spore counts, but all have some deficiencies. Newer methods of identification are being developed using DNA array hybridisation and real-time Polymerase Chain Reaction (PCR) techniques, and using microbial volatile organic compounds (MVOC).

1.5.3. Animal dander and cockroach allergen

Allergic sensitisation to antigens from furred animals and insects (cockroaches) has a complex but well documented effect on asthmatics. There appears to be a dose response relationship to these antigens but that the quantities necessary for sensitivity are not clearly defined, large doses particularly to cat allergen (Fel d1) may decrease the risk of asthma developing (Sporik et al., 1999). Platts-Mills showed an IgG and IdG4 antibody response to cat allergen without asthma sensitisation, suggesting a modified Th2 cell response as a form of tolerance and may explain the lower asthma risk in homes with animals (Platts-Mills et al., 2001). This scenario is complicated by other home allergens including house dust mites (HDM), dog fur (Can f1), cockroaches (Bla g1), particularly in North America, as well as those from moulds and pollens. The individual's sensitivity and possible atopy would also play a role in their immune response. The clothing and hair from cat owners is a major source of allergen in schools and in non-cat homes. It appears that subjects exposed to the lowest and the highest levels of Fel d1 show the least sensitivity (Liccardi et al., 2005). A study noticed in children under 1 year of age, a reduction in allergy to Fel d1 as the numbers of pets (cats and dogs) increased from 0 to >2 and a similar but lesser affect with dog allergen, a similar reduction in allergy sensitisation was found for house dust mites and pollens (Ownby et al., 2002). Multiple exposures to cats, dogs and endotoxins on their fur seem to have a protective effect on asthma and allergy but the association was not clear (Waser et al., 2005). Pet avoidance may also contribute to a protective effect for hay fever, asthma and atopic sensitisation.

1.5.4. Pollens

Pollen grains (~10 to 55μm diameter in size) are the primary source of pollen allergens, which explains why the symptoms of hay fever are located around the eyes, nose and throat (D'Amato, 2001). The pollens are too large to enter the airways but the allergens have been found in the stems and leaves of some allergic plants, which are more friable than the pollen grains. Particles carrying allergens include intact pollen grains, submicron particles and fungal spores (Razmovski et al., 2000). Beggs' review of pollen related papers found that pollen fragments as well as grains can be an asthma trigger and that the antigens are present outside the pollen season (Beggs, 1998). In Madrid Spain, it was found there is an association between pollen levels and asthma related admissions, independent of air pollutants (Tobías et al., 2003). They found there was a time-series effect with pollen counts and asthma admissions increasing through the pollen season. A similar observation was made in Cincinnati, Ohio, USA; concomitant with PM-10 counts but with a significant 3 day delay from pollen emergence (Lierl & Hornung, 2003). Indoor pollen counts are typically 30% of the outdoor values and are usually carried indoors by wind, pets, hair and clothing (Stock & Morandi, 1988).

1.5.5. Food allergens

True food allergy is usually documented as an immunologically mediated hypersensitivity to a specific food within a few seconds to 30 minutes after contact. The symptoms which can be life threatening, include swelling of the throat and closing of the airways. Food intolerance occurs several hours to days after eating and typically involves stomach and skin problems and sometimes headache. The risk of suffering a reaction is a function of the allergen's potency, amount ingested, the sensitivity of the individual and his /her atopic history. IgE food sensitisation is associated with atopic dermatitis (AD) in children, and of those approximately 80% will go on to develop asthma or allergic rhinitis (Hill et al., 2000). The most allergic foods worldwide are wheat, hen's eggs, cow's milk, fish, crustaceans, peanuts, tree nuts and soybeans (Björkstén, 2004 and Sicherer, 2002). Food allergens are thought to affect 6% of children under three years of age and approximately 4% of the general

population (Sampson, 2004). Identification is usually via a double-blind, placebo-controlled food challenge; skin prick testing for food allergens is only 50% effective. A 2004 paper on a new IgG test for antibodies in subjects with irritable bowel syndrome has shown to be an effective indicator of food allergy (Atkinson et al., 2004). The relationship between atopy, asthma and food allergy is complex; it has been proposed that reduction of fruits and vegetables (Devereux and Seaton, 2005) and oily fish (Black & Sharp, 1997) and the increase in consumption of processed foods in industrialised countries may have a role in the increase in asthma over the past few decades. It has been suggested that antioxidant and mineral nutrient supplements may reverse this trend but a 2000 review by Baker and Ayres supported a previous conclusion, 'that there is no proven role of nutritional therapy in the management of asthma' (Monteleone & Sherman, 1997; Baker & Ayres, 2000) but data from the Childhood Asthma Prevention Study (CAPS), an Australian study does identify possible food links; omega-3 fatty acids showed a reduction in cough but not wheezing in asthmatics, (Peat et al., 2004). Exclusion of known food intolerances from homes cooking these foods had considerably improved asthmatic children's symptoms and decreased the use of inhaled steroids (Roberts & Lack, 2003). Seaton & Devereux first proposed that vitamin E supplement during pregnancy can reduce asthma risk in babies (Seaton & Devereux, 2000) and followed this up with favourable results from a continuing cohort studying antioxidants during pregnancy in relation to wheeze and eczema (Martindale et al., 2005). Food allergen avoidance and its effects on asthma may only show positive results later in life, i.e. beyond teenage age so investigation of this complex aspect of asthma continues.

1.5.6. Bacterial Infections

Bacterial endotoxin drives an immune system response in individuals but the mechanisms involved are not clear. Occupational evidence from farm and cotton worker studies show that endotoxin can induce and exacerbate asthma but the pathology does not include sensitisation and eosinophil involvement (Douwes et al., 2002). Bacterial particles inhaled by asthmatics may account for some of their symptoms as endotoxin exposure can determine the severity of asthma for those allergic to HDM; however the data is ambiguous (Kolstad et al., 2002). Tavernier and

colleagues noted that although endotoxin exposure has been shown to have both a pro-inflammatory and protective effect in differing situations they found that it is a risk factor for development of asthma (Tavernier et al., 2005). A number of studies looking at cat, HDM and endotoxin exposures have not found direct links between them (Gehring et al., 2001; Lau et al., 2005) but an earlier paper showed that endotoxin may promote development of type 1 CD4+ T cells, whereas HDM and cat allergens primarily modify the proportion of CD8+ cells of both type 1 and 2 cells (Bolte et al., 2002).

1.5.7. Viral Infections

The decline in infections during preschool years has been suggested as one of the possible explanations for the increase in allergen related respiratory diseases in the West. Viral infections might be an important environmental stimulus for airway injury and remodelling (Gern et al., 2005). Viral respiratory tract infections that cause inflammation in the lower airways may predispose infants and toddlers to asthma development. First-born children have been shown repeatedly and in different settings to have an increased risk of developing asthma (Johnson et al., 2002). It is thought that older siblings introduce many more infections into the home thereby initiating the younger siblings' Th1 cell response, which the eldest do not have. This hypothesis is a simplification; an ECRHS study found that the length and type of exposure to infectious agents can either promote or suppress anti-inflammatory processes that are unrelated to an IgE response (de Marco et al., 2004). Respiratory syncytial virus (RSV) can cause bronchial pneumonia in children and it is one of the most common implicated infections (Lemanske, 2004). Otitis media (a viral or bacterial middle ear infection), another common childhood illness has been linked with mould and an increased risk of asthma in a study, which the authors suggest warrants further investigation to understand this relationship (Pettigrew et al., 2004).

1.6. Non-Biological Air Pollution

Environmental pollutants may also play a role in asthma exacerbation. Beggs' review of experimental and observational studies suggests that further empirical work is necessary to prove what effects indoor and outdoor pollutants have on asthma. Particulates may only aggravate an asthmatic's condition and are not necessarily a causal agent for development of the disease, although a synergistic function seems possible (Beggs & Curson, 1995).

1.6.1. Environmental tobacco smoke (ETS)

Cigarette smoking is equally as common with asthmatics as with the general population (Thomson et al., 2003). Environmental tobacco smoke containing respiratory irritants is probably the most important indoor pollutant contributing to symptoms in diagnosed asthma (Burr, 1999). This includes younger children with asthma who receive passive or second-hand smoke from siblings and parents. Childhood cigarette smoking and ETS at low levels are independently associated with asthmatic symptoms (Sturm et al., 2004). There is increasing evidence showing that smoking can cause asthma in children and that exposure increases the asthma severity (Strachan and Cook, 1998). One study found that a substantial minority of infants with early wheezing episodes were probably related to a predisposition to asthma and/or atopy (had mothers with asthma; $p<0.001$). These children had elevated IgE serum levels during the first few months of life and there were indications that maternal smoking was a risk factor in utero (Martinez et al., 1995). In addition, a more recent study has shown that cumulative and recent ETS exposure increases the risk for adult-onset asthma (Jaakkola et al., 2003).

1.6.2. Volatile organic compounds (VOCs)

Asthma has been associated with formaldehyde and other solvents in the indoor environment and these may increase the risk of developing the disease (Rumchev et al., 2004). It was found that indoor sources of VOCs are higher than outdoor sources associated with traffic-related air pollution (Fischer et al., 2000). Indoor sources of

VOCs include: formaldehyde from wood sheeting products; household products (including paints, paint strippers, and other solvents); wood preservatives; aerosol sprays; cleansers and disinfectants; moth repellents and air fresheners; stored fuels and automotive products; hobby supplies; dry-cleaned clothing; cosmetics. These organic solvents usually act as irritants of the eyes, nose, throat and lungs in all subjects. Significant increases in allergic reactions were noted in a study, resulting from interactions of formaldehyde and ETS indoors (Lebowitz et al., 1992). Although solvent toxicity is biologically plausible, further work is necessary to link specific VOCs with asthma and other respiratory disorders (Schenker & Jacobs, 1996).

1.6.3. Other air pollutants

Air pollution has many sources, most of which derive from the combustion of fossil fuels by industry or motor vehicles. Concentrations of many air pollutants have fallen in the UK but they still have a short and long term impact on health, yet their mechanisms are still unknown (Maynard, 2004). The adverse respiratory effects that can occur from air pollution are frequently noted in the media and from summer 'smog' warnings on weather reports. These warnings are of particular interest and concern to a subgroup of patients with upper and lower respiratory symptoms, including asthmatics. Graham's review focuses on the effects air pollution has on infant children noting their immature lungs are more prone to airway obstruction and compromise due to accumulated secretions. Their greater breathing rate, particularly in infancy and 50% more time spent doing outdoor activity during childhood may worsen the harmful effects of many air pollutants (Graham, 2004). Some children with asthma as well as those without asthma have shown a decrease in lung function after ozone exposure (Etzel, 2003). A study in Baltimore, Maryland, USA showed that indoor levels of individual air pollutants were low but noted the combined insult with indoor allergens could explain the different asthma burden between inner city and rural asthmatics (Breysse et al., 2005).

A 2002 review noted that clinical data have implicated air pollution as associated with an acute inflammatory response in asthmatics but that the chronic effects on the lungs remain unknown. Ozone, NO_2, and PM as more recent air pollutants dominant in western society, may contribute to asthma development (Parnia et al., 2002;

Brunekreef & Holgate, 2002). Diesel exhaust particulates (DEPs) may act as carriers of other allergenic proteins (i.e. pollen fragments) into the airways by adsorbing them onto their surfaces (D'Amato et al., 1998). Ozone, DEPs, and ETS may act as adjuvants to increase the response of allergens, particularly in those with a specific genetic predisposition (Peden, 2005). Bernstein's review reported ozone and fungal spores were cofactors associated with increased asthma symptoms and inhaler use. Other effects noted were that of increased asthma admissions during thunderstorms originally thought due to pollen or fungal spore increases but later attributed to higher ozone levels (Berstein, 2004). Acute respiratory symptoms generally decrease with higher temperatures but this trend is reversed when ozone and PM10 levels are high (Lebowitz et al., 1992).

Air pollution is one of few pollutants whose production could be legislated, the links between it and asthma need further investigation to educate and empower politicians and physicians to act (Trasande & Thurston, 2005).

1.7. Stresses

Psychological problems that can contribute to asthma morbidity and mortality include stress, depression, anxiety, family disturbances including loss and separation, and denial of disease. Liu and colleagues noted that although stress is difficult to objectively define in humans (different stresses have varying affects on individuals) they did find very strong support for the contention that school examination stress promoted some indications of inflammation associated with asthma (Liu et al., 2002). Some stress may simply result from not using asthma medication effectively or having irregular checkups, and others from work related issues, start of school anxiety or examinations etc. Hunt suggests that poor housing conditions are strongly associated with emotional distress and the greater the number of housing issues the more the distress (Hunt, 1990). One study noted that emotional or stress-induced breathing problems are more likely in individuals with intrinsic asthma (Klinnert, 2003). Another study indicated unexpectedly that lung function testing showed less airway obstruction in response to allergens during stress visits than during non-stress, control visits (Laube et al., 2003). A review of asthma and stress suggested that it is time to put together all the possible associations involving asthma, including stress issues, to

better understand the complex systems involved and not fully explained by looking at the individual components (Rietveld et al., 2000).

1.8. Medication

To be successful, asthma management requires adherence to a complex range of essential procedures in order to alleviate potential exacerbations. These include trigger avoidance (HDM, mould, pollen allergens, exercise etc), acute symptom assessment and management, and effective collaboration with health professionals and adherence to the suggested medication use (Walders et al., 2005). Most guidelines follow a stepwise approach to asthma treatment that varies from β agonists alone for very mild intermittent asthma to oral corticosteroids for severe chronic asthma. The type of treatment that is appropriate should be determined by the symptoms, exacerbations and lung function since evidence suggests that none of the drugs in use changes the natural history of asthma (Tattersfield et al., 2002). Inhaled corticosteroids are helpful in patients with mild to moderate asthma. They help improve lung function, reduce symptoms and exacerbations, and have shown to be effective in reducing hospital readmissions, particularly if taken for a 2 week period following a hospital visit (Blais et al., 1998). Short-acting β agonists (i.e. salbutamol or terbutaline) via a pump inhaler are very effective at reducing exercise-induced asthma and for relief of acute asthma attacks. These are not effective with regular use but on a need to use basis. For those asthmatics that use a β agonist inhaler for more than one puff per day then inhaled corticosteroid use is recommended (Tattersfield et al., 2002). Regular use of long acting β agonists (i.e. salmeterol and formoterol) improves asthma control and exacerbations. These long-acting β agonists can be mixed with inhaled corticosteroids for improved patient care. The proportion of asthmatics in the UK regularly using oral corticosteroids (i.e. prednisolone) is small (~1%) but represents 50,000 patients but half a million require at least one dose per year (Walsh et al., 1996). Excessive use requires prophylaxis against osteoporosis and another problem with its use has been shown in one study that indicates cigarette smoking can inhibit the effectiveness of oral corticosteroid therapy in asthmatics (Chaudhuri et al., 2003).

1.9. Summary

Over the last 40 years, asthma has risen progressively to become the most important chronic disease in childhood, particularly in Western countries (Platts-Mills et al., 2000a). The extreme complexity involved in asthma, its identification, possible causes, triggers and treatment has made the disease one of the most costly. It was estimated to cost the USA $6.2 billion in 1990 and the UK figure for the total burden was £843 million per year in 1988 with the health care costs of asthma alone running to £344 million per year (Sculpher & Price, 2003). The challenges in identifying any particular asthma initiator or possible combinations, arises from a vast, unknown mix of mediating factors the individual could be exposed to over time. It is a disease that warrants significant investment both in terms of individual health and societal burden.

Chapter 2 - Literature Review

The Epidemiology of asthma and indoor mould

2.1. Prevalence and environmental antigens

Reported incidence, prevalence and mortality of asthma has in general increased in children over the past four decades, although there has been a decline observed in some international studies (Fleming et al., 2000). The incidence rate is defined as the number of new cases of asthma that occur in a given period of time in a defined population. In the UK, the incidence peaked from a low in the early seventies to a high in 1993, a value 11 times higher than in 1976. The incidence since 1993 has declined in all age groups (Smyth, 2002). A similar result was found in Australian where since the early 1990s there has been a decline in hospitalisation rates and in General Practitioner (GP) consultation rates amongst children with asthma (Poulos et al., 2005). The prevalence rate is defined as the proportion of the population that is affected with asthma at any one time and asthma still affects 10% of the world population. The original International Study of Asthma and Allergies in Childhood (ISAAC, 1994-1995) and European Community Respiratory Health Survey (ECRHS, 1993-1994) provided a global pattern of the asthma prevalence in childhood and adulthood, respectively and this allowed some analysis to be initiated. The authors of one report noted four patterns emerging from the data: *first*, both studies show a particularly high prevalence of reported asthma symptoms in English-speaking countries; *secondly*, in non-English-speaking countries of Western Europe, asthma prevalence is high but in developing regions that are becoming more urbanised the asthma prevalence is also increasing; *thirdly*, ISAAC showed high prevalence in Latin America, particularly in Spanish-speaking regions that were higher than Spain; and *fourthly*, ISAAC, generally showed low asthma prevalence outside the Americas and Western Europe but this was modified by affluence (Japan and Singapore had higher prevalence and Hong Kong had a 12-month prevalence of wheeze of 10.1% and in neighbouring Guangzhou (less affluent), only 2.0%) (Pearce et al., 2000). A cross-sectional study in Spain found that the prevalence of asthma between phase I and phase II of ISAAC (8 years apart) had not changed for those 13 to 14 years of age, whereas it had increased substantially in 6 to 7 year olds (Garcia-Marcos et al., 2004).

The geographical pattern of atopy and bronchial hyper responsiveness supports those found for asthma. Although genetic differences may contribute to the geographical pattern observed, it seems unlikely that they could account for the wide variation found across Europe over a relatively short period of time. Clearly, it is reasonable to assume that variations in asthma prevalence can be attributed to environmental factors as yet undetermined but somehow associated with a modern, western lifestyle (Janson et al., 2001). Phase III of ISAAC in 2002-2003 has followed from the original study as has phase 2 of the ECRHS from March, 2000.

Asthma prevalence has been ascribed to changes in housing design and fitting with carpets, more insulation and lack of chimney ventilation and also changes in lifestyle. Many of these changes have affected the humidity levels within the homes, which then influence the growth of moulds. The quantity of time spent indoors (estimated between 80% and 95%), both at home and at the workplace has increased in the past three decades; this would expose individuals to more antigens for prolonged periods. These large international epidemiological studies and other regional ones including: the German Longitudinal Multicentre Allergy Study (MAS 90) which prospectively investigated the relationship between indoor allergen exposure and the development of asthma (Lau et al., 2000); and the National Asthma Campaign Manchester Asthma and Allergy Study (^{NAC}MAAS) which investigated the relation between genetic predisposition and environmental exposures in the development of asthma, atopy and other allergic diseases (Simpson et al., 2000b), the Childhood Asthma Prevention Study (CAPS), an RCT designed to measure the effects of HDM allergen avoidance as the primary intervention for families with a history of asthma (Peat et al., 2004) to name a few, have helped focus asthma research on allergens, triggers and other factors listed in table 1.1. Childhood asthma usually involves a reaction to an inhaled allergen. There may be more than one allergen and numerous triggers that initiate an asthma attack once the lungs have been sensitised but it seems that the variation in prevalence would correspond to the variation in exposure to the principal allergen dominant in that region. The house dust mite is a known allergen source but there are two main genera (*Dermatophagoides farinae* and *D. pteronyssinus*) only one of which seems dominant in any one area (Gross et al., 2000). Fungi allergens have been associated with respiratory ailments but have yet to be shown as causative for asthma. Probably one of the most difficult areas of allergic asthma and its possible causes to

study is that of looking at asthma's relationship with mould allergens. A few mould--related illnesses were mentioned in the introduction but data from those studies relating moulds and asthma have been less certain than evidence from studies linking asthma with insect and pet allergens. The numerous factors referred to in the introduction and associated with this complex disease could not be reviewed thoroughly in one PhD thesis. This literature review focuses on the effects indoor moulds may have on asthma symptoms, as the main focus of the thesis was a randomised controlled trial investigating the effects upon symptoms of eradication of moulds from homes of asthmatic individuals.

2.2. Aetiological criteria

Epidemiology is the study of how disease is distributed in a population and what the factors are that influence or determine its distribution and eventual control. Epidemiological evidence at some point is implied to be causal. These assertions have long been debated but they are essential if epidemiological evidence is to be used to establish causality. Nine specific criteria were set out by Sir Austin Bradford Hill and reviewed as a guide to show when a causal relation has been demonstrated:

> "None of these nine viewpoints can bring indisputable evidence for or against a cause and effect hypothesis ..... What they can do, with greater or less strength, is to help answer the fundamental question – is there any other way of explaining the set of facts before us, is there any other answer equally, or more, likely than cause and effect?" (Hill, 1971).

The following are the nine conditions proposed by Hill (1965) for determination of causality.

1. Biological plausibility: There is some basis in scientific theory that supports the biological mechanisms by which the exposure could cause the effect.
2. Experimental evidence: Any related research, animal or human based experiments that support a causal inference.
3. Strength of association: The stronger the relationship between the independent and dependent variables, the less likely it is that the relationship is due to an extraneous variable. The relative risk (odds ratio, OR) is used to show the strength of association, the higher the relative risk – the stronger the

association and the less likely other factors are involved. Comparisons between groups are usually shown using OR with a 95%CI (95% Confidence Interval), those values that do not include 1.0 show statistical significance at $p<0.05$.

4. Temporality: Exposure must occur before a disease develops for it to be causal.
5. Consistency: Implies that the findings in one study must be replicated in other studies. The power of the argument for causation builds with subsequent studies in different locations with different patients and differing protocols.
6. Coherence: A cause and effect interpretation for an association does not conflict with what is known of the natural history and biology of the disease.
7. Specificity in the causes: If the exposure is associated with only one type of disease then specificity is met (an ideal situation and not one likely with asthma). Pleural mesotheliomas has only one known cause, asbestos exposure, making it easy to demonstrate a causative relationship between the two.
8. Dose response relationship: This implies that increased exposure to a possible cause is associated with an increased effect.
9. Analogy: A commonly accepted cause-effect in one area can be applied to another area. There is some similarity between accepted phenomena.

Other criteria suggested by Hill are worthy of repeating: a) statistical significance should not be mistaken for evidence of a substantial association. b) an association does not prove causation; there must be other evidence to substantiate it. c) uncertainty about a cause or an association does not mean that some course of treatment cannot commence.

2.3. Biological Plausibility and Experimental Evidence

Epidemiological research is normally preceded and spurred by non-epidemiological evidence of potential health effects associated with a risk factor.

Willem Storm van Leeuwen, professor of pharmacology at the University of Leiden, The Netherlands, suggested in 1924 that inhaled fungal spores, he termed "climate allergens" could cause asthma. He continued this work identifying house dust as a source of allergen and noting how dampness and temperature could play a crucial role in asthma symptomatology (Spieksma & Dieges, 2004). He was one of the more

recent pioneers to make the link between indoor damp air and health suggested by Hippocrates and by authors of the Bible:

> ... If the mildew has spread on the walls, the priest is to order that the contaminated stones be torn out and thrown into an unclean place outside the town. He must have all the inside walls of the house scraped and the material that is scraped off dumped into an unclean place outside the town. Then they are to take other stones to replace these and take new clay and plaster the house.
>
> If the mildew reappears in the house after the stones have been torn out and the house scraped and plastered, the priest is to go and examine it and, if the mildew has spread in the house, it is a destructive mildew; the house is unclean. It must be torn down – its stones, timbers and all the plaster – and taken out of the town to an unclean place. (*Leviticus 14, 39-47*)

Particulates greater than 30μm are removed in the nasal cavity. Particles in the 10-20μm range including pollens, hyphal fragments and smaller inert particles with attached microbes deposit in the primary and secondary bronchi. Those deemed respirable particles from 1 to 5μm, which are the size of most bacteria and many fungal spores; they penetrate to the alveoli and may produce an allergic response (Burrell, 1991).

Aspergillus fumigatus is an opportunistic pathogen to which asthmatics are particularly susceptible. A number of studies have looked at this phenomenon, in one study, two strains of mice were exposed to culture filtrates and mycelial extracts of *Aspergillus fumigatus*, intranasally. The animals received biweekly doses for 6 to 10 weeks and were then dissected 2 weeks after their last dose. Specific antibodies against *Aspergillus fumigatus* were detected in the mice sera. A progressive increase in IgG and IgA antibody isotypes against the culture filtrates and mycelial antigens were detected in both strains but at varying antibody levels. Lung lavage studies showed a relative decrease in the number of macrophages and an increase in the number of lymphocytes after the 6^{th} and 8^{th} instillation of the antigens. The authors conclude that antibody responses and inflammatory changes detected in the lungs of the mice

exposed to the fungal antigens are comparable to allergic bronchopulmonary aspergillosis in humans (Kurup et al., 1990).

The ability of *Aspergillus fumigatus* spores to adhere to pulmonary cells and basement membrane proteins was investigated to determine the mechanisms involved. Both cells of the pulmonary epithelial cell line and purified basement membrane proteins were immobilized on the wells of microtitre plates, where they were exposed to spores of *Aspergillus fumigatus* with and without various treatments. Light microscopy was used to count the adherent spores. Both binding and inhibition of binding occurred dependent on the combination of pre-treatments used. Fibrinogen inhibited spore adhesion to the epithelial cells. Interferon gamma doubled the ability of the spores to bind to the cells. The study suggests that the presence of activated epithelial cells and the exposure of basement membrane that occurs in asthma, together with oxidant stress, may help with the colonisation of the asthmatic's lung tissue with *Aspergillus fumigatus* (Bromley & Donaldson, 1996).

Studies which have been designed to develop new mouse models of lung allergy have demonstrated that *Alternaria alternata* and *Cladosporium herbarum* spores have the ability to induce a type-2 antibody response and subsequent production of IgE and IgG_1 even in the absence of an adjuvant. One study noted that *Alternaria* produced a greater influx of neutrophils when compared to *Cladosporium*, as well as being more diffused throughout the lungs. This difference in cellular activity on exposure to *Alternaria* is possibly one of the reasons for the apparent high level of sensitisation (Havaux et al., 2004).

Another Norwegian study was devised to confirm an adjuvant effect of a β-(1-3)D-glucan, MacroGard® from baker's yeast. Previously, the authors had found that β-(1-3)D-glucan from the cell wall of the mould *Sclerotinia sclerotiorum* increased the allergic response to the model allergen ovalbumin (OVA) in a mouse model. Supposed effects of mould allergen extracts from *Cladosporium herbarum* and *Penicillium chrysogenum* were also investigated using the same model as for the baker's yeast. Groups of eight mice were injected with OVA alone, OVA + extract or OVA + MacroGard® into one footpad. On day 21 they were re-injected with OVA before exsanguinations on day 26. The levels of OVA-specific IgE, IgG_1 and IgG_{2a} in serum were measured using ELISA techniques. Compared with OVA alone, all the OVA plus extracts significantly increased OVA-specific IgE and IgG1 levels ($p<0.05$).

For all groups the levels of IgG_{2a} anti-OVA remained similar to OVA alone. The authors conclude that extracts from the two moulds tested and β-1-3/1-6-D-glucan from baker's yeast have adjuvant effects on the allergic responses in mice (Instanes et al., 2004).

A retrospective review of eleven patients with initial episodes of respiratory arrest (two of which died) was undertaken in a study at the Mayo Clinic, in Rochester, MN, USA, between 1980 and 1989. Skin prick tests to *Alternaria alternata* were positive with 10 patients (91%) compared with 31% of the 99 controls ($p<0.001$). Age was found to be the only confounder amongst sex, distance from clinic and age. After adjustment for age, *Alternaria* skin test reactivity was associated with an increase of approximately 200-fold in the risk of respiratory arrest (OR=189.5, 95%CI: 6.5-5,535.8). All these occurred in the late summer or early autumn, the peak time in the dry Midwest for *Alternaria* and other moulds (O'Hollaren et al., 1991).

Table 2.1: Common Fungal Allergens (adapted from Burrel, 1991.)

Common fungal allergens	
Alternaria	*Ganoderma*
Aspergillus	*Penicillium*
Aureobasidium	*Stachybotrys*
Cladosporium	*Stemphylium*
Epicoccum	*Trichoderma*

Fungi are the principal microorganisms involved in decomposition and they are ubiquitous in human habitation and occupation. As discussed earlier mould spores have been implicated in a number of illnesses, including infections and inflammation, allergies and asthma. The consequences of indoor dampness are mould growth and house dust mite proliferation and possible detrimental effects on individuals' health. The role these play in the exacerbation of asthma has been discussed in numerous studies (reviews by Burr, 2001 and Sidenius et al., 2002). The possible mechanisms of action are complex and currently not well understood but include toxic and immunological effects. More than 400 toxins are produced by a variety of moulds found indoors including: e.g. *Alternaria, Aspergillus, Fusarium, Penicillium, Rhizopus,*

Stachybotrys and *Trichoderma*. Not all mycotoxins are volatile so that some must penetrate humans via inhalation of spores and not directly as the toxin. Patulin, T-2 toxin and penicillic acid have been shown to be acutely toxic to rat alveolar macrophages (AM) *in vitro*, causing membrane damage, inhibition of protein and RNA synthesis, inhibition of phagocytosis and the ability of AM to respond to lymphokines. Aflatoxin is known to cause lesions of varying severity in the lungs of rats. Several mycotoxins experimentally alter immunity, causing inhibition of natural killer cell activity, impaired resistance to pathogenic microorganisms, suppression of antibody response, lymphoid depletion of the thymus associated with delayed hypersensitivity, and functional alteration of bone marrow cells (Sorenson, 1999).

A study of twenty-five staff members with sick building syndrome (SBS) but without type-1 allergic diseases (i.e. hay fever and asthma) from two schools and one indoor swimming pool were examined. Neither the date nor locations were reported in the paper. Three moulds were identified through culture on vegetable juice agar from samples taken within the building structures: *Penicillium chrysogenum*, *Stachybotrys atra* (syn: *S. chartarum*) and *Trichoderma viride*. Blood samples were taken from the patients and leukocytes with approximately 2% basophils were isolated. The presence of fungi-specific IgE in serum was verified by passive sensitisation of basophils acquired from umbilical cord blood cells from other sources. Twelve of the 25 subjects responded to at least one of the mould allergens. Fungi-specific IgE bound to basophils from the patients was examined and confirmed in nine patients showing fungi-specific IgE in serum. These findings demonstrated specific sensitisation to indoor fungi in several patients suffering from SBS without type-1 allergic diseases (Larsen et al., 1997).

Moulds occur the world over, their spores and fragments become airborne in vast quantities and they can elicit sensitising IgE antibodies. Allergy to mould is usually demonstrated with a positive skin prick test or from blood assay. The presence of IgE antibodies does not necessarily infer disease, but it is one way of determining the frequency of sensitisation and its relationship to respiratory symptoms. Unfortunately, there are no standardised fungal extracts so it is difficult to establish epidemiological evidence for consistency in skin prick testing. Not all subjects show a positive skin prick test to specific mould allergens found indoors.

2.4. Epidemiological evidence of associations between moulds and asthma

In January 1984 a postal questionnaire was sent to the homes of 198 children aged 7-8 years registered at a single practice serving one of the most socially deprived areas of Edinburgh, Scotland. It asked how many nights had cough kept the children awake during the previous autumn and how many school days had been lost due to chest ailments. The parents were also asked if their children had ever had instances of breathing while making a high-pitched whistling sound (wheezing) over the past two years. A similar questionnaire was sent in April 1984 asking about the previous winter school term. Additional questions were included on features of the family and home environment considered to be possible risk factors for respiratory disease in this age group:

1. Family history of wheeze: any direct relative who had ever had wheezing.
2. Family size of four or more usually resident at the child's home.
3. Other children under 16, resident at the home.
4. More than one resident per room excluding the kitchen and bathroom.
5. Other people sleeping in the child's bedroom.
6. Child's bedroom unheated in the past 6 months (winter '83-'84).
7. Child's bedroom window left open while sleeping in the past 6 months.
8. Any unvented gas appliances used.
9. Any coal-fired appliances used.
10. Anyone smoking more than 5 cigarettes per day in the home.
11. Is your home affected by damp?
12. Is your home affected by mould or fungus?

Completed responses were received from 165 (83%) parents split evenly between those reporting wheeze (a high prevalence of 50%) and those who did not, suggesting minimal bias. All but 20 children lived in local authority housing. Damp was significantly more common in the coal heated homes (χ^2=7.32, $p<0.01$) but not for gas heating (χ^2=0.56). No association was found between history of wheeze in the family, parental smoking and damp. Smoking was more common in damp homes (χ^2=7.36, $p<0.01$). Of the 50 homes reporting damp, 66% had mould, only 2 reporting mould without damp. Only 10% of the homes with damp or mould had reported it but the

housing authority confirmed those that had. The results were expressed as binary variables using univariate 2x2 tables. Relative odds were used to express the degree of association and chi-square test to assess the significance levels. Nocturnal coughing was significantly associated with a family history of wheeze (OR=2.8, $p<0.05$), damp (OR=4.0, $p<0.001$) and mouldy housing (OR=4.8, $p<0.001$). Multiple logistic regression analysis was also carried out using nocturnal coughing during either season as the response variable, and the child's sex and the 12 home environment features listed above as the explanatory variables. This demonstrated independent contributions from family history of wheeze ($\chi^2=9.93$, $p<0.01$), coal ($\chi^2=4.67$, $p<0.005$) and mould ($\chi^2=11.89$, $p<0.001$). These results remained significant ($p<0.05$) after adding wheeze as an explanatory variable, it made an independent contribution ($\chi^2=11.73$, $p<0.001$) but all of the above remained significant risk factors for nocturnal cough. The authors conclude from this cross-sectional study that damp and mouldy homes, coal fires, and open bedroom windows need further investigation as potential remediable causes of respiratory disease in childhood (Strachan & Elton, 1986). It seems unlikely that open windows could be a possible cause of respiratory disease as other investigations have shown them to be beneficial, noting the greater exchange of fresh dry air.

In November 1986 a random sample of one in three primary schools within the city of Edinburgh resulted in a population sample of 873 children between 6.5 and 7.5 years of age. Parents were sent questionnaires asking about respiratory symptoms experienced by the child in the past year, these included wheezing, chest colds, sore throat, pain and discharges from the ears and hay fever or frequent sneezing. They were also asked how much coughing had kept their children awake through the night, how many days of daytime coughing, blocked or runny nose had occurred in the last month. These were followed by questions about the home environment, the number of smokers, heating and cooking fuels, presence of visible moulds and formation of condensation or damp patches on the walls. Spirometry measurements were made before and after exercise for FEV_1 (forced expiratory volume in the first second). Wheezing in the last year was the symptom with the strongest association with dampness and mould occurrence (OR=3.70, 95%CI: 2.22-6.15). Adjusting this for house tenure, number of occupants and smokers, and gas cooking the results stayed

highly significant (AOR=3.00, 95% CI: 1.72-5.25). At all levels wheeze was reported by the children from mouldy homes. The author cautioned that an awareness of dampness and mould in the home may effect reporting of symptoms and may account for the association between respiratory symptoms and mould growth (Strachan, 1988).

Another earlier study, in the USA looked at the relationship between measures of home dampness and respiratory illness and symptoms in a cohort of 4,625 Caucasian children aged 8 to 12 years and living in six cities between 1983 and 1986. Home dampness was characterised from questionnaire reports of indoor mould or mildew, interior water damage and presence of water in the basement. Respiratory symptoms including bronchitis in the previous year, persistent cough and wheeze, and chest illness were collected from the questionnaires. Lung function was measured by spirometry using forced expiratory volume and vital capacity. Over 50% of the homes in five of the cities reported one or more indicators of home dampness. For prevalence ratios associated with mould the group of children were divided into those who were asthmatics (214), non-asthmatics with wheeze (291) and non-asthmatics without wheeze (3,799). Associations between the measures of home dampness and both respiratory symptoms and non-chest ailments were strong and consistent. Adjusted odds ratios for moulds varied between 1.27 and 2.12, and for dampness between 1.23 and 2.16 all showing an association except for asthma symptoms and mould (AOR=1.27, 95%CI: 0.93-1.74), after adjustment for maternal smoking, age, gender, city of residence, and parental education. A weak relationship was found between home dampness and lung function. The authors conclude that home dampness is common in many areas of the USA and that it is a strong predictor of respiratory and other symptoms in children (Brunekreef et al., 1989).

A cross-sectional study of a random sample of public housing containing at least one child aged 0-15 years was carried out during February to April 1988. Two areas were selected from Glasgow (~500 dwellings) and Edinburgh (~500 dwellings) and one from London (~200 dwellings). Separate and independent assessments of housing conditions were made by a surveyor and health outcomes through structured interview by a trained researcher. Tenants' groups were contacted and their cooperation elicited, and a list of addresses was obtained from the relevant housing departments.

In the 1,220 eligible households, a health-based interview was secured from 891 residents (73%); 156 (13%) refused an interview and 173 (14%) could not be contacted. Surveyors completed housing condition surveys in 597 (48.9%) households. In 139 (23%) dwellings damp was found, 274 (46%) had visible mould growth and the remaining 31% were damp free. Air samples from each mouldy room were collected; a mycologist then estimated the spore count and identified the moulds from Petri dishes in which the air (from a Surface Air Systems sampler) and wall samples were collected. The home type, location, number of rooms, dampness, mould, ventilation, insulation and any renovations were recorded. The findings of the surveyors, mycologist and health researcher were all blind to each other. Confounding factors examined were: smoking in the home, smoking by the individual, net household income, overcrowding, and employment within the home and employment state of the respondent. The results of the housing survey were divided into categorical variables for analysis. Univariate analyses of the relation between each independent variable and dependent variables (e.g. bad nerves, aching joints, nausea-vomiting, backache, blocked nose, fainting spells, constipation and breathlessness) were carried out using chi square (χ^2) tests for categorical variables or one-way analysis of variance (ANOVA) for metric variables. These showed significance for 18 symptoms including: e.g. persistent cough, wheezing, skin problems, blocked nose and breathlessness. In light of the complexity of the subject and because of the issues of confounding, the authors invested considerable effort into ameliorating bias. They discussed and defended investigator, respondent, selection and omitted variable biases. Greater prevalence of wheeze, sore throat, runny nose, cough, headaches and fever were associated with children living in poor conditions compared to those living in dry dwellings. Children in mouldy homes were more likely to have been given medicines (52%) than those in damp (43%) or problem free dwellings (36%), (χ^2=10.82, $p<0.005$). The authors performed a series of logistic regression analyses using the eight symptoms shown to be significantly associated with the housing conditions from above, to examine the association between housing conditions and ill health after controlling for confounders. Having eliminated alternative explanations for the findings, the authors concluded that damp and mouldy dwellings have direct deleterious effects on the physical wellbeing of adults and children (Platt et al., 1989).

A random sample of 1095 children from Edinburgh resulted in 1,000 usable respiratory symptom data, which was collected via postal questionnaire sent to parents. The children had met the criteria that they were 6.5-7.5 years of age in September 1986. Forced expiratory volume in one second (FEV_1) was measured a minimum of two times, once before and twice after running exercise (five minutes apart) while attending school in the first half of 1987. One third of the children (330) were selected for relative humidity and temperature measurements in their bedrooms for 7 days during Jan--Apr 1987. An attempt was made to include all the wheezy children who had reported in the questionnaire visible mould in their homes (114). One in eight randomly sampled parents who did not report either of these factors was also asked to participate. Four visits to sample air using an Andersen volumetric sampler were completed from October 1987 to March 1988 in each home. Samples were taken from the child's bedroom, living room, and a third wherever mould occurred (i.e. other bedroom, bathroom, and hall) or if this was not possible then in the kitchen. Each air sample was collected for 3 minutes (~85*l* total volume) on a Petri dish containing 27ml of malt extract and agar no. 3 with 20 units/ml benzylpenicillin and 0.05 mg/ml streptomycin sulphate to inhibit bacterial growth. The plates were incubated for 7 days at 25°C and then colony forming units (CFU) were estimated from the numbers growing on the plates. Identification was made possible by transferring the fungi to plates of potato dextrose agar at 25°C and then comparing the growths against data from standard texts. Almost 50 species or genera of mould were identified. The total indoor airborne mould counts varied between 0 and 41,000 CFU/m^3 but generally ranged form 50 to 1,500. *Penicillium* and *Cladosporium* accounted for over half of the total mould count and were found in at least one room in every home during all the visits. No correlation was found between the results of the mould air samples taken and the small number of homes showing visible mould observed by inspectors. The fungal spore counts were positively skewed and were converted to natural logs; geometric means were then used in the analysis. The concentration of air borne moulds varied widely between houses and it was expected that the more mouldy homes would show a significant wheeze association but little difference was found. This difference was in contrast to the original study results (Strachan & Elton, 1986)

which may have shown reporting bias or that mild to moderate visible indoor mould growth has minimal influence on the airborne quantities compared with activities that raise large quantities of dust (allergens) like vacuuming and dusting. The airborne spore burden differed little between mouldy and mould-free homes. Mycelia sterilia were present in higher concentrations in homes of wheezy children (geometric mean 2.1 v 0.7 CFU/m^3) compared to those without wheeze. Questionnaire based estimates of mould may be poor indicators of actual mould spore exposure (Strachan et al., 1990).

In March/April 1988, a questionnaire-based study on the health effects of the indoor environment was undertaken in 30 communities across Canada (in six regions). Several screening criteria had to be met including: children aged 5-8 years, no major source of industrial pollution and known family income level. A total of 17,962 questionnaires were sent out and 14,948 (83.2%) were returned. Those living in boats or mobile homes were excluded leaving 13,495 children within the study group. The housing stock was distributed as follows: 81% detached, 6% semi-detached, 13% multiple family dwellings. Moulds were present in 32.4% of the homes, flooding in 24.1% and evidence of moisture in 14.1%. The overall prevalence of home dampness or moulds was 37.6%. There was no standard consensus for such a questionnaire on the burden of household moulds so the authors developed their own from four existing questionnaires. Primary exposure criteria included: number and location of mould sites, record of damp spots and any flooding, any one of these was deemed a positive result. The primary health variables included: persistent cough and/or wheeze, dyspnoea with wheeze, current asthma, chest illness, upper respiratory and non-respiratory symptoms (headache, muscle ache, fever, chills, nausea, vomiting or diarrhoea), and eye irritation. Numerous factors that may influence the primary relation of interest were recorded (i.e. age, sex, education, house type, cooking fuel, hobbies etc). Prevalence values of lower respiratory problems (cough, wheeze, asthma, bronchitis and chest illness) were approximately 50% higher in the damp homes. Adjusted odds ratios ranged from 1.32, (95% CI: 1.06-1.39) for bronchitis to 1.89, (95% CI: 1.58-2.26) for cough. It may have been expected that those reporting allergies would have more symptoms from exposure to the dampness, moulds and house dust mites but this was not the case. A dose-response gradient was shown between the number of mould sites and health outcomes with an odds ratio of 2.26

(95% CI: 1.80-2.83) for cough and presence of two mould sites (Dales et al., 1991). There was no focus emphasised on a specific measure for the outcome, so bias was minimised. Temporal variations in mould spore concentrations occur both outdoors (seasonal variations) and from the occupants' habits that may not have been picked up during the late winter sampling period (sources of spores outdoors are likely to be snow covered and indoor environmental conditions in Canadian winters are usually very dry and not supportive of mould growth).

The second National Health & Nutrition Examination Survey, 1976-1980 (NHANES II) of a white civilian US population, ages 6 to 24 years investigated various allergens and showed that asthma was associated with skin reactivity to house dust (OR=2.9, 95%CI: 1.7-5.0) and *Alternaria* (OR=5.1, 95%CI: 2.9-8.9). The prevalence of asthma and allergic rhinitis increased with increasing number of positive skin prick tests (Gergen & Turkeltaub, 1992).

Five hundred and fifty five children, aged 3 to 17 years, attending the outpatient clinic of St. Göran's Children's Hospital, Stockholm, Sweden, during January 1986 to September 1987, who were diagnosed with any of bronchial asthma, allergic rhinitis or eczema, were entered into the study. They had to show a positive reaction to one of the following allergens via a pre-loaded lancet SPT: *Alternaria alternata*, *Cladosporium herbarum*, *Phleum pratense* (timothy grass), birch, Der p1, Der f1, Fel d1 and Can f1. The positive control was histamine hydrochloride. A wheal at least half the size of the histamine one was considered positive. Three groups were identified; one a HDM sensitised group of 64, another atopic group of 63 sensitised to other aeroallergens and a third, control group including 58 children who had no atopic history. Dust samples were taken for moulds on the living room floors during the winter when the ground outside was approximately 75% covered with snow and a mean temperature of –3.5°C. Parents of the asthmatics were given a filter to attach to their own vacuums and asked to sample a $3m^2$ area for 1 minute. Thirty milligrams of dust was introduced to vegetable-juice agar plates and cultured for 1 week at 26°C. Penicillin and streptomycin were added to eliminate bacterial growth. Fungal growth was quantified as CFU per 30mg of dust and identified microscopically. A questionnaire based upon indoor environmental conditions was posted during April 1988. It had questions about the type of construction, use of air-cleaning devices, ventilation system, carpets

present and housing renovations. Moisture production questions included use of humidifiers, aquariums, amount of plant watering, hours of laundry per week and wet mopping history, number of hours for showers, baths and cooking, from these a moisture index was estimated. Moist or damp areas, mould occurrence and window--pane condensation were also noted. Humidity and temperature readings were taken in March 1988 but no indication of the length of time was given. Mould count differences between the three groups were calculated using the Wilcoxon's two-sample test with two-tailed p values. Any relationships between the CFU and moisture index, and the CFU and mite allergen in the control group were determined with the Spearman's rank-correlation coefficient test. *Penicillium*, *Alternaria* and *Cladosporium* were the most abundant of 31 mould genera found and only one dust sample did not contain any viable moulds. The three previously mentioned moulds plus yeasts and *Aspergillus* comprised 78% of the total CFU count. A positive SPT to either *Alternaria alternata* or *Cladosporium herbarum* was found to be a strong risk factor for asthma (OR=4.1, 95%CI: 1.2-15.3). The mean total number of CFU per 30mg dust was significantly lower ($p<0.02$) in the atopic groups than the control one, which the authors concluded might have been a result of allergen-sanitation procedures. No consistent association was found between viable mould growth and sensitisation to moulds (Wickman et al., 1992). It is unclear from the report just how much of the sampling was completed by the authors and how much the parents of the homes were asked to carry out. Differences in vacuum manufacturers, models and age and the procedures followed by the parents may have been interpreted differently. Temperature and RH loggers were given to parents to locate within the homes and the questionnaires on the built form were completed by the parents and not by an experienced researcher. There were a number of opportunities for bias to be introduced into this study.

An investigation in Helmond, a town of 65,000 inhabitants in the southern part of The Netherlands began previous to publication of the study in 1991 (no study dates are given). Thirty-seven primary schools of students between 6 and 12 years of age were contacted with 4 declining to participate. Parents of 3,344 children returned questionnaires, a 73% response rate. Damp stains were reported by 27% and mould growth by 15% of the study population in the past 2 years. Health outcomes were assessed through questions about cough, phlegm, wheeze, shortness of breath,

asthma, allergy and lower respiratory symptoms. Questions on smoking, and education were included as well as allergy to house dust and pollens but unfortunately not to moulds or pets. After adjusting for smoking, NO_2 and education level, symptoms in both men and women were found to be strongly associated with living in damp conditions. The following adjusted odds ratios resulted: cough (For Men, AOR=2.56; 95%CI: 1.94-3.38 and Women, AOR=1.75; 95%CI: 1.30-2.36), phlegm (Men, AOR=2.26; 95%CI: 1.57-3.24 and Women, AOR=1.66; 95%CI: 1.16-2.38), lower respiratory symptoms (Men, AOR=1.70; 95%CI: 1.38-2.09 and Women, AOR=1.55; 95%CI: 1.27-1.89) and wheeze (Men, AOR=1.63; 95%CI: 1.30-2.06 and Women, AOR=1.43; 95%CI: 1.15-1.77) Interestingly no association was found between damp conditions and allergy to house dust or pollens. Current smoking was strongly associated with cough, phlegm, and wheeze in men and women. The results show that the association between home dampness and respiratory problems previously found in children also applies to adults (Brunekreef, 1992).

A population-based cross-sectional study looking at the occurrence of respiratory symptoms in relation to damp and moulds involved sending a postal questionnaire to a random sample of parents with children (3,200) aged 1 to 6 years in Helsinki (pop. 170,000), Finland, a total of 2,568 responded (80.3%). The questionnaire was modified from the 1978 American Thoracic Society – Division of Lung Disease (ATS-DLD-78-C), but also included questions on personal and environmental determinants (water damage, presence of moisture, visible moulds and mould odour). There was a wealth of personal questions relating to the children: occurrence of respiratory symptoms, allergic diseases, and number of infectious diseases during the past 12 months; parents' education, profession, smoking habits and respiratory and allergic diseases; exposure to ETS during pregnancy and life, type of day care and its environment. The outcomes included cough, phlegm and wheezing, persistent nasal congestion, and nasal excretion during the past 12 months and asthma. The outcome relationships were estimated with logistic regression analysis controlling for age, gender, parents' education, single parent or guardian, ETS, gas cooking, pets and type of daycare. The presence of any determinant within the last year was significant for cough (OR=2.17, 95%CI: 1.39-3.39); phlegm (OR=2.20, 95%CI: 1.27-3.82); wheezing (OR=2.62, 95%CI: 1.39-4.39); nasal congestion (OR=1.94, 95%CI: 1.15-4.98); and

closely associated with nasal excretion (OR=1.43, 95%CI: 0.95-2.17). The rate of asthma symptoms (~2.0%) was similar in both the reference group (n=2,076) and the exposed one (n=492). Mould odour during the past year (OR, 2.38-6.87) and water damage over 1 year ago (OR, 2.54-8.67) had the strongest associations with respiratory symptoms. There appeared to be a dose-response in that the more frequent the mould odour occurred the more symptoms were recorded and there was a time sequence observed between water damage taking place over one year ago and the occurrence of symptoms following that event (Jaakkola et al., 1993).

The respiratory health of 470 (88%) Dutch primary school children aged 6 to 12 years and living in Maastricht was investigated. The group was enrolled from 535 pupils from 2 schools who were given questionnaires asking about respiratory morbidity and housing characteristics to be completed by the parent. Lung function was determined using a forced oscillation technique (a lung resistance measurement) and spirometry (FVC, FEV_1 & PEFR) while attending school. Asthma was determined from the prevalence of chronic cough, shortness of breath (SOB), wheeze, and attacks of SOB with wheezing. In boys all investigated lung function parameters were significantly affected by exposure to passive smoking during their entire life (for shortness of breath: AOR=4.04, 95%CI: 1.21-13.45). Damp stains (yes or no) showed significance against chronic cough (AOR=3.01, 95%CI: 1.31-6.92). All of the asthma symptoms were related to maternal smoking in utero with a trend toward a dose-response relationship, although most were not significant. None of the symptoms showed statistical significance when compared with reported mould growth although chronic cough approached significance (AOR=3.36, 95%CI: 0.80-14.10). Lung function impairment was most consistently related to passive smoking especially in boys and to a lesser degree with home dampness and kitchen gas boilers. The study gives further evidence to the detrimental effects of several indoor home parameters on the prevalence of chronic respiratory symptoms and lung function in children (Cuijpers et al., 1995). No dates were given for the study period.

In 1990 a case-control study was conducted involving a random sample of 7,632 children aged 6 to 12 years. The sample was extracted from 18 schools in the provinces of Noord Holland and 20 in Gelderland, Netherlands. The study looked at

the association between damp housing, childhood respiratory symptoms, and sensitisation to house dust mites and mould allergens. A screening questionnaire was sent to parents regarding respiratory symptoms based on a validated questionnaire, it identified 259 children with chronic respiratory symptoms and 257 control children. The questionnaire also asked about signs of dampness in the home within the last 2 years, these included questions about damp or mould patches in various rooms and also questions about the presence of hairy or feathered pets, smoking, and socioeconomic status. Blood samples were taken and total IgE and specific IgE for Der p1, *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* were determined using RAST. These moulds had previously been identified as dominant in Dutch homes. Pulmonary function was assessed using FEV_1, FVC, PEFR and maximum mid-expiratory flow. In the case group, elevated serum IgE levels for HDM were found in 94 children and in 24 children for any of the mould allergens. For the controls, 31 children reacted to HDM allergen and only 2 to moulds. In both cases and controls home dampness was associated with increased sensitisation to HDM and moulds. There was no relationship shown using odds ratio between dampness and mould within the control group. Restricting the analysis to only those cases that had elevated IgE to HDM and moulds and confining controls to those without any serum IgE response increased the odds ratios with many becoming significant at $p<0.05$. The odds ratio for asthma cases with observed dampness is (OR=3.33, 95%CI: 1.14-9.71) and with observed mould (OR=3.57, 1.15-11.13). The odds ratios for persistent cough ranged from 3.20 in the observed values to a high of 8.79 in the reported figures for both dampness and mould. The authors suggest that sensitisation to HDM and possibly mould, play an important role in the relationship between living in damp housing and childhood respiratory symptoms (Verhoeff et al., 1995).

In 1992, a questionnaire was posted to a random sample of 2,000 individuals, aged 25-64 years, living in Kuopio, Eastern Finland. Seventy-six percent responded and of those, 1,460 were selected for analysis. The aim was to find the prevalence of mouldy homes and find any association with respiratory symptoms and disease in a subarctic climate. Damp and mould criteria were based on four questions:

a. Have you previously had or are you at present able to see visible mould growth on the walls or structure of you home? Resulting prevalence 3.7%.

b. Have you previously or are you currently aware of an odour of mould or cellar-like fusty air in your home? Resulting prevalence 5.5%.
c. Have you previously, or do you currently notice moisture stains in the structures of your home? Yes to any of a, b, or c resulted in a prevalence of 14.7%.
d. Have you previously or are you currently suffering from water/moisture damage in your home? Yes to any of the questions resulted in a prevalence of 23.9%.

A person was defined as being atopic if they answered positively to any one of three questions including: a) ever had physician diagnosed asthma, b) ever had hay fever or other allergic rhinitis and c) ever had infantile or atopic eczema in the hollows of the knees or elbows. Questions on other parts of the body and on stress and depression were included in an attempt to control for bias. Prevalence of damp and mould was given four definitions: Def 1, yes to a., Def 2, yes to b., Def 3, yes to any of a, b or c, Def 4, yes to any. The prevalence of reported infections and symptoms was analysed with logistic regression models. Odds ratios were adjusted for age, sex, smoking, education and dwelling type. As one would expect the results varied with the definition used but they did not change the overall positive pattern of symptoms or respiratory diseases associated with exposure to damp and mouldy homes. Using definition "d" above bronchitis (adjusted odds ratio, AOR=2.04), atopy (AOR=1.63) and allergic rhinitis (AOR=1.66) were significantly more prevalent in occupants of damp or mouldy homes ($p<0.001$) when age, sex, ETS, education years and dwelling type were adjusted for within a logistic regression model. When using definition "b" (i.e. mould odours), the number of colds per year, bronchitis episodes per year, cough, phlegm and rhinitis were all significant to the $p<0.05$ level. The main findings indicate there is a relationship between increased risk of respiratory symptoms and infections and living in damp dwellings, these results remained after adjustment for confounders and questionnaire bias (Pirhonen et al., 1996).

A total of 92 children were enrolled from July to December 1993 including 46 asthmatic children (aged 7 to 15 years who met the American Thoracic Society's criteria for reversible airway disease (i.e., bronchial asthma)), 20 atopic children and 26 non-atopic control children in the Taipei area of Taiwan. All 46 of the asthmatic children had to have at least one positive reaction to any of the following inhaled allergens: HDM, cotton, rice straw, feathers, Fel d1, Can f1, *Candida albicans*, *Aspergillus fumigatus*, *Cladosporium*, *Penicillium*, *Alternaria*, ragweed pollen or Bla g1

were used as controls. The remaining 20 non-asthmatic atopic children had either allergic rhinitis or atopic eczema they were also enrolled. There were no major sources of air pollution within the study area. A questionnaire was developed using a combination of the American Thoracic Society (ATS) – Division of Lung Diseases Respiratory Symptom Questionnaire (DLD), the Harvard Six Cities questionnaire and the Health and Welfare Canada questionnaire for community-based study of children's health. Built form questions included: dampness, visible mould, stuffy odour, water damage, flooding and dampness with all the other factors. Simultaneous air sampling both inside and outdoors were taken with an Andersen one-stage impactor for between 30s and 3min during the summer and 5min during the winter time period. Demographics included: age, gender, education level, as well as number of smokers, home ownership, occupant numbers, humidifier use, air cleaner use, gas cooking, pets, plants, carpets, insect coil burning, and cooking frequency. Total fungal concentrations were significantly higher in the summer in homes of the asthmatics and controls. Indoor mould concentrations were generally high in Taipei, the geometric mean summer (557 CFU/m^3) and winter (402 CFU/m^3) with *Aspergillus*, *Cladosporium*, *Penicillium*, and yeasts the most abundant but no mould allergen variation was observed between the groups in the winter months. Statistically significant concentration differences in the predominant fungus genera were found between the atopic and control groups. Mould concentrations did not vary between damp and dry homes although *Penicillium* values (AOR=0.24, 95%CI: 0.07-0.89) appeared related to damp homes. Home dampness was associated with allergic symptoms in children with asthma and rhinitis. An association was observed between the occurrence of *Cladosporium* and history of asthma symptoms [summer (AOR=1.88, 95%CI: 1.07-3.30) and winter (AOR=4.14, 95%CI: 1.17-14.67)] (Li & Hsu, 1997). Some of the data was unpublished and it was difficult to determine which groups were being discussed possibly due to translation issues.

In Uppsala County, Sweden, data on asthmatic symptoms, other health issues and domestic exposures were collected using a questionnaire sent to 762 pupils 13 to 14 years of age in 11 randomly chosen secondary schools from approximately 130 primary and secondary schools. Current asthma was defined as having a positive response to ever having physician diagnosed asthma, a recent asthma attack or current use of asthma medication. A standardised self-administered questionnaire

based on the ECRHS was mailed to the home of the pupils in January and February 1993. Other questions included: number of respiratory infections in the last 3 months, smoking habits, family composition, other concurrent diseases and symptoms, dampness in the home and past day care attendance. Questions about the school's psychosocial environment were added to determine the stress level of the students. Trained occupational hygienists recorded built form including any smells and signs of damp. Area of open shelving and estimated area of fabrics in relation to room volume were calculated as well as the cleaning regime. Physical measurements were taken in each of 28 classrooms: the air exchange rate, temperature, relative humidity and levels of respirable dust, CO_2, formaldehyde, the sum of VOCs, moulds, bacteria, NO_2 in air and the concentrations of endotoxin, Fel d1, Can f1 and HDM allergens in settled dust. Tracer gas decay using acetone as the source was measured both generally and locally, and the room volume was measured. All measurements were taken using well-established methods. Of the 762 subjects, 627 (82%) competed the questionnaires. Asthma was reported by 40 pupils (6.4%); atopy by 34%, and 18% reported eczema. The most common food reactions were to oranges, tomatoes, shrimps, nuts, eggs and chocolate. Current asthma was more common in those with atopy (OR=3.5, 95%CI: 1.6-7.4), food allergy (OR=5.3, 95%CI: 2.2-12.7) or those who attended day care for several years (OR=1.4, 95%CI: 1.1-1.9). Controlling for these personal factors, current asthma was related to several factors in the school environment. Multiple logistic regression was used to analyse the relationship between asthma and exposures. Current asthma was present in more students of larger schools, with more open shelving, lower room temperatures, higher relative humidity, higher VOCs, and higher concentrations of viable moulds, bacteria levels and cat allergen in settled dust. Only 5% of the homes had reported mould. The authors state that the school environment enhances asthma symptoms. Efforts to decrease dust production and collection should be implemented and school maintenance improved to minimise the allergen burden and moisture damage (Smedje et al., 1997).

All patients with physician diagnosed asthma between 5 and 44 years of age were enrolled from an asthma clinic between November 1992 and February 1993. A total of 102 asthmatics were recruited and twice as many controls were randomly contacted from the Glasgow area, resulting in 196 who agreed to participate. A technician visited

each home and ran through a questionnaire covering respiratory symptoms which included: the presence, frequency and severity of wheeze, chest tightness, cough, and shortness of breath when exercising. Medication use was recorded as were the number of exacerbations requiring steroid use in the past year. Spirometry tests included FEV_1 and FVC. The number of results to questions on asthma was split into categories of mild, moderate and severe. A surveyor was used to assess dampness and mould measuring: temperature and relative humidity outdoors and in each room of the home, electronic resistance using a Protimeter at three points on each wall just above the skirting board and the severity of mould on each wall in each room was graded from 0 to 3 (0=none, 1=trace, 2=obvious but localised and 3=obvious and widespread). The asthmatics were compared in groups rather than as individually matched pairs. Categorical values were compared using odds ratios with confidence intervals and chi square tests for trend where appropriate. Continuous variables were assessed using Student's t-test or Wilcoxon's sum rank test. The dampness and mould severity scores were positively skewed and logarithmic transformations were used for statistical analysis. Linear associations between continuous variables were look at using Pearson correlation coefficient. Logistic regression models were used when controlling for confounders with a significance level of 5%. Self reported dampness in the asthmatics' present home (OR=1.92, 95% CI: 1.18-3.12) and previous home (OR=2.11, 95% CI: 1.29-3.47) was higher than the control homes. The surveyor confirmed dampness in 58 of 90 (64%) dwellings of asthmatics' homes compared with 54 of 132 (41%) homes in the controls (OR=2.62, 95% CI: 1.50-4.55). The severity of asthma score was found to correlate statistically with measures of total dampness (r=0.30, p=0.006) and mould growth (r=0.23, p=0.035) in the home. There was more evidence of airway obstruction in asthmatics from the damp dwellings than in controls (mean difference in FEV_1: 10.6%, 95% CI: 1.0-20.3). There appears to be a weak but significant dose-response relationship between asthma and living in damp housing (Williamson et al, 1997).

During the winter of 1993/1994, data was collected in association with the Pollution Effects on Asthmatic Children in Europe (PEACE) panel study in 14 locations, which looked for an association between home dampness and Peak Expiratory Flow (PEFR) variability, frequency of respiratory symptoms and relief medication (bronchodilators). The children aged between 5 and 13, comprised 896 boys and 718 girls, split from

both urban and rural areas. Criteria for selection involved a parent completed screening questionnaire reporting recent wheeze (without colds), shortness of breath with wheezing, dry cough (without colds) and/or doctor diagnosed asthma ever. The questionnaire included demographics and housing information on moisture stains and mould, gas oven use, unvented heaters, furred or feathered pets, smoking, and age of dwelling to name a few. The number of years the parents were in full education was the only demographic question listed. Incomplete data was excluded from the analysis, and the minimum 2 month PEFR had to have a 60% completion rate, resulting in the total number above (1,614). Skin prick tests involved testing for tree and grass pollens, Fel d1, Der p1 plus histamine hydrochloride and glycerol as the positive and negative controls. Two other allergens were tested for, dependent on the location, these included various moulds and pollen mixes. A 2mm wheal was regarded as positive and a 1mm reaction to the negative control was excluded from the results. Numerous confounders were tested for and those involving home dampness were stratified during analysis because of a possibility of over-adjustment of the association of interest but the correlations were generally low. The results showed that the lowest reported mould was in Oslo, Finland (2.9%) and the highest in Pisa, Italy (36.0%). Extensive analyses were undertaken, those relating to indoor mould showed that PEFR variability in atopic children was associated with reported moulds in homes (OR=1.15, 95%CI: 1.03-1.28), but no relationship was found for non-atopic children. Period prevalence for cough and upper and lower respiratory symptoms was significantly higher in children living in homes with reported mould compared to those in 'dry' homes. Of those children indicating respiratory symptoms or medication use during the observation period, the period prevalence of several respiratory symptoms and bronchodilator use was associated with moisture stains and moulds in the dwelling. These associations (cough, phlegm and bronchodilator use) were generally stronger for atopic children analysed against moulds (OR=1.69, 95%CI: 1.05-2.71), but upper respiratory symptoms in non-atopics was also associated with moulds (OR=1.41, 95%CI: 1.15-1.72). The authors acknowledged that the study was vulnerable to bias as objective home dampness and respiratory symptoms were not collected (Andriessen et al., 1998). The skin prick test procedures used are not common. A 1mm reaction to the negative control would be quite difficult to measure and may be showing dermographism, a reaction to the lancet and not the control.

Usually the negative reaction diameter (if any) is subtracted from the positive one and the resulting number recorded. If the wheal is not symmetrical, the larger and smaller diameters perpendicular to each other are averaged to get a single value.

The association between indoor mould growth and peripheral blood lymphocyte populations in children was investigated in Wallaceburg, Ontario, Canada. During the winter of 1993-1994 dust samples for viable mould in the living room of 400 homes of primary school children were collected. Dust samples were also taken from the bedding of the child closest to 10 years of age in the home and analysed for Der p1 and Der f1 by Enzyme-Linked Immunosorbent Assay (ELISA). A 14 to 20 hour air sample was also collected from the asthmatic's bedroom and assayed for ergosterol, (a fungal cell wall component) to provide a quantitative measure of total fungal mass. A categorical system was initiated by assigning one point for each of the following that occurred:

a) Detectable bedroom ergosterol
b) Detectable living room ergosterol
c) $>10^5$ CFU/g of dust
d) Ratio of (Aspergillus + Penicillium + Eurotium) / (Cladosporium + Alternaria + Epicoccum) >10

Homes were ranked from most contaminated to least. This resulted in studying 39 of the most contaminated homes and 20 from the least contaminated. Some homes were not included because of redecoration, family commitments and aversion to blood sampling. The authors proposed that exposure to fungal antigens or other mould toxins may induce chronic stimulation of lymphocytes, a change of possible significance. Blood samples were taken the following year during February and March 1995 as well as administering a questionnaire and recording the total surface area of mould growth. Peak flow readings were also collected. The blood sampling was repeated one year later and analysed for lymphocyte populations, total serum IgE, and IgE specific to house dust mites, cats and dogs. The more contaminated homes had greater indicators of fungal contamination and the child's bedroom in those homes showed more water-damage, mould growth and humidifier use. House dust mite antigen levels were higher in the more-contaminated homes but did not reach significance at the 5% level. Differences in peripheral blood lymphocyte populations

varied from moderate to large between the more and less contaminated groups. The differences in lymphocyte populations could not be explained by the child's age, HDM in bedding, humidifier use, furred and feathered pets or the presence of self-reported "cold". Follow-up blood sampling a year later (1996) confirmed the results. The authors conclude that the study provided objective evidence supporting previously observed associations in support of a possible causal link between residential fungal exposure and health. The results suggest that residential mould contamination leads to chronic stimulation of the children's lymphocytes (Dales et al, 1998).

Samples from 80 homes in the Latrobe Valley, Victoria, Australia were taken on a bi-monthly basis beginning in March/April 1994 and ending in January/February 1995. Children from these homes between 7 and 14 years of age met the criteria, which resulted in 148 subjects, 53 of whom were asthmatics (43% of the homes had at least one asthmatic). A respiratory health questionnaire was completed, along with PEFR and a SPT was performed. Dust was collected from the beds, asthmatics' bedroom floors and the living rooms and analysed for Der p1 and for viable and total fungal spores. Air samples were collected from the children's' bedrooms, living rooms, kitchens and from outside and analysed for NO_2 and formaldehyde. Airborne fungal spores were determined using a single stage Andersen impactor for viable spores (CFU/m^3) and a Burkard spore trap for total airborne spores (number of $spores/m^3$). The skin prick test was determined after a 15-minute interval using the ratio of the allergen wheal compared with the histamine (positive control) wheal. Results were considered positive if this ratio was ≥ 0.5 after Meinert et al., 1994. Significant risk factors for asthma were: indoor pets (OR=2.68, 95%CI: 1.07-6.70) and gas stoves (OR=3.15, 95%CI: 1.28-7.72). Exposure to gas stoves was a risk factor for respiratory symptoms (OR=2.32, 95%CI: 1.04-5.18) while exposure to *Aspergillus* spores (+10 CFU/m^3) was a risk factor for atopy (OR=1.51, 95%CI: 10.5-2.18). The authors concluded that exposure to gas stoves; pets and fungal spores were statistically significant risk factors for respiratory health in children (Garrett et al., 1998).

A cross-sectional study of 310 randomly sampled houses in four Finnish cities was undertaken using records from building registers. The sample includes houses built in different decades from 1950 to 1980 to represent differing building practices and was

collected between 1993 and 1994. A total of 699 adults over 16 years of age were enrolled. Occupant health data was collected using a self-administered postal questionnaire modified from previous Finnish studies (100% response rate) sent before the home inspection occurred. Exposure was determined both by visual observation by an independent surveyor and by visual and odour based occupant observations. The inspections include signs of leakage, moist spots, paint detachment or other surface material deformation or discolouration. No physical samples were taken. The homes were assessed and classified as either *moisture absent* (control group) or *moisture present* (exposed group). Similarly, the results from the occupant questionnaires on visible mould were defined as *mould absent* (control group) or *mould present* (exposed group). Binary variables were investigated using the chi-square test and the risk related to exposure was assessed by means of multivariate logistic regression models. The results were adjusted for age, sex, smoking, doctor diagnosed allergy, pets and atopic predisposition (any of hay fever, asthma or atopic dermatitis). The occupants were determined as atopic if they reported any atopic diseases (i.e. seasonal allergic rhinitis, asthma and atopic dermatitis). Moisture problems were present in 52% and mould in 27% of the houses. Water vapour exposure was significantly associated ($p<0.05$) with sinusitis (OR=1.92, 95%CI: 1.11-3.30), acute bronchitis (OR=1.98, 95%CI: 1.13-3.48), nocturnal cough (OR=2.11, 95%CI: 1.21-4.98), nocturnal dyspnoea (OR=2.33, 95%CI: 1.09-4.98) and sore throat (OR=1.46, 95%CI: 1.03-2.08). The occupants from the exposed group had significantly more episodes of common cold and tonsillitis ($p<0.05$), atopic predisposition ($p<0.001$) and doctor diagnosed asthma ($p<0.05$) than the control group. Mould exposure was significantly associated with common cold (OR=1.62, 95%CI: 1.08-2.41), cough without phlegm (OR=1.60, 95%CI: 1.01-2.53), nocturnal cough (OR=2.30, 95%CI: 1.32-4.01), sore throat (OR=2.40, 95%CI: 1.56-3.69), rhinitis (OR=1.89, 95%CI: 1.15-3.11). It was found that building related moisture and/or mould increased the risk of upper and lower respiratory infections and symptoms as well as some non-respiratory ones (i.e. fatigue, headache and eye irritation) (Koskinen et al., 1999).

Acute asthma admissions in the Trent region of England were extracted from hospital records between July 1987 and February 1994. Two groups were distinguished by age (0-14 years and ≥15 years) showing radically different annual cycles. No totals

were given but are calculated as: 2,435 days x 10.7 average daily admissions = 26,055 for 0-14 year olds, and 25,811 for the ≥ 15 years group. Simultaneously volumetric spore traps set up around Derby were used as a record of the mould genera. The results identified 30 elementary taxa including 25 of mould plus *Actinomyctes* bacteria, *Myxomycetes* slime moulds, algae and two fern spore groups. Admissions for asthma were adjusted for weekly, seasonal and longer trends by log linear autoregressive models described elsewhere (Newson et al., 1998). These models were used to forecast asthma admissions by region and age group. The spore counts were examined in detail on 6 days of spectacular asthma epidemics. When spore counts for individual taxa were analysed as quantitative variables, two positive and two negative correlations, out of a possible 100, were significant at the 5% level. Splitting the spore count at the 90^{th} percentile produced one negative and eight positive correlations (out of 100) that were significant at the 5% level. The positive associations were all for the group of children and no associations were found for total spore count. Total spore counts above the 90^{th} percentile were significant on four of the six epidemic days (OR=9.92, 95%CI: 1.14-109.84) but were not significantly associated with daily admissions for asthma in either age group in any of the analyses (minimum p=0.083). There was some evidence that spectacular asthma admissions tend to occur on days of high total fungal spore counts but no specific genera were found (Newson et al., 2000).

Associations between home dampness and diagnosed asthma, allergic rhinitis and conjunctivitis, atopic dermatitis, common colds and bacterial respiratory infections were studied amongst 10,667 (a 75% response rate) Finnish university students living in Turku between 1995 and 1996. A postal questionnaire asked: 1. "Have you had mould growth on the surfaces of any of your dwellings during the last year?" 2. "Have you had damp stains, for example, on the walls or on the ceilings of any of your dwellings during the last year?" and 3. "Has there been a leak or water damage in any of your dwellings during the last year?" The results were then divided into two categories, a mould only one and another including all three answers. Validation of the procedures was through a clinical comparison of a subset of individuals in this study compared to a previous questionnaire (Kilpeläinen, et al., 2001a). Interactions between atopy and dampness were also investigated. Water damage, stains or visible mould were recorded in 15% of the dwellings. Odds ratios were calculated for visible

mould only and for answers to all three questions above against asthma, allergic rhinitis and conjunctivitis, atopic dermatitis, common colds (≥4 times/yr) and other respiratory infections. The strongest associations were found between visible mould exposure with current asthma (OR=2.15, 95%CI: 1.48-3.12) and common colds (OR=1.49, 95%CI: 1.18-1.87). Other questions incorporated in multivariate analysis were: present and past smoking, education level achieved, pets including birds, carpets present, heating system type, number of occupants, location of residence (i.e. rural, urban or farm) and housing type (flat or other). The strongest adjusted odds ratios for visible mould was for asthma (AOR=2.21, 95%CI: 1.48-3.28) and for colds (AOR=1.48, 95%CI: 1.17-1.88). Current asthma, allergic rhinitis, allergic conjunctivitis and atopic dermatitis were significantly negatively associated with the presence of pets (AOR 0.33-0.68) and carpets (AOR 0.40-0.69). The association of parental atopy and home dampness on current asthma symptoms was statistically significant (p=0.033) and there was a weak negative association between home dampness and residence in a flat (OR=0.83, 95%CI: 0.74-0.95). The risk of current asthma in damp homes was highest among atopic individuals. The risk of current asthma symptoms, allergic rhinitis and atopic dermatitis were higher in damp dwellings, and there was an increased risk of common colds (Kilpeläinen, et al., 2001b).

In a follow-up study to the original ECRHS, thirty-five young adults from Melbourne, Australia with current asthma and sensitisation to moulds were visited four times over a period of a year, from 1997 to 1998. Current asthma was defined as having wheeze in the past 12 months, plus having bronchial hyper-reactivity, which was determined by having a PD_{20} FEV_1•<2mg methacholine in an inhalation bronchial challenge. At each home visit a respiratory questionnaire was administered, bedroom floor dust and air samples were taken and medication use was recorded. Bedroom temperature (T) and relative humidity (RH) were also recorded (no time periods are given) using a standardised procedure. Absolute humidity was calculated from the relative humidity and temperature values. Sensitisation to allergen was defined as having a wheal diameter >3mm from a skin prick test, with a negative reaction to saline solution (control). The allergens included: Der p1, *Cladosporium herbarum*, *Aspergillus*

• PD_{20} FEV_1: a provocative dose of methacholine required to cause a 20% fall in FEV_1 from a baseline value.

fumigatus, *Alternaria tenuis*, *Epicoccum nigram*, and *Penicillium* (species name not given), with a positive (histamine) control. Diaries of PEFR were recorded diurnally for 14 days as well as symptoms of asthma, wheeze, cough, sleep and restriction of activities. These were assessed and scaled from zero (asymptomatic) to three (severe). The PEFR results were recorded as peak flow variability (PFV), defined as the difference between the highest morning and evening daily values expressed as a percentage of the highest value. Ergosterol, a fungal membrane lipid was assessed as an indicator of total fungal biomass from the dust samples. Identification and abundance of viable fungal propagules suspended in the air samples were collected using an Andersen sampler with potato—dextrose agar plates and a standardised procedure. Continuous outcomes, not normally distributed were log transformed and summarised by season with the geometric means and geometric standard deviations. The temperature, relative and absolute humidity, Der p1 levels, presence of a cat, smoking and medication use were considered as possible confounders. Where outcomes could not be changed to normality, non-parametric two-way ANOVA with factors of season and household were applied. Significant seasonal variations were observed in viable airborne moulds (0 to 6,105 CFU/m^3, with a median of 421 CFU/m^3), ergosterol levels in floor dust and PFV. *Cladosporium* and *Penicillium* were highest in the summer and *Aspergillus* and *Alternaria* had the highest levels in winter. The association between visible mould and daily peak flow variability (PFV) (OR=1.5, 95% CI: 1.03-2.30) was independent of season, smoking and the dose of reliever medication. No association was found between total fungi, specific fungi or ergosterol and PFV. Der p1 levels had no significant influence on asthma, even with those sensitised to HDM. The authors concluded that mouldy homes adversely influence asthma in those sensitised to fungi (Dharmage et al, 2002).

A prospective cohort of 1,002 enrolled infants born in Connecticut and Massachusetts between 1996 and 1998 looked at the risk of increased incidence of respiratory symptoms after exposure to particular fungal genera. Complete data was recorded for 880 infants through the first year of life, all of whom had an older sibling with asthma. Information gathered included days of wheeze or persistent cough, maternal allergy and asthma, socioeconomic variables and housing characteristics. A Burkard portable air sample running at 20*l*/min was used for 1 minute in the home to collect airborne

mould samples within 4 months of the child's birth concurrently with an interview. The fungi was identified to genus level and recorded as CFU/m^3 and divided into 4 categories (none, low, medium and high representing 0, 1-499, 500-999 and ≥1000 counts). Regression analysis was used to examine the effects of mould on wheeze and persistent cough, adjusting for potential confounders. The most common moulds found in homes were *Cladosporium* (62%) and *Penicillium* (41%). *Cladosporium* was associated with reported mould ($p<0.02$) and water leaks ($p<0.003$). The relative risk (RR) of persistent cough was associated with reported mould (RR=1.49, 95%CI: 1.18-1.88). High rates of wheeze (RR=2.15, 95%CI: 1.34-3.46) and persistent cough (RR=2.06, 95%CI: 1.31-3.24) were associated with the highest counts of *Penicillium* in models controlling for maternal history of asthma and allergy, socioeconomic status, seasonal mould collection and housing characteristics. The authors conclude that infants exposed to high concentrations of *Penicillium* spores are at significant risk of developing wheeze and persistent cough (Gent et al., 2002).

From September 1997 to March 2000, a population-based incident case-control study was undertaken with adults aged 21 to 63 years of age living in the Perkanmaa Hospital district of South Finland. All new asthma cases identified within this district were recruited from the 441,000 inhabitants. Controls were also selected randomly from this population. The study consisted of 521 asthmatic adults and 932 controls. Environmental exposure assessment was by questionnaire asking information on water damage, damp stains, structural dampness, visible mould and mould odour at home and at work. For all but the last indicator the occurrence was noted as; in the past year, 1 to 3 years or more than 3 years ago. For mould odour, it was daily, 1 to 3 days per week, 1 to 3 days a month, less than 1 day per month or never. Lung function measurements involved baseline spirometry of forced vital capacity and flow volume, a bronchodilation test using salbutamol, a diurnal peak expiratory flow test for at least 2 weeks. For those subjects suspected of having asthma but who had negative results from the previous tests, a two-week oral steroid treatment was undertaken, if these individuals showed improvement with the medication use they were declared as having asthma symptoms and they were then retested. Odds ratio was used to quantify exposure relations and outcomes as well as adjusted odds ratio in logistic regression analysis. Numerous covariates were used to adjust for

confounders: sex, age, parental atopy or asthma, education level, ETS exposure, pets, dampness and mould problems at home and work, and occupational exposure to dusts, fumes etc. After adjustment, it was found the risk of asthma was related to the presence of visible mould and/or mould odour in the workplace (AOR=1.54, 95% CI: 1.01-2.32) but not to water damage or damp stains alone. None of the exposure indicators in the home were related to risk of asthma. The fraction of asthma risk attributable to workplace mould was estimated to be 35.1% (95% CI: 1.0-56.9%) amongst those exposed. The authors speculate that the lack of an association at home may have been because at home more attention is paid to moisture and damp problems, which may be ignored or not noticed at work. They suggest that indoor mould problems constitute an important occupational health hazard (Jaakkola, et al., 2002).

In a random general population sample from 38 centres, involving the 1994 ECRHS, data was extracted for analysis looking at the effects home dampness, house dust mites and mould exposure have on adult asthmatics. The interviewer led questionnaire asked questions all related to the previous 12 months on; wheezing with breathlessness, wheezing without colds, being woken by shortness of breath, having an asthma attack and whether asthma medication had been used. This was followed by a housing related questionnaire on: the age and type of home, heating and ventilation systems, glazing type, type and location of floor coverings, water damage and presence of moulds. For the subjects, forced expiratory volume in 1 second (FEV_1) plus other lung function indicators were measured as were the individuals serum IgE levels for HDM (>0.35 kU/l)[□] and *Cladosporium herbarum*, and skin prick tests for HDM, *Cladosporium herbarum* and *Alternaria alternata* with wheals >0mm. Odds ratios were adjusted for sex, age group and ETS. The results of this cross-sectional community-based study showed that reporting of mould in the last year was associated with wheezing without colds (AOR=1.44, 95%CI: 1.30-1.60), wheezing and breathlessness (AOR=1.34, 95%CI: 1.18-1.51), current asthma symptoms (AOR=1.28, 95%CI: 1.13-1.46) and bronchial hyperresponsiveness (AOR=1.14, 95%CI: 1.01-1.29). Increase in current asthma was stronger for those individuals sensitised to *Cladosporium herbarum* (OR=2.41, 95%CI: 1.32-4.39), in addition, those centres with

[□] (For total IgE protein, a mass unit (U) is defined by the World Health Organisation (WHO) International Reference Preparation 75/502 as: 1 IU is equal to 2.4ng IgE.)

a high community prevalence (NZ, AU, USA & UK) of asthma also reported high indoor mould exposure. This association was consistent across the 18 countries and was found in asthmatics sensitised to mould allergens (Spearman r_s=0.46) but this association was also reported by non-asthmatics (r_s=0.54). The authors suggest that the observed relation between mould exposure and asthma at the individual level is unlikely to be explained by over-reporting of mould by asthmatics or by under-reporting by symptom-free subjects (Zock et al., 2002). The information given on skin prick testing was inadequate and any wheal greater than zero is not standard practice for a positive result (usually any wheal >3mm against a negative saline or glycerol control is considered positive). Skin prick testing for additional common indoor mould allergens (any of *Aspergillus*, *Penicillium*, *Cladosporium* and *Stachybotrys*) would have also been useful.

In order to assess whether asthma severity is associated with sensitisation to airborne mould spores rather than to other seasonal or perennial allergens, a 30 centre wide epidemiological survey, part of the ECRHS (Burney et al., 1994), was undertaken. Asthmatic participants between the ages of 20 and 44, numbering 17,089 were randomly sampled from all the centres. At stage 1, a short postal questionnaire was completed pertaining to asthma and asthma-like symptoms. Stage 2 involved a 20% random sample of responders who were invited to a local test centre to complete a detailed questionnaire and undergo SPT and blood tests, lung function by spirometry and airway challenge with methacholine. In addition to this subset, other respondents were asked to join if they had: been woken by shortness of breath, had an asthma attack in the past 12 months or were currently taking asthma medication (totalling 14,098). The detailed questionnaire included questions on smoking status, occupation, social status, home environment, medication use and use of medical services. Allergen coated lancets included: *Alternaria alternata*, *Cladosporium herbarum*, *Phleum pratense* (timothy grass), birch, olive, *Parietaria judaica* (Pellitory-of-the-wall), *Ambrosia artemisifolia* (Common ragweed), Der p1 and Fel d1 were used in the SPT. An uncoated lancet was used as a negative control. After 15 minutes the spots were measured in millimetres, a wheal diameter >3mm larger than the negative control was considered positive. Baseline FEV_1 and forced vital capacity (FVC) were measured using standardised procedures. Asthma was defined by the sum total of 3 categorical scores. Each of the variables was given an ordinal value of increasing

severity: a score of 1, 2 or 3 based on: FEV_1 results (mild >80% (1), moderate 70%--80% (2) and severe <70% predicted (3)), the number of asthma attacks in past 12 months (2, 3-6, >6), the number of admissions to hospital for breathing related problems (0, 1-2, >2) and whether inhaled oral corticosteroids had been used in the past 12 months (1 or 2). The forth variable (medication use) was given a score of 1 or 2. The sum of the variables was then assigned an asthma severity classified (after Ronchetti et al., 1997) as mild (scores from 4 to 5), moderate (6) and severe (≥7). After dropouts and missing data, the analysis was completed on 1,132 participants. The associations between severity of asthma symptoms and categorical variables were assessed using the chi square (χ^2) test. No heterogeneity was found between regions in the association between severity and sensitisation to allergens ($p>0.30$). Asthma severity for severe ν mild asthma increased significantly with increasing sensitisation to *Alternaria alternata* and/or *Cladosporium herbarum* (OR=2.34, 95%CI: 1.56-3.52). This association existed across all regions although the frequency varied. No association was found between asthma severity and sensitisation to pollens or cats. In multivariate logistic regression analysis for sensitisation to moulds, pollens, Der p1 and cats simultaneously, the OR for sensitivity to moulds were 1.48 (0.97-2.26) for moderate ν mild asthma and 2.16 (1.37-3.35) for severe ν mild asthma ($p<0.001$ for trend). The results showed that sensitisation to moulds are a major risk factor for severe asthma symptoms in adults (Zureik et al., 2002).

From September 1996 to the end of 1998 in five hospitals in Connecticut and Massachusetts, United States, a cohort of 849 infants with an asthmatic sibling were enrolled at birth to look at relationships between wheeze and persistent cough against Der p1 & f1, cockroaches (Bla g1), Fel d1, Can f1, NO_2, and moulds. A research assistant visited the homes and administered a questionnaire on demographics (including family smoking habits) when the enrolled child was 2-4 months old. The mothers were asked to fill out a symptom card and they were contacted by phone at 3, 6, 9 and 12 months for the results. A record of gas oven and wood-burning fire use was noted. Dust samples were collected from the main living area floor and furniture using a standardised protocol (Leaderer et al., 2002). Fungal spores were collected using a Burkard portable air sampler for 1 minute at 20*l*/min in the main living area and

from the asthmatic's bedroom. NO_2 was measured using Palmes diffusion tubes from the living room over 10-14 days and was found to be associated with persistent cough. Among infants whose mothers were asthmatics, exposure to cockroach allergen ≥2U/g modestly increased the risk of wheeze (OR=1.87, 95%CI: 0.94-3.71), and for Fel d1 (OR=0.6, 95%CI: 0.35-1.03). Persistent mould affected both infants with asthmatic mothers (for wheeze, OR=2.27, 95%CI: 1.27-4.07 and cough, OR=1.83, 95%CI: 1.04-3.22) and those mothers without asthma for cough only (OR=1.55, 95%CI: 1.04-2.31). Measured mould was associated with wheeze (OR=1.23, 95%CI: 1.01-1.49) (Belanger et al., 2003), but no mention of what mould types, quantities found or methods of examination were given.

The relationship between building dampness and respiratory symptoms in 1,853 (89%) young adults randomly selected from 2,084 (between 20 and 44 years of age) were investigated in 1993. Three centres were included in the study based in Västerbotten, Uppsala and Götenborg, Sweden. As part of a random sample for the ECRHS stage II, subjects were asked to participate in a detailed interview-led questionnaire, spirometry, methacholine challenge and blood sampling for specific and total IgE. The interview questions included building related problems of water damage, leaks, surface moulds within the past year as well as health related ones on current asthma, wheezing, being awoken by a tight chest or shortness of breath and other shortness of breath attacks when at rest and after exercise. Bronchitis-related questions asked about nocturnal cough, long-term cough and long-term phlegm production. Smoking (>1 cigarette/day for 1 year) results were split into three groups of never, ex and current smokers. Spirometry included FEV_1, FVC and methacholine challenge as per ECRHS methods. Blood was tested by the CAP® system (Gleeson et al., 1996) in all centres; specific IgE was measured against Der p1, Fel d1, grass and birch pollens, and *Cladosporium herbarum*. Water damage was reported by 136 (7.4%) subjects, visible mould by 318 (17.3%) and both by 74 (4%) in the last year. Of those participants, reporting mould and water damage more of them had attacks of breathlessness both at resting (AOR=3.24, 95%CI: 1.44-7.29) and after exertion (AOR=2.76, 95%CI: 1.36-5.60) compared with subjects without these problems. The same group also had significantly more long-term cough (AOR=2.23, 95%CI: 1.24-4.00). The results were adjusted for age, sex, ETS history, and IgE responses to the allergens noted. The authors conclude that building dampness is a common problem

in Sweden and that it is related to an increased prevalence of some respiratory symptoms (Gunnbjörnsdottir et al., 2003). It is unfortunate that only one type of mould was tested for although it appears to be one of the most common indoor moulds but one that does not generally provoke a reaction to its antigens.

In New York City, a study looking at the relationship between mould hypersensitivity and asthma in a group (>900) of patients evaluated for allergic disease at a single medical centre took place between 1993 and 2001. Asthma diagnosis consisted of a history of wheezing, use of bronchodilators and prior diagnosis of asthma by another doctor. Serological testing identified 531 patients for mould-specific IgE and other IgE specific aeroallergens, analysis was performed and associations were analysed using bivariate and multivariate methods. Three distinct groups of allergens were identified using factor analysis (Group 1 included those who responded to Fel d1 and Der f1, Group 2 to tree, grass and ragweed pollens and Group 3 to the *Ascomycetes* moulds [*Cladosporium herbarum*, *Alternaria tenuis* and *Aspergillus fumigatus*]). Asthmatics had a highly significant increase in the incidence of hypersensitivity to groups 1 and 3. Adjusted odds ratios using these selected group variables as predictors of asthma showed cat or dust mite allergen gave a stronger response (AOR=3.5, 95%CI: 2.2-5.9) than for moulds (AOR=1.8, 95%CI: 1.1-3.1). Analysis showed hypersensitivity to either *Alternaria tenuis* or *Cladosporium herbarum* had a significant independent association with asthma (χ^2=7.55, p=0.006) after adjustment for Group 1 and for other clinical factors. Pollen hypersensitivity was not associated independently with asthma. Mould hypersensitivity and Fel d1/Der f1 hypersensitivity were strongly correlated in non-asthmatics (Lin & Williams, 2003).

In 1,405 family homes around metropolitan Boston, Mass, USA, infants were screened for a family history of asthma or having one parent with allergy. This reduced the prospective birth cohort to 499 families with 505 infants. A trained technician made home visits after the infant was 2 to 3 months old but no actual study dates are given. These included a questionnaire on socioeconomic characteristics and on the household environment including air and floor dust sampling. Air samples were collected using a Burkard culture plate sampler rated at 45*l*/min on to Dichloran glycerol agar (DG18) Petri dishes. The infants bed and bedroom floor area were vacuumed for 5 minutes. Moulds were cultured for a minimum of 10 days but the

methods are not given. Telephone questionnaires were used as follow-up every two months asking about respiratory symptoms and illnesses since the previous interview as well as questions dealing with home characteristics and day care attendance. Again no specific information is given for the length of time this went on for but can be inferred from the paper to be one year. The object of this study was to look at the contribution fungal exposure has on lower respiratory illnesses (croup, pneumonia, bronchitis and bronchiolitis) in the infants' first year of life. *Penicillium* was the genus found in the most number of homes in air (422) and was second in dust samples (367 along with yeasts). *Aspergillus* was the most often found in dust samples (373) but yeasts had the most spores at 58,000 CFU. The most common indoor fungi identified were: yeasts, *Aspergillus*, *Cladosporium*, *Penicillium*, *Aureobasidium*, and *Alternaria*. No correlation between mould genera was found. Multivariate analysis found significant increased relative risk between lower respiratory illnesses and high levels (>90th percentile) of airborne *Penicillium* (RR=1.73, 95%CI: 1.23-2.43), dust-borne *Cladosporium* (RR=1.52, 95%CI: 1.02-2.25), Zygomycetes (RR=1.96, 95%CI: 1.35-2.83) and *Alternaria* (RR=1.51, 95%CI: 1.00-2.28) after controlling for sex, presence of water damage or visible mould, winter birth, breastfeeding and exposure to siblings friends. The relative risk of lower respiratory illnesses was elevated in homes with any mould at a level greater than the 90th percentile (RR=1.86, 95%CI: 1.21-2.88). Exposure to high fungal levels increased the risk of lower respiratory illnesses in infancy, even for children without wheeze (Stark et al., 2003). The time intervals for sampling, their frequency and start dates are missing.

All children between 1 and 9 years of age with asthma or allergic rhinitis attending a university clinic in Rome between January 1990 and December 1997 were enrolled, totalling 6,840. Diagnosis was established through family and personal history, physical examination, SPT or RAST for inhalants including *Alternaria alternata*. Skin prick testing was graded from 1 to 4 with only those achieving a three or four rating considered positive. RAST results were also graded from 1 to 4 with 3rd class =IgE levels between 0.7 IU/ml and 17 IU/ml and 4th class greater than this amount. Of the total number of children, 213 (3.1%) had sensitisation to *Alternaria alternata* and other aeroallergens (not stipulated) and 89 (1.3%) to *Alternaria alternata* only. Sensitisation to the allergen was at 4 years of age in males and 5 in females. The SPT and RAST

results were in accordance with each other. Family history of asthma was positive in 83% of the children. *Alternaria alternata* reactions in asthmatics are noted as life threatening by the authors and although the percentage of individuals that are allergic to it are small (3.3%) those sensitised only to *Alternaria alternata* (1.3%) are a significant (p=0.0001) proportion. They suggest that all asthmatics be tested for *Alternaria alternata* followed by suitable treatment (specific immunotherapy) (Cantani & Ciaschi, 2004).

Beginning in the summer of 1998, a stratified cross-sectional study of 274 schools in Denmark enrolled 1,053 children aged 13 to 17 years. The pupils answered a questionnaire on building related symptoms and other relevant health issues. Exposure measurements within the schools included: room temperature, relative humidity and CO_2 levels as well as assessing for mould infestation. Dust samples were collected from the floors, air and ventilation ducts during a regular working day and examined for endotoxin and cultured for viable moulds. The number of CFU of mould was divided into low (833 to 11,364), medium (11,965 to 64,350) and high (67,698 to 3,113,207) levels. No positive association between building related symptoms and the extent of visible mould and moisture damage was found, although the strongest independent association was found when using moulds in floor dust. Five of the eight building related symptoms (throat irritation, headache, eye irritation, lack of concentration and dizziness) were significantly and positively related to CFU of mould in floor dust. After adjustment for potential confounding factors, all but concentration problems remained significant. In none of the analyses was mould growth the strongest covariate, being secondary to any one of the following: asthma, hay fever, recent airway infection or psychosocial factors (Meyer et al., 2004).

Research looking at how the indoor environment impacts on adult asthma and rhinitis began in 1992 in Northern California. Three separate samples of subjects with asthma or rhinitis were enrolled into a cohort by various means up until 1999. In total 548 eligible subjects completed interviews and of these 226 (58%) were re-interviewed in 2002/2003 for this study. Forty-five minute telephone interviews were conducted approximately 22 months from the initial call. The questionnaire covered medication use, income, employment status and smoking exposure. Health status was assessed using the Short-Form 12 (SF-12) Physical Components Scale (PCS) medical

questionnaire and asthma severity by a previously validated method (the Severity and Asthma Score (SAS) by Eisner et al., 2000), which included current asthma symptoms, medication use and health care utilisation (a maximum score of 28 reflects severe asthma). Those subjects who had not been previously diagnosed with asthma were subjected to another validated telephone (Marks Asthma Quality of Life, QOL) questionnaire (a maximum score of 60 reflects greatest adverse effects of asthma on quality of life). Spirometry measurements of FEV_1 were taken along with blood samples and serum cotinine swabs within 8 weeks of the initial contact. The samples were assayed for IgE antibodies to Fel d1, Can f1, Bla g1, Der p1 and Der f1. Five minute indoor air measurements were taken in the subject's bedroom, main living area and the kitchen for CO, CO_2, RH and T using various equipment. Palmes' diffusion tubes were used in the kitchen for NO_2. Monitoring badges were worn for 1 week and analysed for aldehyde, acrolein and VOCs. Bedroom pillows and mattresses were vacuumed for 8 minutes at approximately 400l/min. The kitchen floor was sampled for 2 minutes and the living room floor for 6 minutes. The dust samples were analysed for the same allergens as measured in blood plus endotoxin and glucan. House dust mite was the most prevalent allergen (36%) and was significantly higher in homes of those sensitised ($p<0.001$). Pet allergens were the next highest but no relationship was found between quantity and sensitisation ($p=0.69$, Fel d1; $p=0.46$, Can f1). Endotoxin and glucan were significantly correlated ($r=0.35$; $p<0.001$). Both endotoxin and glucan were significantly higher in homes with dogs ($p=0.008$, $p=0.001$, respectively), whereas no relationship was found for cats. Most of the volatiles were below detection with the exception of formaldehyde but values here were low (mean 15ppb). House dust associated exposures together with some indoor air quality (IAQ) variables were related to FEV_1% predicted ($r^2=0.24$; $p=0.0001$). Looking at a multivariate model for FEV1% predicted, including all of the variables listed above that met the $p<0.20$ threshold, two showed a negative association, for dog ownership (-9.7 ± 3.5%; $p=0.006$) and for total dust collected from bed mattress (-1.8 ± 0.6%; $p=0.003$). Using the same model but substituting SAS as the dependent variable (model $r^2=0.18$; $p=0.007$), found that dog ownership (3.3 ± 1.1; $p=0.002$), dog antigen (-0.5 ± 0.2; $p=0.03$) and presence of an air filter (2.3 ± 0.9; $p=0.01$) all statistically significant. Der p1 was the only statistically significant variable ($r^2=0.15$; $p=0.02$) associated with the SF-12PCS questionnaire (3.4 ± 1.7; $p=0.04$) in the model. Heating with coal or wood

was negatively associated with the QOL questionnaire (r^2=0.14; p=0.02) (-7.9 ± 3.3; p=0.02) when it was substituted into the model. This study found that the components of house dust had a moderate relationship to lung function and asthma severity. Multiple factors of the indoor environment were associated with lung function, disease severity, health status and quality of life in adults with asthma and rhinitis (Blanc et al., 2005).

A longitudinal population based study of 1 to 7 year old children looking at the joint effects of parental atopy and exposure to moulds in homes on the development of asthma began in 1991. The study was located in Espoo, a suburb of Helsinki, Finland and enrolled all births between January 1984 and December 1989. A parent administered baseline questionnaire was distributed in March 1991 to a random sample of children with an 80% response rate of 2,568. A follow-up survey was conducted 6 years later in March 1997 receiving 1,984 (77% of the baseline population) questionnaires. Those who had asthma at baseline or who had missing data were excluded from this study leaving 1,916 children enrolled. The study determinants were parental allergic diseases and four indicators of exposure at baseline including: water damage history, presence of moisture or visible mould and perceived mould odour within the home. A total of 138 (7.2%) children developed asthma within the study period giving an incidence rate of 125 per 10,000 person-years (95%CI: 104-146). Covariates used in analysis included: age, sex, breast feeding duration, parental education, maternal smoking during pregnancy, ETS exposure, gas cooking, presence of furry and feathered pets and child care during the previous year. Incidence rate ratios (IRR) were calculated using Poisson regression analysis adjusting for the covariates above, for both parental atopy (adjusted IRR=1.52, 95%CI: 1.08-2.13) and for the presence of mould odour (AIRR=2.44, 95%CI: 1.07-5.60), they were both independent determinants of asthma incidence but no apparent interaction was observed. This cohort study with assessment of exposure before onset of asthma strengthens the evidence for the independent effects of parental atopy and mould exposure on the development of asthma (Jaakkola et al., 2005).

A pilot study was undertaken in a general practice and at University Hospital, Salford, Greater Manchester, UK. Recruitment of 181 Caucasian asthmatic patients was evenly distributed over 30 months from January 1996 to June 1998. Inclusion criteria

included an asthma diagnosis, age from 16 to 60 and informed consent. Exclusions included a COPD diagnosis, non-European ethnic group and consumption of antihistamine within 48 hours of interview. A questionnaire was presented concerning respiratory symptoms, smoking status and known allergies. Skin prick tests were performed for 5 moulds (*Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium*, and *Candida*) and 4 other common inhalant allergens (Der p1, grass pollen, Fel d1, and Can f1). Subjects were ranked into three groups as to the number of lifetime hospital admissions for asthma (82 never admitted, 53 had one admission and 46 had multiple admissions (MA)). Those with multiple admissions reacted the most to all allergens. Moulds and dog allergen had the strongest correlation and HDM the weakest (76% of MA reacted to at least one mould compared with only 16%-19% of other patients, chi square, $p<0.0001$). Two of the most severe asthma attack patients attending the Intensive Care Unit were only allergic to one mould, one to *Aspergillus* and the other to *Penicillium*. Multiple mould reactions were highest in the MA group (50%) with the others at 5% and 6%; $p<0.0001$. The number of asthma admissions was related to the size of the wheal to mould (Spearman Correlation Coefficient, $r=0.60$, two-tailed $p<0.0001$) and less strongly correlated to the number and size of non-mould SPT ($r=0.34$, two-tailed $p<0.0005$). The year was divided into 4-month seasons, March to June deemed the pollen period, followed by the mould one and lastly from November to February the infection period. On this basis the hospital admissions for asthmatics between 16 and 40 was highest from July to October (moulds) and those over 40 peaked in November to February (infections, chi square, $p<0.02$). This study supports the findings that mould sensitisation may be associated with severe asthma attacks requiring hospital admission (O'Driscoll et al., 2005).

In 1998, children aged between 4 and 12 years attending one of two schools: one with documented indoor mould problems and another without any such problems located within one mile of each other were enrolled. Both schools, located in El Paso, Texas, USA had similar built form, evaporative cooling systems and numbers of pupils. One school had been built in 1959 and the other in 1975. A self-administered questionnaire was given to the 1,142 students, which included questions on demographics, medical history and any building related complaints from the school or from home. Questionnaire response rates were 78% for the contaminated school and 64% for the 'clean' one. Medical complaints were split into respiratory symptoms, skin symptoms,

systemic problems and nosebleeds which were also given a frequency response of never, rarely, sometimes, often and very often. Nurse records relating to possible mould related symptoms from the children in the contaminated school were labelled as 'Before' (first term) and 'After' (second term) when the mould problem was publicised. Air sampling showed that the "clean" school had no fungal contamination and the indoor genera were the same as the outdoor air but with much lower concentration. The contaminated school contained abundant *Penicillium sp.* and *Aspergillus niger.* One room also had *Stachybotrys* genera. Absenteeism was higher in the contaminated school compared with the clean one ($t=4.25$, $p<0.001$). Adjusting for age and gender showed absences were significant (AOR=0.95, 95%CI: 0.93-0.98). All symptoms measured were higher in the contaminated school as were the number of nurse visits. Frequencies between the contaminated ν clean school showing significance were: cough and/or wheeze (75 (9.5%) ν 35 (5.1%), $p<0.001$), headache (127 (16.1%) ν 90 (13%), $p<0.001$) and joint pains 7 (0.9%) ν 1 (0.1%), $p<0.005$). All symptoms except for nosebleeds showed significance after the mould problem was publicised. In conclusion, the authors state that this study lends support to the concept of sick building syndrome (SBS) for buildings contaminated with *Penicillium sp.* and *Stachybotrys sp.* (Handal et al., 2004) but makes no comment about the increased frequency of symptoms after publicising the problem.

2.5. Physical factors and moulds

Fungi as well as bacteria found indoors are usually saprotrophs; they obtain their nutrients from dead organic matter. They acquire food from the breakdown of cellulose, pectin, starches, various mineral-based building products, textiles, emulsion paints and fats (from cooking) and occasionally from living organisms. *Aspergillus fumigatus* is one such fungus that can grow as a parasite or opportunist pathogen, which can invade the body of immunocompromised individuals. The factor that most determines fungal growth is the availability of moisture. The substrates, which support the mould growth absorb moisture at different rates and can hold water in differing quantities. The amount of free or available water in a substrate is usually designated using the term water activity (a_w), which is the ratio of the vapour pressure exerted by the water in the material to the vapour pressure of pure water at the same temperature

and pressure. The a_w conditions for filamentous mould growth at 25°C have been divided into extremely xerophilic (a_w<0.75), moderately xerophilic (a_w=0.75-0.79), slightly xerophilic (a_w=0.80-0.89) and hydrophilic (a_w≥0.90), (Flannigan & Miller, 2001). *Aspergillus* and *Penicillium* fall to the dry end of the scale, *Alternaria* and *Cladosporium* are slightly xerophilic and *Stachybotrys* and *Rhizopus* to the wet end. These values are optimum and those found in dwellings may vary considerably because they are affected by temperature, see table 2.2.

Table 2.2: The effect of temperature on the minimum water activity of selected moulds collected from the air and walls and grown on emulsion painted woodchip wallpaper (Grant et al., 1989).

Genera	a_w at 12°C	a_w at 18°C
Aspergillus versicolor	0.87	0.79
Penicillium brevicompactum	0.87	0.83
Penicillium chrysogenum	0.87	0.85
Cladosporium sphaerospermum	0.93	0.92
Ulocladium consortiale	0.94	0.92
Stachybotrys atra (chartarum)	0.98	0.97

From an exhaustive literature review the growth limits of six genera have been formulated in terms of the RH and T necessary for growth on building substrates. The moulds were split into six categories ranging from xerophilic (A) to hydrophilic (F) (*Aspergillus repens* (A), *Aspergillus versicolor* (B), *Penicillium chrysogenum* (C), *Cladosporium sphaerospermum* (D), *Ulocladium consortiale* (E), and *Stachybotrys atra* (F)). The authors state that these two parameters are the most important in governing the free water available to hygroscopic building materials and possible mould growth. These limits were incorporated into a simulation design tool in order to predict likely mould infestations. The system has been tested against monitored data and mycological samples taken from a mould infested home. Moulds collected from a 1940's house in Edinburgh were pressed on to malt-extract agar plates and incubated at 25°C under 8 different relative humidities ranging from 67.8% to 98.7% for 120 days. The plates were examined daily. There was no growth detected below 74.5%

RH at 25°C and the minimum values for growth were in good agreement with the predicted model for the more xerophilic moulds. The authors note that the system was only tested at 25°C, which is quite warm compared with most British homes and that the culture medium may have been too high in nutrient value (Clarke et al., 1999).

From a previous study (Hunter et al., 1988), nineteen species of fungi were identified in 10% of all indoor air samples, these were assessed for their ability to grow under different levels of water activity (a_w:a_w= RH/100). A subset of moulds was selected for a more detailed analysis of a_w on a range of building substrates including paint and woodchip wallpaper. Mould laden swabs from wall samples were used to inoculate malt extract agar (MEA) and incubated at 25°C for identification. The water activity was controlled using saturated salts and solutions of potassium hydroxide to produce atmospheres of specific relative humidity within the incubation chambers. The temperatures used varied between 20 and 25°C. A surface temperature probe was used to measure actual wall temperatures on the inner surface of external walls of a West London home, and from these results, values of 5, 12 and 18°C were selected for the subset part of the study. The lowest a_w recorded using the MEA was 0.76 for *Aspergillus repens*, while that for *Aspergillus versicolor* and *Penicillium sp* was 0.79. On the wallpaper painted with emulsion, the minimum a_w was found to be 0.79 at 25°C for *Aspergillus versicolor* and for *Penicillium chrysogenum*. At 12°C, closer to actual field measurements, the a_w rose to 0.87 for these two moulds. Increased temperature and nutrient content lowered the a_w necessary for mould growth. It is beneficial to keep the relative humidity and temperature of a dwelling at levels that maintain a a_w below 0.80 to curb mould growth (Grant et al., 1989).

There are thousands of genera of moulds all with differing growth requirements and predicting their likely growth in buildings seems daunting. Moulds are found in homes much cooler than the laboratory conditions used in the model. The humidity requirements for growth could easily be met through occupancy and minimal ventilation but may not show the available free water held in the building material. Sources of water include, water ingress, clothes drying, bathing and showering, combustion gas heaters, plants, pets and people (overcrowding), resulting in condensation on walls and windows.

2.6. Indoor humidity, ventilation and mould growth

The term 'dampness' has been used to characterise a variety of moisture problems in homes including those with high humidity levels, condensation, water ingress, and other signs of excess moisture, such as fungal growth. While studies report that dampness is prevalent in residential housing in a wide range of climates, attempts to understand its scale and significance are hampered by the fact that there is no generally accepted definition of 'dampness' or of what constitutes a 'dampness problem'. The primary sources of indoor dampness differ across climates, geographic areas, and building types. Although dampness problems appear to increase as buildings age and deteriorate, the experience of building professionals suggests that some modern construction techniques and materials and the presence of air-conditioning also increase the risk of dampness problems (Institute of Medicine, 2004). The existence today of these problems suggest that what is known about their causes and prevention is often ignored in building design, its construction, maintenance, and use. Also the worst quality buildings can be found in areas with the most severe climates (Blane et al., 2000).

Air sampling for viable mould propagules was carried out in 15 homes in England (High Wycombe and London) and 47 in Scotland (Edinburgh, Glasgow and Stirling) during three winters between 1983 and 1986. The sampling was sporadic due to access problems to the homes. A 6-stage Andersen sampler was operated from 10 seconds to three minutes depending on the quantity of airborne propagules present. Malt extract agar filled Petri dishes were used to collect the fungi and were incubated at 25°C for four days. Spores were identified by colony morphology if possible and either left to grow further or removed for culture on to potato dextrose agar. Swabs were also taken where possible and cultured for identification. The number of CFU ranged from <12 to 449,800 CFU/m^3. Thirty-seven filamentous fungi were isolated from the samples. In the Scottish dwellings, *Penicillium* (96%) was the most frequently identified followed by *Cladosporium* (89%), *Aspergillus* (75%), *Ulocladium* (62%), *Geomyces pannorum* (57%) and *Sistotrema brinkmannii* (51%). Yeasts were found in most (94%) homes with an average abundance of 13%. The dominant moulds identified from the swabs were: *Cladosporium*, *Penicillium* and *Ulocladium*, *Aspergillus*

versicolor, *Phoma herbarum* and *Geomyces pannorum* were prominent amongst these. Only *Penicillium* (27.6%), *Cladosporium* (15.3%) and *Aspergillus* (1.7%) were quantified from the smaller English part of the study. *Stachybotrys atra*, though found infrequently occurred in large numbers (~17,900 spores/m^3) when observed. High numbers of spores from the air samples were associated with the swab results and with construction work. Vacuuming caused considerable but temporary increases in the airborne spore counts (Hunter et al., 1988).

A controlled trial involved 40 houses selected from asthma patients visiting a Southampton asthma clinic. Those willing to participate were asked to fill in a questionnaire about their home, which was later scrutinised externally for suitability. Those approved were examined between March and May 1994. Twenty homes were allocated for mechanical heat recovery ventilation units being installed and 20 as controls where nothing was done. Relative humidity and temperature measurements were made in a number of locations within each asthmatic's bedroom for a period of one year. The bedroom height was also recorded. External loggers were also used in two locations on the north face of buildings protected from rain. Weather data was also collected from the Southampton Weather Centre and showed good agreement with the external loggers. Humidity was described as moisture content in grams of water vapour per kilogram of dry air (g/kg), which is called the mixing ratio (w). The WHO working hypothesis was that a w below 7g/kg was sufficient to inhibit HDM growth. In the paper the term absolute humidity is used incorrectly to describe this quantity, which is actually a density measure (g/m^3). Air leakage was measured using a standard fan pressurisation test at 50 Pa pressure difference. HDM, other allergen levels and indoor air pollutants were also measured and any clinical changes. The results of these were reported in another paper. The mechanically vented bedrooms had a mean mixing ratio of 6.75 g/kg during the winter and the controls (7.53 g/kg: $p<0.001$). Fifteen of the homes had mean w below the WHO limit compared with only three of the control homes. Humidities were lower in the leakier homes but only in the mechanically vented homes did it show significance ($p<0.001$) (Stephen et al., 1997).

An overview of the British housing stock was presented as a paper at a 1997 ASHRAE conference, reporting the lack of ventilation and resulting moisture problems.

Ventilation in British homes had not been an issue, it was assumed that it just happened through cracks in the building envelope. Until the late 80s almost half the building stock predated World War II and had working chimneys and open fires. Changes to the building fabric were first recorded following the English House Condition Survey 1988, which reported that 35% of the houses had condensation problems and 17% had mould problems. Homes were being tightened up with chimney removal following central heating installations and new PVC window installations. This led to more stringent ventilation regulations in the Approved Document to Part F of the Building Regulations of England & Wales in 1990, requiring background ventilation, extraction units and the recognition of whole house mechanical ventilation but it was not made a requirement. The relative small size of British dwellings, high occupant density, low internal temperatures and minimal ventilation has led to a major moisture problem that continues. Extraction from wet areas (bathroom and kitchen) and increased background ventilation has improved the situation modestly but concerns about HDM levels and other respiratory issues continue (Woolliscroft, 1997).

During March 1993, fifty-nine single storey detached homes within a small area of Stockholm, Sweden were investigated. These naturally vented homes were all of similar design and built in two stages between 1968 and 1970. In 22 of the homes mechanical supply and exhaust ventilation units were installed. Measurements were taken of the ventilation rate, indoor air temperature and humidity, mattress allergen levels and total indoor VOC levels. Inclusion criteria included ≥ 2 persons resident, and that residency had to have been continuous from a previous study (originally 70 homes) in 1991 including use of the same mattress for sampling. No changes to the ventilation system or building fabric could be made. Passive tracer gas techniques were performed using perfluorocarbon tracer (PFT) to assess the air change rate. Temperature was logged in the living room and one bedroom for 14 days as was the average relative humidity using lithium chloride hydrate as an absorption substance in diffusion tubes. Absolute humidity was calculated in grams of moisture per kilogram of dry air (actually the mixing ratio). Outdoor values were collected from the Swedish Meteorological and Hydrological Institute for the same time period. Mattress dust samples were analysed using ELISA techniques. Indoor VOC were measured from diffusion tube samplers. Only five of the homes with the mechanical ventilation had air

change rates below the minimum accepted level of <0.5 ACH, compared with 24 of the 29 naturally ventilated homes (OR=0.06, 95%CI: 0.01-0.20). None of the 23 homes with an ACH ≥0.5 had a mixing ratio of 7g/kg (from WHO) or more compared with 10 of 36 homes with ACH<0.5 (p=0.01). A similar result was found when comparing the HDM levels. The study showed that mechanical ventilation increases the possibility of achieving ACH≥0.5 which protects against high humidity levels contributing to HDM survival as well as high indoor pollutant levels in the winter months (Emenius et al., 1998).

A matched case-control study was carried out on a cohort of 3,754 children born in Oslo, Norway between 1992 and 1993 and followed for two years. The case series involved 251 children with bronchial obstruction. The control was matched one to one by birth date. The aim of the study was to determine the effective total air change rate (ACH) per hour of the participants' residences. The measurements were compared with those from other countries with a similar climate. The ACH was measured in 344 homes using the PFT technique over a two-week period. The Norwegian national building code requirements of 0.5 ACH was met by 36% of the homes tested. Despite similar building practises in other Nordic countries, Norwegian homes seem better ventilated. The general perception that building practises have improved over time was supported by the findings of this study. A linear regression with ACH as the dependent variable and construction year (continuous) as the independent variable, shows a linear trend with slope β=-0.002, $p<0.05$ until 1987 when a revision of the building code occurred. The trend looks to continue beyond this date but due to the small numbers, did not show significance. The authors conclude that the results support the hypothesis that new building regulations and construction methods and the implementation of energy conservation measures have decreased the effective air change rate in Norwegian homes until 1987 (Øie et al., 1998).

Beginning in 1996, floor dust and air samples were collected from 485 bedrooms of participants over a one-year period to investigate the prevalence of moulds and the influence the residential characteristics had on the levels of fungi. This was a follow-up study to the ECRHS undertaken in Melbourne, Australia in 1992 from an original random sample of 3,200 adults between 20 and 40 years of age. An interviewer administered a questionnaire relating to the home environment and recorded

temperature and relative humidity data. The dust samples were analysed for ergosterol, a marker of total fungal biomass exposure. Total and genera-specific fungal propagules were identified from the air samples. Viable moulds exceeding 500 CFU/m3 were found in 55% of homes. *Cladosporium* (90%) and *Penicillium* (76%) were the most prevalent and abundant genera found indoors. The median ergosterol level in bedroom floor dust was 3.8 μg/g of dust. Old carpets had the greatest effect on the ergosterol levels. The ergosterol results did not correlate with specific or total fungi. The mean temperature in the bedrooms was 18°C and the mean relative humidity was 60%. Total fungal propagules ($p<0.001$), *Cladosporium* ($p<0.001$) and ergosterol ($p=0.01$) were significantly associated with the season in which they were sampled. The absolute humidity was a significant independent predictor of total viable spore counts but not for ergosterol. The authors conclude that high indoor fungal exposures were associated with poor ventilation, infrequent vacuuming, and the presence of pets, visible mould and old carpets (Dharmage et al., 1999). No actual values for absolute humidity are given it is just noted that it is a combination of the temperature and relative humidity.

Mould related problems were identified in 23 public buildings in Denmark. A pilot study was initiated to elucidate these problems from 72 mould-infected building materials sampled from these buildings. Water damage was found to have occurred over many months, mainly through roof leakage, but also via rising damp and defective plumbing, which resulted in the mould growth. The materials most vulnerable to mould infestation were organic products containing cellulose (i.e. jute, wallpaper, cardboard and wooden materials). The mould genera most frequently observed were *Penicillium* (68%), *Aspergillus* (56%), *Chaetomium* (22%), *Ulocladium* (21%), *Stachybotrys* (19%) and *Cladosporium* (15%). The mould species most frequently occurring were *Penicillium chrysogenum*, *Aspergillus versicolor* and *Stachybotrys chartarum*. In field measurements, several trichothecenes (secondary metabolites of moulds) were detected in each of four building materials (wood, insulation materials with canvas, linoleum, and gypsum boards) infected with *Stachybotrys*. The team also tested for mould derivatives in the laboratory and measured production of satratoxin H and G from *Stachybotrys* and carcinogenic mycotoxins from *Aspergillus versicolor*, both of

which were grown on gypsum boards. Water activity (a_w) was the most important factor for mould growth (Gravesen et al., 1999).

Using the same data set from an earlier study, (Emenius et al., 1998) the authors looked at whether windowpane condensation (on double-pane windows) and indoor vapour contribution ≥3g/m^3 could be used as indicators of minimal air change, high humidity and HDM allergen concentrations in mattress dust. During a 14-day period temperature, relative humidity, ventilation rate and VOC were measured simultaneously. Mattress dust was also collected. The RH and T were converted to a vapour ratio using the Mollier diagram and the moisture expressed in g/kg of dry air. Water vapour both inside and from outdoors was also expressed as absolute humidity in g/m^3. The difference between these concentrations constituted the indoor vapour contribution. Defective ventilation was considered any value below 0.5 ACH; excessive humidity as values ≥7g/kg and ≥45%RH, and HDM concentrations higher than or equal to 2μg/g in mattress dust. The authors found that windowpane condensation and low indoor vapour contributions less than 3g/m^3 during the Swedish winter are true markers of a dwelling without humidity or HDM problems. These markers correctly classified more than 70% of the homes (Emenius et al., 2000).

Sixteen dwellings were used to investigate the effects positive input ventilation (PIV) units had on the indoor environment. The units were set to a fixed flow rate of 38*l*/sec (a ventilation rate of approximately 0.7 ACH). One house was used as a test house (timber-framed end terrace) at the British Research Establishment (BRE) site in Garston. The other 15 homes were located in Merthyr Tydfil and Aldershot. Temperature, humidity and the weather were monitored for several months at all locations. In the test house the unit was tested over four periods with internal doors either open or closed, and the fan on or off, to investigate any influence on performance this may have. A tracer-gas decay test (using sulphur hexafluoride) was used to indicate indoor air movements and ventilation rates. In the occupied homes, participants were asked about any condensation and ventilation problems both before and after installation of the fans. Excess vapour pressure was used to indicate humidity changes; it is the difference between the indoor partial vapour pressure and the outdoor value. The cycle of three weeks on and three off was interrupted by the tenants limiting access or by them not wanting the units turned off. The PIV was found

to be effective in reducing the RH in the tightly built test house by 10%. When looking at the absolute humidity excess the PIV was found to be effective throughout the home when doors were open but only effective upstairs when doors were shut. In the occupied homes, the effect was less clear. It appeared that the homes with the highest initial humidities (in Merthyr Tydfil) benefited the most (less window and wall condensation) and the newer, initially drier homes in Aldershot the least but the values varied within the homes from room to room. In all the houses, the attic space, which was the source of the input air, had higher humidity levels than the outdoor air indicating some leakage from within the homes. The occupants were more impressed with the PIV units than the results indicated (Stephen, 2000).

Forty homes of asthmatics attending an asthma clinic in Southampton were enrolled during 1994. The object of the study was to determine if mechanical ventilation and high efficiency vacuuming could reduce HDM levels in the homes. The homes were randomised to four categories based on baseline humidity levels: both, the ventilation unit and vacuuming, ventilation alone, vacuuming alone and no intervention. The homes were monitored for 12 months and included: RH & T measurements, mite numbers and Der p1 concentration. Homes using the mechanical ventilation units achieved significantly lower humidity levels than those without ($p<0.001$) with associated reduction in mite numbers ($p<0.05$) and Der p1 levels. The addition of vacuum cleaning enhanced the effect. Unfortunately, the improvements were not sufficient enough to see improved symptoms (Warner et al., 2000). The authors used g/kg of water vapour to indicate the differences between indoor values before and after compared with outdoor values but referred to this as absolute humidity, which is a term usually given to a density measure (g/m^3). No actual start and end dates are given for the study period.

A case study looked at HDM-sensitised children who were selected from 30 single-family homes living in the same area of Stockholm, Sweden. Samples and inspections were taken during a 2-week period in December 1989. The homes were split into 13 homes with HDM≥2,000ng/g of mattress dust and 17 with low levels (≤1,000ng/g dust). Each home was inspected for building type and internal environment was examined for ventilation, thermal climate, and air quality (using formaldehyde and total volatile organic compounds) and for water damage or mould growth. Measurements of

ventilation over one week were made using a passive multiple tracer gas method. From these, ventilation rates were calculated as an air change rate per hour (ACH). Air temperature and relative humidity measurements were taken twice over a one-week period. Absolute humidity levels were calculated for indoor and outdoor air and the difference recorded as Additional Humidity (AH). The home ventilation rates were very low with a mean ACH of 0.2 with a range from 0.1-.06. There was a significant association between the AH and the concentration of HDM allergen in the mattress dust (r_s=0.49; p=0.008) but not in the bedroom floor dust samples. Nine of 11 homes in the high infestation group lived in a single storey dwelling as opposed to 2 of 18 in the low infestation group (p=0.001). Single storey homes were significantly associated with higher concentrations of HDM in mattresses (p=0.002) and in floor dust ($p \leq 0.0001$) and had a tendency toward lower ventilation rates than multiple storey homes (p=0.08). For regions with a cold winter climate the air change rate of the home and infiltration of outdoor air into the bedroom appear to be important for HDM infestation (Sundell et al., 1995).

2.7. Measuring moulds

Measurement of moulds is difficult. There is at the present no universally accepted method of determining the quantity of mould in the air or on a surface. With these difficulties in mind it is problematic to then attempt to make correlations between moulds and how they may affect asthmatics.

Whether measured in air or from surfaces the following need to be considered: a) what to measure (spores, fragments of moulds, glucans, ergosterol, DNA, MVOC); b) the time scale, how long to measure for and on what media to culture the fungi on if necessary, and c) the diurnal and seasonal effects both indoors and from outside. These are all features of fungi identification problems that need further investigation.

Studies of spores in the air in the UK were pioneered by Hirst (1953) and Gregory and Hirst (1957) at Rothamsted, where surveys were run for short periods of time and their particular interests were in the dispersal of plant pathogens.

The main methods of airborne spore measurement have depended upon entrapment on agar media and then incubating them so that counts can be made of the developing colonies. However, the proportions of propagules that are non-culturable vary with

each species and the total that can be cultured can be as little as a few percent. Non-culturable spores may be as effective as culturable spores in triggering allergenic and non-immunological mechanisms. It is prudent and necessary for analysis to account for some measure of total fungal burden, such as measuring ergosterol or β-D-glucan concentrations.

Once the spore numbers have been counted they can be transferred to new plates for further growth and with sufficient expertise can be identified to species level. The problems with this gravitational method are that it favours the larger spored moulds, which have a higher deposition rate. A volumetric sampler is therefore preferred, such as an Andersen sampler (Andersen, 1958), in which the air is drawn onto the culture plate causing the spores to be impacted onto the agar surface. To achieve a high level of sampling efficiency, air is drawn through the sampler at a rate of 28.3 *l*/min so that sampling has to be restricted to relatively short periods of time (usually <10min), to avoid the culture plate becoming overloaded. If just one sample is taken in a day then it is possible to miss changes in spore concentration associated with diurnal fluctuations or changes in meteorological conditions. The choice of agar and the humidity can also have an influence on the types of spores that will germinate as moulds vary in their growth requirements. This can be used to advantage when studying a particular mould (Mullins, 1994), but can lead to difficulties when trying to take a census of all fungal spores in the air. Growth on culture plates is also restricted to imperfect or asexual stages of fungi, which results in the absence of most Ascomycetes, Basidiomycetes and parasitic fungi which only grow on host tissue.

The alternative method of conducting surveys of airborne fungi is to collect the spores onto glass slides for microscopic identification. As with the previous example, a suitable apparatus, such as a Hirst trap or Burkard trap (Hirst, 1952) must be used to impact the spores on to a layer of adhesive on the slide surface. These units can run for 24 hours or for a seven-day period and thus have one advantage as the results are not biased diurnally or by meteorological variations. It is however, difficult to identify the spores as many are smaller than 10μm in diameter, which limits the specificity of the technique. It does however allow for identification of Ascospores, Basidiospores and spores of parasitic fungi such as rusts, smuts and mildews. Burge et al. (1977) compared spore concentrations obtained by spore trapping with those obtained using culture plate techniques and found that as *Cladosporium* spore concentrations

(assessed by spore trapping techniques) rose, culture plate methods progressively underestimated prevailing spore concentrations, giving low estimates (20-40%) at levels below 100 spores/m^3 and falling to 5% at levels above 500 spores/m^3. The Composting Association has published a protocol for sampling of airborne microorganisms which targets mesophilic bacteria and the fungus *Aspergillus fumigatus* as appropriate indicators of the composting process (Composting Association, 1999).

The first part of a study of 11 homes in the autumn of 1987 looked at determining what the optimal method for the enumeration and identification of viable fungi sampled from within these homes was. A comparison was made between six commercial available air samplers using four culture media, see table 2.3, below.

Table 2.3. Examples of indoor mould samplers. All are mains operated except where noted.

Name of sampler	Description
Slit	Air drawn via pump through a slit orifice directly onto a rotating media.
N6 Andersen	Air drawn via a pump through a sieve plate over a media.
Surface Air System (SAS)	A portable unit: air is drawn through a single stage sampling head over culture medium.
Reuter Centrifugal (RCS)	Air drawn through a fan and the spores are impacted onto a medium coated strip inserted on a drum surrounding the fan.
Gelatin Filter (GF)	5cm diameter gelatin filters are place in a holder connected to a pump.
Open Petri dish (OPD)	10cm diameter dishes containing culture media are exposed to the air over time.

To prevent overloading, the length of time sampled and the rate of flow of the units varied as per sampler type. The four culture media were: Malt extract agar (MEA); Dichloran Glycerol agar (DG18); Oxytetracycline Glucose Yeast Extract agar (OGYA) and Dichloran Rose Bengal Chloramphenicol agar (DRBC). All samples were incubated for 4 days at 25°C and the number of CFU counted. After counting, spores

or mycelium were transferred to appropriate media for species identification. The Slit and N6 Andersen samplers in combination with DG18 and MEA gave the best precision and the highest yields of CFU/m^3 and number of species isolated. In the second part, 46 homes were investigated for dampness using the N6 Andersen sampler with DG18 media and with a questionnaire to assess the home conditions. All the measurements were re-sampled five weeks after the initial period to assess the variability over time. In total, 19 mould genera were found. Six genera were found in almost all homes: *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Penicillium* and *Wallemia*. Variation was found to be much smaller between homes than within them (Verhoeff et al., 1990).

The authors followed up this study with a second one in May 1989 using similar sampling methods but adding 84 randomised homes to the sample. The original 46 homes all contained visible mould. Sampling was done by the N6 Andersen both indoors and outside. This time, three species dominated: *Cladosporium*, *Penicillium* and *Wallemia*. The results had a low predictive value lacking reproducibility, which limits their use in epidemiological studies of the relationship between exposure to moulds and respiratory symptoms. The number of CFU/m^3 varied considerably between genera and between indoors and outdoors. The presence of mould was only weakly related to dampness. Highly statistically significant values were found between CFU yield obtained with the OPD and the CFU/m^3 yield obtained from the N6 Andersen sampler using ln-transformed data with the Pearson correlation coefficient (0.78 living room; 0.65 main bedroom). The number of species isolated from OPD was significantly lower than from the pump operated N6 Andersen (Verhoeff et al., 1992).

Mould growth in buildings can be considered a major health issue but most investigations of indoor air spora use culture-based methods for identification and quantification. Culturable organisms comprise only a small fraction of the total quantity of particulates in the air and thus many are not being quantified. Long-term measurements of airborne fungal biomass may be more relevant to epidemiological studies than using the total spore count. Airborne biomass has been measured using (1-3)-β-D-glucans found in hyphae and spores, and ergosterol (a sterol from the fungal membranes) as an indicator of exposure. Glucans are known to cause inflammation reactions in lymphocytes, affect interleukin-1 secretion via T-lymphocytes, stimulate

bacterial and tumour defence mechanisms and cause a decrease in numbers of pulmonary macrophages. Ergosterol is stable under air-dry conditions and can thus be collected on polycarbonate filters. Other methods included in some case studies, patients' serum has been used to detect specific spores on slides from samplers, and both highly specific and less specific antisera could be used either with a fluorescent antibody technique or with ELISA. Solid-phase polymerase chain reaction (PCR) could be used to detect pathogens of harmful species and in some fungal groups secondary metabolites, volatiles and mycotoxins may be used for identification (Flannigan, 1997).

During the winter of 1998-1999, seventeen homes from Quebec in eastern Canada were enrolled because of self-reported health problems following water damage in their homes. This period was used to minimise the influence of outdoor spores, which were snow covered. Samples were taken from the main living area or from the ill occupant's bedroom. Mould growth sources were analysed in every room of the sampled storey. A six stage Andersen microbial sampler was used to collect the air samples about one metre from the floor. The sampler was loaded with rose Bengal agar and with malt extract agar. The flow rate was set at 28.3*l*/min and ran for 5 and 20 minutes with each media. These were then incubated for 7 to 10 days at 30°C. Moulds were identified from the media to genus or group level. Temperature and humidity were recorded. Surface samples were taken using scotch tape, cotton swabs and on contact plates (Replicating Organism Direct Agar Contact, Rodac). When available, bulk samples of the infected substrate were sampled. The study demonstrated that, surprisingly, some of the wet moulds (e.g. *Stachybotrys*) were found more in air samples than from surface samples and some dry spores (e.g. *Aspergillus fumigatus*) were more easily isolated from surfaces. The authors concluded that both air and surface samples are necessary to evaluate mould diversity in water-damaged homes and that the number of mould growth areas is a good predictor of airborne mould concentration (Duchaine & Mériaux, 2001).

Summary

The factor that most determines fungal growth is the availability of moisture but temperature is also important. The growth limits for different genera in terms of RH and T and for different substrates have been extensively investigated. It is beneficial to keep the relative humidity and temperature of a dwelling at levels that maintain a water activity below 0.80 to curb mould growth (Grant et al., 1989). There are thousands of genera of moulds all with differing growth requirements and predicting their likely growth in buildings seems daunting. Moulds are found in homes much cooler than the laboratory conditions used in modelling their growth. The humidity requirements for mould growth are met through occupant behaviour and minimal ventilation. These factors affect the available free water held in the building materials (i.e. plaster, brick, gypsum board, wood products, furnishings etc).

The English Housing Condition Survey reported mould to be present in 15% of homes surveyed in 1996 (ODPM, 1996). Mould is quite common in British homes, and has been reported in 20-30% of the homes of people with asthma or recent wheeze (Williamson et al., 1997; Burr et al., 1988; Strachan, 1988). The relative small size of British dwellings, high occupant density, low internal temperatures and minimal ventilation has led to a major moisture problem that continues. Extraction from wet areas (bathroom and kitchen) and increased background ventilation has improved the situation modestly. The scientific literature provides evidence that ventilation has an effect on indoor humidity levels. A number of studies examined the effect of mechanical ventilation and in general the proportion of homes with higher indoor humidity were significantly greater in homes with the number of air changes per hour were less than 0.5 compared to those with ACH >0.5. There is also good evidence that the occupancy of a dwelling and the water generating behaviour of the occupants has a marked effect on the indoor humidity levels.

There are also problems with monitoring airborne microbial populations to assess the indoor air quality as the methods used are lacking in precise, accurate and representative estimates of bioaerosol exposure. Air sampling for bioaerosols, including fungi has been used for many years using air samplers followed by analysis of the culture using microscopy on various agar media (Buttner et al., 2002). However, air sampling cannot guarantee that the air is free from contaminants as the organisms

may become re-distributed from floors and other surfaces through daily activities. It is thus prudent to sample surfaces in addition to air sampling to locate and identify any biocontamination (Stetzenbach et al., 2004). The growth of these cultures can be affected by the sampling procedure, transportation system, storage method, and laboratory culture. Thus, it is possible to underestimate the bioaerosol populations. Fungi are capable of causing health effects whether they are culturable or sterile mycelium or spores (Levetin, 1995). Other methods of assaying bioaerosols using (1-3) β-D-glucan or ergosterol can give an indication of fungal contamination but not of specific moulds. As a result of the problems mentioned previously, it is necessary to develop new sampling and analysis techniques for bioaerosol detection.

In a review of the epidemiological evidence, the American National Academy of Sciences concluded: "There is sufficient evidence of an association between fungal exposure and symptom exacerbation in sensitized asthmatics. Exposure may also be related to non-specific chest problems." (IOM, 2000). It cannot be assumed that these associations are directly causal; a considerable number of confounders such as lifestyle, socioeconomic status, house dust mite infestations, cold interior temperatures, reporting bias or even publication bias could contribute to the results. In one study, the exposure of individuals to *Cladosporium* had doubled within two years and they were more likely than others to have had an asthma attack in the last twelve months. This provides better evidence of a causal relationship than a cross-sectional association, although a deteriorating indoor environment was a marker for some other factors that could cause or aggravate asthma (Matheson et al., 2005).

The Government white paper "Saving lives: our healthier nation" recognises housing as a key health determinant (SSH, 1999). Clearer evidence of moulds' health effects could be provided by a controlled trial and would be useful in providing advice for patients with respiratory diseases. It may also be useful in providing local authorities with guidelines for prevention of moulds and highlight the need for building regulations to address the hygrothermal conditions determining mould growth.

Chapter 3 - Methodology

3.1 Sample selection and randomisation

This study was devised to evaluate, by application of a randomised controlled trial, the effect of mould remediation upon the health of asthma sufferers in domestic residences. Ethical approval was gained through the Bro Morgannwg and Bro Taf local ethics committees. The members of the Ethics Committee raised no objections to the proposed study but did suggest some modifications to the study Information Sheet and the sheet giving Informed Consent.

The majority of suitable participants were identified through their own general practitioners (GPs). A General Practice was identified through connections with the Heath Hospital, Cardiff and from that practice other surgeries were identified. The eleven General Practices involved in the study identified all patients on their practice asthma register, and then wrote to each individual using a letter supplied by the researcher. This letter introduced them to the study and invited them to contact the researcher should they wish to participate and if they believed their home had evidence of mould (Appendix 4).

Letters were also sent to people listed on a Neath and Port Talbot (NPT) County Borough Council housing register as residing in a home with mould contamination. Finally letters were sent to a random sample of households in the NPT Borough Council Area and the letter sent out asked householders to complete and return a short questionnaire, which enquired if they suffered from asthma or wheeze and if they had any visible signs of mould or condensation in the home (Appendix 4). Also a number of possible participants contacted the research team directly as a result of publicity surrounding the project. The numbers of households contacted, replying and assessed as eligible from these sources are listed in Figure 3.1. Of those who replied the majority were ineligible on the basis of the mould status of their home.

In total 209 possible participants (i.e. those having agreed to participate, reporting having asthma and mould in the home) were then contacted by the research team. The purpose of this call was to confirm their eligibility, outline the purpose of the study and the commitment they would have to make to the study (i.e. stay in the study for one year). A home visit was then booked for those confirmed as eligible and who agreed to be included in the trial. As many of the participants had work

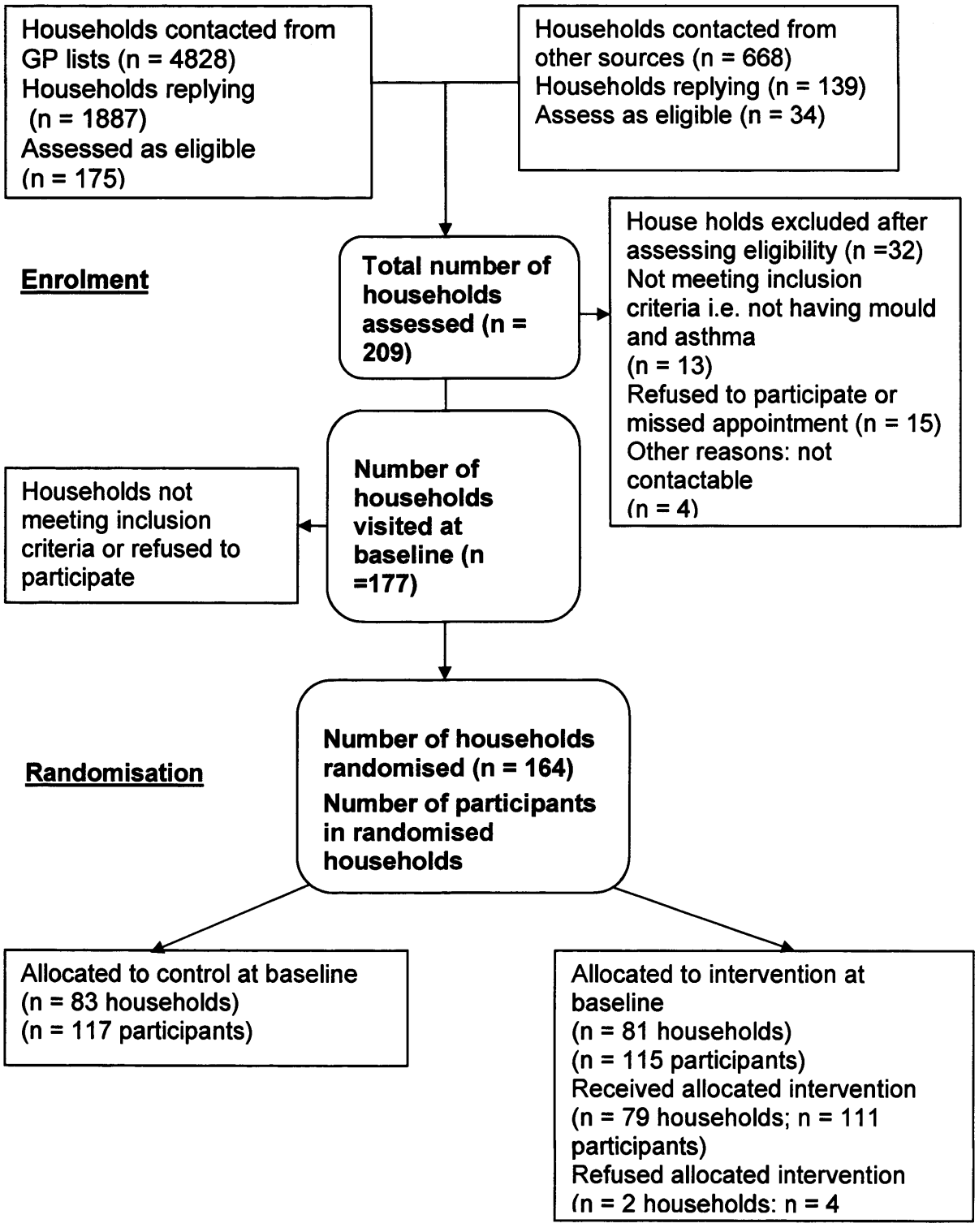
and family commitments, arranging an appointment was often difficult, this also meant that for many a suitable time was after they had returned from work, and due to the wide geographical study area home visits often involved a great deal of travel.

During the home visit the research team further discussed the study outlining the design and its goals. If subjects agreed to the visit continuing and were eligible then informed consent (Appendix 4) was obtained from each asthma sufferer for participation in the study, or in the case of children parental consent. For those who did consent a standard house visit was conducted, where a structured health symptom questionnaire (Appendix 1), relating to chest symptoms, and a site sheet (Appendix 5), relating to housing conditions, were used to interview subjects. Also a skin prick test was administered to determine their allergic status, as well as taking a 24 hour air sample. In total 177 possible households were visited of which 92.7 % were found to be eligible, consent was obtained and were entered in to the trial.

Upon completion of the baseline visit the 164 households recruited, containing 232 asthma sufferers were randomised after the first visit to either the control or intervention groups. A randomisation process was carried out by a departmental statistician. The randomisation process was stratified by three housing types (namely; semi-detached and detached, terraced or flats), by means of serially numbered sealed envelopes.

For each stratum a list of paper slips were produced labelled to treat or not treat, with 50% allocated to each stratum but divided into blocks of 10 so that each block was split between 5 treat and 5 not treat. This was to ensure that whenever recruitment stopped the sample would be reasonably balanced within each stratum as well as overall. The stack of envelopes from each stratum was kept in a locked cabinet. At all times the researchers were blind to this randomisation process receiving an envelope dictating whether to treat or not to treat. Accordingly the householder was then informed of the group to which they had been assigned, and their details recorded on an Access database.

Figure 3.1. Flow diagram representing the enrolment and randomisation of households and asthma sufferers to the trial.



3.2. Baseline Measurements

The following were recorded at baseline:

3.3. Chest Questionnaire and Peak Flow Measurement.

3.4. Skin prick tests (SPT).

3.5. Visual assessment of mould.

3.6. Measurement of mould.

3.7. Measurement of Der p1.

3.8. Measurement of Relative Humidity and Temperature.

3.9. Household Built Form and Characteristics, and People Behaviour.

3.3. Chest Questionnaire and Peak Flow Measurements

The chest questionnaire used in this RCT was based on the ISAAC study questionnaire (ISSAC 1993 in Asher et al., 1995) [Appendix 1]. It was designed to record current symptoms of wheezing and asthma as well as medication use. Some additional questions were added from the ECRHS and others, which included presence of hay fever, eczema and frequency and history of smoking in the home. There were three versions used based upon the baseline questionnaire administered by the researcher at the participant's home. The other two had additional questions added both for the six-month postal questionnaire and the 12-month research administered one. The additional questions included information about any changes to the home, any redecorating, as well as modifications to flooring, improvement of windows etc. as well as information on changes to the asthmatic's bed. The participants were given the questionnaire on the first day visited and asked to fill it in by the following day's visit and it was then checked for completeness by the researcher.

A standard peak flow diary card was used for two weeks to record the peak expiratory flow rate (PEFR) of the participants. The highest of three exhalations is considered to be the best indicator of PEFR variability. Three readings were taken in the morning before any asthma medication was used and three times in the evening just before going to bed, following standard procedures (Klein et al., 2001); the highest value recorded was used in analysis. A Clement Clarke Standard Mini-Wright peak flow meter was used for adults and a Clement Clarke Low Range Mini-

Wright was used for the children. The flow rate marker on the unit is first set to zero, the unit held horizontally in front of the mouth whilst standing. A full breath is taken and with the lips firmly around the mouthpiece, a fast hard out breath moves the marker. This value is recorded for the appropriate date and the procedure followed two more times. Any symptoms of cold, cough or wheeze were recorded for each day the PEFR was taken. This procedure was performed at baseline, six months and at 12 months. The asthmatics were given a self-addressed stamped envelope in which to return the diaries once they had completed them. Follow-up phone calls were occasionally necessary to attempt to increase the number of returns. As an indication of asthma severity, the variability of each asthmatic's airway resistance was expressed as the coefficient of variation (CV) of these values, both for the morning and then for the evening exercises (Andriessen et al., 1998).

3.4. Skin Prick Tests (SPT)

It was decided early in the RCT that skin prick testing was to be used over serum blood IgE sampling as a means of identifying allergen reactivity in the individuals. This was in part due to the relative ease of taking a SPT and the difficulties associated with obtaining a blood sample from children. Subjects underwent a skin prick test to assess sensitivity status for various purified allergens including Der p1, *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, cat dander, and grass pollens plus a negative (saline solution) and positive (histamine) control.

After giving consent, fine pen marks were made down the inside of the forearm of each subject approximately 10 to 15 mm apart and a drop of allergen placed next to each numbered pen mark, in order to identify any reaction. A new, sterile lancet was used on each patient to gently prick (abrade) the skin through the drop of each of the allergens. The lancet was wiped with a clean tissue in between each allergen, so to avoid cross-contamination. After the skin had been pricked at the site of each allergen, the skin was dabbed with a tissue to absorb any remaining allergen. The subject was then left for a period of approximately 15 minutes for any reaction to develop, during which they were informed not to scratch the area. After about ten minutes the area was examined for any reaction – a reaction would appear red with a white raised bump (wheal). If no reaction was observed, it was left for another five minutes. Any wheals identified where then measured.

Specifically, the diameter of each wheal was measured in millimetres (mm) using a transparent scale with fine millimetre circles engraved on it and placed against the wheal; this information was then recorded on the site sheet. A positive reaction was considered as a wheal greater than 3 mm, when the reaction to the negative control was subtracted. This has frequently been suggested and accepted as a positive reaction to an allergen (Ronchetti et al., 2003; Jones et al., 2002; Chinn et al., 1996). The wheals would usually subside within a few hours. Anaphylaxis was discussed but no known reactions have been recorded from skin prick testing.

3.5. Visual Assessment of Mould

Areas of visible mould were identified anywhere within the interior of the home. Most of the fungi were found on walls, ceilings, window frames and occasionally on the floors of the structures. Other sources of mould growth included carpets, sofas, furniture and occasionally clothing or shoes. Removal of wallpaper from walls was necessary in some cases with permission from the occupants. Digital photographs were taken as and where possible. A scale rule with colour chart was used as a reference alongside the mould. A written description also accompanied the photograph, which was numbered as to the visit date and room involved. A close-up and wide-angle photograph were taken if possible to show the extent of the fungal growth. From these, the areas of mould were estimated and recorded in square metres. In addition, a photograph of the exterior was taken at this time to help with later identification of the home. From this data and with the help of notes taken a mould density classification was developed using a modification of a mould density classification developed by Williamson et al., 1997, into 1 of 6 categories. Areas with minimal density of mould fall into the first 3 categories (0, Absent; 1, Trace; 2, Spotty). These can be found around window frames, pipes, skirting boards, shoes etc. Those areas in the higher density levels (3, Obvious and discrete; 4, Obvious and patchy; 5, Obvious and widespread) are very noticeable areas, which tend to occur on walls, ceilings, furniture, floors etc. Example photographs of each mould level can be found in Appendix 3. As well as looking at the estimated area of coverage and the mould density scale separately, a visual mould index was created using the product of these. All the figures will be minimal values as areas behind wallpaper, cupboards and under painted surfaces were hidden and were therefore not included in the assessment.

3.6. Measurement of Mould

3.6.1. Swab Samples

Upon arrival at the property, the householder directed the researcher to the room/rooms infected by mould. A visual inspection of the property was carried out by the researcher to ascertain if any other areas of the home were affected by mould. Each separate area of mould in each room affected was sampled using a sealed sterilised swab (manufactured by Copan). Using a sweeping action the swab was rolled over the area of mould in order to collect an adequate sample for analysis. The swab was then re-sealed and labelled appropriately; detailing the house identification number and the room the swab was taken from, together with the date taken and whether the sample was taken at the baseline visit or the 12 month revisit. Samples were transported back to the department, where they were stored at approximately 5°C until they were sent for analysis. The samples were then couriered to a mycologist on a weekly or fortnightly basis depending on the quantity of samples collected. The swabs were plated out onto corn meal agar Petri dishes and incubated at room temperature (~21°C) until the moulds had developed adequately for identification; this took from a week to one month. Corn meal agar was used as it has the advantage that moulds sporulate readily on this media and few moulds fail to sporulate. Identification was carried out using microscopic techniques at X100 magnification and where necessary spores were removed using sterile needles and mounted for examination at X400 magnification. The following references were used for identification: Barnett, 1960: Ellis, 1971: von Arx, 1974. The mycologist then estimated the relative amount of each mould genera present from each swab sampled.

3.6.2. Ergosterol measurement and analysis

A sound insulated box containing a Gast centrifugal pump was situated and run in the living room of each property sampled for a period of approximately 24 hours. The pump and the box had been inspected and tested against electrical and mechanical failure using thermal cut-outs and surge suppression features by the appropriate university body (see Appendix 6). In a clean room, Millipore 0.4 µm

polycarbonate filters (37mm diameter) were removed from in between non-reactive protective papers and inserted into the holders in advance of the visits. This work was conducted in batches, with filters removed from their supply container and placed in the plastic canister using plastic tweezers. The filter was used as the substrate on which to collect air particulates, and was held in place using a plastic filter canister, which was attached to the sampler via vinyl tubing. The tubing was fed through a tripod, which held it securely in place, the stand was adjusted to a height of approximately one meter, and the filter canister was then connected to the tubing using a nylon attachment. The pump was adjusted to sample at a rate of 12 litres per minute gauged against a calibrated flow meter (Environmental Monitoring Systems: 1-20 *l*/min range). The pump was fitted with a timer, so the correct sample period was always recorded. After approximately 24 hours the researcher returned to the property to collect the equipment. The flow meter was reconnected, so to record the current sample rate and then the pump was switched off. This together with the pump run time was recorded on the site sheet (Appendix 5). The filter which remained in the canister was then disconnected from the pump and labelled appropriately; detailing house identification number, visit number, date, sample duration and the flow rate at the start and the end. Samples were then transported back to the department and stored at approximately 5°C until they were prepared and sent for analysis. The samples were removed from the fridge and taken to the clean room in preparation for mailing to the laboratory for analysis. Carefully, they were removed from the canisters and placed between two of the protective papers in which they were supplied. The three layers were then sandwiched in a jig specifically designed and built for the purpose of cutting the filters exactly in half. It was thought worthwhile holding back half the sample for future analyses. Each half was placed in an aluminium canister with a lid and labelled as 'a' or 'b' plus the house identification and sample date. One was stored back in the fridge and the other sent to CABI Biosciences in Surrey for analysis of ergosterol.

Ergosterol High Performance Liquid Chromatography (HPLC) analysis.

Ergosterol is the major sterol component of fungi but it is only found to a limited extent in higher plants (Weete, 1973). Since the initial suggestion by Seitz et al. (1977), ergosterol has been used as a bio indicator of the level of fungal contamination throughout many systems. The measurement of ergosterol in

samples requires extraction using a solvent system and then measurement using HPLC or Gas Chromatograph and Mass Spectrometer (GC/MS) systems. For the purposes of this project the method used was based on that devised by Young (1995) using a microwave assisted extraction procedure combined with an HPLC detection. This application of microwaves during the extraction has enabled greatly reduced samples to be used in the measurement of the ergosterol concentration.

Materials and equipment

Petroleum ether analytical grade (99%, 60–80°C): Ergosterol Sigma E-1650: Methanol (HPLC grade): Sodium Hydroxide, all from Fisher Scientific.
HPLC column Novapak C18, 205nm.

Experimental Procedure

The filter is placed in culture test tubes fitted with Teflon lined caps. To the filter 2 ml of methanol is added. To this suspension 0.5 ml of 2M Sodium Hydroxide is added and the sample vortexed for a few seconds. The samples are then microwaved for 60 seconds using a 600W microwave oven and allowed to cool to room temperature before proceeding. To the samples 2 ml of petroleum ether (60/80) is added and the samples vortexed. This is repeated three times until a total of 6ml has been added. The samples are evaporated to dryness, sealed with para-film and stored in the fridge. Prior to HPLC measurement the samples are re-suspended in 200 µl of methanol.

HPLC conditions

Solvent	HPLC grade methanol
Flow Rate	2ml/min
UV	282 nm
AUFS	0.005
Injector Volume	100 µl

Three standards of 90% Ergosterol were run prior to each test and these were used to calibrate the machine. Standards were 0.1 µg/ml, 1.0 µg/ml and 2.25 µg/ml. Ergosterol retention time was approximately 8.5 minutes.
On the basis of the data provided by CABI the approximate precision of the assay is 4% and the lower limit of detection is 0.005 µg. The paper from which the

method was taken quotes a detection limit of 500 pg or 0.0005 µg, and a method detection limit of 0.002 µg/sample. The quoted coefficient of variation for the precision was 4%.

3.6.3. Hirst Trap and Spore Identification

Standard glass microscope slides were exposed in a Hirst Automatic Volumatic Spore Trap (Hirst 1952). The mounting agent used was glycerine jelly (Appendix 9) and this was also used to mount cover slips on the slides after they had been exposed. Air was drawn through the equipment at 10 *l*/min so that this transect width enabled a conversion factor of 10 to express spore counts as spores per cubic metre of air. The slides were examined under a microscope at 400x magnification, and a longitudinal transect 37 microns wide was scanned and all pollens and spores seen were identified and counted.

Pollens and spores were identified according to their morphological features, with reference to: Nilsson (1982): Gregory (1973): Barnett (1960) and Hyde & Adams (1958).

Two locations were identified for placement of the Hirst trap. The first was on the roof of the Neath Civic Building in Neath, South Wales. The second was on the roof of one of the buildings on the Llandaff site of the University of Wales Institute Cardiff (UWIC). Samples were collected from the sites once per week to coincide with home visits. A mycologist advised the team that a weekly sample was sufficient to have a satisfactory cross-section of the pollens and spores present throughout the three-year sampling period. He also thought that two locations were adequate for our needs in supplying a good example of the current fauna throughout South Wales.

The more portable Burkard unit using a stick tape system has now replaced the aging Hirst trap that has been used as the standard volumetric pollen and spore trapping method for decades.

3.7. Measurement of Der p1

The bed is often cited as the most important domestic location for HDM exposure. The factors leading to this are the relatively high concentration of allergen at the site, the closeness of the head to the allergen source, the proportion of time spent in the bed and the opportunity for dust disturbance (Sidenius et al, 2002; O'Meara & Tovey, 2000; Dreborg, 1998; Frederick et al, 1997). Exposure from this source is low grade and chronic, occurring mainly overnight (Custovic et al, 1999). Although the methods currently used to assess exposure are in their infancy, sampling of reservoirs of house dust and subsequent measurement of the concentration of Der p1 allergen in dust samples by species specific monoclonal antibody ELISA test are at present the best index of exposure available (Platts-Mills, 1992; Tovey et al, 1981). The most prevalent species of house dust mite in the UK is *D. pteronyssinus*, and the major allergen this species produces is Der p1 (Arlian et al, 2002). On the basis of the above information it was therefore decided that these methods would be used in this RCT.

Using a hand held portable Dirt Devil vacuum cleaner ('Handy-Zip' model), a one square metre area of uncovered mattress was vacuumed for a period of two minutes. The one square metre area was marked off using a collapsible jig. Dust samples were also collected from the floors of the asthmatics' bedroom using similar procedures. The vacuum bag was carefully removed from the appliance and placed in a 'zip-seal' plastic freezer bag, which was then labelled and transported back to the department, where it was immediately stored at –20°C until it was prepared to be sent for analysis. This procedure was followed at baseline and at 12 months although only baseline samples were analysed. Prior to samples being sent for ELISA analysis, all baseline mattress dust was transferred from the paper vacuum bag into the plastic bag, which was then vigorously shaken to fully mix the sample. The bedroom floor samples were frozen in storage for later analysis as funding permits. The samples were then split into three aliquots of similar amounts. After separation into the aliquots, one was then packaged to be sent to the Building Research Establishment (BRE) for analysis for the concentration of Der p1. The further two aliquots remain stored at –20°C for any future investigations. Der p1 was analysed using an ELISA plate procedure based on the method of Luczynska et al. (1989). Briefly, 100µl of diluted allergen standard or house dust was added to Anti-Der p 1 mAb coated plates and

incubated for 1 hour at room temperature. The wells were washed with PBS-T and incubated for 1 hour at room temperature with diluted biotinylated anti-Group 1 mAb. The wells were then washed with PBS-T and diluted Streptavidin – Peroxidase was added. The mixture was incubated for 30 minutes at room temperature. The wells were then washed with PBS-T and the assays developed by adding 100 µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 containing a 1/1,000 dilution of 30% H_2O_2 (i.e.10µl/10ml ABTS). Finally the plate was read when the optical density at 405nm reached 2.0-2.4. The sample optical density was then compared to the control curve of the allergen standard for determination of the Der p1 concentrations.

3.8. Measurement of Relative Humidity and Temperature

Relative humidity and temperature measurements were taken using Tiny Tag Ultra (TGU-4500) data loggers (Omni Instruments, Dundee, UK). These data loggers are able to store 7,900 data readings over 5 months at 15 minute intervals. They are able to record temperatures using a 10K NTC thermistor over the range of -30°C to 50°C (accuracy ± 0.2°C between 0 & 50°C; resolution 0.25°C at 0°C). The relative humidity is measured using a capacitive sensor over the range of 0% to 95% (accuracy ± 3% at 25°C; resolution in excess of 0.5% relative humidity). Readings were taken at 30 minutes intervals over a minimum of 2 weeks, with the logger being placed between 1.0m to 1.5m above floor level. The loggers were placed in each of the asthmatics' bedrooms as well as one in the living room. After the elapsed time the loggers were picked up from the participants and the data downloaded as text files, which were then uploaded using a purpose built macro in Excel (Appendix 2). Concurrently, information was taken of the outdoor temperature and relative humidity levels, wind direction and strength, and precipitation records from equipment on the roof of the Welsh School of Architecture (WSA) in Cardiff. Average daily atmospheric pressure, temperature and relative humidity records were collected from MET data in Cardiff and The Mumbles (Swansea), and from other web-based sites in the UK, and checked against each other for accuracy. The Mumbles humidity values were always higher as would be expected from a shoreline based station. A correlation test was used comparing the two data sets and it was found that both data sets are very strongly and positively correlated (0.942). It was thought more appropriate to use the

Cardiff MET data since most of the study house locations were located inland. The Cardiff MET data was used to calculate the mixing ratio, a value often used in meteorological work measured in grams of water vapour per kilogram of dry air.

3.9. Household Built Form and Characteristics, and People Behaviour

A site sheet questionnaire was developed that asked questions about the house environment and how the occupants perceived that environment. It included questions on the occupants habits as well as recording the location and necessary details about the equipment used for sampling in the home (i.e. placement of loggers, start and end flow rate of the air sampling pump, skin prick test results, etc.). Questions about the family history of asthma were also written down as well as the number of occupants; house type, storey height, number of smokers, tenure, etc. see Appendix 5 for further details. A similar questionnaire was used at baseline and at 12 months. Skin prick test information was not necessary to repeat at 12 months. Most questions were repeated as a means of observing consistency in the responses.

3.10. Mould Eradication and Intervention

All visible mould was removed from the homes using a proprietary fungicidal treatment supplied by Mould Growth Consultants Ltd. (MGC, Surrey). This involved two surface treatments, RLT Bactdet (a brown aqueous liquid consisting of Sodium dichlorophen plus detergent) for cleaning and sterilising the surface and RLT Halophen (an aqueous liquid consisting of Dialkyl dimethylammonium Chloride plus synthetic polymer emulsions) to penetrate the substrate killing the roots of the mould and forming a fungicidal barrier. MGC Fungicidal Additive was used to add to wallpaper paste or paint as a final surface treatment to stop new fungal growth. All the chemicals were supplied with COSHH data sheets and a notice of approval from the Health and Safety Executive last tested in June 1999. All products were brushed or rolled on the surfaces using the supplied safety equipment. The first coat is diluted as per the directions of one part solution to four parts warm water and applied to the surface. This is left to dry for a minimum of one hour. The second treatment follows using the same mixing and application procedure. The third treatment was left to the occupants to apply during redecoration.

In addition to the eradication it was deemed prudent to install a whole home input ventilation unit (sometimes called a positive pressure fan) as a means of reducing the moisture levels in the homes. An assessment was made by the researcher and by a representative of Nuaire Home Ventilation of Caerphilly, Wales; following this, a Positive Input Ventilation (PIV) unit (Drimaster) supplied by the same company was installed in the attic of the home usually above the stairs (see Appendix 6). If installation there was not feasible, a wall unit (Flatmaster) was installed, usually within the kitchen. If the bathroom conditions showed excessive moisture levels and mould growth then an extraction unit (Genie) was also installed in the bathroom. Qualified engineers supplied by the company installed all the equipment. The units were self-regulating, if excessive heat or cold was detected than the units shut off automatically. The PIVs needed no input from the occupants but a manual was left with them should problems occur. The units run continuously unless the upper and lower temperature limits are met. The engineer adjusted the input speed according to the size of the dwelling. The Drimaster has two washable G4 grade filters that remove pollens and other contaminants from the air; they should not need replacement for 5 years.

3.11. Repeat Measurements – six months

The chest questionnaire and the PEFR diary sheet were mailed to the participants at 6 months with a self-addressed stamped envelope in which to return them. A phone call was made to ascertain receipt and to encourage their return. No other information was collected at this time.

3.12. Repeat Measurements – 12 months

The 12-month return visits were identical to the baseline ones except that no skin prick test was performed. Peak flow diaries were picked-up at the same time as the loggers and the researcher returned to the home if the PEFR sheet had not been completed. The individuals were informed that they would be given the results on completion of the study and thanked for their participation. When all the data was collected the controls received a mould eradication kit (the same as used for the intervention) sufficient to deal with the problem in their home.

3.13. Schedule of RCT Data Entry and Checking

Each household and the occupants partaking in the study were assigned unique identifiers. Four field visits per day were then organised by phone mostly in the evening for the ~200 homes. Between eight and twelve visits per week were desirable but not always achievable. A 24-hour air sample was taken which meant returning to the home the following day around the same time. Various equipment was installed and then collected during the second visit, along with site sheets and health questionnaires, which were transferred to either an Excel or an Access database with password protection or data was downloaded directly from the equipment by the author. The researcher returned to the homes after two to three weeks to retrieve the data loggers and PEFR sheets. Those sheets not finished by this visit date were asked to be returned by pre-paid post. In addition, the data was double entered by secretarial staff and then checked for accuracy once more by a third individual. The database contained information on all the data obtained, although there are missing values for most of the variables. Once the data was entered into a database, a variety of programs were used to tease out the information required. These include proprietary software for the data loggers which required transposing into Excel, then to Visual Basic to run a macro and finally to SPSS for analysis and to Word for printing.

The whole procedure was repeated a year later but with fewer participants due to individuals dropping out or moving.

Chapter 4 - Results

Project Schedule

The study began in September 2001 with literature research, equipment development and testing. Questionnaires, procedures and equipment were tested in January 2002, with baseline measurements starting in March 2002. The last baseline visits were in October 2003 and the last 12 month visits in October 2004.

4.1. Study Population

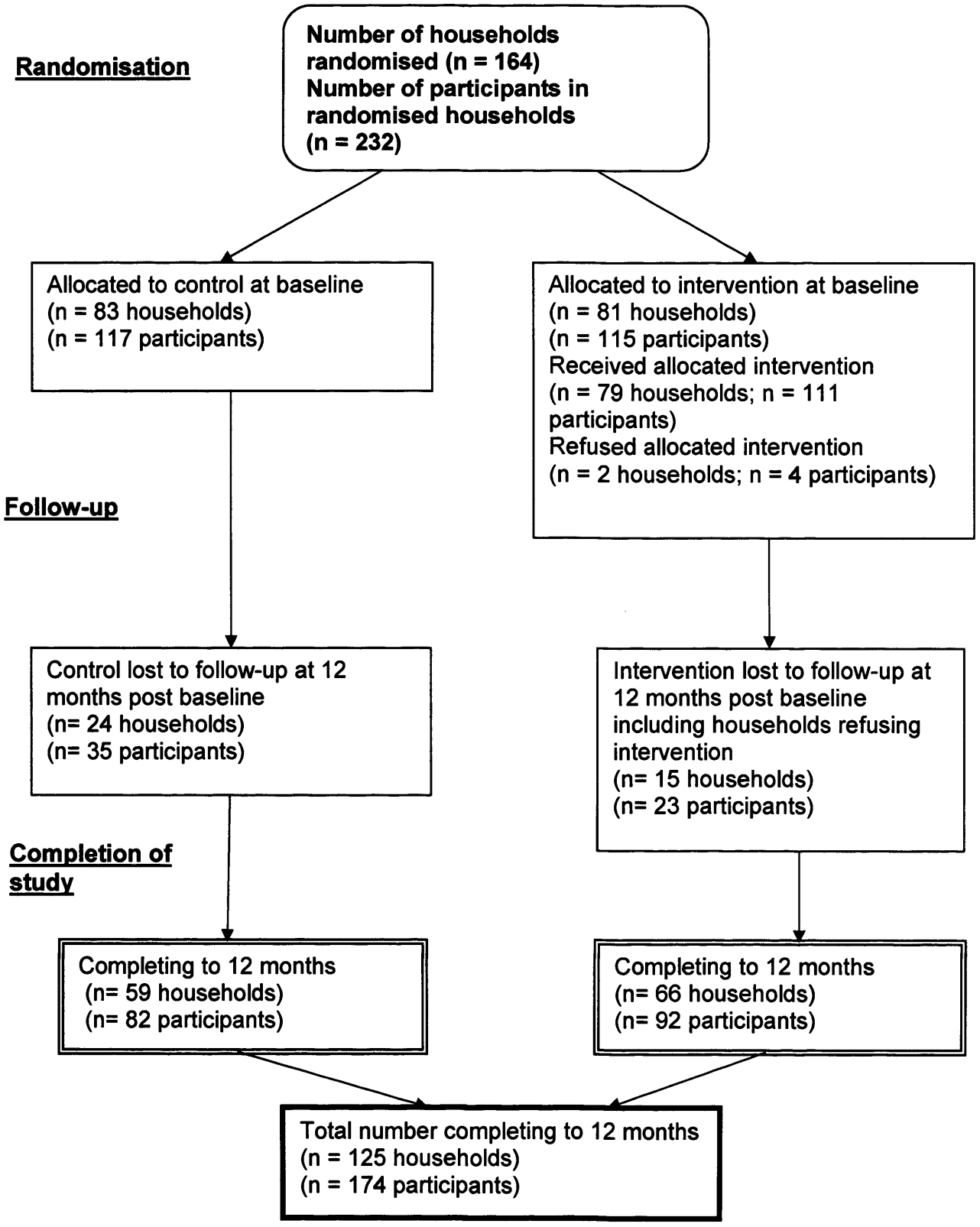
Eighty-eight percent (n = 204) of the subjects were recruited from the asthma registers of General Practitioners, a further 14 subjects (6%) were sourced from the Neath Port Talbot County Borough Council's Housing Register, 3 subjects (1%) from the questionnaire sent to a random sample of homes in the borough, and a further 11 (5%) were recruited by word of mouth (e.g. through being introduced to the study by friends and neighbours who were existing participants) as shown in table 4.1.

Table 4.1. Subjects recruited for the study

Source	Subjects No. (% of total subjects)	Males No. (% of males)	Females No. (% of females)	Children (<12 years) No. (% of children)	Adults (12 + years) No. (% of adults)
General Practice	204 (88)	85 (91)	119 (86)	57 (89)	147 (88)
Borough Council	14 (6)	2 (2)	12 (9)	5 (8)	9 (5)
HANAH	3 (1)	2 (2)	1 (1)	0 (0)	3 (2)
Other	11 (5)	4 (4)	7 (5)	2 (3)	9 (5)
Total (% of total subjects)	**232 (100)**	**93 (40)**	**139 (60)**	**64 (28)**	**168 (72)**

Figure 4.1 below details the flow of participants and homes through the study. In total 164 households containing 232 asthma sufferers were randomised, resulting in 83 homes (117 participants) being allocated to the control group and 81 homes (115) participants being allocated to the intervention group. There were a number of households that left the study before completing to 12 months, mainly due to house moves or no longer wishing to have further home visits. There were a few more drop outs from the control group but no gross differences between those who dropped out after randomisation and the groups from which they were derived; so

Figure 4.1. Flow chart showing the number of households and participants randomised and the number followed up and completing the trial



selective bias does not appear to have been present at this stage. For the control group 71.1% of households (70.1% of control participants) completed to 12 months, whereas 81.5% of households (80.0 % of intervention participants) completed the trial to 12 months in the intervention group. Of the households randomised, 76.2% (n = 125) and 75% of participants (n = 174) completed to 12 months. Approximately one quarter of households left before completing the full trial.

Figure 4.2. Bar Chart showing the frequency distribution of subjects by gender across the age groups at baseline

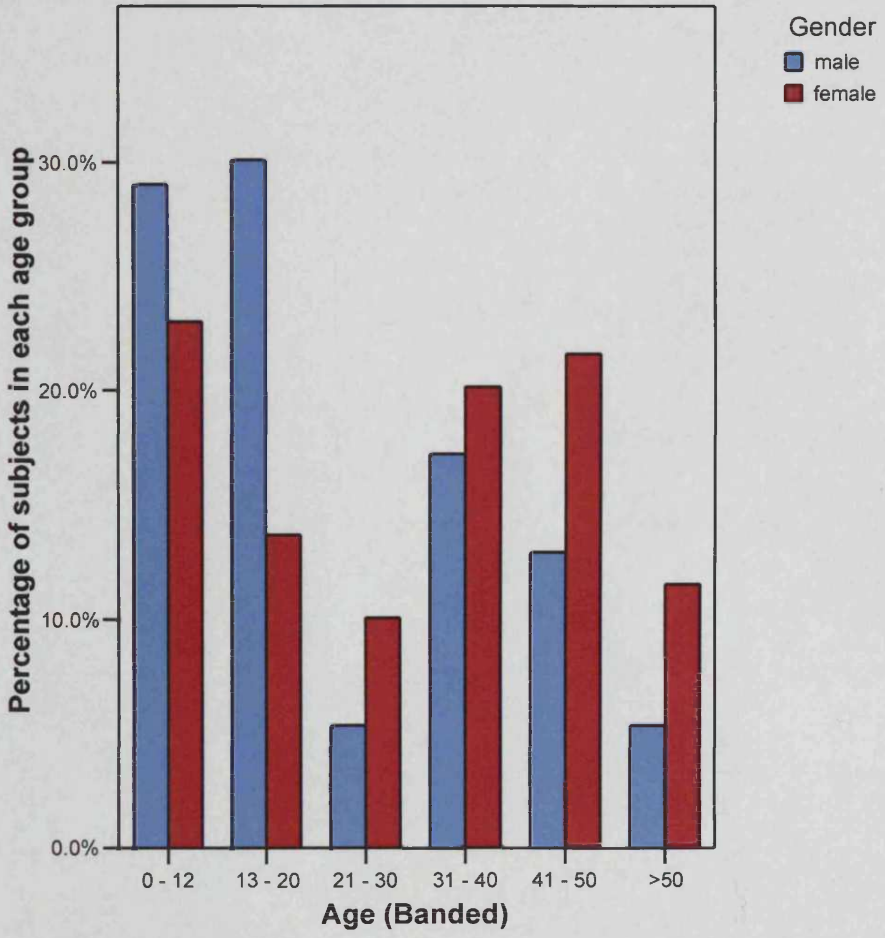


Figure 4.2 shows the distribution of participants in relation to age group and gender. The study population consisted of 232 subjects suffering from asthma with ages ranging from 3 to 61 years, the mean age (SD) being 26.78 (16.04) years; 27.5% (64/168) were children (under 12 years of age), with a mean age (SD) of 8.36 (2.28) years, 72.4% (168/232) were adults, with a mean age (SD) of 33.81 (13.21) years.

4.2. Effects of the intervention upon health

All health outcomes were analysed on the basis of 'intention to treat', i.e. subjects were considered to belong to the group to which they were randomly allocated regardless of whether a) an intervention was not implemented or b) a control acted subsequently to eradicate the mould.

Table 4.2. Characteristics of the intervention and control groups at baseline

	Intervention group: no mould at 12 months	Intervention group: mould at 12 months	Control group: visited at 12 months
No. houses	39	27	59
No. containing a smoker (%)	18 (46.2)	12 (44.4)	27 (45.8)
No. subjects (n) at baseline (m, f)	55 (20,35)	39 (16,23)	84 (33,51)
No. of Children: n (%)	15 (27.3)	14 (35.9)	22 (26.2)
Mean age (SD)	26.6 (15.7)	24.3 (16.5)	27.6 (16.2)
Recent wheeze n (%)	42 (76.4)	30 (76.9)	66 (78.6)
Wheeze disturbs sleep: n (%)			
n/a	13 (24.1)	8 (20.5)	17 (20.2)
Never	10 (18.5)	3 (7.7)	9 (10.7)
Less than weekly	5 (9.3)	10 (25.6)	23 (27.4)
At least weekly	26 (48.1)	18 (46.2)	35 (41.7)
Wheeze limits speech: n (%)			
n/a	13 (24.5)	8 (20.5)	17 (20.2)
yes	11 (20.8)	7 (17.9)	12 (14.3)
no	29 (54.7)	24 (61.5)	55 (65.5)
Wheeze affects activity: n (%)			
n/a	13 (23.6)	8 (20.5)	17 (20.2)
Not at all	7 (12.7)	4 (10.3)	17 (20.2)
A little	23 (41.8)	13 (33.3)	32 (38.1)
Moderately	10 (18.2)	10 (25.6)	12 (14.3)
A lot	2 (3.6)	4 (10.3)	6 (7.1)
Rhinitis: n (%)			
n/a	14 (25.5)	8 (20.5)	31 (36.9)
yes	39 (70.9)	26 (66.7)	51 (60.7)
no	2 (3.6)	5 (12.8)	2 (2.4)
Rhinoconjunctivitis: n (%)			
n/a	16 (29.1)	12 (30.8)	33 (39.3)
yes	22 (40.0)	16 (41.0)	27 (32.1)
no	17 (30.9)	11 (28.2)	24 (28.6)
Rhinitis affects activity: n (%)			
n/a	16 (29.6)	12 (30.8)	33 (39.3)
Not at all	8 (14.8)	10 (25.6)	11 (13.1)
A little	18 (33.3)	9 (23.1)	29 (34.5)
Moderately	9 (16.7)	5 (12.8)	7 (8.3)
A lot	3 (5.6)	3 (7.7)	4 (4.8)

Numbers do not always add up to totals because of missing data. n/a – not applicable

Table 4.2 shows the randomisation resulted in similar numbers of subjects of both sexes with the same mean age in both intervention and control groups, and each group contained approximately the same percentage of children. The same percentage of households contained a smoker in each group. Nineteen subjects (16.5%); and 27 subjects (23.1%) currently smoked cigarettes in the intervention and control groups, respectively. The two groups showed similar cross-section with respect to these characteristics and further to this, the prevalence of symptoms at baseline was comparable in the two groups.

Chest Questionnaires (see Appendix 1) were administered at baseline, and then at 6 months and 12 months after randomisation. The answers to questions were compared for each symptom with those at baseline; subjects who improved or deteriorated were defined as those whose reporting of that symptom had changed, either by occurring on one occasion and not on the other, or in respect to its severity. In each group, the net percentage who improved was calculated (the number who improved minus the number who deteriorated, as a percentage of all with information on both occasions). The difference of the net percentage (intervention-control) was then expressed, with 95% confidence intervals.

After 6 months in the study, information was obtained from 67 subjects in the intervention group and 62 in the control group, and the percentage change in symptoms calculated as shown in table 4.3.

Referring to table 4.3 the difference in the net percentage of subjects showing improvement in recent wheeze between the intervention and control groups was 8.7% although this was not statistically significant. The difference between the groups in the net percentage of subjects who were adversely affected by recent wheeze was higher e.g. 9.8% for wheeze limiting speech, 15.5% for wheeze disturbs sleep and 23.2% for wheeze affecting physical activities but only the latter achieved statistical significance. The difference between the groups in the net percentage of subjects showing improvement in symptoms of rhinitis and rhinoconjunctivitis was 9.1% and 15.6% respectively but neither was statistically significant.

The subjects were also asked about changes in breathing and medication usage in the six months since baseline. Between the two groups there was a large and statistically significant difference with respect to both of these. The difference in the net percentage whose breathing improved since baseline was 52.2% (33.6, 70.8) and for reduction in medication was 64.9% (39, 90.8).

Table 4.3. Net improvement of symptoms in intervention and control groups at 6 months when compared to baseline results

	Intervention group					Control group						
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)	
(2) Have you had wheezing or whistling in the chest in the last 4 weeks?	67	15	7	45	11.9	62	7	5	50	3.2	8.7 (26, -8.6)	
(4) In the last 4 weeks, how often, on average, has your sleep been disturbed due to wheezing?	67	25	6	36	28.4	62	19	11	32	12.9	15.5 (38.1, -7.1)	(Inc all participants). If answered 'no' to Q2, answer to this Q was treated as 'Never woken with wheezing'
(5) In the last 4 weeks, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths?	65	13	1	51	17.9	62	8	3	51	8.1	9.8 (21.7, -7.5)	(Inc all participants). If answered 'no' to Q2, answer to this Q was treated as 'No'.

Table 4.3. Net improvement of symptoms in intervention and control groups at 6 months when compared to baseline results

	Intervention group					Control group						
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)	
(7) In the last 4 weeks, how much did this wheeze interfere with your daily activities?	67	27	6	34	31.3	62	16	11	35	8.1	23.2 (45.5, 1)	(Inc all participants). If answered 'no' to Q2, answer to this Q was treated as 'Not at all'.
(8) In the last 4 weeks, have you had wheezing or whistling in the chest when you did not have a cold or flu?	66	23	4	39	28.8	62	14	4	44	16.1	12.7 (31.5, -6.1)	(Inc all participants). If answered 'no' to Q2, answer to this Q was treated as 'No'.
(9) How is your breathing compared with how it was 6 months ago?	67	39	4	24	52.2	59	12	12	35	0	52.2 (70.8, 33.6)	
(11b) In the last 6 months have you used your medication less, the same or more than/as previously?	63	36	9	18	42.9	50	7	18	25	-22	64.9 (90.8, 39)	If answered 'no' to Q11a, they were not included in this Q.
(11c) In the last 4 weeks, have you taken any treatment for wheezing or asthma?	63	1	5	57	-6.3	59	4	4	51	0	-6.3 (5.7, -18.3)	(Inc all participants). If answered 'no' to Q11a, answer to this Q was treated as 'No'.

Table 4.3. Net improvement of symptoms in intervention and control groups at 6 months when compared to baseline results

	Intervention group					Control group						
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)	
(13) During the last 4 weeks, have you had a cough on most days?	67	13	7	47	9.0	62	11	7	44	6.5	2.5 (21, -16)	
(14) During the last 4 weeks, have you brought up phlegm from your chest on most days?	66	11	6	49	7.6	62	9	10	43	-1.6	9.2 (27.5, -9.1)	
(16) During the last 4 weeks, have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu? (Rhinitis)	65	15	7	43	12.3	62	9	7	46	3.2	9.1 (27.8, -9.6)	(Inc all participants). If answered 'no' to Q15, answer to this Q was treated as 'No'.
(17) During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? (Rhinoconjunctivitis)	65	15	8	42	10.8	62	6	9	47	-4.8	15.6 (34.3, -3.1)	Inc all participants). If answered 'no' to either Q15 or Q16, answer to this Q was treated as 'No'.

Table 4.3. Net improvement of symptoms in intervention and control groups at 6 months when compared to baseline results

	Intervention group					Control group						
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)	
(18) During the last 4 weeks, how much did this nose problem interfere with your daily activities?	65	16	10	39	9.2	62	18	13	31	8.1	1.1 (24.3, -22.1)	(Inc all participants). If answered 'no' to either Q15 or Q16, answer to this Q was treated as 'Not al all'
(20) In the last 4 weeks, have you taken any treatment for a runny or blocked nose problem or hay fever?	66	4	9	53	-7.6	59	10	5	44	8.5	-16.1 (-0.4, -32.6)	
(22) In the past 4 weeks, have you taken any treatment for an itchy rash or eczema?	66	8	4	54	6.1	59	7	6	46	1.7	4.4 (20.1, -11.3)	

Note: *Questions 9 and 11 b above are a comparison between baseline and six months.*

**Difference = value in intervention group minus value in control group.*

Referring to table 4.4 the difference between the groups in the net percentage whose breathing has improved since baseline is still large (29.9%) and statistically significant but not as large as at 6 months (52.2%). Comparing responses regarding medication usage in the previous 6 months at six months and at twelve months shows that between these sampling dates there was a further improvement in medication reduction in the intervention group but that this was not statistically significant.

The difference between the groups in the net percentage of subjects showing improvement in recent wheeze observed at 6 months has decreased at 12 months i.e. at 12 months the difference is 2.9% (18.7, -12.1). The difference between the groups in the net percentage of subjects who were adversely affected by their wheeze was 7.8% for wheeze limiting speech and 15.8% for wheeze disturbing sleep, which is almost identical to the effect size seen at six months, but again not statistically significant. However, the net percentage difference in those reporting wheeze affecting physical activities is 5.6% and is not statistically significant compared to the 23.2% seen at six months. The difference between the groups in the net percentage of subjects showing improvement in symptoms of rhinitis or rhinoconjunctivitis is both large, 18.4% and 13.1% respectively, and statistically significant for the former.

To further understand the effects the moulds may have had on the asthma subjects' health, the chest questionnaire data was broken down into those homes that had intervention where the mould remained absent, and those that had intervention but in which the mould reappeared, compared with those in the control group. Although the numbers of participants split into these groups are small there were significant differences between these three groups and those looking just at the intervention versus control groups. The data can be seen in Appendix 11. At 6 months there was almost 3 times the improvement in wheeze affecting speech in the homes without mould (29.4%) compared to those where mould returned (10.3%) matched against the control group (6.8%). There was a considerable and significant difference in net improvement in wheeze affecting daily activities (36.6%), improved breathing (72.2%) and decrease in medication use (82.2%) in those homes where mould did not return. Medication use was still reduced in the

Continued on page 107.

Table 4.4. Net improvement of symptoms in intervention and control groups at 12 months when compared to baseline results

	Intervention group					Control group					Difference in net % better * (95% CI)	
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement		
(2) Have you had wheezing or whistling in the chest in the last 4 weeks?	89	22	7	60	16.9	81	20	4	57	19.8	2.9 (18.7, -12.1)	
(4) In the last 4 weeks, how often, on average, has your sleep been disturbed due to wheezing?	89	30	10	49	22.5	81	35	4	42	38.3	15.8 (34, -2.4)	(Inc all participants). If answered 'no' to Q2, answer to this Q was treated as 'Never woken with wheezing'
(5) In the last 4 weeks, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths?	87	11	1	75	11.5	81	6	3	72	3.7	7.8 (18.1, -2.5)	(Inc all participants). If answered 'no' to Q2, answer to this Q was treated as 'No'.

Table 4.4. Net improvement of symptoms in intervention and control groups at 12 months when compared to baseline results

	Intervention group					Control group					Difference in net % better * (95% CI)	
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement		
(7) In the last 4 weeks, how much did this wheeze interfere with your daily activities?	90	36	11	43	27.8	81	30	12	39	22.2	5.6 (25.9, -14.7)	(Inc all participants). If answered 'no' to Q2, answer to this Q was treated as 'Not at all'.
(8) In the last 4 weeks, have you had wheezing or whistling in the chest when you did not have a cold or flu?	90	28	6	56	24.4	81	20	4	57	19.8	4.6 (20.7, -11.5)	(Inc all participants). If answered 'no' to Q2, answer to this Q was treated as 'No'.
(9) How is your breathing compared with how it was 12 months ago?	90	51	5	34	51.1	80	25	8	47	21.25	29.9 (41.1, 11.7)	
(11b) In the last 6 months have you used your medication less, the same or more than/as previously?	70	33	13	23	28.2	68	11	16	23	-7.4	20.8 (44, -2.4)	If answered 'no' to Q11a, they were not included in this Q.
(11c) In the last 4 weeks, have you taken any treatment for wheezing or asthma?	89	25	5	59	22.5	81	8	4	69	4.9	17.6 (31.5, 3.7)	(Inc all participants). If answered 'no' to Q11a, answer to this Q was treated as 'No'.

Table 4.4. Net improvement of symptoms in intervention and control groups at 12 months when compared to baseline results

	Intervention group					Control group					Difference in net % better * (95% CI)	
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement		
(13) During the last 4 weeks, have you had a cough on most days?	90	16	13	61	3.3	81	15	8	58	8.6	-5.3 (11.1, -21.7)	
(14) During the last 4 weeks, have you brought up phlegm from your chest on most days?	89	15	9	65	6.7	81	11	8	6.2	3.7	3 (18, -12)	
(16) During the last 4 weeks, have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu? (Rhinitis)	67	22	2	43	29.9	52	7	1	44	11.5	18.4 (34.5, 2.3)	(Inc all participants). If answered 'no' to Q15, answer to this Q was treated as 'No'.
(17) During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? (Rhinoconjunctivitis)	45	8	7	30	2.2	55	6	12	37	-10.9	13.1 (35.6, -9.4)	(Inc all participants). If answered 'no' to either Q15 or Q16, answer to this Q was treated as 'No'.

Table 4.4. Net improvement of symptoms in intervention and control groups at 12 months when compared to baseline results

	Intervention group					Control group					Difference in net % better * (95% CI)	
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement		
(18) During the last 4 weeks, how much did this nose problem interfere with your daily activities?	43	10	17	16	-16.3	55	14	24	17	12.7	-29 (2.6, -60.6)	(Inc all participants). If answered 'no' to either Q15 or Q16, answer to this Q was treated as 'Not al all'
(20) In the last 4 weeks, have you taken any treatment for a runny or blocked nose problem or hay fever?	90	15	9	66	6.7	80	9	11	60	-2.5	4.2 (19.4, -11)	
(22) In the past 4 weeks, have you taken any treatment for an itchy rash or eczema?	89	10	4	75	6.7	77	20	4	53	20.8	-14.1 (0, -28.2)	

Note: *Question 9 above is a comparison between baseline and 12 months.*

Question 11b above is a comparison between six months and 12 months.

**Difference = value in intervention group minus value in control group. Calculation of these confidence intervals is shown in Appendix 8.*

Continued from page 102.
intervention group with mould (34.8%) compared to controls but the values were not as substantial. Rhinoconjunctivitis was significantly improved in homes without mould (20.6%); there was no improvement in those homes where the mould returned (0.00%).

At 12 months the improvements are not as dramatic but there are changes. Medication use in both intervention groups declined between 6 and 12 months but was not significant. The net percentage difference better for runny nose problems (28.1%) and for rhinoconjunctivitis (24.2%) improved significantly in the intervention group without mould compared to the other groups (15.5% in both). In both the intervention groups, treatment for itchy rash or eczema increased compared with the control group.

The measure of variability of PEFR used for each subject was the coefficient of variation (CV). It is the ratio of the standard deviation divided by the mean, multiplied by 100, so that it is expressed as a percentage. Since the CV provides a measure of the relative variation and is scale-free, it is useful for comparing the difference between two sample sets. To calculate the CV the highest PEFR out of three for a particular morning was used for the two-week period of readings (14 values), similarly for the evening readings. In tables 4.5, 4.6 and 4.7 the mean CV for each group was calculated for morning and evening measurements separately and on each occasion of baseline, 6 months and 12 months. In each group the variability of both morning and evening readings tended to decline when comparing 6 month and 12 month readings respectively to baseline. There were no significant differences between the changes in the two groups at either six months or twelve months.

Table 4.5. Variability of PEFR at baseline

Group	Baseline		
	No.	Mean	SD
CV of morning PEFR			
Intervention	107	7.85	5.75
Control	104	8.55	5.23
CV of evening PEFR			
Intervention	107	7.00	5.09
Control	104	8.04	5.67

Table 4.6. Effects upon variability of PEFR at six months

Group	Change 0-6 m			Difference*
	No.	Mean	SD	(95% CI)
CV of morning PEFR				
Intervention	41	-0.42	4.44	1.78
Control	41	-2.20	4.74	(-0.23, 3.80)
CV of evening PEFR				
Intervention	41	-1.59	4.61	0.27
Control	40	-1.86	5.14	(-1.89, 2.42)

**Difference = change in intervention group minus change in control group*

Table 4.7. Effects upon variability of PEFR at twelve months

Group	Change 0-12 m			Difference*
	No.	Mean	SD	(95% CI)
CV of morning PEFR				
Intervention	82	-1.62	6.47	0.46
Control	66	-2.08	6.30	(-1.58, 2.50)
CV of evening PEFR				
Intervention	82	-1.30	6.04	1.42
Control	67	-2.72	6.30	(-0.58, 3.43)

**Difference = change in intervention group minus change in control group*

The participants PEFR were split into the same three groups as the chest questionnaire data. Referring to tables 4.8, 4.9 and 4.10, there were no changes in the PEFR difference after splitting the intervention group into those homes where mould returned and those where it did not at 6 and at 12 months.

Table 4.8. Variability of PEFR at baseline for three groups

Group	Baseline		
	No.	Mean	SD
CV of morning PEFR			
Intervention No Mould	54	7.83	6.78
Intervention Mould	39	7.80	5.81
Control	83	8.46	5.39
CV of evening PEFR			
Intervention No Mould	54	7.57	5.81
Intervention Mould	39	6.66	4.76
Control	83	8.23	6.18

Table 4.9. Effects upon variability of PEFR at six months for three groups

Group	Change 0-6 m			Difference*
	No.	Mean	SD	(95% CI, compared to Control for each intervention group)
CV of morning PEFR				
Intervention No Mould	22	-0.66	4.91	1.54 (-1.00, 4.08)
Intervention Mould	19	-0.13	3.94	2.07 (-0.44, 4.57)
Control	41	-2.20	4.74	
CV of evening PEFR				
Intervention No Mould	22	-3.15	4.94	-1.29 (-3.98, 1.40)
Intervention Mould	19	0.21	3.50	2.07 (-0.54, 4.68)
Control	40	-1.86	5.14	

**Difference = change in intervention group minus change in control group*

Table 4.10. Effects upon variability of PEFR at twelve months for three groups

Group	Change 0-12 m			Difference*
	No.	Mean	SD	(95% CI, compared to Control for each intervention group)
CV of morning PEFR				
Intervention No Mould	48	-1.66	7.04	0.42 (-2.00, 2.84)
Intervention Mould	34	-1.56	5.67	0.52 (-1.94, 2.97)
Control	66	-2.08	5.96	
CV of evening PEFR				
Intervention No Mould	48	-1.90	5.66	0.82 (-1.44, 3.08)
Intervention Mould	34	-0.44	6.54	2.28 (-0.39, 4.95)
Control	67	-2.72	6.30	

**Difference = change in intervention group minus change in control group*

4.3. Effects of the Intervention on Mould

4.3.1. Effects as assessed by visual presence or absence of mould

As shown in Figure 4.3. and Table 4.11, there is a significant reduction in the percentage of homes with visual mould at 12 months especially in the intervention group (60% reduction), compared with homes in the control group. The odds of getting mould in these homes are five times the odds of getting mould in the intervention homes at 12 months (OR: 5.11; CI: 2.33, 11.23).

Figure 4.3. Bar chart comparing the percentage of homes with any visual mould

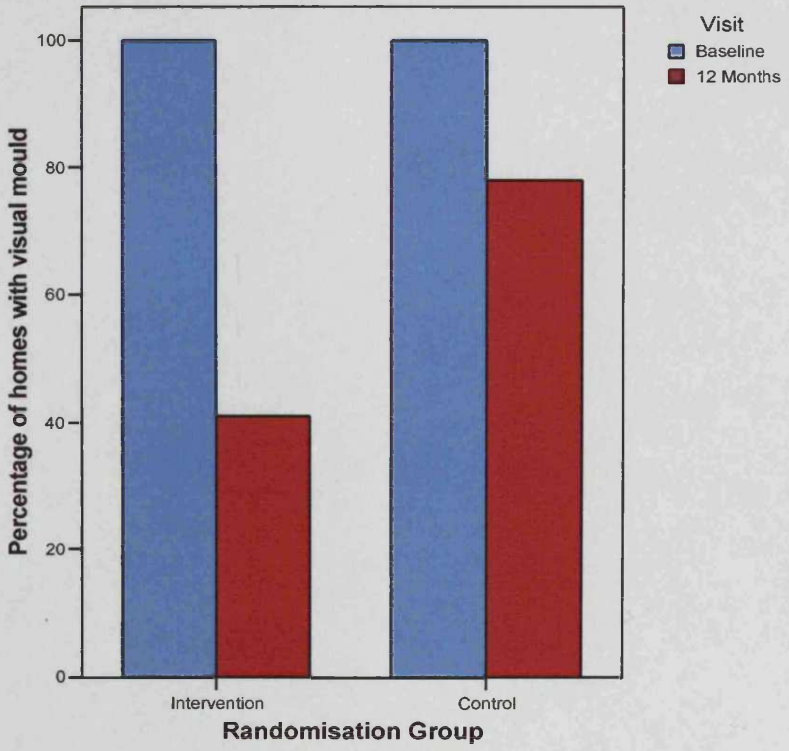


Table 4.11. Comparison of the number of homes with any visual mould at baseline and 12 months by randomisation group (n = 126) (as assessed by researcher)

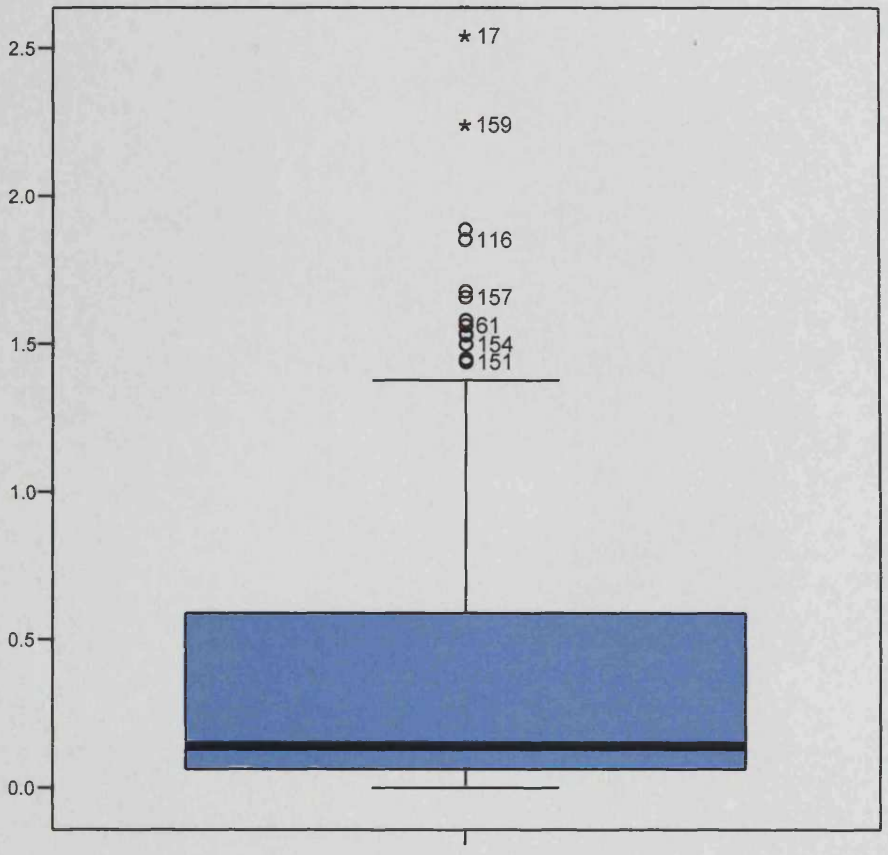
Group	No mould at 12 months	Mould at 12 months	OR	95% CI	p-value (significance of OR compared to OR = 1)	Pearson's Chi squared for cross tabulation with continuity correction
Intervention	39	27	5.11	2.33-11.23	p<0.001 0.00006	p<0.001 0.00004
Control	13	46				

A box plot (see figure 4.4) is a useful means of visualising the distribution of univariate variables; it shows the median value (thick dark bar) and the spread of the data. Table 4.12 defines the various parts making up a box plot; used when comparing the distribution of two or more sets of data. The larger the length of the box the greater is the spread of the data. If the median line is not centred within the box then the data has a skewed distribution. If the upper whisker of a box plot is much longer than the lower one, this gives the impression of positive skewness. All data points are compared with the entire group of data and if they are found to be outside the 50% of all values they are termed outliers or extreme values. These may be isolated values far from the spread of the data or values that do not follow the general pattern of the others. They may influence the results of various statistical techniques used to investigate a set of data and thus the box plot is a good means of observing how the data is distributed before doing further analyses.

Table 4.12. Description of a box plot (Tukey, 1977)

Box	Top of the box - 75th percentile (upper quartile) Bar - median (50th percentile) / With equal number of data points either side Bottom of the box - 25th percentile (lower quartile)
Whiskers	Upper – largest value, which is not an outlier or an extreme value Lower - smallest value, which is not an outlier or an extreme value
Outlier (o)	A value that is more than 1.5 box lengths above or below the box
Extreme Value (*)	A value that is more than 3 box lengths above or below the box

Figure 4.4. An example of a box plot showing outliers (°) and extreme (*) positive values with a skewed distribution

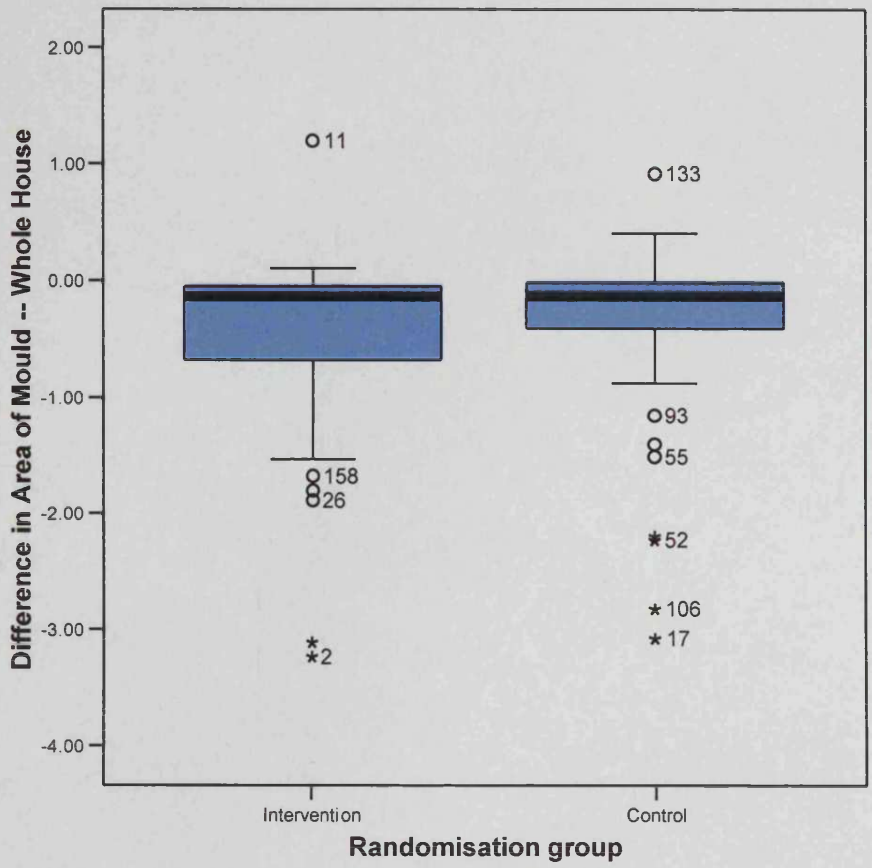


If a continuous variable has a normal distribution in two groups then the null hypotheses (that there is no difference between the two means) may be tested parametrically using the t-test.

The Mann-Whitney U test is the non-parametric alternative to the t-test for independent samples but instead of comparing the means of two groups, the Mann-Whitney test compares the medians. It converts the scores on the continuous variable to ranks across the two groups and then evaluates whether the ranks for the two groups differ significantly. Non-parametric tests can be used instead of parametric tests if the data is skewed. A number of variables are tested below to see if they differ between two independent groups. A decision was taken to conduct comparisons by means of t-test and Mann-Whitney both for the sake of completeness and to allow comparisons between the results using different approaches.

4.3.2. Effects as assessed by a decrease in the total areas of mould

Figure 4.5. Box plot comparing the difference in area of mould at baseline and 12 months for whole house by randomisation groups *



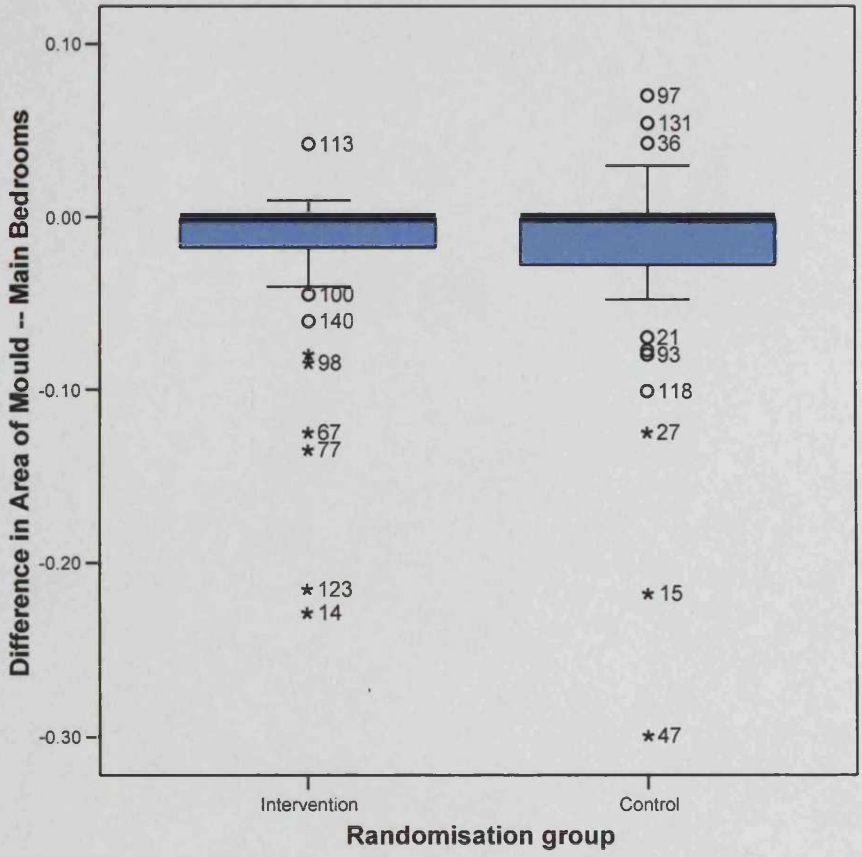
*Two extreme negative values have been excluded from the Intervention box plot for presentation purposes (-5.50 & -15.3).

	Intervention	Control
N	66	59
Mean	-0.75	-0.37
Median	-0.13	-0.13
t-test p value	0.167	
Mann-Whitney p value	0.245	

Figure 4.5 shows the difference in the area of mould at baseline and 12 months are similar in the two randomisation groups (n = 125). The intervention group (n = 66) and the control group (n = 59) have exactly the same median value (-0.13), the

inter-quartile range is wider in the intervention group, as is the overall range of values (excluding outliers and extreme values). As the table shows; the mean is slightly lower in the intervention group (-0.75) than in the control group (-0.37). The differences observed between means and medians are not statistically significant (t-test p-value = 0.167, Mann-Whitney p-value = 0.245).

Figure 4.6. Box plot comparing the difference in area of mould at baseline and 12 months for main bedroom by randomisation groups *



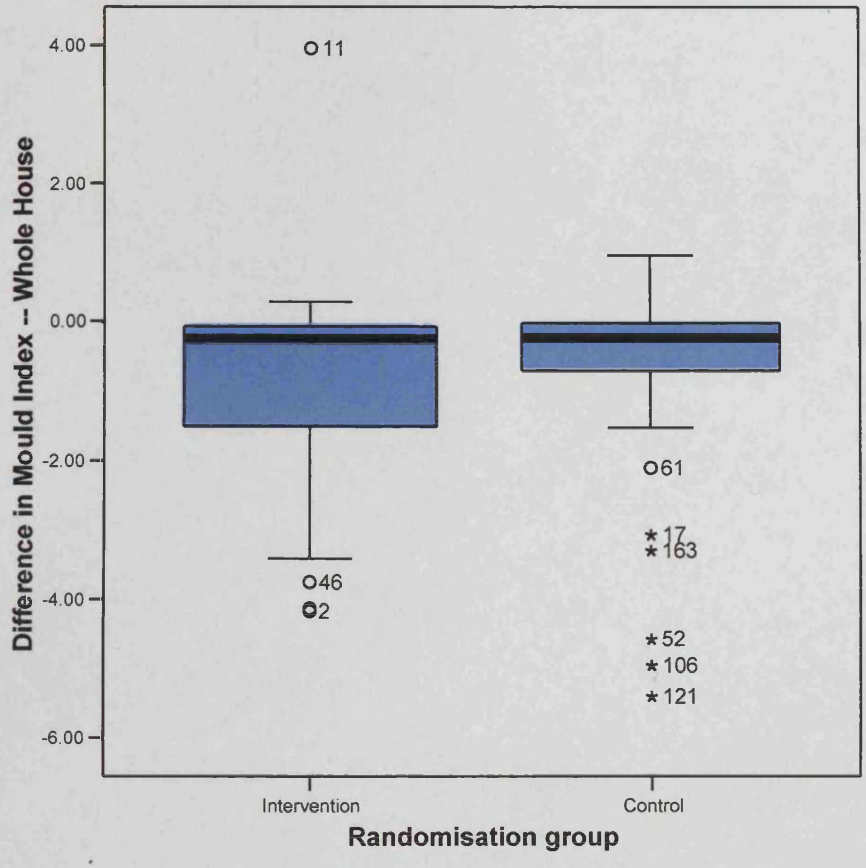
*Three extreme values from each group (Intervention; -0.50, -0.54 & -0.64: Control; 0.35, -0.68, -0.71) have been removed for presentation purposes.

	Intervention	Control
N	66	59
Mean	-0.18	-0.08
Median	0	0
t-test p value	0.384	
Mann-Whitney p value	0.40	

As shown in Figure 4.6 the difference in the area of mould at baseline and 12 months are similar in both randomisation groups' main bedrooms (n = 125). The intervention group (n = 66) and the control group (n = 59) have the same median value (0), the inter-quartile range is of similar width in the two groups. The overall spread of values (excluding outliers and extreme values) is wider in the control group. As the table shows, the mean is slightly lower in the intervention group (-0.18) than in the control group (-0.08). The differences observed between means and medians are not statistically significant (t-test p-value = 0.384, Mann-Whitney p-value = 0.40).

4.3.3. Effects as observed by assessment of visual mould index

Figure 4.7 Box plot comparing the difference in mould index at baseline and 12 months for whole house by randomisation groups *

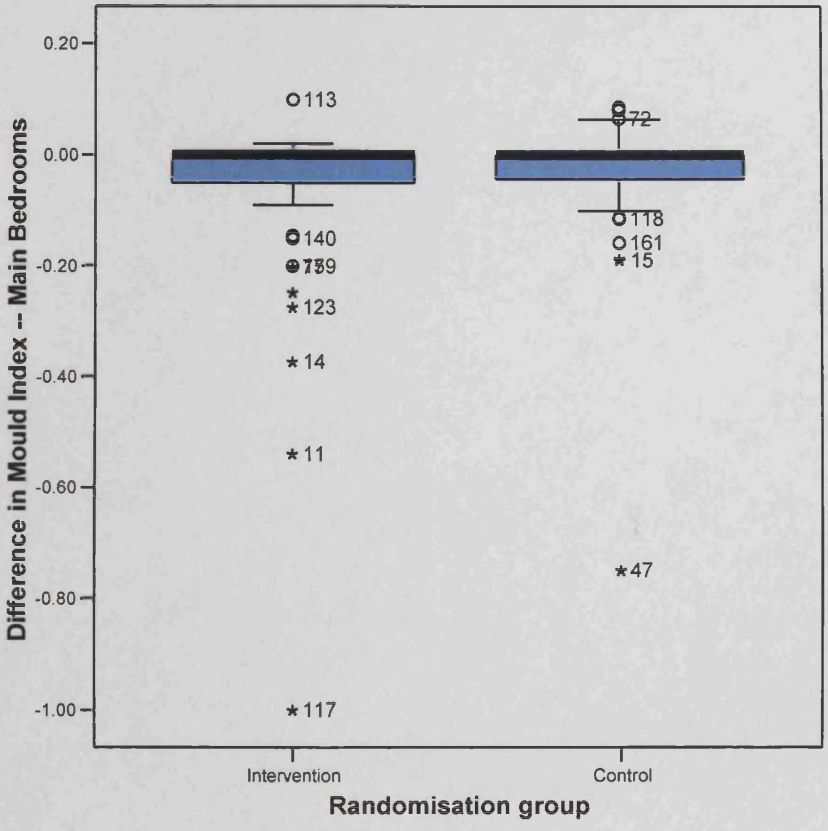


*Two negative extreme Intervention values are not shown for presentation purposes (-10.5 & -25.6).

	Intervention	Control
N	66	59
Mean	-1.27	-0.64
Median	-0.24	-0.22
t-test p value	0.174	
Mann-Whitney p value	0.205	

As shown in Figure 4.7 the difference in the index of mould at baseline and 12 months are similar in both randomisation groups (n = 125). The intervention group (n = 66) and the control group (n = 59) have an extremely similar median value (-0.24 and -0.22 respectively), the inter-quartile range is wider in the intervention group, as is the overall range of values (excluding outliers and extreme values). As the table shows, the mean is slightly lower in the intervention group (-1.27) than in the control group (-0.64). The differences observed between means and medians are not statistically significant (t-test p-value = 0.174, Mann-Whitney p-value = 0.205).

Figure 4.8. Box plot comparing the difference in mould index at baseline and 12 months for main bedroom by randomisation groups *



*The following results have been removed from the graph for clarity (Intervention; -1.6, -1.8, -3.8 & -12.8: Control; 0.8, -1.3, -1.5, -1.7 & -2.8).

	Intervention	Control
N	66	59
Mean	-0.36	-0.14
Median	0	0
t-test p value	0.334	
Mann-Whitney p value	0.334	

As shown in Figure 4.8 the difference in the index of mould at baseline and 12 months are similar in both randomisation groups (n = 125). The intervention group (n = 66) and the control group (n = 59) have the same median value (0), the inter-quartile range is of similar width in the two groups, as is the overall range of values (excluding outliers and extreme values). As the table shows, the mean is slightly lower in the intervention group (-0.36) than in the control group (-0.14). The differences observed between means and medians are not statistically significant (t-test p-value = 0.334, Mann-Whitney p-value = 0.334).

4.4 Effects of the Intervention on decreasing corrected mixing ratios

A review in 1997, which used the English House Condition Survey 1988 and 1991 as the sources for some of the figures, showed that 35% of the housing stock in Britain was affected by condensation and 17% by mould growth (Woolliscroft, 1997). The two are intimately associated with each other and at the present, the means of recording the atmospheric conditions within the homes is through temperature (T), relative humidity (RH) and ventilation measurements. The temperature measurement is fairly straightforward but there are several methods used to express the amount of water vapour in air. For comparison, a number of ways of describing the mean quantity of water vapour in air and how they relate to mould growth can be seen in table 4.13.

Table 4.13. Comparison of mean water content in air for Cardiff at 12 months

	Intervention group without mould	Intervention group with mould	Control
Relative Humidity %	56.7%	57.4%	58.2%
Temperature (°C)	20.8	20.5	20.6
Absolute humidity (g/m^3)	50.2	50.4	51.3
Mixing Ratio (g/kg)	8.53	8.48	8.65

The most common is relative humidity, which is the ratio (as a percent) between the water vapour actually present and the amount necessary for saturation to occur at a given temperature. Relative humidity alone gives no indication as to the actual amount of water in the air but more importantly, the value is temperature dependent. Warm air can hold more water vapour than cold air as observed when condensation droplets form around a cold glass of beverage as the air immediately around the glass cools. The density of water vapour in air is called the absolute humidity (p); it is the mass of water vapour present per unit volume of space usually expressed in grams per cubic metre (g/m^3). Absolute humidity can be calculated as follows:

$p = e/(461.5*273.15)$ where e is in pascals (Pa) and the result is in kg/m^3.

Multiply by 1,000 to get g/m^3. The equation for e follows, below.

Moisture within the air can also be expressed as the mass of water vapour contained in a unit mass of air (dry air plus the water vapour); this is called the specific humidity (q) and is usually expressed as grams per gram or grams per kilogram (g/kg). It is mass dependent and as such is unaffected by changes in T or

pressure (P) unless the quantity of water vapour changes. As mentioned earlier, warm air can hold more moisture at constant pressure than cold air so the saturation specific humidity is greater at higher temperatures. Moist air is less dense than dry air at constant T, therefore a parcel of air has a greater q at saturation if the P is low compared with when the P is high. The mixing ratio (w) is defined as the ratio of the mass of water vapour to the mass of dry air usually expressed as grams per kilogram (g/kg). It is very close to the specific humidity but it is always slightly larger. Importantly, it is unaffected by changes in temperature. The maximum amount of water vapour the air can hold at any given T by weight is referred to as the saturation mixing ratio. The RH is equal to the mixing ratio divided by the saturation mixing ratio, multiplied by 100. The water vapour pressure (e) divided by the saturation vapour pressure (e_s) times 100 also equals the RH.

Where the saturation vapour pressure (e_s) is:

e_s = 0.61078*Exp((T*17.2694) / (T+237.3)) where T is the temperature (°C)

The partial vapour pressure (e) of the water vapour equals:

$e = e_s * (RH/100)$

And the mixing ratio (w) equals:

w =0.62197*(e/(P+(e*(0.62197-1)))) where the gas constant for water vapour is 0.62197

The saturation mixing ratio (w_s) equals:

$w_s = e_s/P$, where P is the atmospheric pressure.

$RH = 100* w / w_s$ and also $100*e/e_s$

(after: Wallace & Hobbs, 1997)

The mixing ratio is typically a few to several grams per kilogram at mid latitudes but in the tropics it can reach values of 20g/kg. If neither condensation nor evaporation takes place then the mixing ratio of a parcel of air will be constant.

The mixing ratios used to derive the table 4.14 and figures 4.9, 4.10 & 4.11 were calculated using the formulas above in a macro program (see Appendix 2). The mixing ratio can also be visualised and found using the psychrometric chart, see Appendix 8.

The atmospheric or outdoor mixing ratio was calculated and it was subtracted from the indoor mixing ratio, resulting in the corrected mixing ratio (CMR). A one-week average value was used starting on the first day of the visits at baseline and at 12

months. The indoor values are always higher as a result of water vapour being added through occupant activities such as bathing, cooking, washing etc.
As can be seen from table 4.14, the average outdoor mixing ratios for each of the three years of study were: Year 1, 6.44g/kg; Year 2, 6.21g/kg: Year 3, 6.38g/kg (305 days only).

Table 4.14 Variation in annual mean mixing ratio in Cardiff over the study period

Year	Minimum	Maximum	Mean
2002	2.21	11.51	6.44
2003	1.91	14.22	6.21
2004	1.63	12.42	6.38

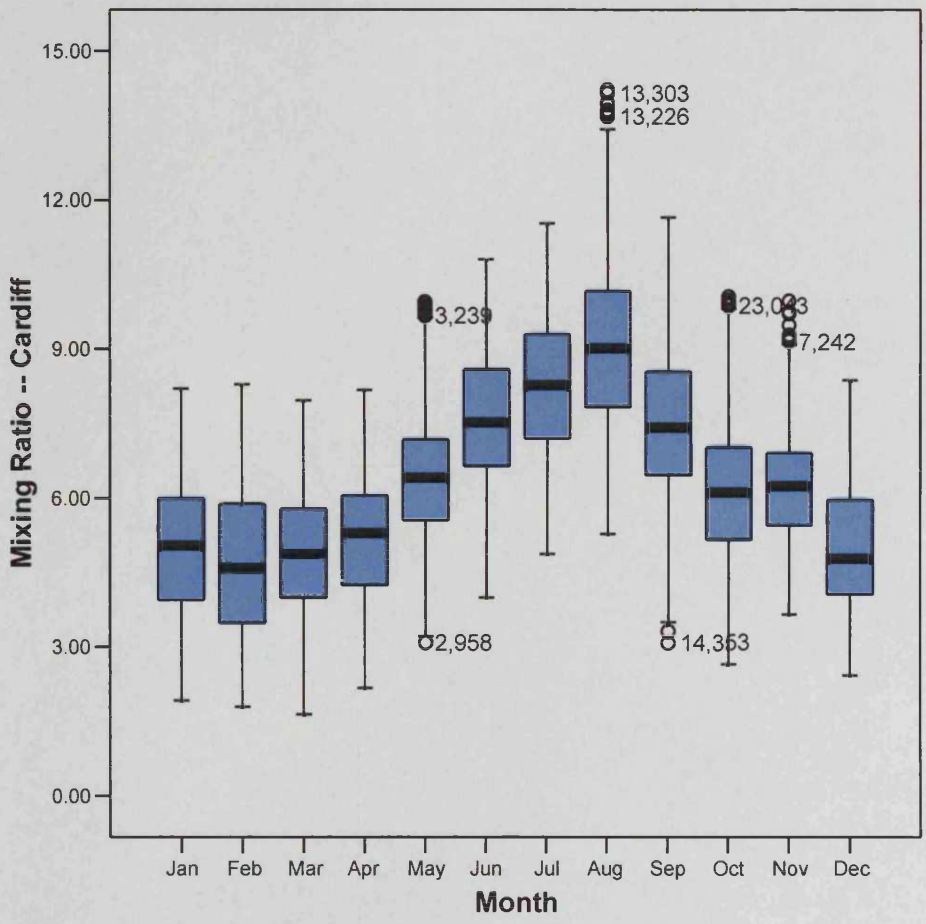
Table 4.15 Seasonal split in outdoor mixing ratios for Cardiff over the three year sampling period*

			Mean	Median	Minimum	Maximum
Winter Summer Split	Winter	Outdoor Mixing Ratio	5.78	5.79	1.76	11.92
	Summer	Outdoor Mixing Ratio	6.91	6.82	1.60	14.33

* Winter months include: October to March and Summer months: April to September.

There is a 20% increase in the mean outdoor mixing ratios in the summer months compared with the winter months. Although the winter months are considered damper, the actual humidity values are much lower.

Figure 4.9 Mean monthly outdoor mixing ratios for Cardiff over the sampling period



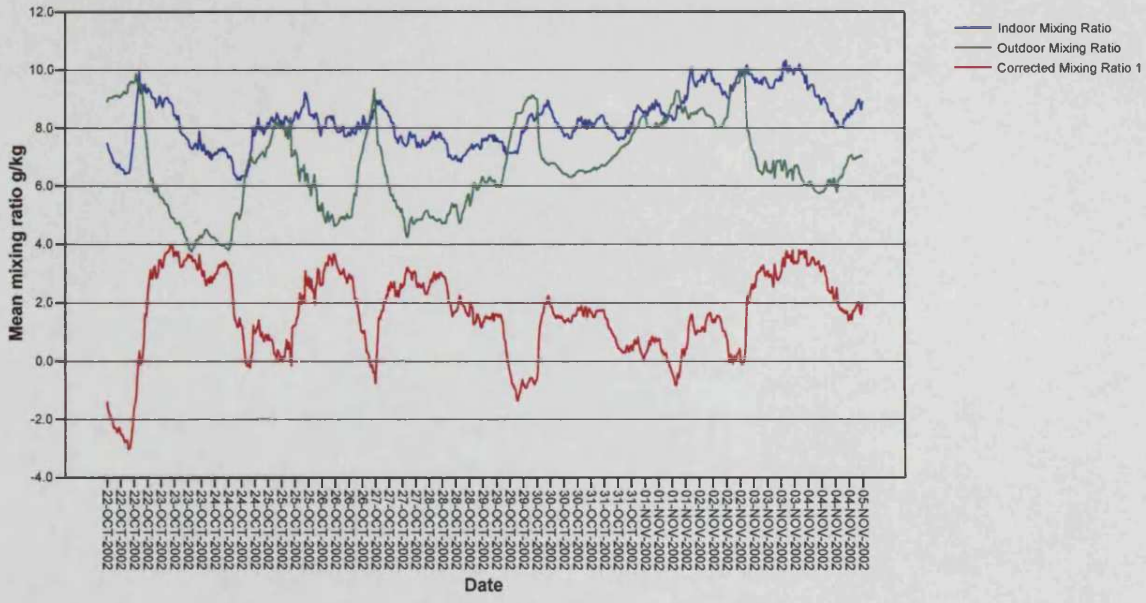
As shown by figure 4.9 there is a substantial variation in the mixing ratios throughout the year with the low values (~4.5g/kg) falling in February and December and the high (~9.0g/kg) occurring in August. The summer value is double the winter one and probably needs some consideration when assessing the intervention method. Referring to table 4.14, there is a ~0.5g/kg variation in the year on year variation in the mean outdoor mixing ratio.

Figures 4.10 and 4.11 are two randomly chosen graphical examples of the mixing ratio observed for a two-week period in the intervention and control groups at baseline and at 12 months (see additional graphs in Appendix 2). The uppermost line is the indoor mixing ratio, which has the highest water vapour content in all the cases. The values are quite erratic but overall the indoor and outdoor patterns mirror each other showing the influence the outdoor value has on the indoor mixing ratio. In the baseline intervention graph the difference in the indoor and outdoor appears to fluctuate diurnally within a range of about 6g/kg. At 12 months, the diurnal effect is much less and the range of variation has dropped to about 3g/kg.

The peaks and troughs do not always coincide, showing that there is a lag time within the home of the effects of the outdoor conditions. Similarly, for the control home there are a large number of tight peaks and troughs, which can be seen in both the baseline and 12 months graphs. The very high peak on the 12-month graph is probably an anomaly the logger picked up and not an actual atmospheric effect. There is minimal change in the control graphs.

Figure 4.10. Difference in mixing ratios in an intervention home.

At baseline:



At 12 months:

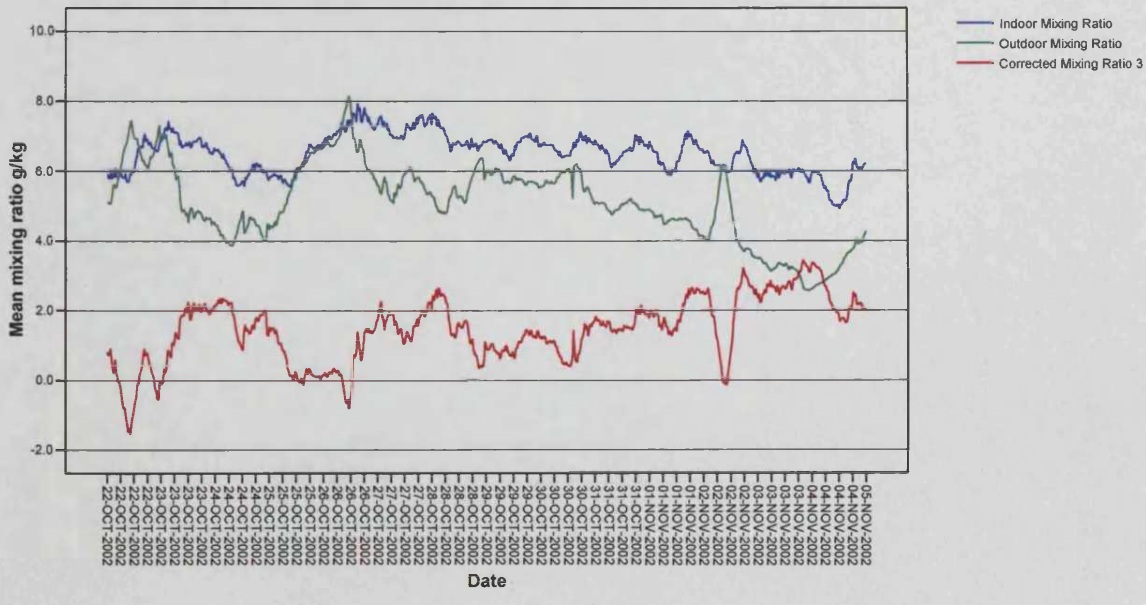
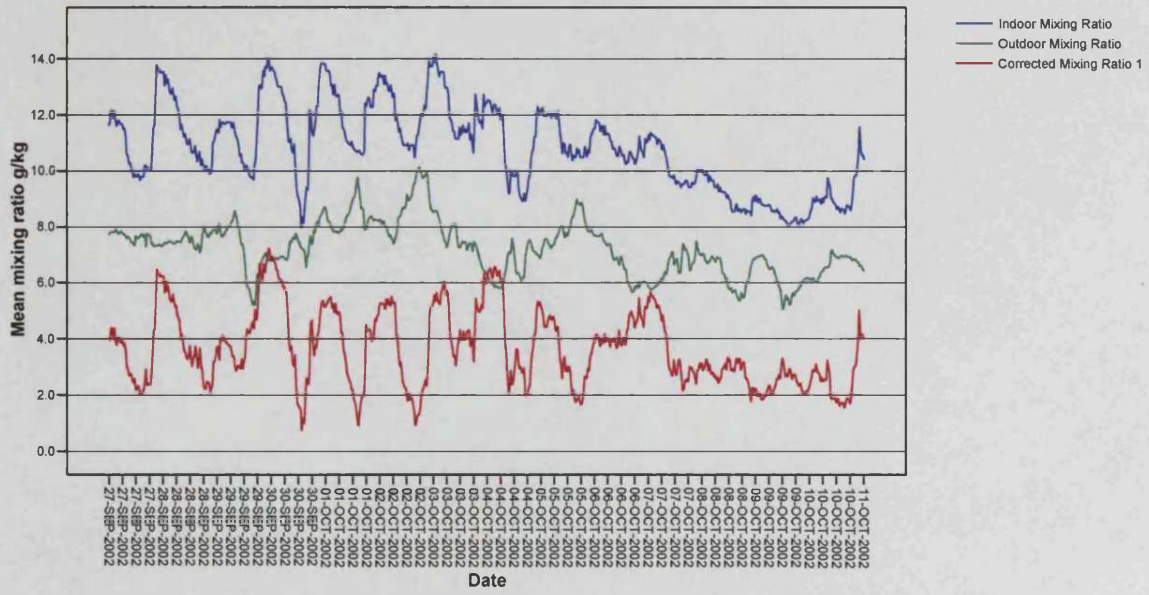


Figure 4.11. Difference in mixing ratios in a control home.

At baseline:



At 12 months:

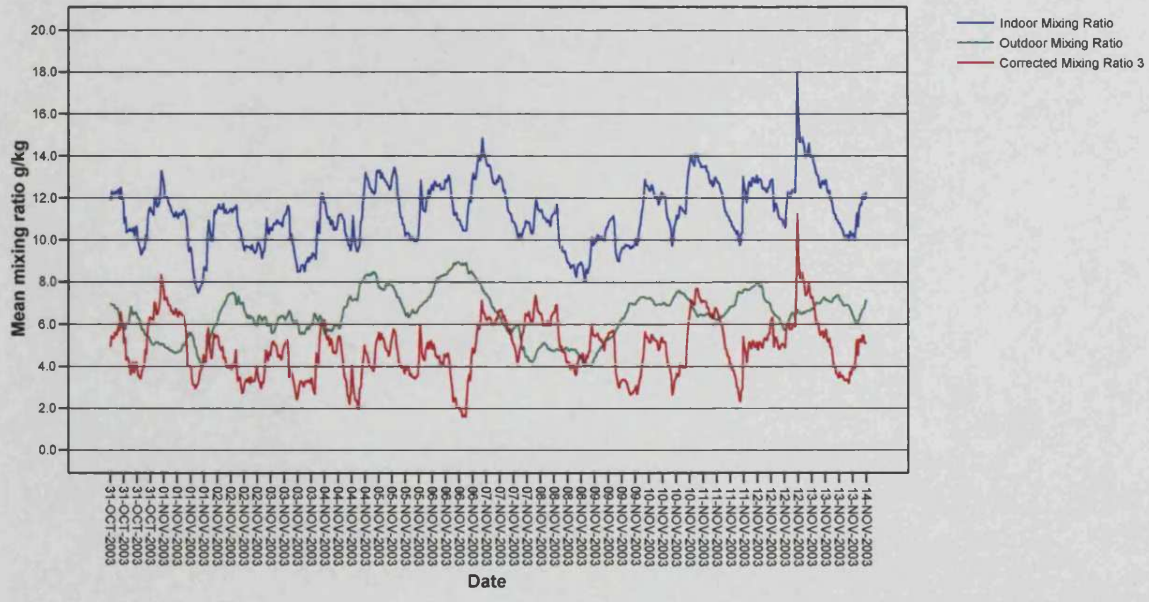


Figure 4.12. Box plot comparing the difference in corrected mixing ratios at baseline and 12 months by randomisation group

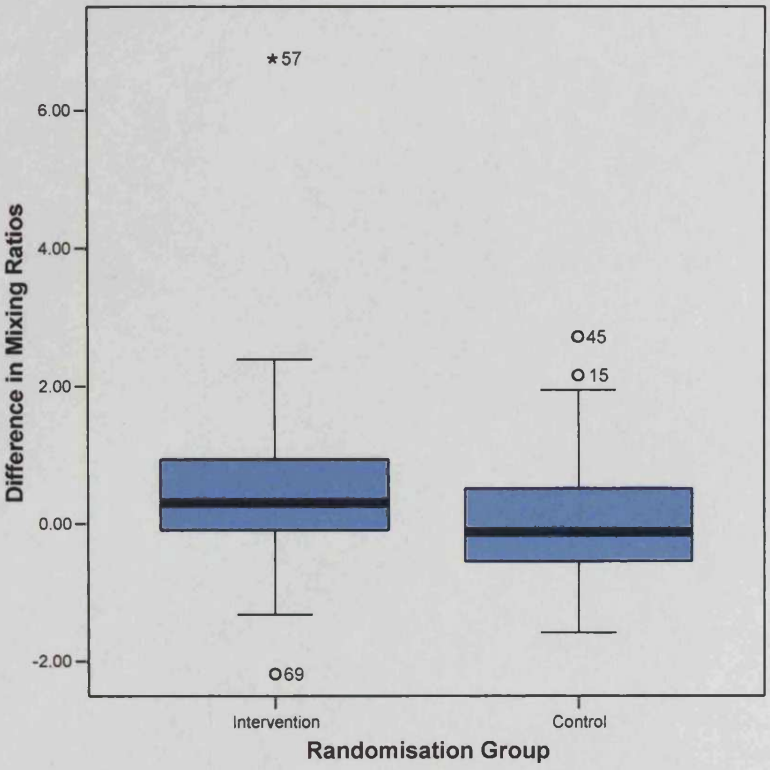
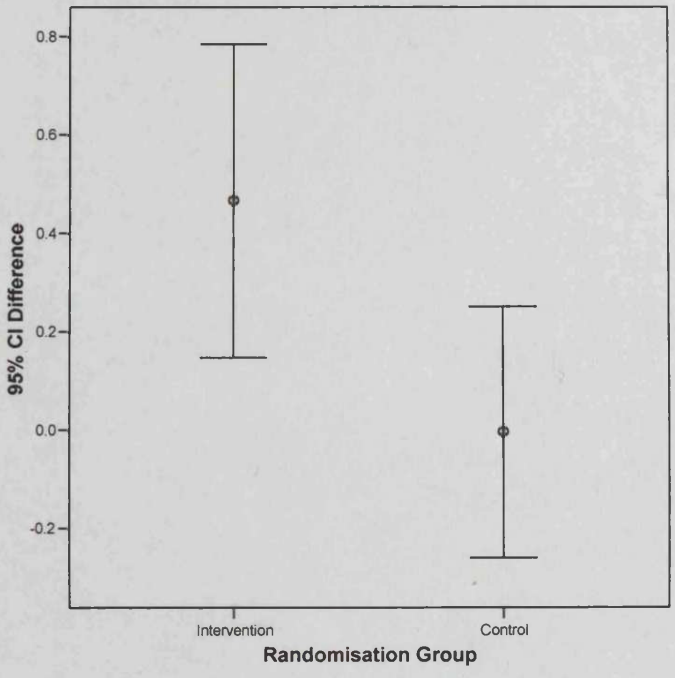


Figure 4.13. Graph comparing the mean difference in corrected mixing ratios between baseline and 12 month visits by randomisation groups



	Intervention	Control
N	64	58
Mean	0.50	0.02
Median	0.35	-0.09
t-test p value	0.011*	
Mann-Whitney p value	0.003*	

As shown in Figure 4.12, there is a significant difference between the two randomisation groups (n = 122) in the corrected mixing ratio at baseline and 12 months. The intervention group (n = 64) has a significantly higher median (0.35) than the control group (n = 58, median = -0.09), the inter-quartile range is of similar width in the two groups, as is the overall range of values (excluding outliers and extreme values). Figure 4.13 displays the means for the two groups, showing that the mean is significantly higher in the intervention group (0.50) than in the control group (0.02). The differences observed between means and medians are, statistically significant (t-test p-value = 0.011, Mann-Whitney p-value = 0.003). These findings suggest that the intervention introduced into the intervention homes has had a positive effect on reducing the corrected mixing ratios (as the difference is largest in this group). The overall percentage drop in the intervention group is 21%, compared to 0.8% increase in the control group.

Table 4.16 Outdoor mixing ratios matching the dates samples were taken

		Mean	Median	Minimum	Maximum
Intervention	Outdoor Mixing Ratio -- Baseline	6.32	6.22	4.00	9.65
	Outdoor Mixing Ratio -- 12 Months	6.54	5.97	4.32	9.42
Control	Outdoor Mixing Ratio -- Baseline	6.41	6.16	4.05	9.20
	Outdoor Mixing Ratio -- 12 Months	6.53	6.37	3.80	9.43

The outdoor mean and median values rose between baseline and 12 months, yet the indoor levels (as measured by corrected mixing ratios) dropped in the intervention group and rose slightly in the control group.

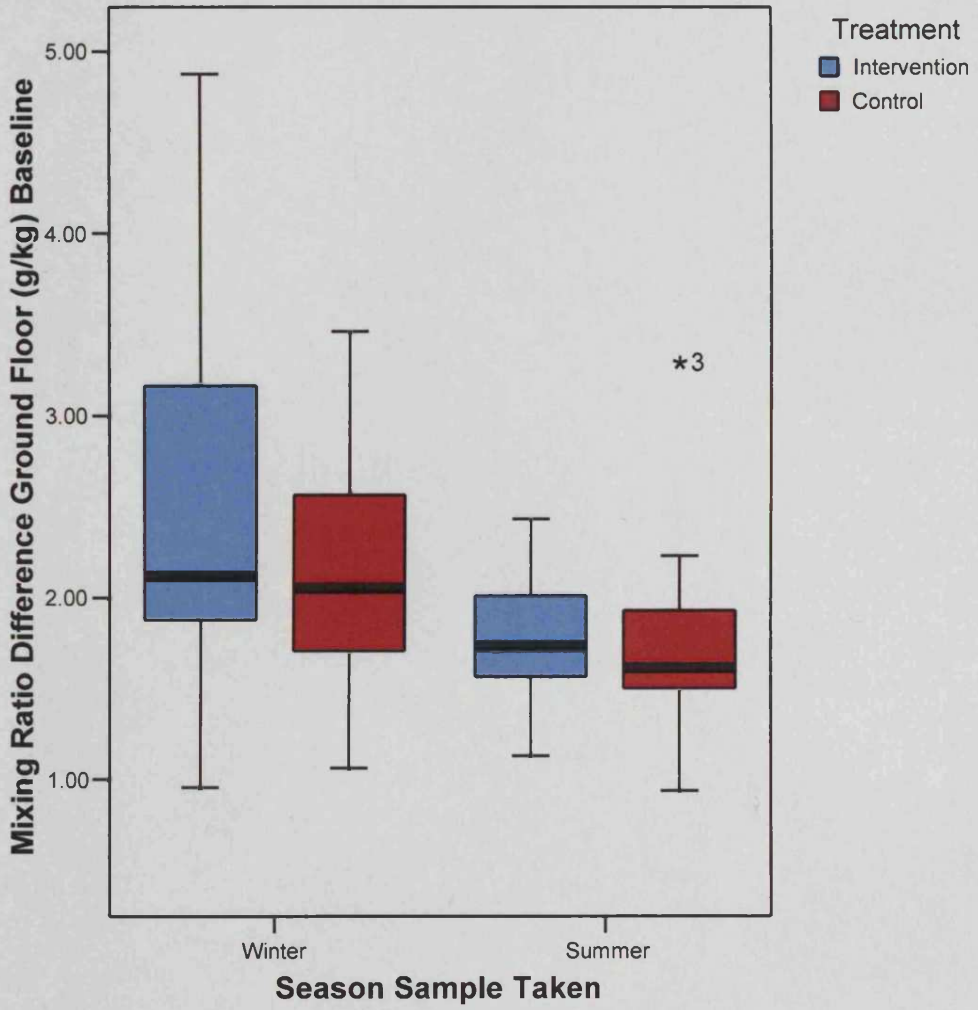
Table 4.17 Outdoor mixing ratios over entire study period – Jan 2001 to Oct 2004 *

		Mean	Median	Minimum	Maximum
Winter	Outdoor Mixing Ratio	5.78	5.79	1.76	11.92
Summer	Outdoor Mixing Ratio	6.91	6.82	1.60	14.33

* Winter months include: October to March and Summer months: April to September.

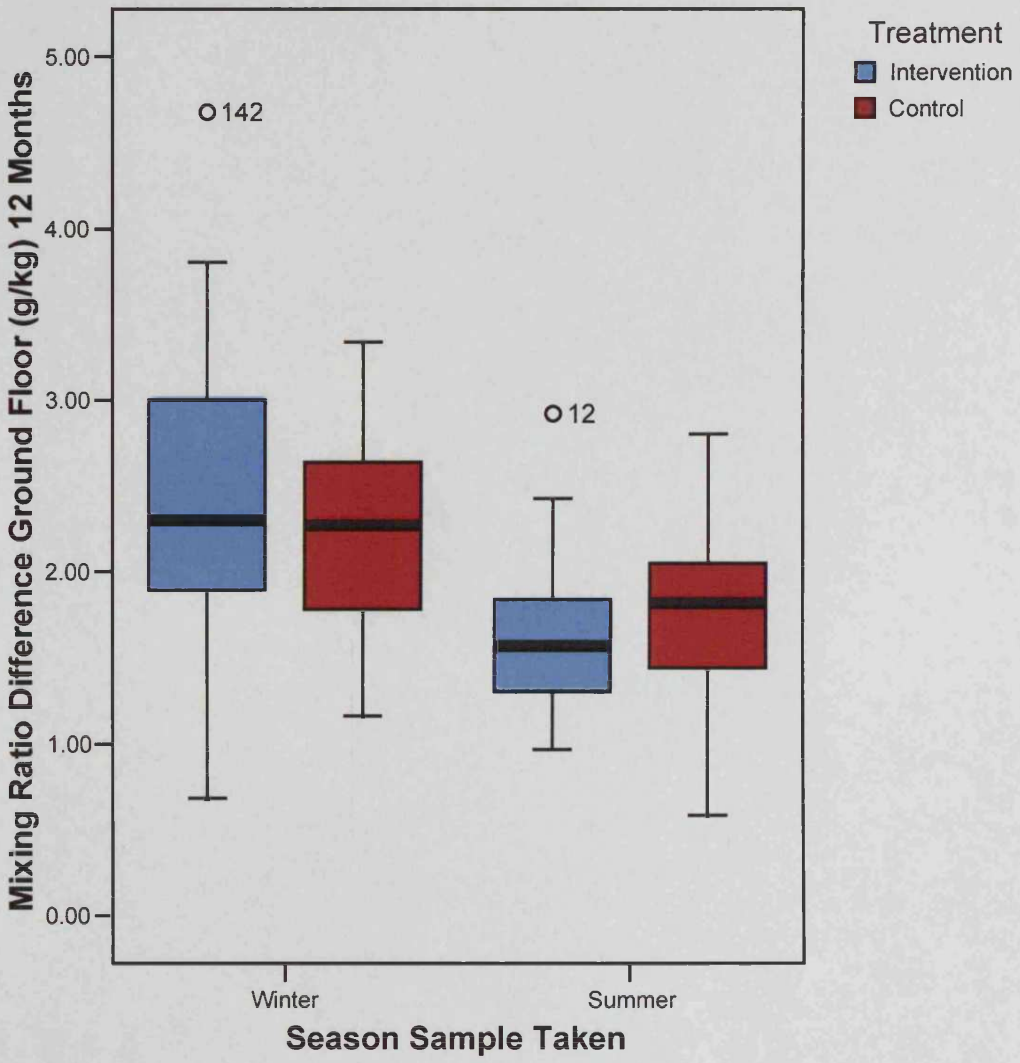
The range of outdoor mixing ratio values is much larger than those obtained on the actual sampling dates.

Figure 4.14 Corrected mixing ratio comparison for ground floor rooms at baseline split by seasons *



* Winter months include: October to March and Summer months: April to September.

Figure 4.15 Corrected mixing ratio comparison for ground floor rooms at 12 months by season*



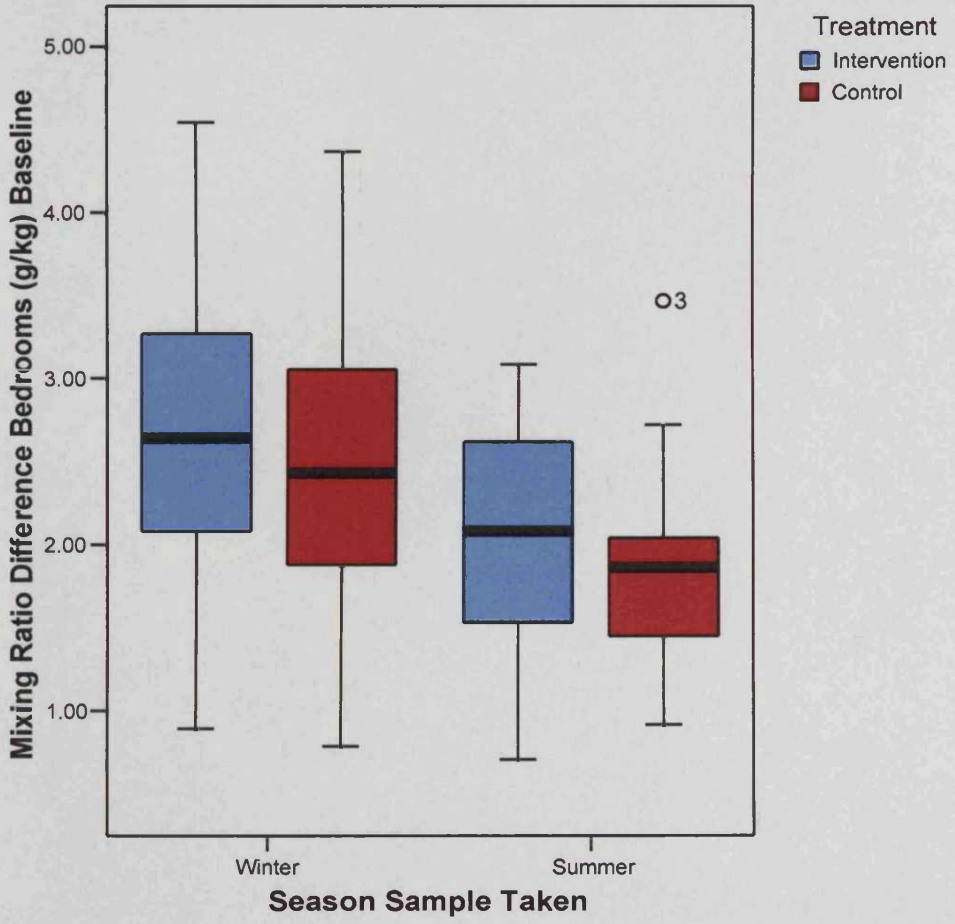
* Winter months include: October to March and Summer months: April to September.

Splitting the sample set into two seasons and between upper and lower floors where applicable shows some significant results. At baseline (see figures 4.14 & 4.16) the median and mean values are almost identical, which is to be expected as the samples had recently been randomised between the two groups, however the bedrooms show more variation. The sample sizes varied with approximately ten more homes during the winter months than the summer ones. On the ground floors, there is little change between the intervention and control groups but with the intervention mixing ratio difference dropping slightly in the summer season.

Table 4.18 Comparison of the mean difference in corrected mixing ratio between intervention and control groups on the ground floor

	Intervention	Control
N – ground floor rooms	44	39
Mean – ground floor rooms	0.12	-0.04
Median – ground floor rooms	0.17	-0.02
t-test p value – ground floor rooms	0.176	
Mann-Whitney p value – ground floor rooms	0.243	

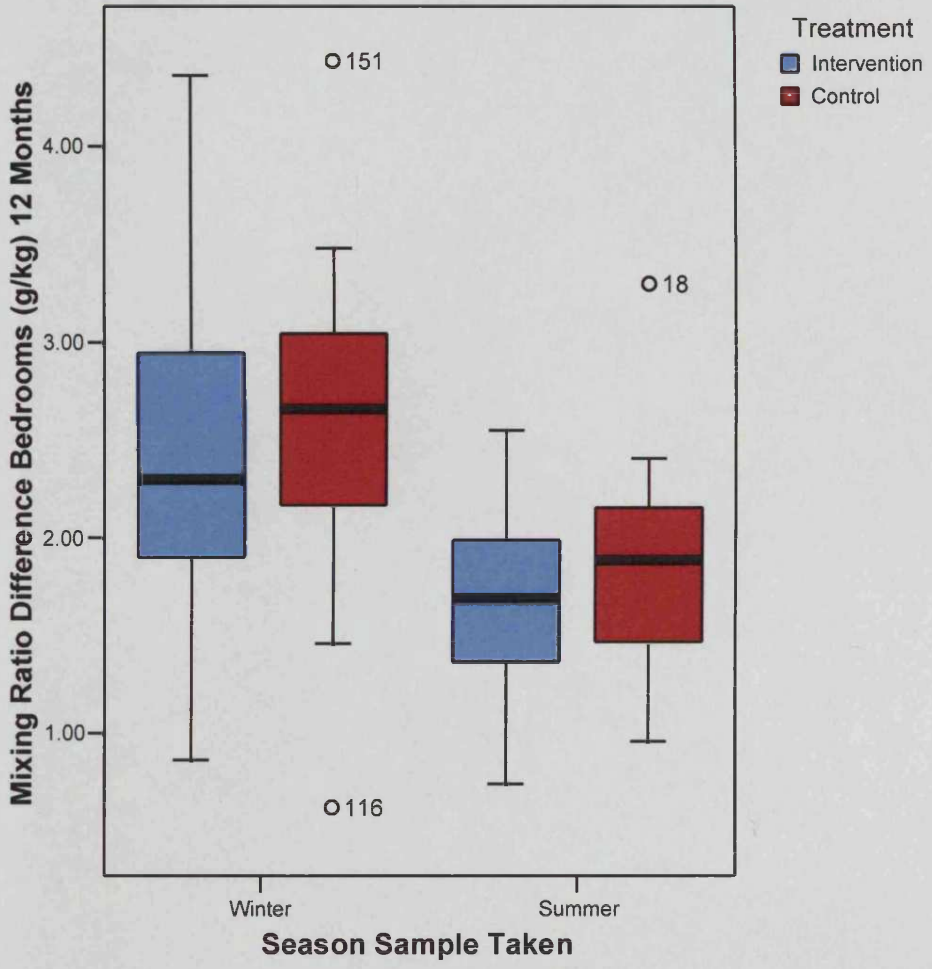
Figure 4.16 Corrected mixing ratio comparison in bedrooms at baseline by season *



*Winter months include: October to March and Summer months: April to September.

In the bedrooms, there is considerable change at 12 months compared to baseline however, with the intervention values dropping in both seasons and the control values staying constant (see figure 4.17). The drops in the intervention values in the winter are greater than those in the summer but are not statistically significant. The difference between summer and winter was significant at the $p<0.05$ level for all groups. There was a statistically significant difference ($p<0.05$) in the corrected mixing ratios between the intervention and control homes in the bedrooms ($p=0.006$) but not in ground floor rooms ($p=0.176$), see tables 4.18 and 4.19. When considering the whole house the t-test value returned was close to significance with $p=0.052$ and the Mann-Whitney $p = 0.005$.

Figure 4.17 Corrected mixing ratio comparison in bedrooms at 12 months *



Ταβλε 0.┌ Winter months include: October to March and Summer months: April to September.

Table 4.19 Comparison of the mean difference in corrected mixing ratio between intervention and control groups in bedrooms

	Intervention	Control
N – all bedrooms	43	39
Mean – all bedrooms	0.33	-0.05
Median – all bedrooms	0.22	-0.06
t-test p value – all bedrooms	0.006*	
Mann-Whitney p value – all bedrooms	0.005*	

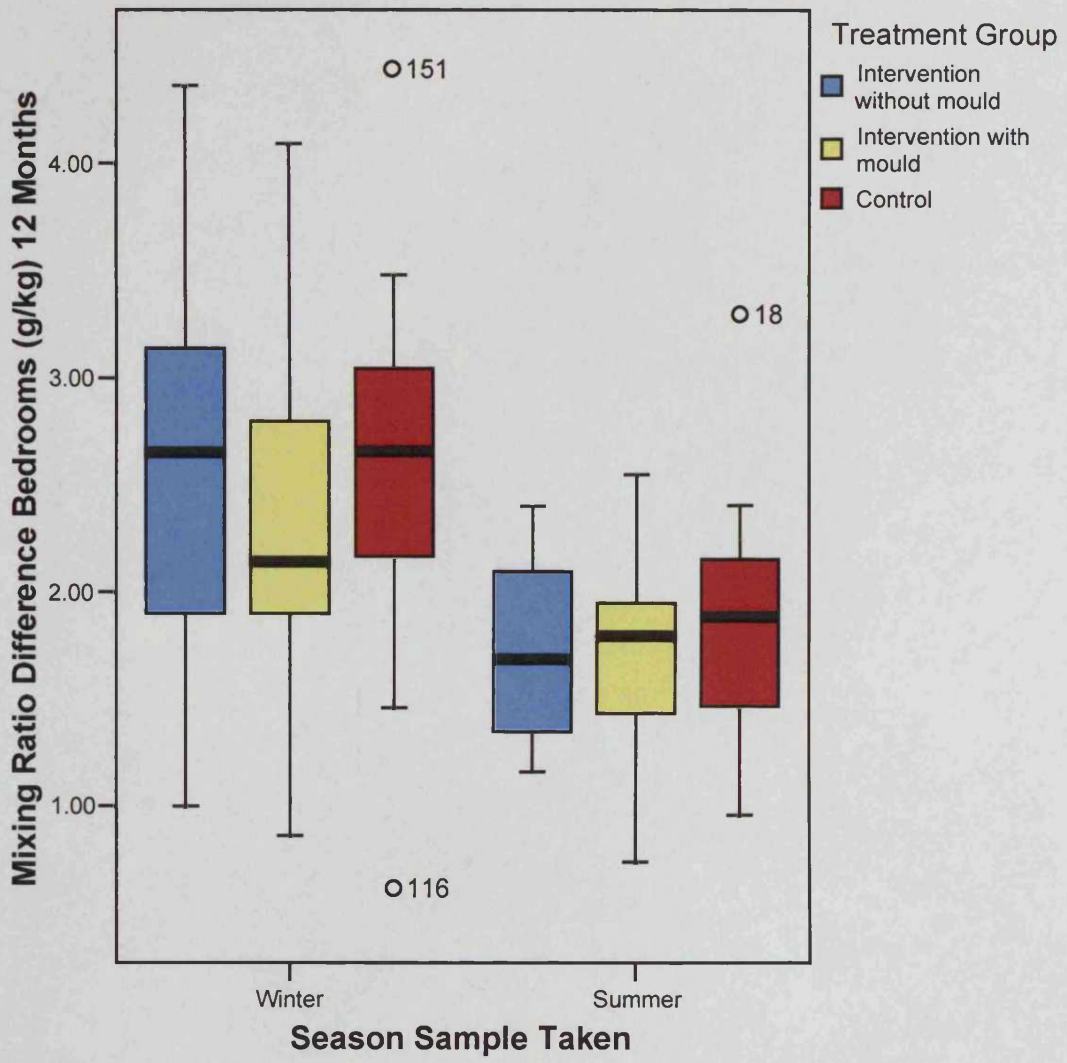
Table 4.20. The difference in the corrected mixing ratio values (g/kg) between the randomisation groups; for the entire year and also split between winter and summer months.

	Intervention	Control	Difference (n% improvement)
Whole House -- Year	0.16	-0.04	0.20 (2.5%)
Whole House -- Winter	0.20	-0.03	0.23 (3.0%)
Whole House -- Summer	0.12	-0.04	0.16 (1.8%)
All bedrooms -- Year	0.33	-0.05	0.38 (4.8%)
All bedrooms -- Winter	0.27	-0.03	0.30 (3.8%)
All bedrooms -- Summer	0.43	-0.07	0.50 (5.6%)
Ground Floor Rooms -- Year	0.12	-0.04	0.16 (2.0%)
Ground Floor Rooms -- Winter	0.15	-0.03	0.18 (2.3%)
Ground Floor Rooms -- Summer	0.07	-0.05	0.12 (1.3%)

Larger differences show greater improvement.

Referring to table 4.20 the positive effects of the ventilation units on the mixing ratios is diluted by looking at either the means of the whole house or the yearly averages.

Figure 4.18 A comparison of intervention without mould, those where mould returned and control homes for all bedrooms by season.*



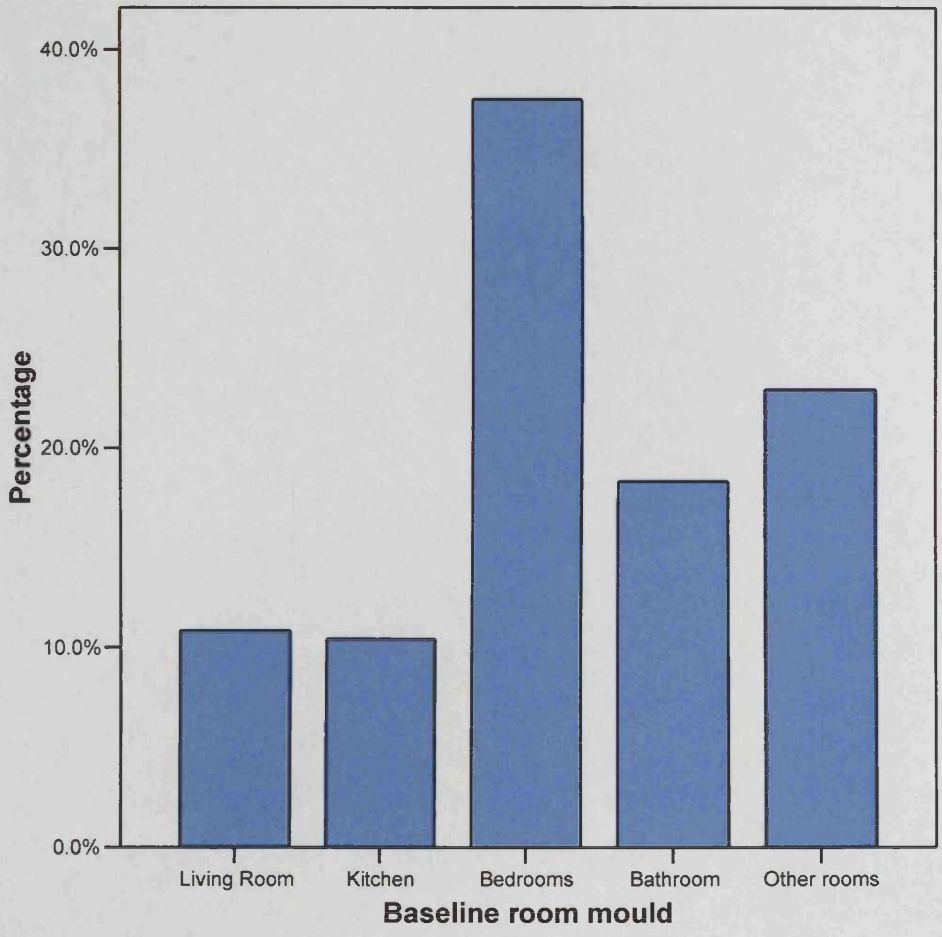
* Winter months include: October to March and Summer months: April to September.

The summer months' high outdoor mixing ratio has an overwhelming affect on the indoor mixing ratio as shown by the small reductions and variability in the three groups. There was a greater drop in the difference in corrected mixing ratio in the intervention homes where mould returned than in the other groups during the winter months.

4.5. Distribution of visible mould at baseline

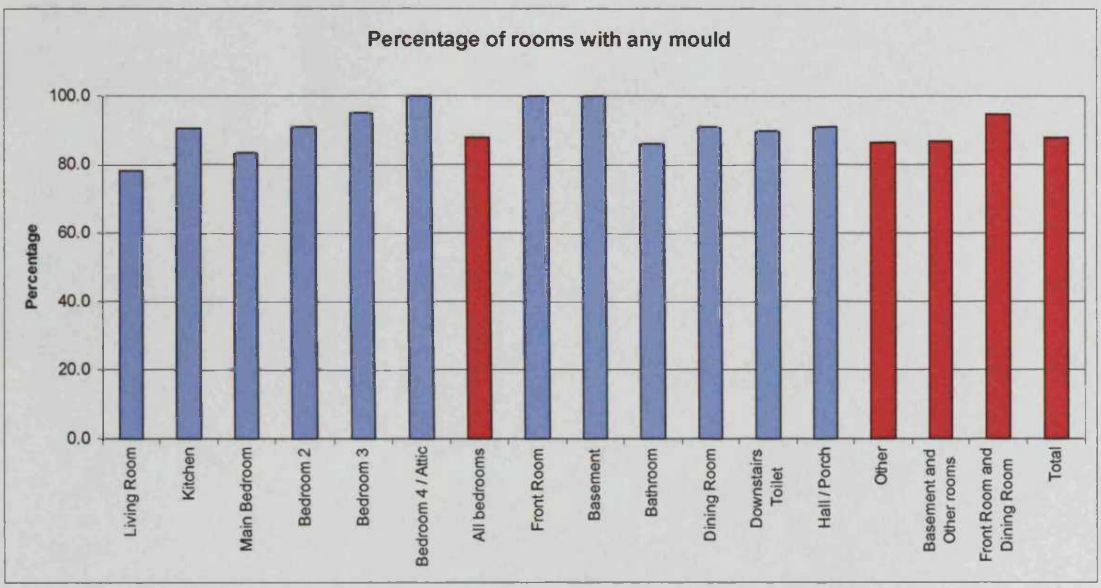
4.5.1. Percentage of rooms with visible mould in total sample

Figure 4.19. Bar chart showing the percentage of rooms from the total sample with observed mould (n = 240)



As shown in Figure 4.19 above, visible mould was most commonly found in bedrooms (38%), followed by other rooms (23%), then bathrooms (18%) followed by kitchens (10%) and living rooms (11%). The bedrooms percentage includes all bedrooms with mould, not just the bedrooms of asthmatics. Other rooms includes; dining rooms; front rooms; halls; porches; basements and conservatories. Bathrooms includes; both upstairs and downstairs bathrooms and toilets.

Figure 4.20. Bar chart showing the percentage of rooms sampled by swab with any mould



Room combinations are displayed in red

For each room with visible mould a swab sample was taken, which was then cultured. Of all the rooms sampled by swabs, just less than 90% were culturable on cornmeal agar media. All the attic rooms, front rooms and basements sampled had viable mould spores. Approximately 78% of living rooms sampled had positive swabs. Spores from the swabs were not always culturable; this may be caused by the use of household detergents, which can inhibit the mould spores from developing. This may occur around window frames and in bathrooms where moulds are unsightly and easily removed.

4.5.2. Variation in mould genera (as identified by swab)

Table 4.21. Variation in positive swab samples by genera and by room type

Room	Total rooms	No mould (0)	*Absidia*	*Alternaria*	*Aspergillus*	*Aspergillus niger*	*Aureobasidium pullulans*	*Chaetomium*	*Cladosporium*	*Gliocladium*	*Penicillium*	*Phoma*	*Rhizopus*	*Stachybotrys*	Sterile mycelium	Yeasts
Living room	46	10	1	0	3	2	2	0	11	0	25	1	0	8	1	3
Kitchen	43	4	0	0	2	0	1	0	15	0	22	1	0	8	3	3
Main bedroom	73	12	0	1	7	0	4	0	36	4	41	2	20	11	0	3
Bedroom 2	55	5	0	0	5	0	2	0	20	1	30	0	0	9	2	3
Bedroom 3	21	1	0	0	1	0	1	0	4	0	16	1	0	2	1	1
Attic/ Bedroom 4	2	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0
All bedrooms	151	18	0	1	13	0	7	0	60	5	88	4	20	23	3	7
Front room	8	0	0	0	1	0	1	0	4	1	5	1	0	1	0	0
Basement	1	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0
Bathroom	78	11	0	2	1	0	3	0	30	1	34	2	0	17	6	1
Other	22	3	7	5	6	6	4	0	9	3	14	1	0	2	1	0
Basement and other rooms	23	3	7	5	7	6	4	0	10	3	15	1	0	2	2	0

Room	Total rooms	No mould (0)	*Absidia*	*Alternaria*	*Aspergillus*	*Aspergillus niger*	*Aureobasidium pullulans*	*Chaetomium*	*Cladosporium*	*Gliocladium*	Penicillium	*Phoma*	*Rhizopus*	*Stachybotrys*	Sterile mycelium	Yeasts
Hall/ Porch	33	3	0	0	4	1	0	1	7	0	19	1	1	4	1	3
Dining room	11	1	0	0	1	0	1	0	2	0	5	0	0	2	0	1
Front room and dining room	19	1	0	0	2	0	2	0	6	1	10	1	0	3	0	1
Downstairs toilet	10	1	0	0	0	1	0	0	1	0	4	0	0	3	0	1
Total	596	73	15	14	54	16	32	1	216	19	330	17	41	96	21	27
Overall % of each mould		12.25	2.52	2.35	9.06	2.68	5.37	0.17	36.24	3.19	55.37	2.85	6.88	16.11	3.52	4.53

The mould genera found most often from swab samples is *Penicillium* (55.4%) followed by *Cladosporium* (36.2%), *Stachybotrys* (16.1%), *Aspergillus* (9.1%), *Rhizopus* (6.9%), and *Aureobasidium pullulans* (5.4%). More than half the swabs taken contained *Penicillium* and just over two-thirds had *Cladosporium*.

Figure 4.21. Bar chart showing the variation in mould genera for homes sampled by swab

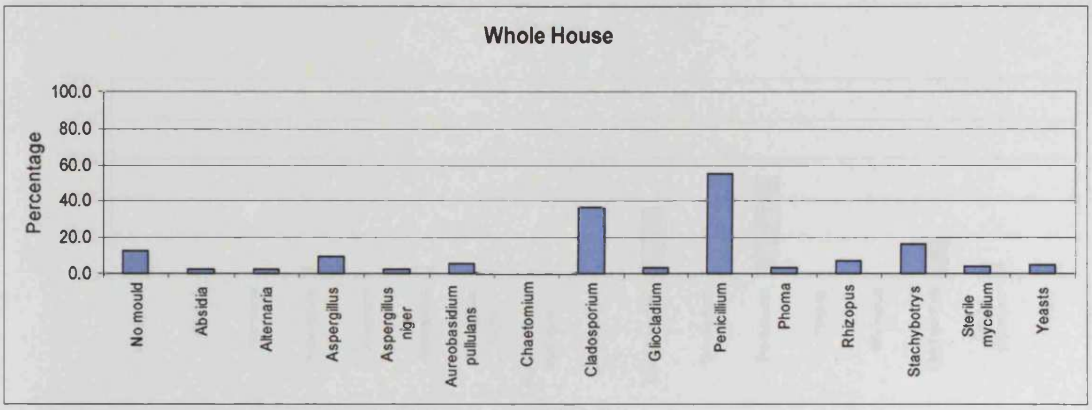


Figure 4.22. Bar chart showing the variation in mould genera for bathrooms sampled by swab

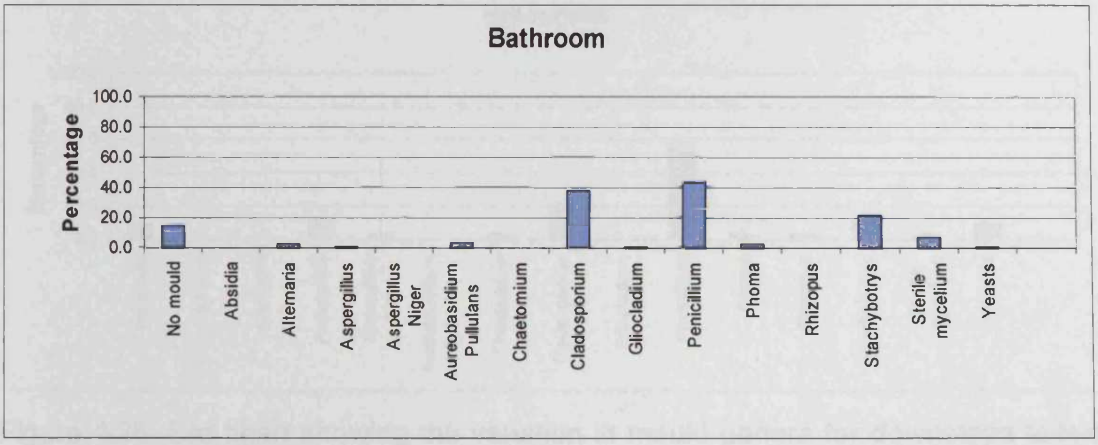


Figure 4.23. Bar chart showing the variation in mould genera for livings rooms sampled by swab

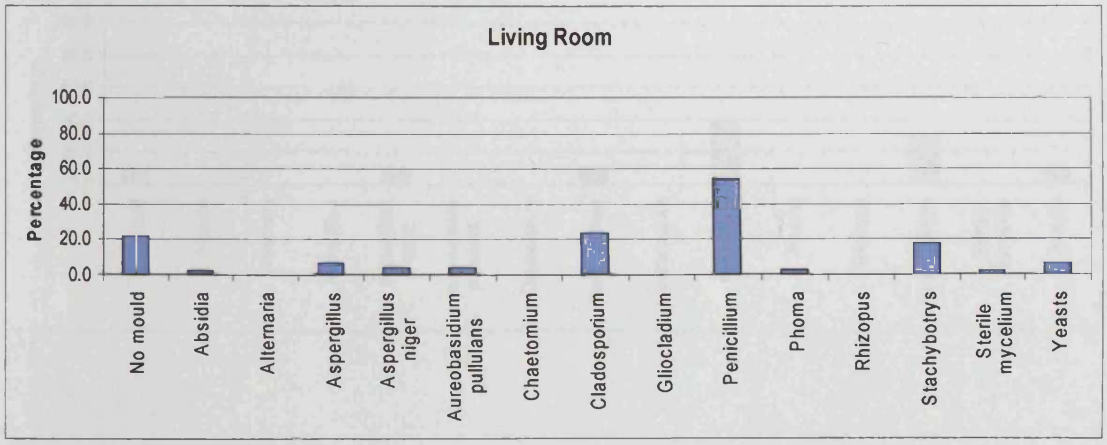


Figure 4.24. Bar chart showing the variation in mould genera for kitchens sampled by swab

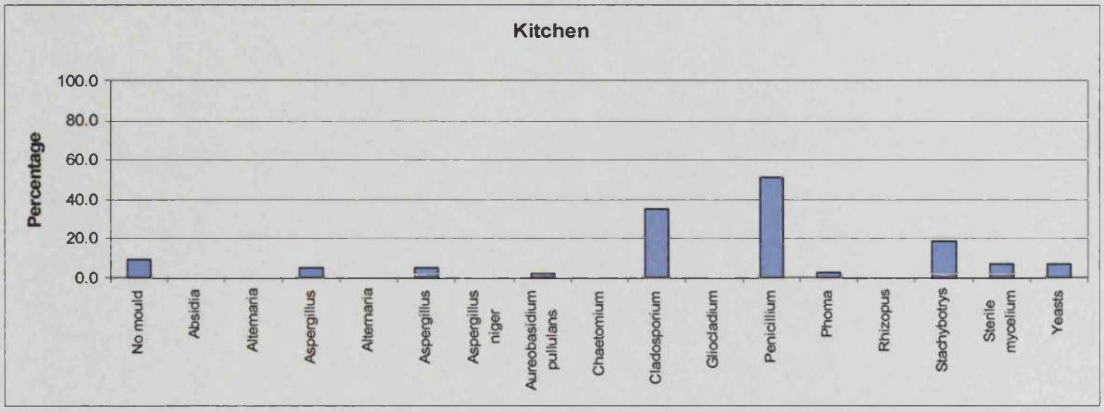


Figure 4.25. Bar chart showing the variation in mould genera for halls and porches sampled by swab

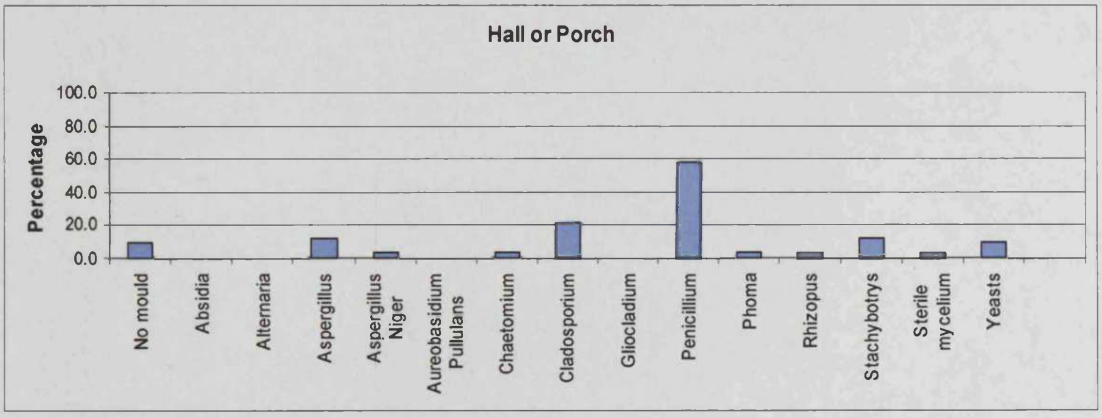


Figure 4.26. Bar chart showing the variation in mould genera for downstairs toilets sampled by swab

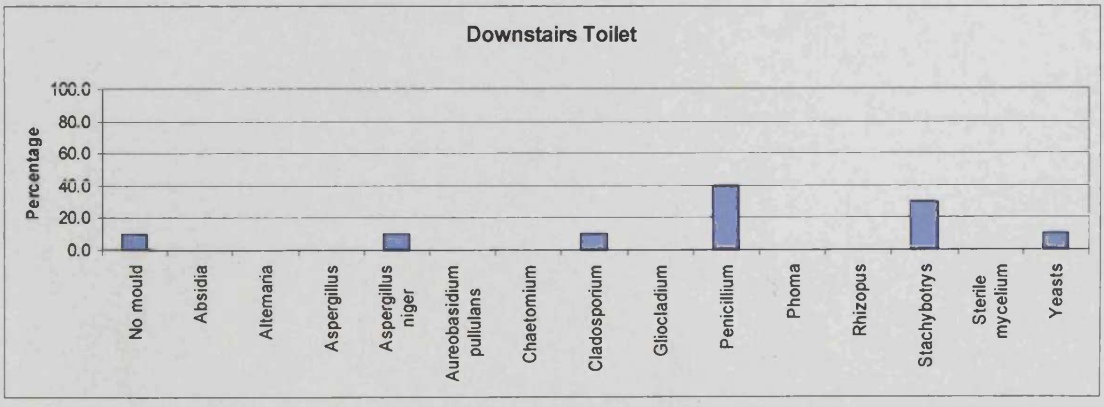


Figure 4.27. Bar chart showing the variation in mould genera for all bedrooms sampled by swab

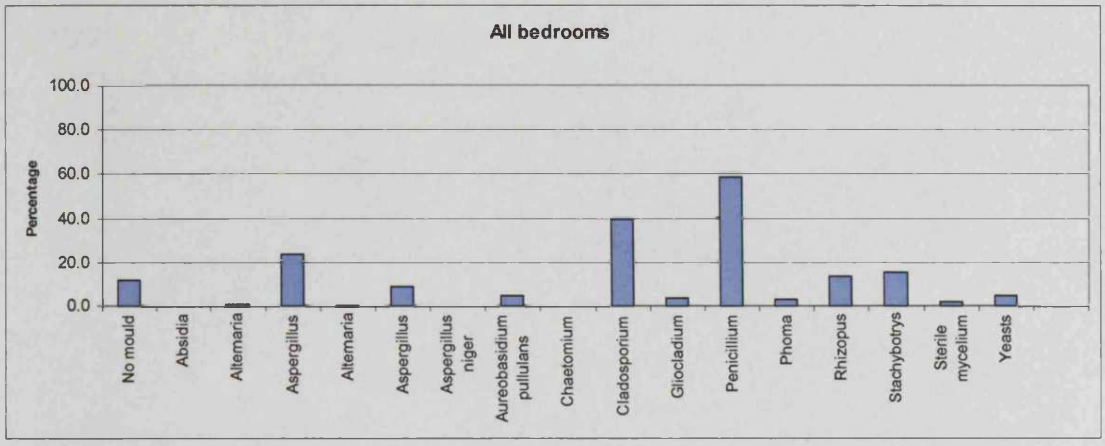


Figure 4.28. Bar chart showing the variation in mould genera for all basements and other rooms sampled by swab

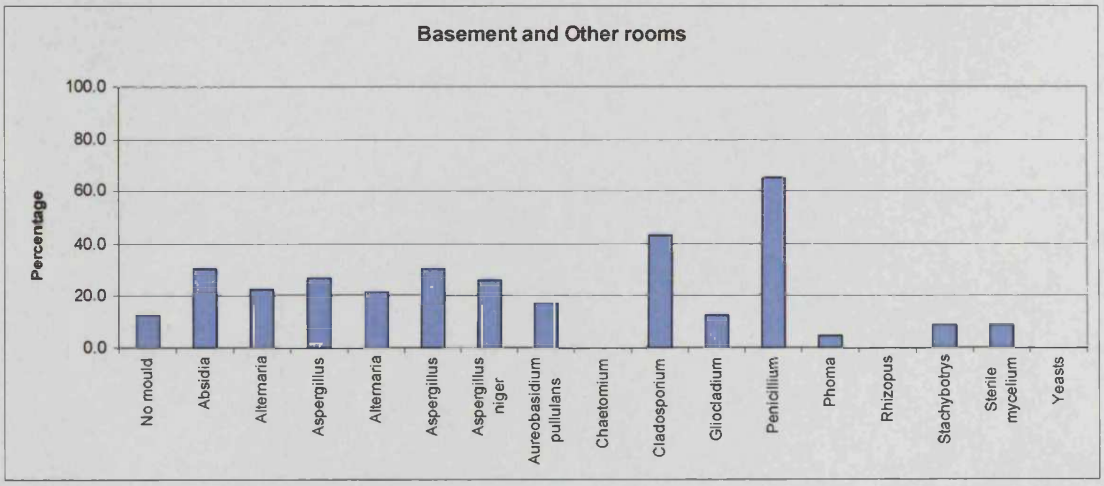
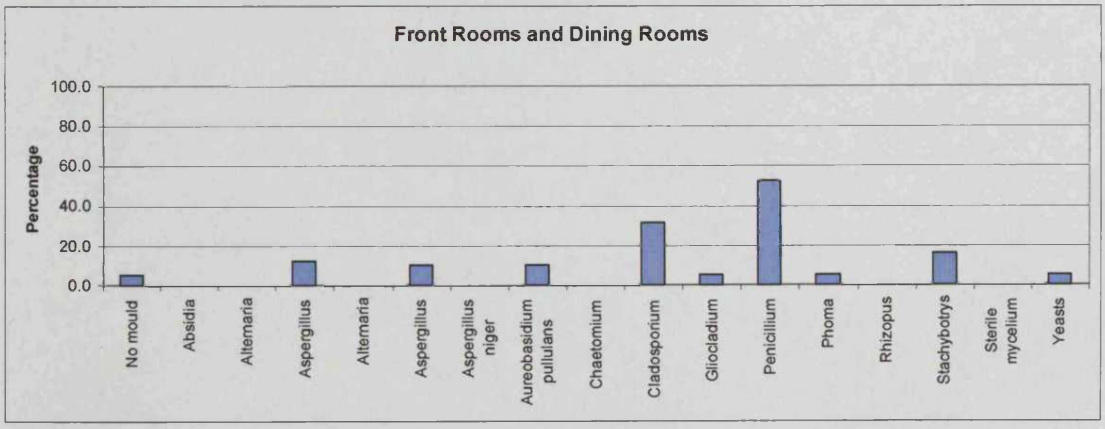


Figure 4.29. Bar chart showing the variation in mould genera for all front rooms and dining rooms sampled by swab

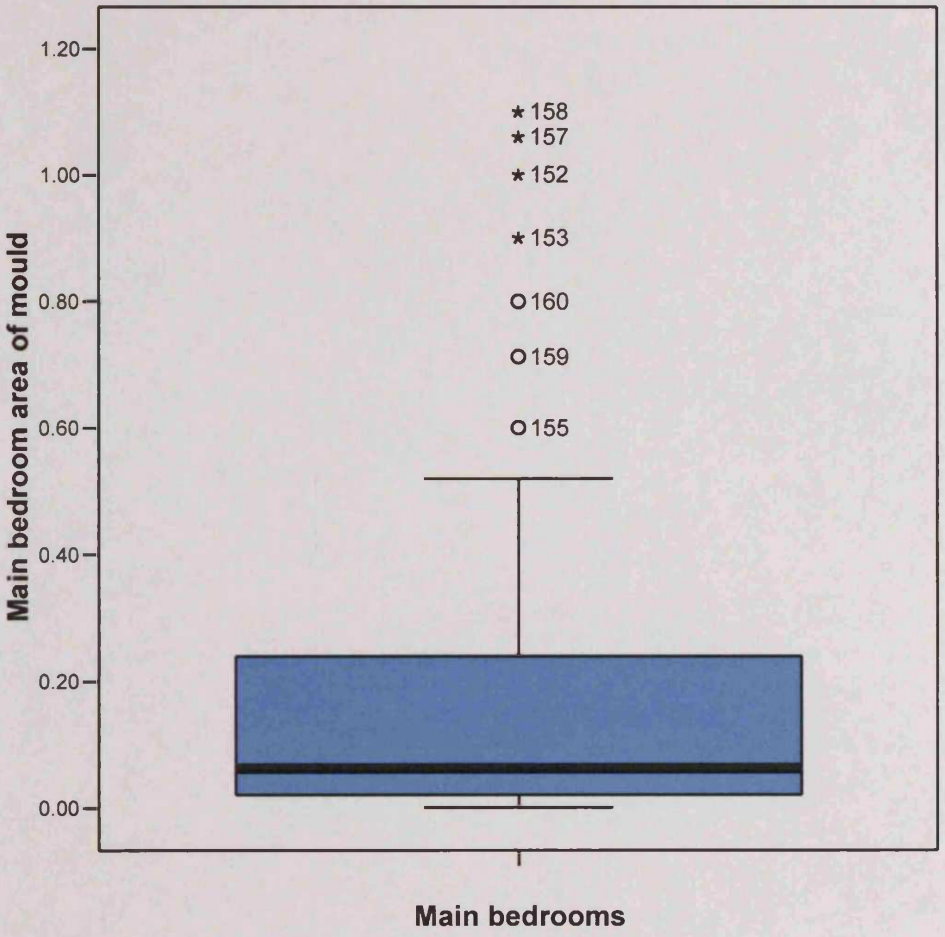


As shown in the previous table, *Penicillium* is the most frequent mould found. As seen in the graphs it is the dominant mould in all the rooms shown. In the damper

environments, (i.e. the bathroom and basements) *Cladosporium* almost equals the *Penicillium*. For the downstairs toilet and the bathroom, *Stachybotrys* is very abundant.

4.5.3 Variation in area of mould

Figure 4.30. Box plot showing the variation in the Area of Mould (m^2) in main bedrooms – excluding bedrooms with no mould *

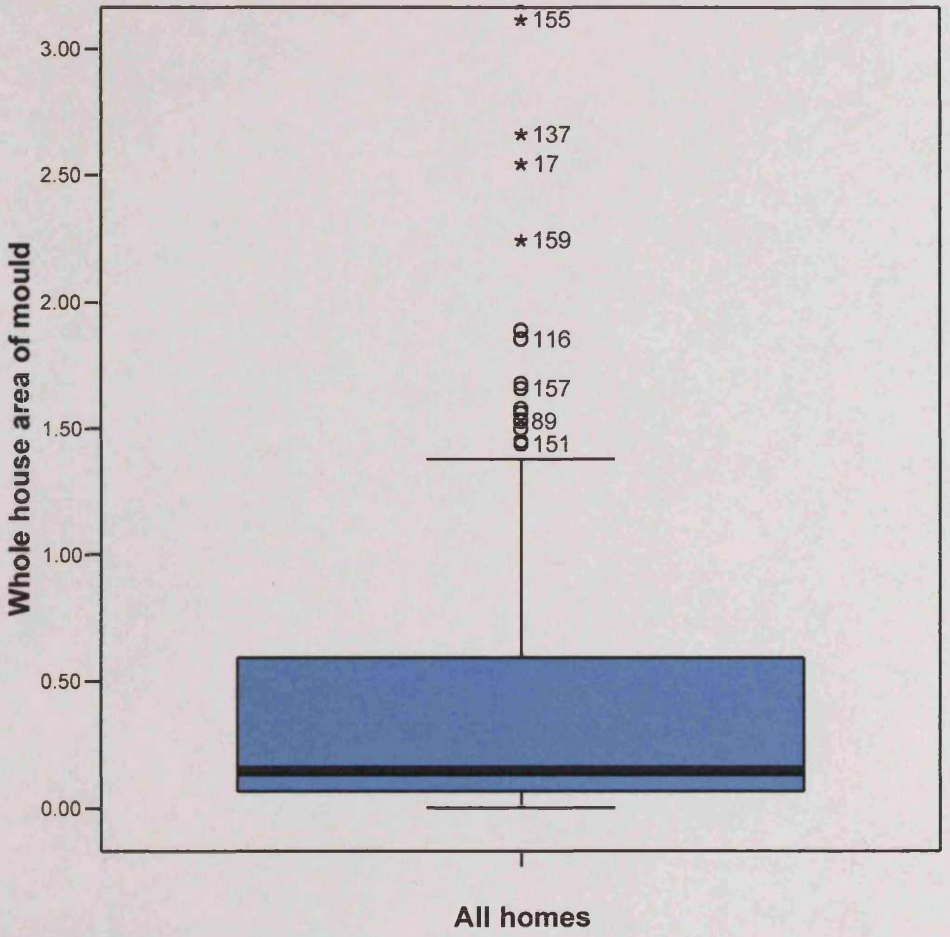


* Three extreme values (1.6, 4.9 & 6.4) have been removed to better display the results.

N	74
Mean	0.3521
Median	0.0620

Forty-five percent of main bedrooms sampled had mould (from a total n = 164). Of these 74 bedrooms with mould, the mean area of mould in these bedrooms was 0.35 m^2, and the median area was 0.06 m^2. The median value is a better indicator of the distribution as the very large outliers and extreme values skew the mean value. The median area of mould is very small in relation to the surface area of the typically room (~40-55m^2).

Figure 4.31. Box plot showing the variation in the Area of Mould (m^2) in whole houses *



* For presentation purposes, five of the extreme values are not shown (3.2, 3.8, 4.8, 5.4 & 15.3).

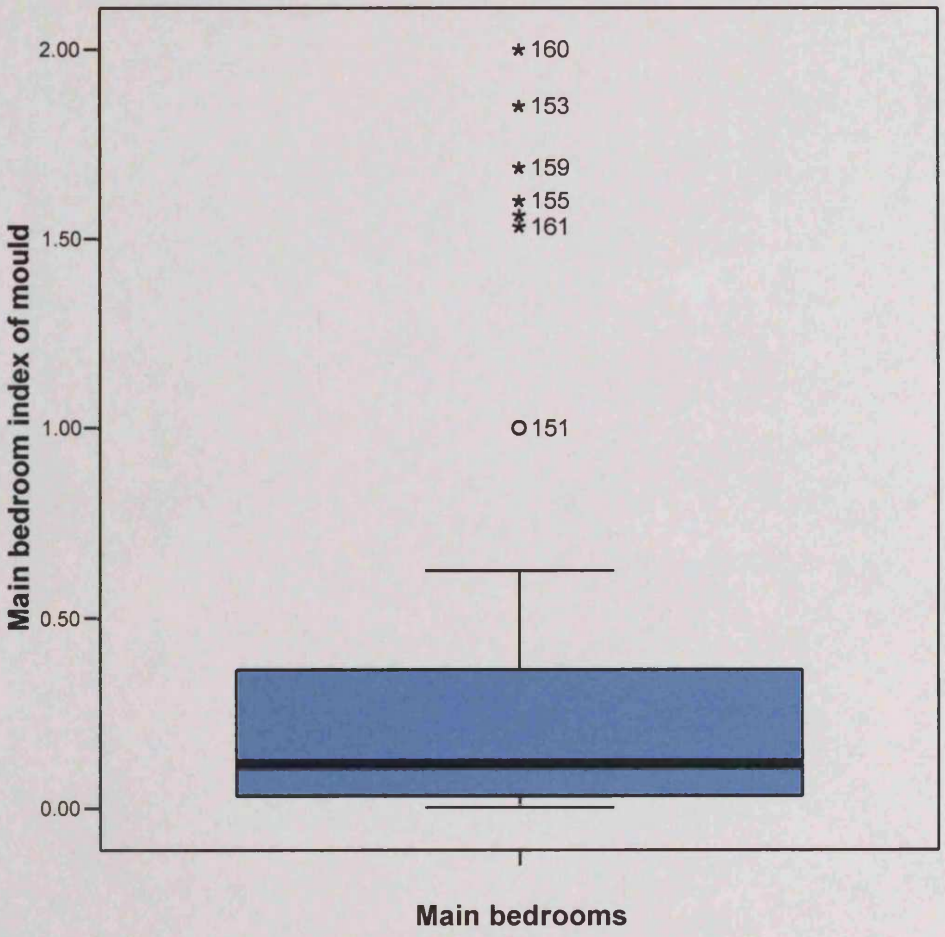
N	164
Mean	0.6360
Median	0.1450

Of the 164 homes sampled, the mean area of mould was 0.64 m^2, and the median was 0.15 m^2. Again, extreme values have skewed the result shown by the large difference between the median and mean values.

4.5.4 Variation in Mould Density Index

As discussed in the methodology section; the mould index is a product of the mould area multiplied by the categorical variables of mould density (1-5).

Figure 4.32. Box plot showing the variation in the Index of Mould in main bedrooms – excluding bedrooms with no mould



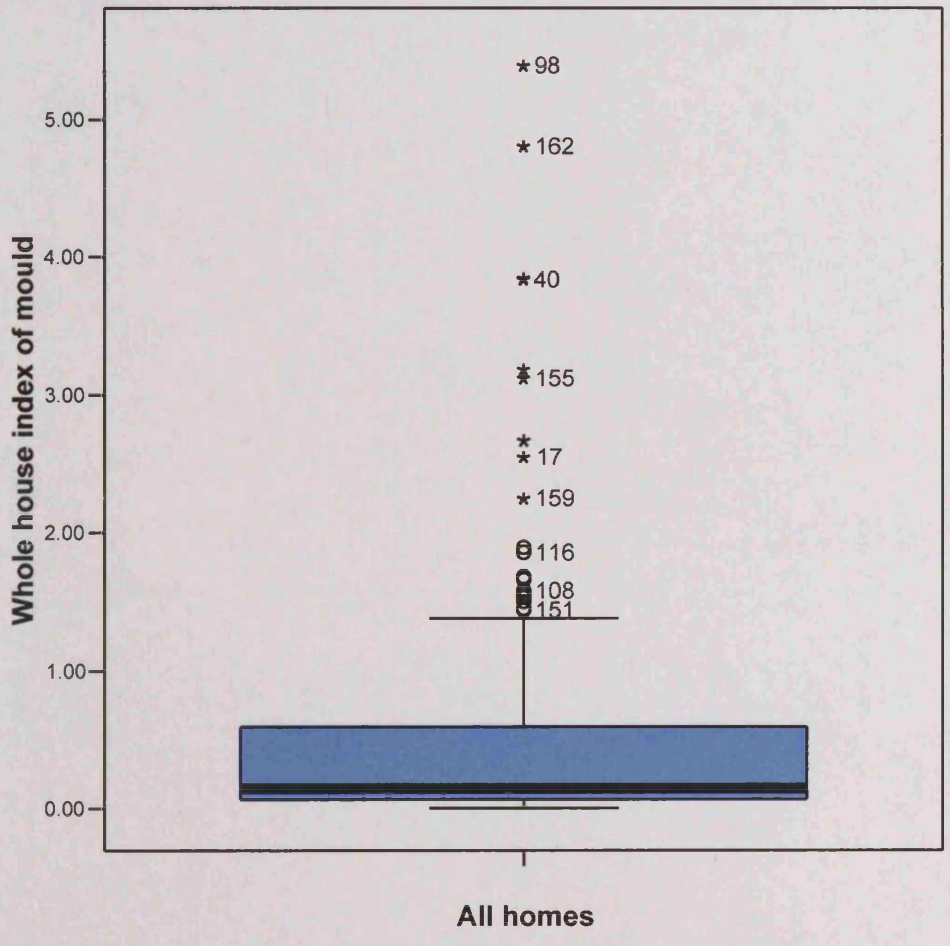
- The extreme values (2.5, 2.8, 3.8, 9.6 & 12.8) are included but not shown for presentation purposes.

N	74
Mean	0.7032
Median	0.1163

Of the 74 (45%) bedrooms sampled with mould, the mean index of mould was 0.7, and the median was 0.12. Multiplying the area by a number has exaggerated the spread of values.

There were 50 percent of homes with small absolute areas of mould (less than 0.05 m^2). However, the area gives no indication of the density or the degree of colonisation of the mould growth. This density was assigned a categorical value, which is then multiplied by the area of mould coverage. The resulting index appears to be a better indicator of the degree of mould contamination than area alone.

Figure 4.33. Box plot showing the variation in the Index of Mould in whole houses *



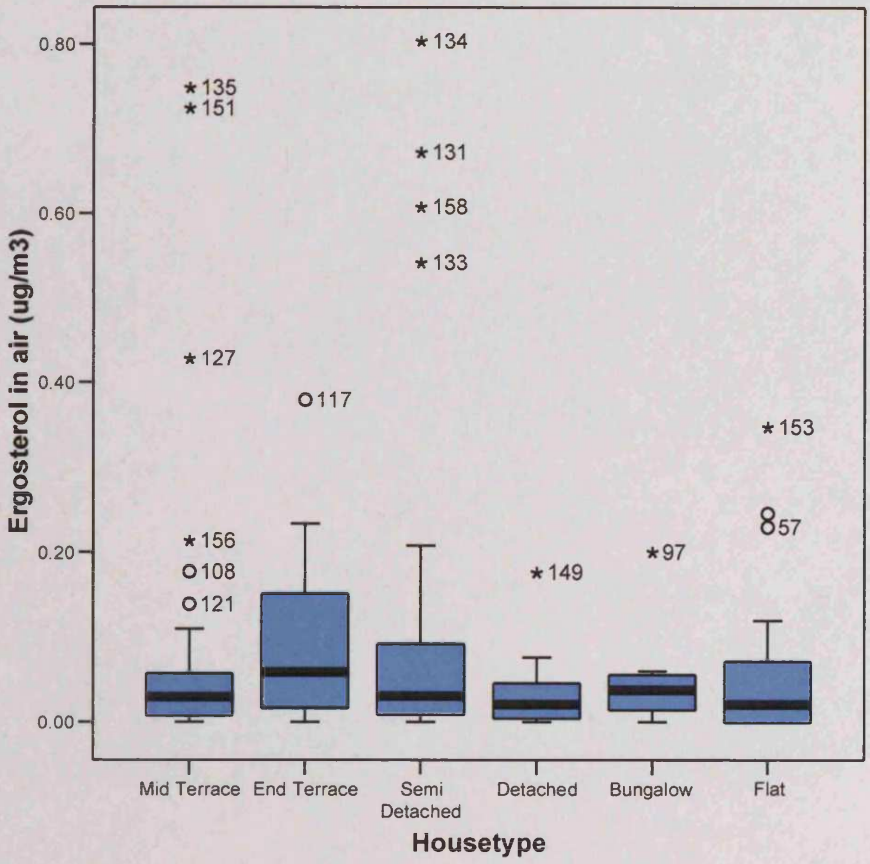
*One extreme value of 15.3 has been removed for display purposes.

N	164
Mean	1.1635
Median	0.2700

Of the 164 homes sampled the mean index of mould was 1.16, and the median 0.27. The range for the entire house is even more exaggerated than for the bedroom. The mean value is approximately four times the median value emphasising the skewed data with many lower values and a few very high ones.

4.5.5. Ergosterol results

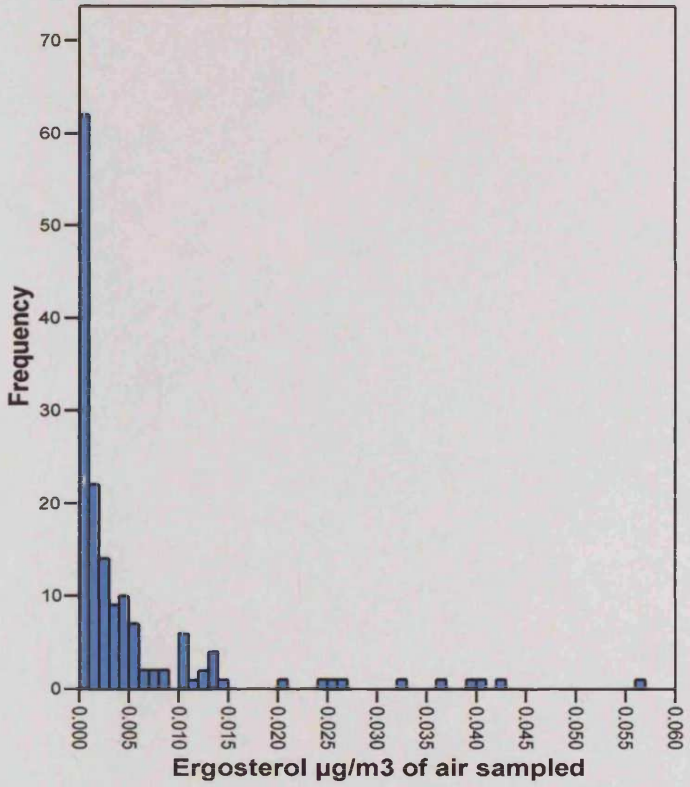
Figure 4.34. Box plot showing the range of ergosterol values (µg/m^3) of air sampled by house type for all homes sampled at baseline (N = 154)



The above figure shows the distribution of ergosterol µg/m^3 of air sampled. The end terrace and semi detached homes show the highest levels of ergosterol. There were no significant differences between the groups testing by either ANOVA or

Kruskall-Wallis at the 5% significance level (p = 0.774 and p = 0.606 respectively). The mean values (µg/m^3) for each house type are: mid terrace .005, end terrace .006, semi .006, detached .002, bungalow .003, flats .004.

Figure 4.35. Histogram showing the distribution of ergosterol of air sampled for all homes sampled at baseline (N = 154)

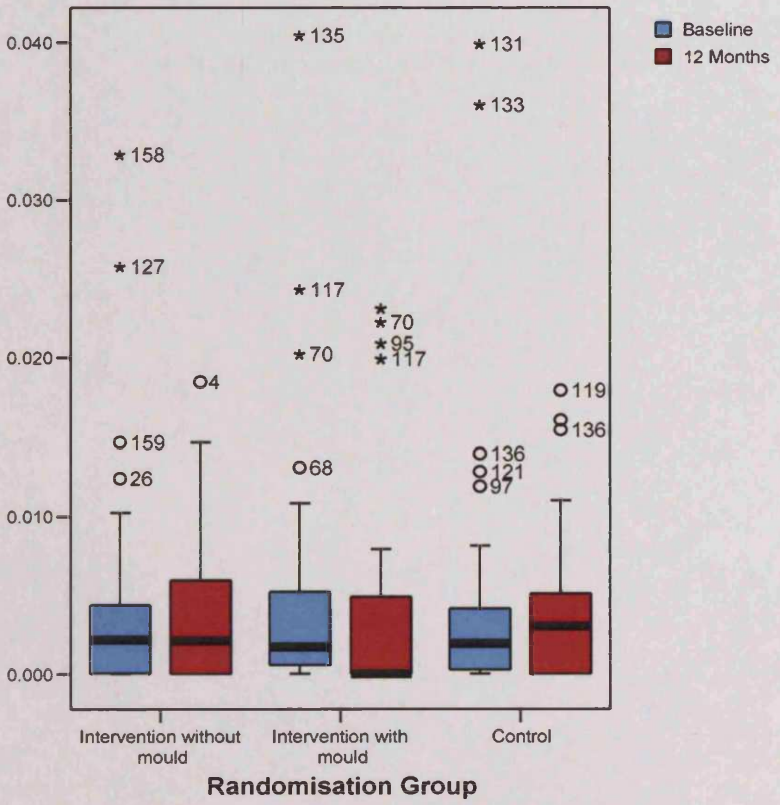


The histogram above shows the distribution in ergosterol µg/m^3 throughout the homes sampled (n = 154 homes) at baseline. In total, there were 10 missing homes primarily due to problems in the analysis during experimentation at CABI, also 2 samples were misplaced during the course of the trial. It can be seen from the above distribution that samples ranged from 0.000 – 0.056 µg/m^3, the majority of samples (76.0%) showed low levels of ergosterol in the range of 0.00 - 0.005, the highest level of ergosterol being 0.056 µg/m^3. The mean ergosterol (SD) and median ergosterol (quartiles) levels were respectively 0.0049 µg/m^3 (0.009 µg/m^3) and 0.0018 µg/m^3 (0.002 µg/m^3, 0.0049 µg/m^3).

	Intervention	Control
N	62	55
Mean	-0.0017	0.0003
Median	-0.0003	0.0006
t-test p value	0.324	
Mann-Whitney	0.288	

The analysis above describes the difference in ergosterol levels measured during the trial between baseline and 12 months, comparing the control and intervention homes. Neither the t-test or Mann-Whitney U statistics showed any significant difference (p = 0.324 and 0.288 respectively) on comparing the median or mean differences in ergosterol, although there was a drop of 5.2% in the mean ergosterol levels detected in the intervention homes as compared to a 7.6% increase in the mean level in the control homes.

Figure 4.36. Box plot showing the difference in ergosterol of air sampled between baseline and 12 month samples for all homes (N = 117) *



* One extreme value in the baseline group (Intervention without mould: 0.056) and one in the 12 month control group (0.044) are not shown due to the scale chosen.

	Intervention without mould	Intervention with mould	Control
N	40	27	59
Mean	0.0054	0.0073	0.0038
Median	0.0018	0.0018	0.0019
t-test p value	0.350	0.498	
Mann-Whitney	0.501	0.253	

The analysis and box plot shown above describes the difference in ergosterol levels measured during the trial comparing the difference between baseline and 12 months in each of the control and both intervention groups. At baseline, there were no differences in the mean ergosterol level in all the groups. At 12 months, there was a change with the intervention group without mould staying constant, the group with mould dropping and the control group increasing. Neither the t-test or Mann-Whitney U statistics showed any significant differences ($p = 0.350$ and 0.498 for t-test and $p = 0.501$ and 0.253 for Mann-Whitney, respectively) on comparing the median or mean differences in ergosterol.

4.5.6. Outside spore counts

Figure 4.37. Scatter plot showing the quantity of tree pollen spores over the sample period

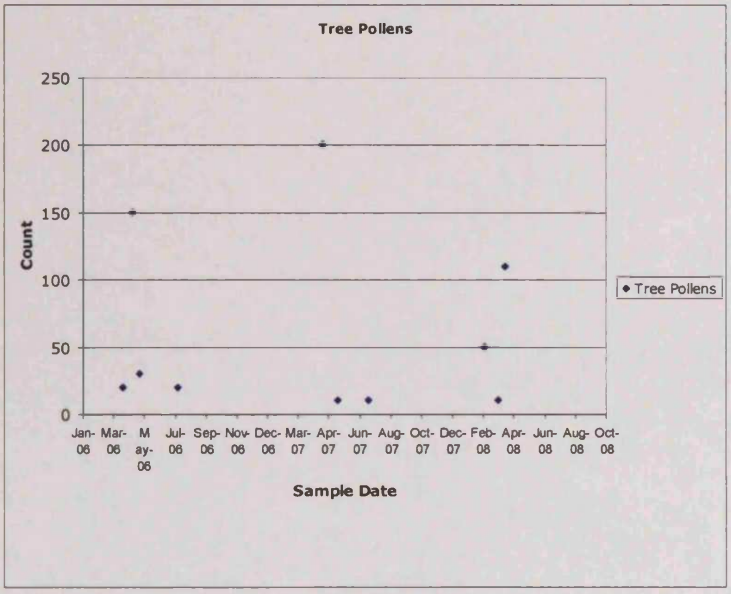
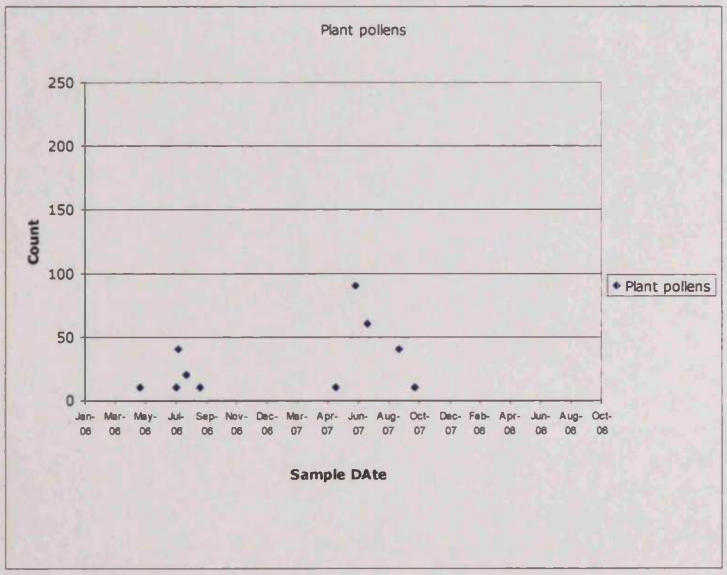
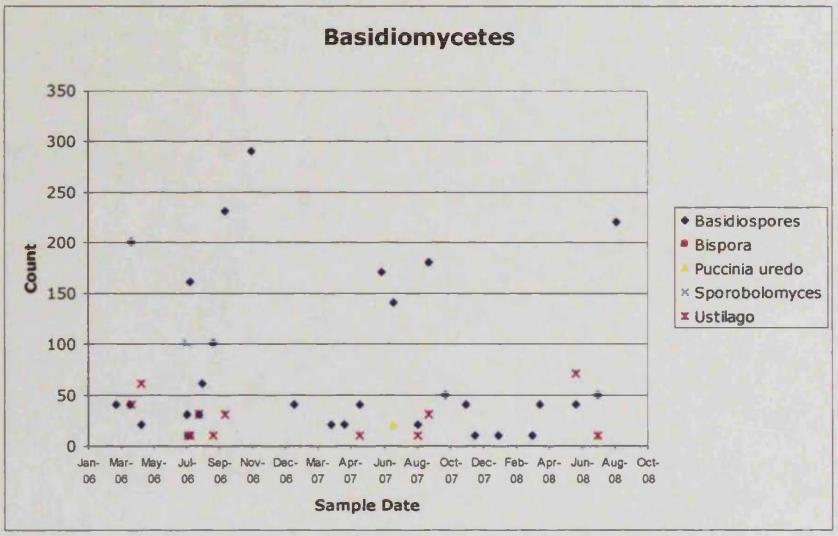


Figure 4.38. Scatter plot showing the quantity of plant pollen spores over the sample period



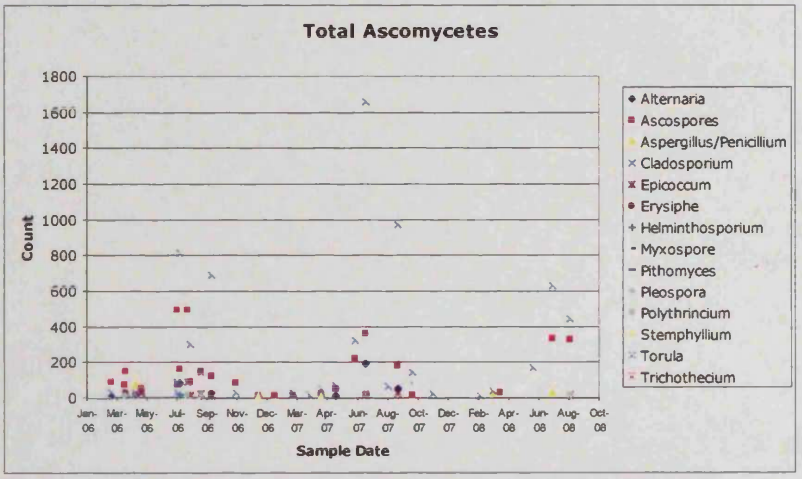
Both the tree and plant pollens show a definite frequency of release with the tree pollens earlier in the year centred around March. The plant pollens show two periods of release, one around April/May and other about July/August, one is probably grass pollen season.

Figure 4.39. Scatter plot showing the quantity of basidiomycetes spores over the sample period



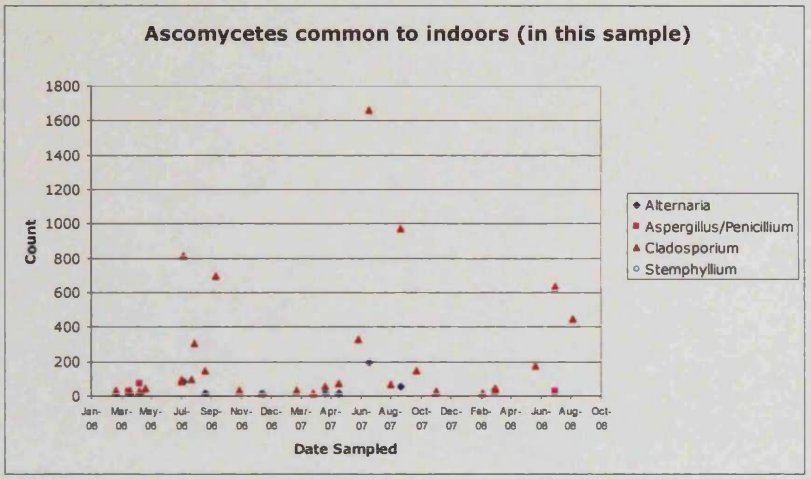
Basidiospores are the most abundant and the most frequent. *Ustilago* is the next most dominant in the outdoor spora.

Figure 4.40. Scatter plot showing the quantity of all ascomycetes spores over the sample period



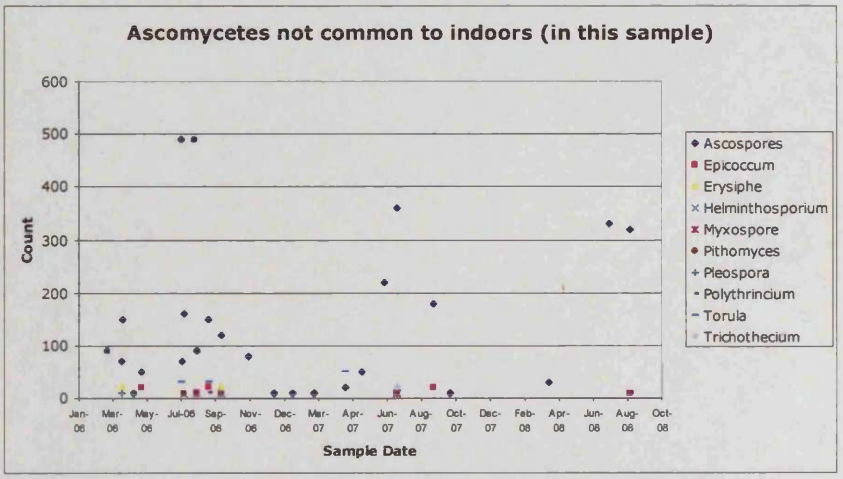
Ascomycetes include those moulds most often found indoors (i.e. *Aspergillus* and *Penicillium*, *Alternaria*, and *Cladosporium*). *Cladosporium* is present in the greatest numbers and it is appears to peak in the summer/autumn months.

Figure 4.41. Scatter plot showing the quantity of ascomycetes spores common to the indoor environment (in this sample) over the sample period



March to May is the period of the year when present outdoor *Cladosporium* is the most abundant fungal spore but there also appears to be a peak in late summer.

Figure 4.42. Scatter plot showing the quantity of ascomycetes spores not common to indoors (in this sample) over the sample period



Ascospores are the most abundant and most frequent mould.

Many of the slide results are missing due to apparatus malfunctions, poor weather conditions and some results were not received.

4.6. Factors determining mould growth

4.6.1 Occupant effects on mould growth

From the RCT, there was a complete set of data on occupant habits and built form features for 164 homes. To ascertain how certain factors may influence the mould growth, the assessments of mould area and mould density were assigned to one of two binary categories. Areas of mould were assigned to category 1 if they were $<0.15m^2$ or category 2 if $\geq 0.15m^2$; $0.15m^2$ is the median value of the data. The mould density was assigned to category 1 if the scale values were ≤2 or to category 2 if they were ≥3. A visual mould index was calculated as a product of the mould area and the mould density; the results were also assessed with respect to various environmental factors. The mould index was also assigned a binary value with category 1, a visual mould index <2 and category 2, a visual mould index ≥2.

Table 4.22. Significance of building or occupant characteristics affecting mould area.

Factors which may affect mould area	Number of dwellings (n)	Mould area $<0.15m^2$	Mould area $\geq 0.15m^2$	Chi-Square significance values for the proportions of households in the two different mould areas
Number of Occupants				
1 to 2	51	57%	43%	
3 to 4	102	50%	50%	p = 0.394
5 and more	9	33%	67%	
Open or closed windows				
Sometimes or never	109	42%	58%	p = 0.002*
Always open	14	86%	14%	
Dehumidifier use				
No	45	58%	42%	p = 0.026
Yes	4	0%	100%	
Heating				
Gas central heating	151	52%	48%	
Electric storage	4	75%	25%	p = 0.016*
Room gas heating	7	0%	100%	
Two band storey height				
≤2.41m	80	58%	42%	p = 0.084**
>2.41m	82	44%	56%	
Fish tank or not				
No fish tank	150	53%	47%	p = 0.130
Fish tank	13	31%	69%	
Drying on the line				
Never	20	40%	60%	
Always	67	58%	42%	p = 0.056**
Sometimes	37	35%	65%	
Carpet in bedroom				
No	26	33%	67%	p = 0.084**
Yes	137	54%	46%	
Glazing type				
Single	29	59%	41%	p = 0.341
Double	133	49%	51%	

Those values with * (p<0.05) show significance and those with ** p>0.05 although not statistically significant are approaching significance.

The results of analysis show some statistically significant relationships. Opening windows, dehumidifier use and heating type were found to be significant for mould area (table 4.22, above).

Table 4.23. Significance of building or occupant characteristics affecting mould density.

Factors which may affect mould density	Number of dwellings (n)	Category 1 Low density	Category 2 High density	Chi-Square significance values for the proportions of households in the two different categories of mould density
Number of Occupants				
1 to 2	51	69%	31%	
3 to 4	102	64%	36%	p = 0.699
5 and more	9	56%	44%	
Open or closed windows				
Sometimes or never	109	59%	41%	p = 0.015*
Always open	14	93%	7%	
Dehumidifier use				
No	45	73%	27%	p = 0.295
Yes	4	100%	0%	
Heating				
Gas central heating	151	64%	36%	
Electric storage	4	75%	25%	p = 0.843
Room gas heating	7	71%	29%	
Two band storey height				
≤2.41m	80	57%	43%	p = 0.040*
>2.41m	82	73%	27%	
Fish tank or not				
No fish tank	150	65%	35%	p = 0.739
Fish tank	13	69%	31%	
Drying on the line				
Never	20	63%	37%	
Always	67	69%	31%	p = 0.309
Sometimes	37	54%	46%	
Carpet in bedroom				
No	26	67%	33%	p = 0.864
Yes	137	65%	35%	
Glazing type				
Single	29	79%	21%	p = 0.092**
Double	133	62%	38%	

Those values with * (p<0.05) show significance and those with ** p>0.05 although not statistically significant are approaching significance

Opening windows and dwelling storey height (up to 2.4m compared with storey heights above this value) were found to be significant for mould density (table 4.23).

Table 4.24. Significance of building or occupant characteristics affecting visual mould index.

Factors which may affect visual mould index	Number of dwellings (n)	Visual mould index <2	Visual mould index ≥2	Chi-Square significance values for the proportions of households in the two different categories of visual mould index
Number of Occupants				
1 to 2	51	88%	12%	
3 to 4	102	84%	16%	p = 0.005*
5 and more	9	44%	56%	
Open or closed windows				
Sometimes or never	109	80%	20%	p = 0.064**
Always open	14	100%	0%	
Dehumidifier use				
No	45	93%	7%	p = 0.199
Yes	4	75%	25%	
Heating				
Gas central heating	151	83%	17%	
Electric storage	4	75%	25%	p = 0.655
Room gas heating	7	71%	29%	
Two band storey height				
≤2.41m	80	83%	17%	p = 0.943
>2.41m	82	83%	17%	
Fish tank or not				
No fish tank	150	85%	15%	p = 0.034*
Fish tank	13	62%	38%	
Drying on the line				
Never	20	80%	20%	
Always	67	90%	10%	p = 0.090**
Sometimes	37	73%	27%	
Carpet in bedroom				
No	26	81%	19%	p = 0.808
Yes	137	83%	17%	
Glazing type				
Single	29	90%	10%	p = 0.275
Double	133	81%	19%	

Those values with * ($p<0.05$) show significance and those with ** $p>0.05$ although not statistically significant are approaching significance

High occupancy; where only 12% of 1 and 2 occupant dwellings and 16% of 3 to 4 occupant homes had a high visual mould index compared with 5 or more occupant dwellings with a high visual mould index (56%) see table 4.24. The presence of a considerably sized fish tank (9% of homes had fish tanks) increased the percentage rate of visual mould index from 15% without a fish tank to 38% with one. Occupants of homes, who recorded that they always left windows open, never had a high visual mould index compared with a 20% value for those who sometimes or never opened their windows.

None of the following were significant at the $p<0.05$ level for mould area, mould density or visual mould index: tenure, drying on the radiator or in a tumble dryer,

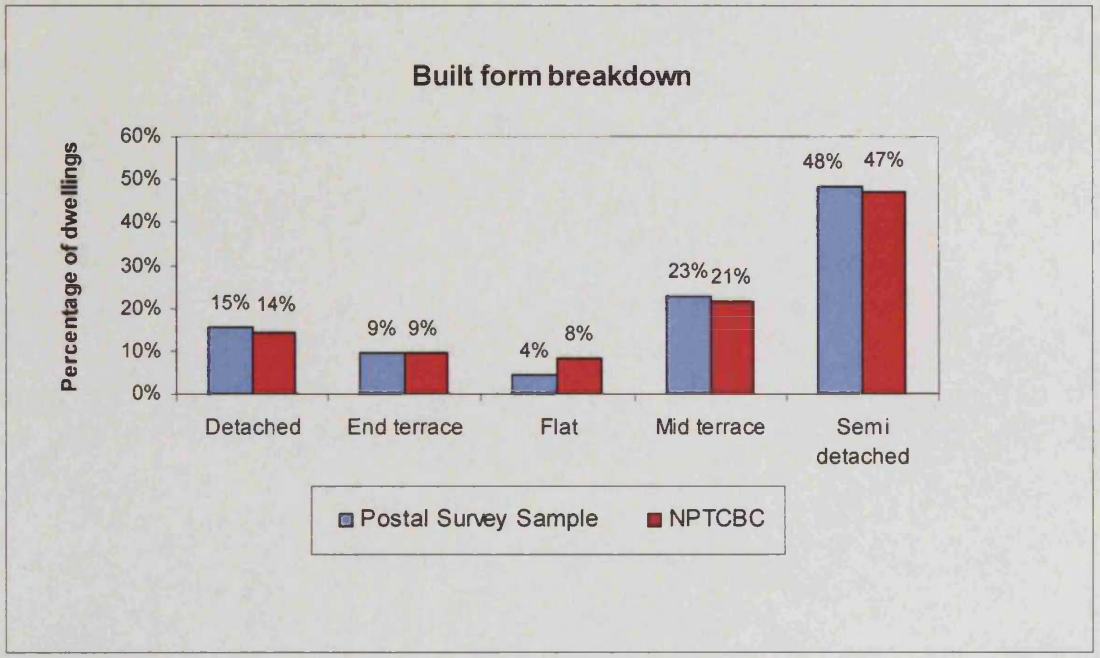
smoking, carpet in the living room, presence of dogs or cats, house type, number of storeys, window frame type, window area, floor area, or age of the heating system (results in Appendix 10).

Any association between the design or built form of the homes visited and the fungal growth found within them was investigated. The 209 stratified homes in the RCT were insufficient in numbers to attempt to make any conclusions regarding the built form. It was fortuitous however; that the Welsh School of Architecture (WSA) had a much larger data set of 55,000 homes in one of the same areas (NPT) from which homes for the RCT were recruited. The data held by the WSA used a Geographical Information System (GIS) based model called the Environmental and Energy Prediction (EEP) Model. The EEP model was originally used to predict energy consumption in the NPT homes. The information from this database included housing characteristics (i.e. size of household, age of property, number of storeys, window area, floor area, and built form i.e. mid terrace, end terrace, semi-detached, detached or flat).

Previous to the start of the RCT, a postal survey was undertaken where a large number of short questionnaires (4,828) were sent to households enquiring about mould status, which resulted in 1,887 responses (39%). Of these, 977 were matched to the EEP database and from these, 152 homes (16%) had self-reported mould. Since the addresses were known the individual built form of each home was obtained from the EEP database.

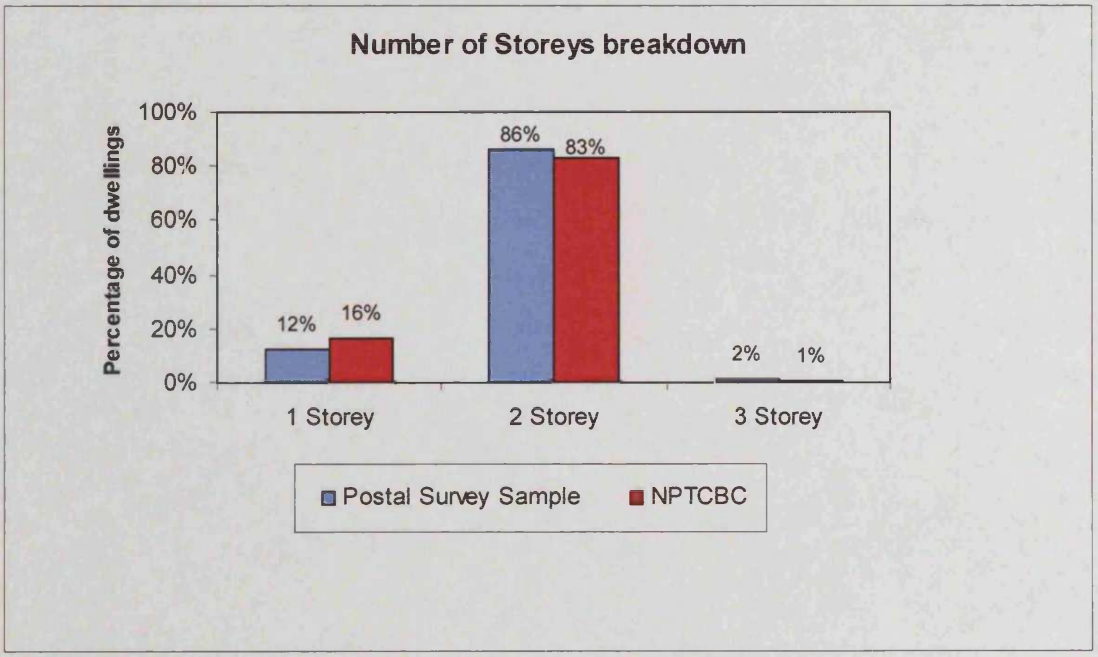
Using the 977 households, the author compared the occurrence of built form types with the sample of all households in the EEP database, in order to check the representativeness of the RCT sample. Only data from the NPTCBC area was used from the RCT, as the EEP model did not include Cardiff in its remit.

Figure 4.43. Built form breakdown for dwellings responding to postal survey questionnaire compared to households in NPTCBC (n=977)



In figure 4.43, there is a very close correlation between all the housing types except that flats are under represented in this postal survey.

Figure 4.44. Number of storeys breakdown for dwellings responding to postal survey questionnaire compared to households in NPTCBC recorded from EEP.



From the figure above, again there is close correlation between the number of storeys in both study samples except for the slightly smaller number of flats, which are represented by single storey dwellings.

The occurrence of reported mould in each of the homes from the postal survey was investigated and compared with built form characteristics recorded for these individual properties from EEP (as shown in Table 4.25). Mould was found to vary significantly ($p<0.01$) with the number of storeys. Twenty-three percent of the single storey homes and 33% of three storey homes had mould present, which was much higher than the two storey dwellings (14%). Floor area also showed significance ($p=0.012$) with 22% of those with small areas showing mould growth and only 14% of the rest of the building stock. Built form also shows significance when looking at mould growth within the home, over 20% of flats, end terraces and bungalows had mould compared with only 11% of detached homes and 14% of the rest of the housing stock.

Table 4.25. Significance of built form characteristics.

Analysis undertaken on built form characteristics	Chi-Square significance values for the proportions of dwellings with self-reported mould
Number of storeys 1 storey, 23% have mould 2 storey, 14% have mould 3 storey, 33% have mould	0.008 *
Is it small floor area or larger Small floor area (<85m^2), 22% have mould Medium/large floor area, 14% have mould	0.012 *
Built form End terrace 23% have mould Mid terrace 14% have mould Semi detached 14% have mould Detached 11% have mould Flats 21% have mould Bungalow 24% have mould	0.035 *
Window area to front façade area wall ratio	0.064 **
Built pre 1919 or post 1919	0.068 **

Those values with * ($p<0.05$) show significance (the results are shown) and those with ** $p>0.05$ although not statistically significant are approaching significance.

4.6.2 Multiple Regression of Bedroom Mould Area

Mould area in bedrooms was investigated using multiple regression analysis. Variables identified during the various analyses throughout this thesis were looked at initially as single factors possibly affecting mould area. These included: mixing ratio, corrected mixing ratio, temperature, relative humidity, number of openings, number of occupants, house type, floor area, storey height, number of storeys, fish tanks, and clothes drying regimes. The correlations identified as significant between individual variables and visible bedroom mould area resulted in three significant factors showing linearity, namely: window and door openings (correlation coefficient (cc) = 0.250, p = 0.029), RH (cc = 0.254, p = 0.027) and floor area (cc = 0.309, p = 0.009). These were subsequently put into a multiple linear regression model, which are summarised in tables 4.26 to 4.28.

Table 4.26 Results of multiple regression analysis – Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.168(a)	.028	-.010	19785.996

a Predictors: (Constant), Openings, Main Bedroom humidity - Visit 1, Floor area

Table 4.27 ANOVA results

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	867347657.143	3	289115885.714	.739	.532(a)
	Residual	29752909647.857	76	391485653.261		
	Total	30620257305.000	79			

a Predictors: (Constant), Openings, Main Bedroom humidity - Visit 1, Floor area
b Dependent Variable: All bedrooms mould area

Table 4.28 Coefficient results

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-4689.465	18961.053		-.247	.805
	Floor area	39.317	48.479	.095	.811	.420
	Main Bedroom humidity	6656.581	31840.041	.024	.209	.835
	Openings	1421.112	1568.868	.108	.906	.368

a Dependent Variable: All bedrooms mould area

These results (R-squared = 0.028 and Adjusted R-Squared = -0.01) showed that this model does not predict the observed values. This is emphasised by the F statistic, which is not significant (p = 0.532). Therefore, it is not possible to reject the null hypothesis that there is no linear relationship between the dependent variable (bedroom mould area) and the independent variables (floor area, RH, and number of openings). This may suggest that visible mould area is not a good indicator of mould contamination, as none of the environmental measurements recorded showed any significant results in the regression model. Other reasons may include; the small sample size, the possible and likely correlation between the numerous independent variables, and the limited range of the visible mould areas observed.

4.7. Moulds and Der p1 in mattress dust

The water vapour available in the air can effect both the HDM population and the mould growth. Both require modestly high mixing ratio values ≥7g/kg, (WHO, 1984) for development to occur and both are known problems in damp housing (Peat et al., 1998). The relationship between Der p1 concentration (µg/g) in mattress dust samples and the area of mould / mould index as determined by examination of photographs by whole house and by specific bedroom was examined.

The distribution of Der p1 in mattress dust for the subjects included in the RCT has already been published (Davies, 2005). As shown in table 4.29 there is a difference in the Der p1 concentration (µg/g) between the two groups (n = 119) split at the median value; the homes with an area of mould of less than 0.1625m^2 (n =60) has a lower median value (10.15) than the homes with an area of mould of 0.1625m^2 or more (n = 59, median = 13.28). The mean Der p1 is higher in the homes with an area of mould of 0.1625m^2 or more (16.16) than the other homes (11.21). The differences observed between means and medians are statistically significant (t-test p-value = 0.032, Mann-Whitney p-value = 0.013).

Table 4.29. Area of Mould in whole house and Der p1 Concentration

	Area < 0.16m^2	Area ≥ 0.16m^2
N (119)	60	59
Mean Der p1 conc.	11.21	16.16
Median Der p1 conc.	10.15	13.28
t-test p value	0.032	
Mann-Whitney p value	0.013	

Using the 75th percentile value for the area of mould, the distribution of Der p1 can be seen in table 4.30. There is a significant difference in the Der p1 concentration (µg/g) between the two groups (n = 119), the homes with an area of mould using a value of less than 0.58m^2 (n =90) have a significantly lower median (10.99) than the homes with an area of mould using a value of 0.58m^2 or more (n = 29, median = 13.29). The mean is slightly higher in the homes with an area of mould of 0.58m^2 or more (15.78) than in the other homes (12.98). The differences observed between means and medians show statistically significance using the Mann-

Whitney, p-value = 0.029 but not using the student t-test, p-value = 0.300, table 4.30).

Table 4.30. Mould Area for whole house and Der p1 Concentration

	For an area < 0.58m^2	For an area ≥ 0.58m^2
N (119)	90	29
Mean Der p1 conc.	12.98	15.78
Median Der p1 conc.	10.99	13.29
t-test p value	0.300	
Mann-Whitney p value	0.029	

Figure 4.45. Box plot comparing the Der p1 concentration in bedrooms with and without mould

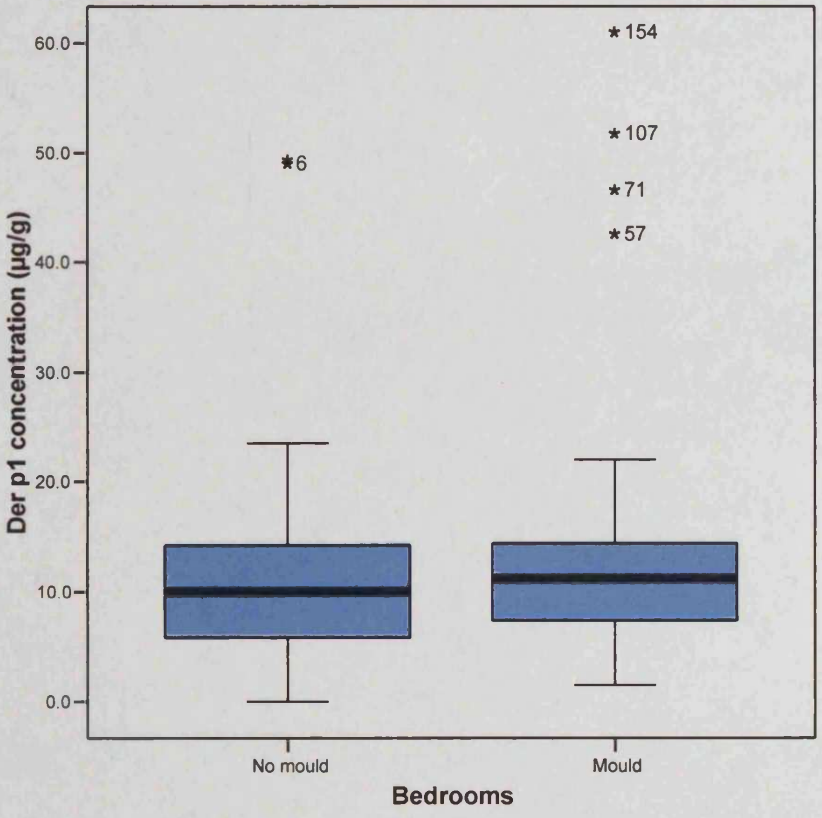


Figure 4.46. Graph comparing the mean Der p1 concentration in bedrooms with and without mould

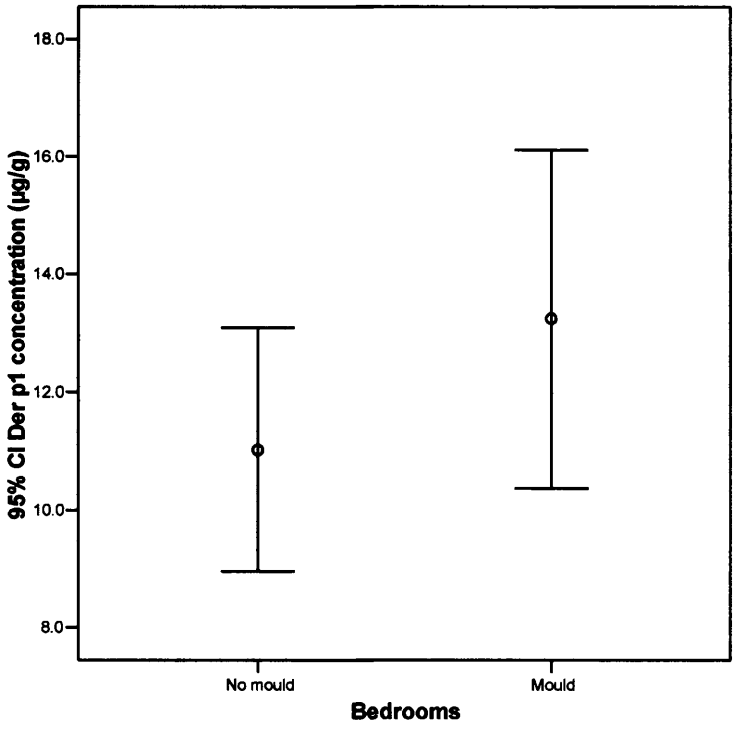


Table 4.31. Der p1 concentration in bedrooms with and without mould

	Bedrooms with mould	Bedrooms without mould
N	87	67
Mean Der p1 conc.	13.24	11.02
Median Der p1 conc.	11.21	10.03
t-test p value	0.239	
Mann-Whitney p value	0.348	

As shown in Figure 4.45 there is minimal difference in the median Der p1 concentration (µg/g) between the two groups (n =154; value number 136 at 100µg/g dust is not shown), the bedrooms with mould (n = 87) have a slightly higher median (11.21) than the bedrooms without mould (n = 67, median = 10.03), the inter-quartile range is slightly narrower for bedrooms with mould, as is the overall range of values (excluding outliers and extreme values). Figure 4.46 displays the means for the two groups, showing that the mean is slightly higher in the bedrooms with mould (13.24) than the bedrooms without mould (11.02). The differences observed between means and medians are, not statistically significant (t-test p-value = 0.239, Mann-Whitney p-value = 0.348).

4.8. Skin sensitivity of subjects

Table 4.32 describes the prevalence of skin prick positive results in the study population who gave a valid skin prick test, i.e. a wheal diameter of 3mm or greater after correcting for the negative control and a positive test for histamine. In relation to the skin prick testing 15 participants declined to be involved, 17 showed a reaction to the negative control or no reaction to the positive control.

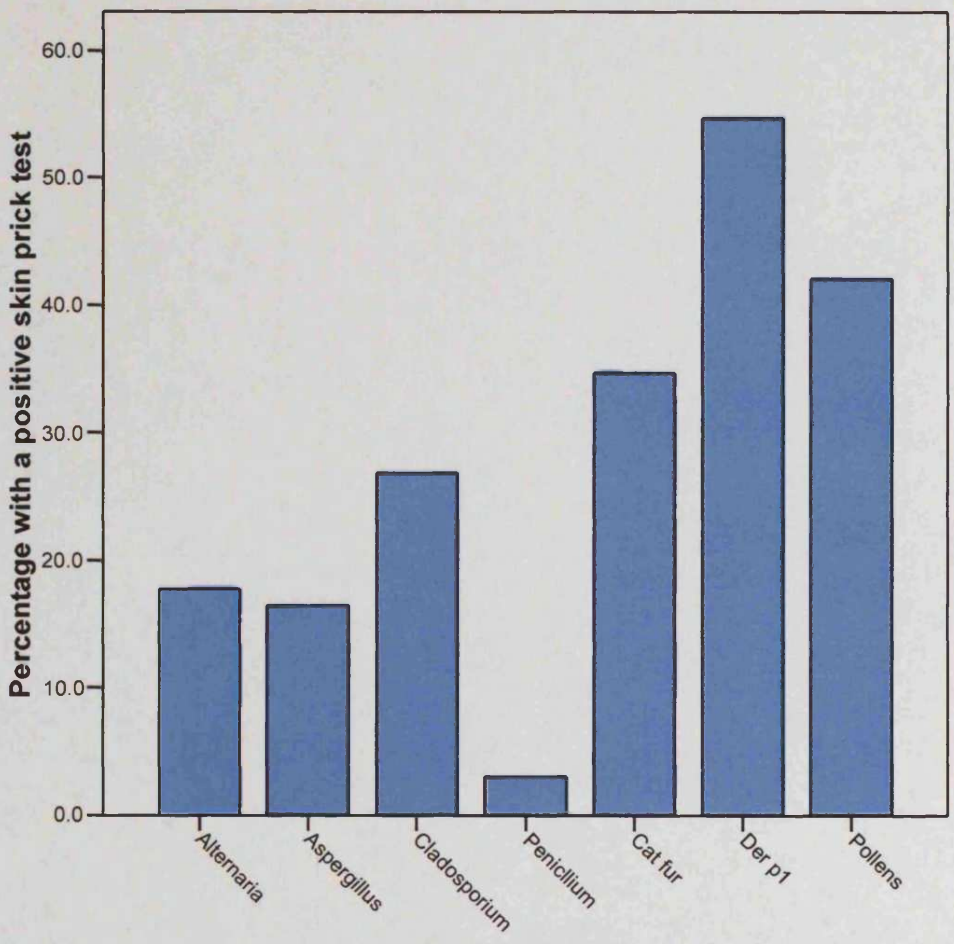
Of the remaining 200, the ages ranged from 3 to 61 years; taking 12 years of age as the cut off point for a child, 56.3% (27/48) children and 75.0% (114/152) adults tested positive to one or more skin prick agents (see also tables 4.34, p170 and 4.35, p171). In both groups, the highest levels of sensitisation were found to be house dust mites (64% adult and 46% children). In relation to the mould extracts tested the most common positive reaction was to *Cladosporium*, 21% (10/48) in children and 32% (48/152) in adults. *Alternaria* and *Aspergillus* showed the same level of sensitisation in children with 10% (5/48) giving a positive reaction to both. In adults the level of positive reaction was doubled compared to children with 20% (31/152) of adults sensitive to *Alternaria* and 22% (33/152) producing a reaction to *Aspergillus*. In both adults and children, *Penicillium* showed the lowest frequency of sensitisation with only 1% of adults and 4% of children displaying a positive result to this extract.

Similar results were observed on dividing the study group by gender. Although notably males were found to exhibit a higher degree of atopy than females, with 73% (60/82) of males and 69% (81/118) of females giving a positive skin prick result (see also tables 4.32 and 4.33 on page 170). As previously the *Cladosporium* extract was the most common mould result with 23% (27/118) of females and 38% (31/82) of males giving a positive result. Also *Alternaria* and *Aspergillus* showed similar levels of positive reaction.

Table 4.32. Number allergic to each mould type, dust mites, cat fur and pollen out of all the participants in the study with a valid SPT

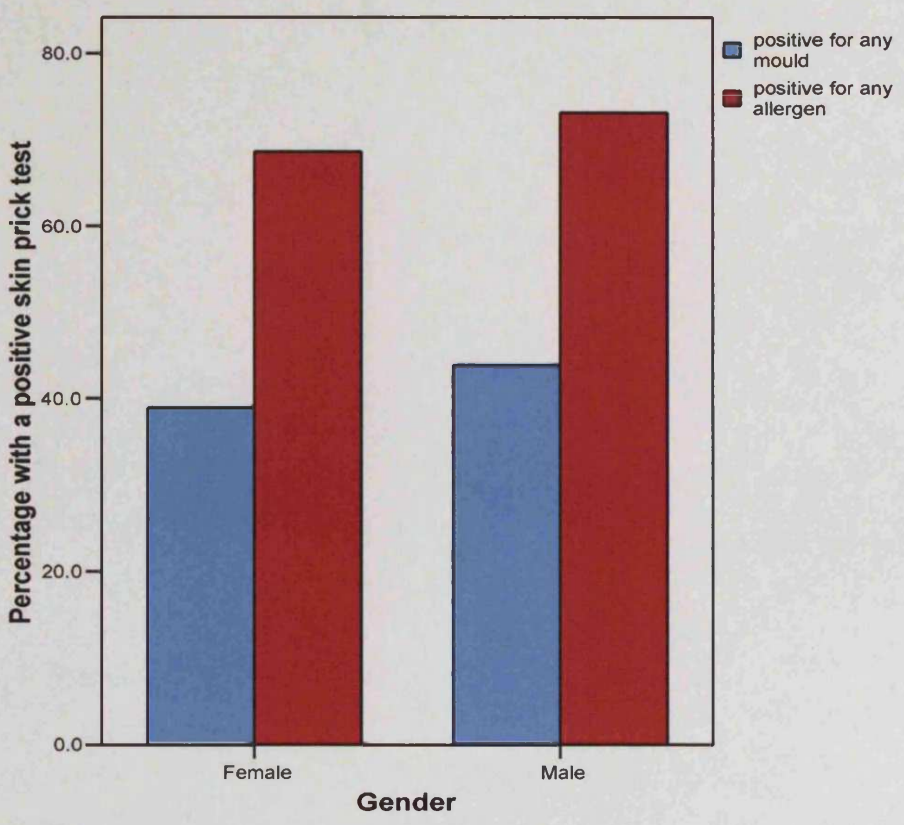
	Total	Allergy to *Alternaria*	Allergy to *Aspergillus*	Allergy to *Cladosporium*	Allergy to *Penicillium*	Allergy to cat fur	Allergy to house dust mites	Allergy to pollens
All (n)(%)	200 (100)	40 (20)	38 (19)	58 (29)	7 (3.5)	76 (38)	120 (60)	95 (48)
Male (n)(%)	82 (41)	17 (21)	15 (18)	31 (38)	4 (5)	38 (46)	56 (68)	42 (51)
Female (n)(%)	118 (59)	23 (19)	23 (19)	27 (23)	3 (2.5)	38 (32)	64 (54)	53 (45)
Adult (n)(%)	152 (76)	31 (20)	33 (22)	48 (32)	6 (4)	67 (44)	98 (64)	81 (53)
Child (n)(%)	48 (24)	5 (10)	5 (10)	10 (21)	1 (2)	9 (19)	22 (46)	14 (29)

Figure 4.47. Allergies in those that had a valid SPT result



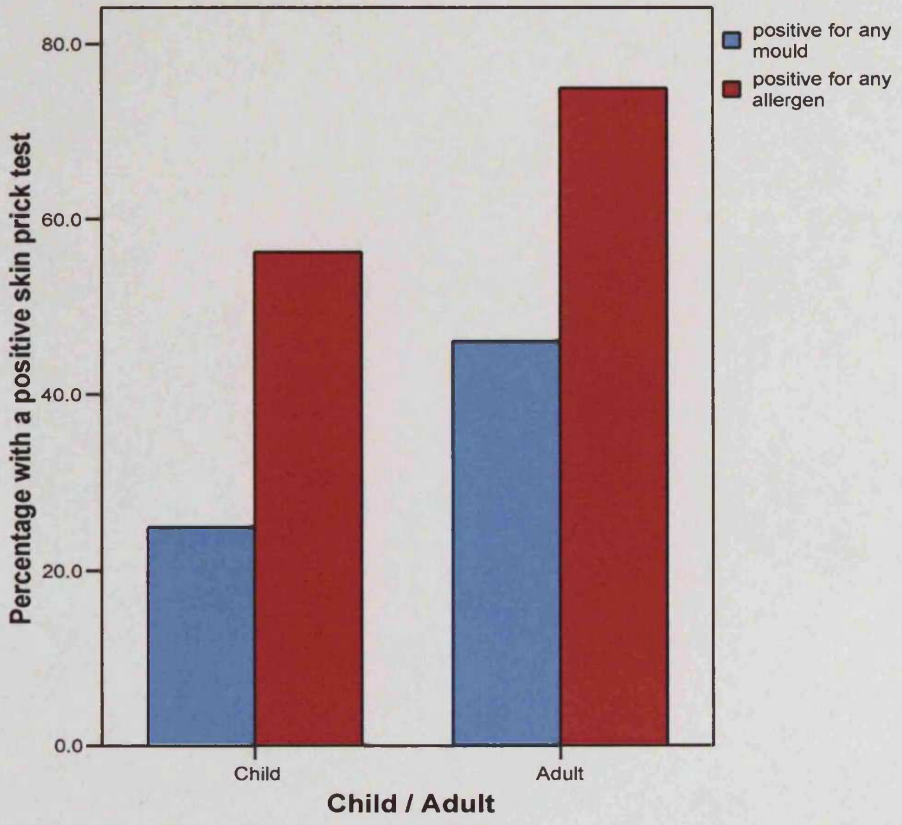
The above bar chart graphically describes the skin prick data detailed in table 4.32 in relation to the trial participants. As can be seen the highest levels of sensitisation are observed with respect to house dust mite (Der p1) and grass pollen allergens, *Cladosporium* is the most common mould extract for which a positive skin prick test was observed. *Alternaria* and *Aspergillus* extracts gave a similar level of positive result.

Figure 4.48. Percentage of females and males with positive SPT for any mould and for any allergen (from those with a valid skin prick test result)



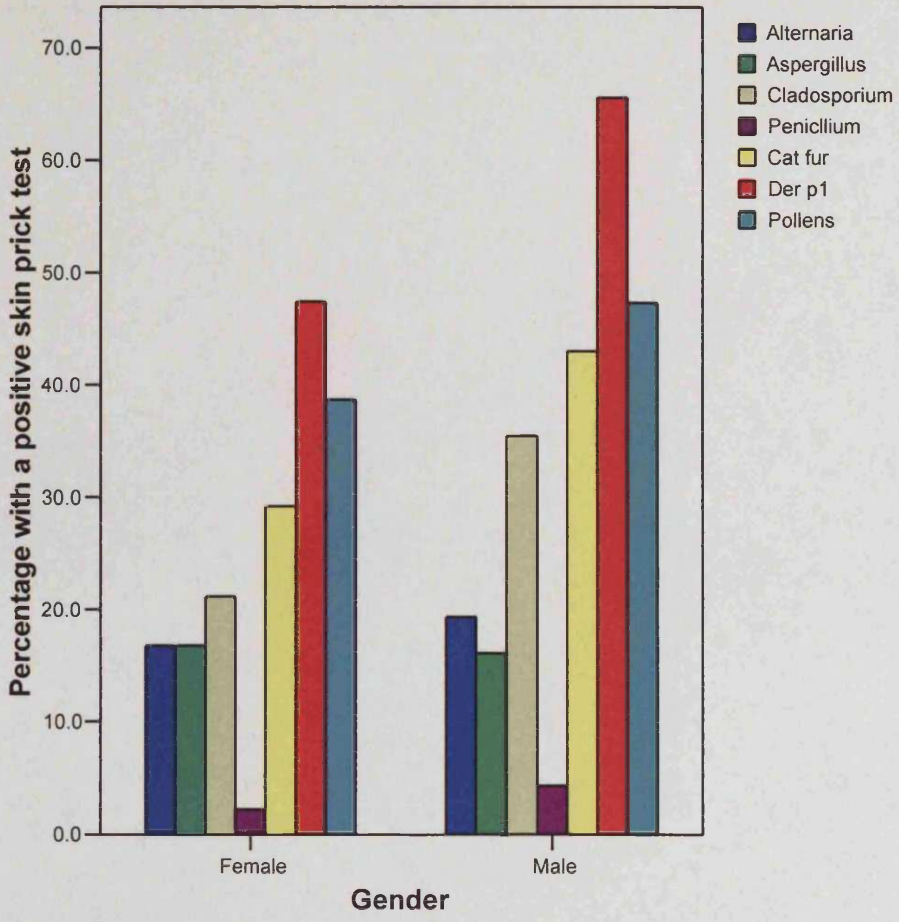
The above figure 4.48, demonstrates that although males in the study group show slightly higher levels of atopy there is little difference in the levels of positive reaction between males and females. On testing by Chi-squared analysis, there was no significant difference between males and females, either in relation to a positive mould test or to any of the allergens, at the 5 % level of significance ($p = 0.629$ or $p = 0.311$ respectively).

Figure 4.49. Percentage of adults and children with positive SPT for any mould and for any allergen (from those with a valid skin prick test result)



The above bar chart (figure 4.49) further clarifies the observation detailed in table 4.32 on page 162, that adults are more likely to give a positive skin prick result than children, this is statistically significant on using a Chi-squared analysis at the 5% level ($p < 0.001$) when comparing positive reactions to any allergen tested or solely to mould extracts.

Figure 4.50. Female / Male split in those that had a valid SPT result



The figures 4.50 and 4.51 graphically represent the distribution by gender and child -- adult groupings for skin prick positive results in study participants giving a valid skin prick test. As previously described Der p1 and grass pollen are the most common allergens to which these groups give a positive result, with *Cladosporium* showing the highest frequency with respect to the mould allergens tested. *Alternaria* and *Aspergillus* show similar levels of positive reactions, although few participants were reactive to *Penicillium*.

Figure 4.51. Child / Adult split in those that had a positive SPT result

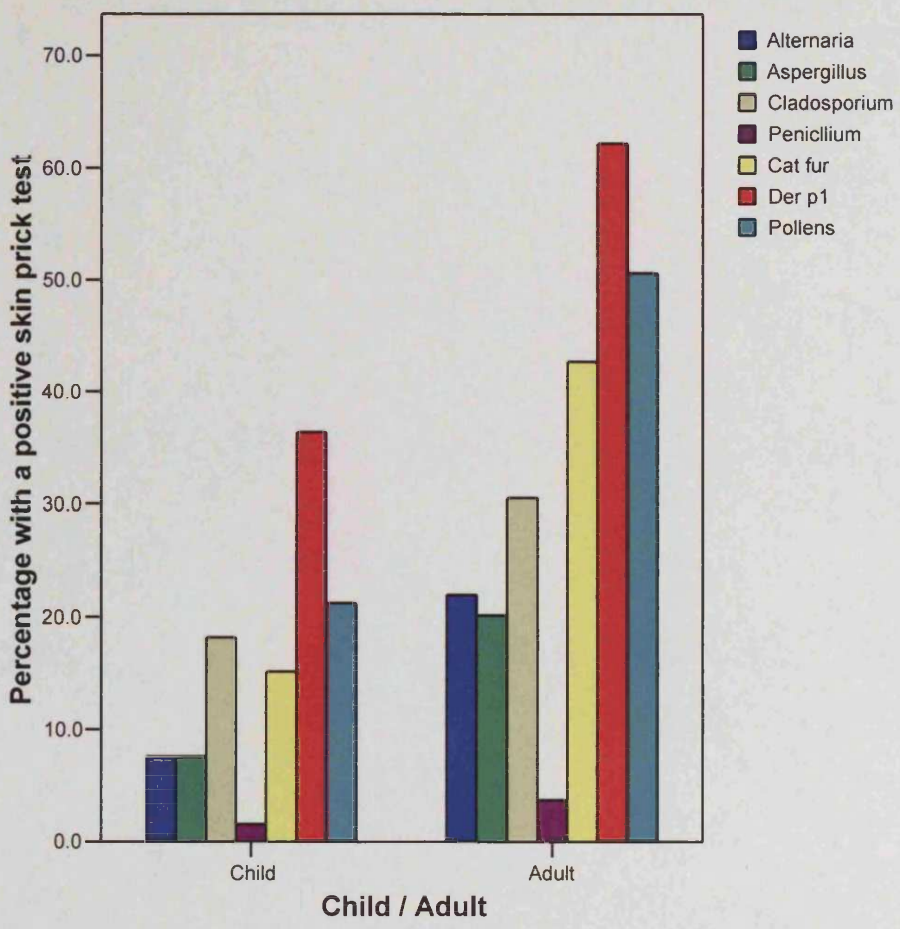


Figure 4.52 and Table 4.33, further breaks down the skin prick test results by examining the distribution in relation to those that gave a positive result, thereby excluding any negative skin prick results, hence only those who are atopic in relation to the testing regime applied in this study are evaluated. As noted previously, pollen and Der p1 allergens were the most common ones to elicit a positive result, with *Cladosporium* showing the highest levels of the mould extracts, with the lowest level being found for *Penicillium*.

Figure 4.52. Bar chart showing the distribution by percentage of allergy status of participants designated allergic by having a positive skin prick result to any extracts tested

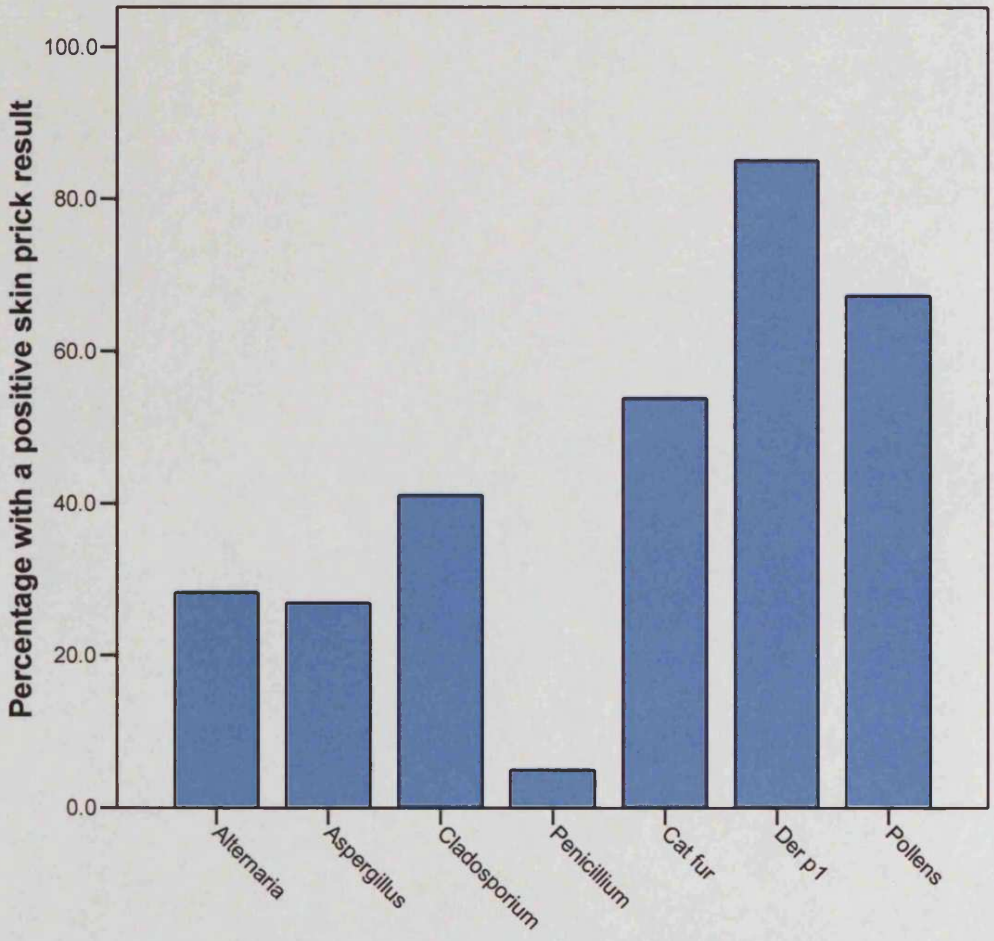


Table 4.33. Number allergic to each mould type, dust mites, cat fur and pollen out of those participants that had a positive skin prick test

	Positive SPT	Allergy to *Alternaria*	Allergy to *Aspergillus*	Allergy to *Cladosporium*	Allergy to *Penicillium*	Allergy to cat fur	Allergy to house dust mites	Allergy to pollen
Participants with positive SPT (%)	141 (100)	40 (28)	38 (27)	58 (41)	7 (5)	76 (54)	120 (85)	95 (67)
Male (%)	60 (43)	17 (28)	15 (25)	31 (52)	4 (7)	38 (63)	56 (93)	42 (70)
Female (%)	81 (57)	23 (28)	23 (28)	27 (33)	3 (4)	38 (47)	64 (79)	53 (65)
Adult (%)	114 (81)	35 (31)	33 (29)	48 (42)	6 (5)	67 (59)	98 (86)	81 (71)
Child (%)	27 (19)	5 (19)	5 (19)	10 (37)	1 (4)	9 (33)	22 (81)	14 (52)

The table 4.34 below, describes the level of allergy to fungi in the adult study population. Overall, of those displaying a positive skin prick result 61% (70/114) were allergic to any of the moulds tested. Again, the highest numbers are observed in the *Cladosporium* group closely followed by *Aspergillus* and *Alternaria*. Although more individuals that were sensitive *Alternaria* gave a positive reaction to two or more of the other fungal species (80%, 28/35), *Cladosporium* and *Aspergillus* have similar levels in relation to two or more fungal species (69%, 33/48 and 67%, 22/33, respectively). Interestingly, although only a few participants gave a positive result to *Penicillium* the majority (83%, 5/6) were also sensitive to the other three types of fungi.

Table 4.34. Prevalence of fungal allergy based on skin prick testing of the adult population with asthma (152 adults with a valid SPT)

	SPT Positive (any allergen)	Any Fungi	One FS	Two FS	Multiple FS
Total: n (%)	114 (100)	70 (61)	34 (49)	22 (31)	14 (20)
Alternaria: n (%)	35 (31)	35 (100)	7 (20)	15 (43)	13 (37)
Aspergillus: n (%)	33 (29)	33 (100)	11 (33.33)	10 (30.33)	12 (36.33)
Cladosporium: n (%)	48 (42)	48 (100)	15 (31)	19 (40)	14 (29)
Penicillium: n (%)	6 (5)	6 (100)	1 (17)	0	5 (83)

FS = Fungal Spore

Table 4.35 overleaf, describes the prevalence of fungal allergy in children demonstrating a positive reaction to any of the allergens tested. Similar to the adult group, of those children displaying a positive skin prick result 44% (12/27) were allergic to any of the moulds tested. As described in the previous figures *Cladosporium* is the mould to which children are most commonly sensitised (37%, 10/27), followed by *Alternaria* and *Aspergillus* (19%, 5/27) with *Penicillium* eliciting a positive reaction in only 4% (1/27) of participants showing a positive reaction to

any of the allergens. Also the one participant that was sensitive the *Penicillium* was also positive for all the other three mould types tested. Unlike the adult group sensitisation to two or more of the fungal extracts was more common in the *Aspergillus* and *Alternaria* groups (80%, 4/5) with *Cladosporium* showing lower levels of multiple sensitisation (50%, 5/10), although it should be noted that the number of children tested was significantly fewer than that of the adults.

Table 4.35. Prevalence of fungal allergy based on skin prick testing of the child population with asthma (48 children with a valid SPT)

	SPT Positive (any allergen)	Any Fungi	One FS	Two FS	Multiple FS
Total: n (%)	27 (100)	12 (44)	7 (58)	2 (17)	3 (25)
Alternaria: n (%)	5 (19)	5 (100)	1 (20)	1 (20)	3 (30)
Aspergillus: n (%)	5 (19)	5 (100)	1 (20)	1 (20)	3 (60)
Cladosporium: n (%)	10 (37)	10 (100)	5 (50)	2 (20)	3 (30)
Penicillium: n (%)	1 (4)	1 (100)	0	0	1 (100)

FS = Fungal Spore

Chapter 5 - Discussion

Improvement in breathing, medication usage and symptoms

A striking feature of the results is that subjects from the intervention group reported large improvements in their breathing compared to controls both at 6 months and 12 months albeit the improvements reported were diminished at 12 months compared to six months. Similarly, subjects from the intervention group reported a large reduction in medication usage in the previous six months and this effect remained and was slightly stronger at 12 months.

Comparing the questionnaire responses with those obtained at baseline showed that there was a greater tendency for those in the intervention group to report improvements in recent wheezing. There was some indication of a modest improvement in recent symptoms of wheeze in the intervention group at six months but this had lessened by 12 months. Further to this, for those subjects who also reported that wheeze was disturbing their sleep, affecting their activities or limiting their speech (i.e. those displaying more severe symptoms) the difference between the groups was larger. These improvements in the intervention group are perhaps surprising in the context of the marked reported reduction in medication usage in the intervention group compared to the control group. Thus at six months members of the intervention group had not only shown large relative reduction in medication usage but also modest relative improvement in wheeze with relative improvements greater for those whose wheeze affected them in some way.

The difference in the net percentage of subjects showing improvements in symptoms of rhinitis and rhinoconjunctivitis was of similar effect size to that for wheeze. Improvements of symptoms of rhinoconjunctivitis and rhinitis at six months in the intervention group showed further improvement at 12 months.

Splitting the intervention group into those in which mould remained absent and those in which mould returned showed some interesting results. At 6 months there was almost 3 times the improvement in wheeze affecting speech in the homes without mould than in those where mould returned compared with the control group. There was a significant difference in net improvement in wheeze affecting daily activities (36.6%), improved breathing (72.2%) and decrease in medication

use (82.2%) in those homes where mould did not return. Medication use was still reduced in the intervention group with mould (34.8%) compared to controls but the values were not as substantial. Rhinoconjunctivitis was significantly improved in homes without mould (20.6%); there was no improvement in those homes where the mould returned.

At 12 months the improvements are not as dramatic but they are present. Medication use in both intervention groups declined between 6 and 12 months but was not significant. The net percentage difference in those that got better for runny nose problems and for rhinoconjunctivitis improved significantly in the intervention group without mould compared to the other groups. These results suggest that there is some evidence, albeit not significant, of greater improvement in those intervention homes in which mould remained absent compared with those where it reappeared.

The clinical effect size observed for improvements in self-reported breathing and medication usage were considerable, i.e. 52% difference between groups and 59% difference respectively, at six months. The clinical effect size regarding symptoms although less marked was also significant to health, i.e. a difference of 25% in wheeze affecting activities between the two groups.

There was no observed difference between the groups with respect to the variability of peak expiratory flow. However it should be noted that peak flow variability was comparable in the intervention group for markedly lower medication usage.

The experimental design ensured that smokers were equally distributed between the groups (i.e. control group 25% and intervention group 36%). It is reasonable to expect that other confounders such as house dust mites would be similarly distributed. Previous ecological studies have not been capable of allowing for confounders in this way.

Observed Mould

It is usual for individuals to believe that the bathrooms in a home are the most likely to promote fungal growth. But in this study, moulds were found most often in the bedrooms (38%), followed by other rooms (23%), bathrooms (18%) and then the kitchen (10%) and living room (11%). This suggests that the bedroom is a potential problem area. It is a contained space which would minimise ventilation. Main

bedrooms may show up as worse because they often have two occupants producing moisture. It is possible that lower bedroom temperatures would create ideal conditions for condensation, followed by mould growth. Examining the swab samples most of the exposed swabs produced some fungal growth. Only living rooms fell just below the 80% mark. The samples from the attic room, front room and basement produced mould growth in 100% of the samples. The reasons for this are varied but it is likely that all the rooms lack sufficient ventilation. The basement is probably a moist environment because it is below ground level and is unlikely to be heated. The walls will wick moisture to the interior in an effort to reach equilibrium with the surroundings. The front rooms are often closed off from the rest of the home and not heated thus the cooler stagnant air in the room cannot hold as much moisture and releases some of it to the surrounding walls, ceiling and furniture. The attic is a different case in that it has mould growth probably because warm moist air from the rooms below rises due to the stack effect, to meet a possible cold exterior wall and ceiling where again the water vapour condenses out and helps supply the necessary moisture for fungal propagation.

Penicillium (55%) was the most abundant mould found overall from the swab samples followed by *Cladosporium* (36%), *Stachybotrys* (16%), *Aspergillus* (9%) and *Rhizopus* (7%). *Cladosporium* is the most abundant mould in the outdoor environment and the second most abundant in the interior environment. It is most common in the bedrooms and bathrooms of the swabs taken. *Penicillium* and *Stachybotrys* were also abundant in these rooms. It is not expected to find *Stachybotrys* in the bedrooms as it is highly hygrophilic (Flannigan & Miller, 2001) and requires a water activity (a_w) above 0.90 to grow. *Penicillium* is a primary coloniser and it usually favours xerophilic conditions with a_w between 0.75 and 0.85. *Cladosporium* falls in to the mesophilic range with a_w between 0.85 and 0.90. These three fungi are all the most abundant moulds found by swabs in the main bedrooms and bathrooms. *Alternaria alternata* which was the other mould skin prick tested for is also hygrophilic but appears much less frequently.

One key point to have arisen from the study is that although there was no statistical correlation between mould levels as assessed by area or mould index and ergosterol, there was an obvious drop in the level of ergosterol at 12 months in the intervention homes albeit not statistically significant. Interestingly the levels of ergosterol were lowest in flats, detached homes and bungalows as compared to semi-detached and terraced houses. Although these differences were not

significant, they seem to show an inverse relationship to the mixing ratio values as determined at baseline. This might suggest that ergosterol sampling by air is a poor biomarker of fungal biomass, although other studies suggest that it is a robust procedure for mould evaluation if sufficiently long sampling periods are used (Foto et al., 2005). It is possible that built form or the number of storeys which can affect the air change rate needs to be considered when applying air sampling methods. Further analysis of other biomarkers of fungal contamination such as β-glucan may be useful, and it is hoped that future work will include assaying some of the samples saved for such markers.

There is in principal a possibility for personal fungal exposures in settings other than the home to lead to misclassification of exposure status. However, in the UK, schools are almost universally free from mould as are most workplaces.

Improvement in the presence of mould post intervention

Moulds are often dark in colour and easily identified in homes. As part of the RCT digital photographs of any mould that occurred in the home were recorded along with a description of the area. The control homes were five times more likely to have mould at 12 months than the intervention homes. A comparison of the visual presence of mould shows in the two groups based at baseline and at 12 months that there was a decrease in both the intervention and the control groups, although there was a much larger and statistically significant reduction in the intervention group (~40% difference). The overall reduction may be a reflection of a seasonally drier period compared with the same period at baseline. However, the year long outdoor mixing ratio averages are within 3.6% of each other which suggests that the drop in the indoor mixing ratios of the intervention group is likely a result not of the weather but of the PIV units which also appear to have reduced the available moisture to inhibit mould growth in the intervention group. It is also possible that just by visiting the homes it has made the occupants more aware of their home and they may have inadvertently 'improved' the environment between the two visits.

There were minimal differences between the intervention and control groups when looking at all the mould areas or the mould index values for the main bedroom and the whole house. This may be explained because the values for mould area are for the most part small and looking at the difference between these produces a change which is even smaller. The mould index is more useful than just area of mould as it

incorporates the mass and the degree of colonisation of mould growth, amplifying potential problem areas.

Ergosterol sampling

In addition to sampling surfaces by swabs it was decided to use a biomarker of mould levels in air, namely ergosterol. Ergosterol is the key sterol found in the cell membranes of filamentous fungi, as well as being found in yeasts (Pasanen et al., 1999). As a result it has been used as a biomarker for fungal biomass although it should be noted that the levels of ergosterol in fungi vary between fungal species and that the application of this method to air samples needs to be viewed with caution. In this study, living room air samples were investigated for ergosterol. Samples ranged from 0.000–0.056 $\mu g/m^3$, the mean ergosterol (SD) and median ergosterol (quartiles) levels were respectively 0.0049 $\mu g/m^3$ (0.009 $\mu g/m^3$) and 0.0018 $\mu g/m^3$ (0.002 $\mu g/m^3$, 0.0049 $\mu g/m^3$). These levels are at the detection limit of the assay. Studies have investigated dust samples for ergosterol which may give a more accurate assessment of the indoor mould levels (Matheson et al., 2005) but these too require at least 30g of dust to be useful. However, for the purposes of this project it was decided that the actual airborne concentration of mould was more important in relation to the possible levels of exposure experienced by the participants. Other studies have suggested that a better method is to assess air samples over a longer period of time. It would have been desirable to have also assessed the mattress samples for levels of ergosterol; however costs to the project and the necessity to determine Der p1 levels prohibited that from taking place.

Mixing Ratio

The indoor mixing ratio values are always higher than outdoor values as a result of water vapour being added through occupant activities such as bathing, cooking, washing etc. These activities can add up to 12 kilograms (Yik et al., 2004) of moisture per day for a family of four. The outdoor mixing ratio has a substantial effect on the indoor values, and there is a substantial seasonal variation in the outdoor mixing ratios. The year on year variation is quite small. The maximum value that occurred during the sampling period over the three years was 9.43g/kg and the minimum 3.80g/kg, which would make considerable changes to the conditions in the home but these extremes are only present for a short period of

time (hours). The actual high mixing ratio for the three calendar years was 14.33g/kg and the low was 1.60g/kg.

The corrected mixing ratio represents that quantity of moisture that the occupants have added to the air and it is this value that the intervention reduced. The magnitude of the difference between the improvement in the intervention (21%) and the decrease in the control (-0.87%) is quite substantial (t-test: p=0.011, Mann-Whitney p=0.003), showing that the PIV unit had a significant effect on reducing the humidity in the homes. As mentioned earlier, the variation in the outdoor mixing ratio is only a few percent so that the change indoors cannot be explained through weather changes alone.

Built Form

The growth of various fungi is dependent on the available water either in the air as water vapour or in the substrate on which the mould is growing (Miller, 1992; Wilkins et al., 2000) and this is directly dependent on the ventilation rate within the home, the type of home and the habits of the occupants.

The link between the design or built form of the homes visited and the fungal growth found within them was investigated, but the number of homes in the RCT was insufficient to attempt to make any significant conclusions. It was fortuitous that the WSA had a much larger data set of 55,000 homes in one of the same areas (NPT) from which homes for the RCT were recruited. The information from this database included many housing characteristics. The large number of short questionnaires sent to households initially enquiring about mould status resulted in 977 responses and of these 16 % had self reported mould. Since the addresses were known the individual built form of each could be obtained from the EEP database.

From the EEP database the presence of visible mould was found to be higher in small floor area homes, flats, bungalows and end terraces. No significant relationship was found between visible mould and many other dwelling characteristics (see Appendix 10). As in a Finnish study, the age of the dwelling had little significance on the presence or quantity of mould (Nevalainen et al., 1998). From statistical analysis of the site visit data, it shows that the number of occupants has a significant effect on the visible mould growth as does owning a fish tank presumably by adding large volumes of water vapour to the air. The opening of windows and doors was significant for both mould area and mould

density and approached significance for mould index, pointing to the positive effects of ventilation inhibiting mould growth. The location of clothes drying had some effect but it was not significant with the numbers available. Storey height below 2.4m and those above this value had a significant effect on the mould density but not on area or index of mould. This may be related to minimal ventilation but cannot explain why the area of mould does not show a positive result. From this study, the presence of mould is clearly linked to the built form of the dwelling. There is a storey effect on mould growth and the rating of mould growth by the researcher shows that the number of occupants and window opening also contribute to the quantity of mould. Moisture production from the occupants, number and type of pets, window opening and clothes drying habits all play a role in the growth of moulds. Higher occupant density may explain the higher mould growth in smaller dwellings but the storey height may also have a role. It appears that the design of the building can have an effect on the interior environment but this can be modified by the habits of its occupants.

Der p1 sampling

In terms of environmentally important allergens one of the first to be identified, and often viewed as the most important is Der p1, derived from the faeces of the dust mite *Dermatophagoides pteronyssinus*. This is the predominant house dust mite found in the UK and its key allergen, Der p1, is a protein with a molecular weight of 24 kD (kiloDaltons) derived from its gastrointestinal tract (Sporik et al., 1990). In the UK, 48% to 85% of patients with asthma are allergic to mite allergen compared to 5% to 30% of the general population (Platts-Mills et al., 1989). In line with these findings this randomised controlled study found 60% of the asthma patients surveyed gave a positive skin prick result to Der p1. As part of this trial investigating asthma in relation to the home environment, Der p1 levels were measured in mattress dust taken from 145 homes in the Cardiff, Neath and Port Talbot areas of South Wales, UK. Although cross-sectional in nature it was hoped that this part of the study would add information about the interplay between dust mites and mould in the homes of asthma sufferers.

Inhaled allergens, and in particular those from dust mites and moulds, are known to exacerbate asthma symptoms in sensitized individuals, and until recently, were implicated in the development of the condition (O'Donnell and Frew, 2002; Custovic et al., 1996; Dharmage et al., 2001). Studies have demonstrated that avoidance

strategies to dust mites can improve the respiratory function of such individuals (Ehnert et al., 1992). It has also been suggested that methods to reduce allergens may also affect microbial products such as endotoxin that may prove a benefit in reducing symptoms or even preventing asthma (Grimshaw and Warner, 2005). Both mould and dust mites are influenced by the microclimatic conditions of the dwelling (Crowther et al., 2001; Davies et al., 2004). Furthermore the materials used in the home can have definite effects, these include mattresses, carpeting and soft furnishings. Of key importance are both the humidity and temperature levels found in the home. House dust mites are known to be particularly reliant on the humidity levels found in the home, although there is still much conjecture as to the threshold limits for their survival. It is also known that mould and dampness are often associated in the home with poor ventilation, and this can result in increased levels of water vapour. Humidity levels are therefore of critical importance to both the levels of mould and Der p1 in the homes. Although it should be noted that these hygrothermal conditions are not alone in determining the populations of dust mite and moulds, as the building structure, occupancy and cleaning regimes used in the dwelling may also play an important role (Matheson et al, 2003; Crowther et al, 2001; Davies et al 2004)

As a result, the levels of Der p1 were compared for homes and bedrooms in relation to the amount of mould present. Mould index and area were compared by examining homes with levels of mould below $0.16m^2$ with those in excess of this median value. Statistically significant differences were found in relation to the mould area, the mould index level demonstrated that homes classified as having $0.16m^2$ or more mould had statistically higher levels of Der p1 (mould area: t-test p-value = 0.032, Mann-Whitney p-value = 0.013). The mould index method developed during the course of this study may provide a more accurate method of determining mould level compared to area covered alone, as there is some association between mould index and the levels of Der p1. This is as expected based on various studies that link higher levels of humidity and temperature in homes with greater prevalence of mould and house dust mites (Shelton et al., 2002; Mamoon et al., 2002; Davies et al., 2004; Arlian and Platts–Mills, 2001).

This is an important result considering that fungal spores and fungal breakdown products, such as β-D-glucans, not only elicit an allergic reaction but also have the ability through various reactions to act as adjuvants, increasing the degree of allergic reaction to other sensitizers as shown in animal models (Instanes et al.,

2004; Havaux et al., 2004). It would, therefore, seem logical that as high levels of house dust mites and mould contamination are often found in the same home this may not only increase the symptoms of asthma sufferers but also lead to increased sensitisation in susceptible individuals. Hence, further clarification of the ability of the PIV intervention to reduce both levels of mould and Der p1 contamination is warranted, and samples have been taken during this study to compare levels in control and intervention homes. However, this work was not carried out during the trial owing to funding and time constraints, although it is hoped to secure further funds to undertake this important analysis.

Skin Prick data

In order to assess the allergic (atopic) status of the asthma sufferers enrolled in this RCT, skin prick testing was carried out. A positive reaction demonstrating the presence of atopy is usually acknowledged as a result of the development of an IgE response to the antigen. The allergens included house dust mites and pollens, in addition to a range of mould extracts, namely *Alternaria alternata*, *Aspergillus fumigatus, Cladosporium herbarum* and *Penicillium notatum*.

Of the 200 participants who gave a valid skin prick test to (after allowing for the negative control and where there was a positive reaction to the histamine control), 71% (141/200) were found to be positive to any one of the allergens tested. Forty-one percent (82/100) had a reaction to at least one of the mould extracts. As might be expected the most common positive test was to house dust mites closely followed by pollens. The proportion of asthma sufferers classified as atopic varies greatly between geographical regions and study population 45% to 75% (Pearce et al., 1999). Similarly, the levels of asthmatics classified as atopic to moulds varies between 17% (Dharmage et al., 2001) and 50% (O'Connor et al., 2004). Our results suggest, however, that in homes contaminated with mould there are a high proportion of atopic asthma sufferers, with a similarly high level of these patients having a positive test to various mould extracts.

The data was further evaluated in the results by examining the levels of a positive skin prick result by group in relation to male and female or child and adult. In terms of the gender breakdown the relative levels of sensitisation were found to be 73% (60/82) for males and 69% (81/118) for females, which when tested by non-parametric or parametric means showed no statistical significance in these groups at the 5% level. However, in relation to the split by child/adult, adults showed

higher levels of sensitisation 75% (114/152) where as only 56% (27/48) of children gave a positive reaction. This difference between adult and children was significant at the 5% level (Chi-squared $p<0.001$, t-test $p<0.001$). The increase in sensitisation between adults and children demonstrates an important age effect most likely as a result of the ongoing maturation of the immune system through childhood. One study in Hungary, in relation to skin prick testing and moulds, showed an increase in prevalence from 10.6% to 38.5% in a cohort of asthmatic children over a ten year period (Szantho et al., 1992).

Similar results were found in relation to the data gathered with respect to the skin prick testing of the various mould extracts. In this case, the relative levels of sensitisation were 44% (36/82) in males and 39% (46/118) in females; again there was no significant difference in the groups. However, as in the testing of all skin prick results, of the adult group, 46% (70/152) gave a positive reaction which was significantly higher than the child group in which 25% (12/48) were positive (Chi-squared $p<0.001$, t-test $p<0.001$). *Cladosporium* showed the highest prevalence of positive skin prick testing, followed by *Alternaria* and *Aspergillus* and finally *Penicillium*. Analysis investigating the relationship between sensitisation to the various moulds showed that multiple sensitisation was more common in those sensitised to *Alternaria* or *Aspergillus* in children (80% of those sensitised showing multiple sensitisation) only *Alternaria* showed a similarly high level of sensitisation in adults (80%). Although few participants showed sensitisation to *Penicillium*, it was commonly associated with multiple fungal sensitisations, 86% of all *Penicillium* positive participants gave a skin prick positive test to two or more fungal extracts. In addition, in line with other studies the level of skin prick positive testing to moulds in the atopic asthmatics was 30% (Kurup et al., 2000).

The observation that *Cladosporium* was the most common allergen to which participants were allergic may well demonstrate higher levels of this mould type in homes in the geographical region studied. The results from the swab analyses of the homes showed *Cladosporium* and *Penicillium* (approximately 38% and 58%, respectively) to be the most prevalent types of mould found in these households. Although *Alternaria* is a more common sensitizer, fewer homes provided any evidence of its presence compared to *Penicillium*, to which few participants were allergic. This may be explained by there being higher levels of *Alternaria* outdoors and there is some evidence of the presence of *Alternaria* in house dust samples in the absence of environmental mould spores (Becker et al., 1996). This would

suggest that *Alternaria*, *Cladosporium* and *Aspergillus* are more potent sensitising moulds than *Penicillium*. In this regard, studies designed to develop new mouse models of lung allergy have demonstrated that *Alternaria alternata* and *Cladosporium herbarum* spores have the ability to induce a type-2 antibody response and subsequent production of IgE and IgG_1 even in the absence of an adjuvant. In addition, the study also noted that *Alternaria* produced a greater influx of neutrophils when compared to *Cladosporium*, as well as being more diffused throughout the lungs. This difference in cellular activity on exposure to *Alternaria* may therefore be one of the reasons for the apparent high level of sensitisation (Havaux et al., 2004). Also, another study has shown that extracts from *Cladosporium herbarum* and *Penicillium chrysogenum*, as well as β-1,3/1,6-D-glucan (a cell wall component of various moulds) from bakers yeast had the ability to increase the allergic response acting as an adjuvant in ovalbumin sensitised mice (Instanes et al., 2004).

Overall, the skin prick data shows that the levels of sensitisation to various moulds are in the region of 30%, highlighting there importance as possible factors in exacerbating the symptoms of allergic asthmatics. In addition the mould's ability to cause sensitisation in susceptible individuals and the relatively high levels of mould contamination found throughout the UK makes remediation a priority to asthma sufferers.

Conclusion

The RCT approach was adopted to circumvent the reporting biases which occur with observational studies. There was no way of 'blinding' participants in respect to whether they were in the intervention group or control. Therefore in principle their responses to questions could be biased by their perceptions. However the consistency of responses to questions between 6 months and 12 months for a single individual indicates that this probably has not occurred appreciably since individuals are not likely to remember their responses six months previously.

Relative to the control group, patients in the intervention group reported improvement in their chest symptoms, especially in the first 6 months, and also a reduction in medication use. These results were much stronger (especially for wheeze) when comparing those participants in homes where the visible mould remained absent. At 12 months the effect on chest symptoms was less consistent than at 6 months. Perhaps this was because the difference in mould exposure between the two groups was likely to be less than at 6 months, given that it was 100% initially and only 38% at 12 months. The effect on rhinitis and rhinoconjunctivitis may have been more evident at 12 months because of the seasonal nature of these conditions in many people, the questionnaire being completed at the same time of year as previously recorded. PEFR variability, expressed as the CV of these readings (an index of asthma severity associated with indoor mould in another study: Andriessen et al., 1998), declined in both groups in both morning and evening, with no significant differences between the groups. Thus the intervention appeared to produce an improvement in symptoms consistent with previous evidence, but without a corresponding effect on an objective index of asthma severity.

Although it is often believed that bathrooms in a home are the most likely to promote fungal growth, in this study visible mould was most prevalent in bedrooms (38%) and was also found in living rooms (11%), bathrooms (18%), kitchens (10%) and in the category of all other rooms (23%).

In general, the prevalences of different genera were similar in all the rooms with the highest prevalences observed for *Penicillium* (50%), *Cladosporium* (30%), and *Stachybotrys* (20%). In the damper environments (e.g. bathrooms) *Cladosporium* concentrations approached those of *Penicillium*. A number of other studies have reported *Penicillium* and *Cladosporium* as prevalent in

homes in temperate climates; these have not reported *Stachybotrys* at the frequency that was observed in this study. It was somewhat unexpected to find *Stachybotrys* at this prevalence, particularly in bedrooms as it is highly hygrophilic and requires a water activity above 0.9 to grow.

Assessment of the area covered by visible mould showed that these were not particularly large. The overall distribution of areas affected covered a wide range (Inter Quartile Range 0.02 - 0.32m^2) and were non-Gaussian. The mean and median of all areas affected was 0.34m^2 and 0.07m^2, respectively. Mould area was correlated weakly with floor area, (correlation coefficient (cc) = 0.309 and p = 0.009); RH, (cc = 0.254, p = 0.027); and number of openings, (cc = 0.250, p = 0.029). Comparison of homes with an area affected by mould less than the median value with similar homes having more than the median value only showed statistically significant differences between the means for heating type and frequency of open windows.

A multiple regression analysis of various factors (difference in mixing ratio, number of openings and occupants, house type, floor area, storey height, presence of fish tanks, and clothes drying regime) which may potentially affect bedroom mould area showed that none of the these independent variables have a significant effect on the visible mould present. Each variable was independently tested against bedroom mould area as well as living rooms and whole house mould areas. Those factors having a linear or almost linear slope against bedroom mould area included; floor area, difference in mixing ratio, house type grouping and openings but none showed significant results (although floor area was almost significant, p = 0.07). It is possible that the numbers available (58) were too small or that visible mould area is a poor indicator of mould contamination.

Studies report that dampness is prevalent in residential housing but there is no generally accepted definition of dampness. Its possible causes include a breach of the building envelope, failure of a water-using device, excessive indoor water vapour generation, and inadequate ventilation. The availability of moisture is the primary factor that controls mould growth indoors since the nutrients and temperature range that they require are usually present. The RH and T were recorded continuously for between 14-21 days in each of a minimum of two rooms at baseline and at 12 months in each home (64 intervention and 58 control homes). The mean indoor absolute humidity

measured for all the rooms at baseline was 51.1g/m^3 within a range of 35.0g/m^3 to 62.3g/m^3. Indoor absolute humidity concentrations ranged diurnally over a range of approximately 27.3g/m^3. Water vapour levels in the homes increased particularly over the evening hours until midnight and then fell overnight reflecting activity in the home. The mean outdoor level of humidity was 50.4g/m^3 and subtracting this from the indoor levels gives a measure of the water vapour generated by the occupants (i.e. mean difference = 0.7g/m^3).

Twelve months after eradication of mould in the intervention homes it was again present as visible mould in 40% of these homes. However in only five of these homes had the mould reappeared on the surfaces treated with fungicide and in all but one the quantity was minimal; in the other houses in the group the new mould occurred on surfaces that were previously unaffected.

It is difficult to assess the relative contributions of mould removal, fungicidal treatment and improved ventilation to these homes. The fungicide appeared to prevent mould re-growth, and the fan to reduce atmospheric humidity, but do not know what would happen if each element of the intervention had been omitted. Increased ventilation may result in a greater reduction in mould growth, although this would be offset with a loss of heat during the winter months.

At baseline the mean humidity was 8.67g/kg (SD 1.28) in the intervention homes and 8.51g/kg (SD 1.54) in the control homes; at 12 months it was 8.44 (1.67) and 8.76 (1.49) g/kg, respectively. The difference between these changes was statistically significant (p=0.011) with a net reduction of 0.48g/kg in the intervention group; 95%CI, 0.11-0.85, and a net increase in the control group (0.02g/kg). The seasonal effects on the ventilation and possibly the stack effect on the storeys could be used to better tease out a more suitable intervention regime.

Prevention of indoor mould requires the control of the temperature and water vapour concentration indoors, which in turn relate to the housing conditions. Many occupants are not able to make improvements unaided and the Government is addressing this, in part, through the national Home Energy Efficiency scheme known as Warm Front (DTI, 2001). Targeted at vulnerable households, this scheme provides grants for the improvement of home insulation levels and heating to tackle fuel poverty.

Mould sensitivity is quite common in patients with asthma symptoms, occurring in 43% of the participants in this trial. By comparison, an international (mainly European) survey found sensitivity to *Alternaria alternata* or *Cladosporium herbarum* in 5.9% of a random sample of young adults (Zock et al., 2002), the prevalence rising successively in groups with increasingly severe asthma (Zureik et al., 2002). Thus the benefits of mould eradication may be partly attributable to reducing exposure to allergens in mould-sensitive persons. Some of the participants in this trial had negative skin prick tests to mould spores, so that an additional mechanism is probably operating. Moulds can also emit mycotoxins and other volatile compounds that are irritants to the mucous membranes and thus may provoke asthma and rhinoconjunctivitis (Flannigan et al., 1991; Kilburn, 2003).

The intervention was effective in reducing the indoor humidity and also the presence of mould. The results of this trial suggest that homes with poor hygrothermal conditions containing patients with asthma and rhinitis would be well advised to eradicate mould from their homes, and that mould eradication should be incorporated into asthma control programmes, which tend to be largely medication based. These initiatives should be targeted as a Public Health issue. Mould removal and fungicide application may need to be repeated as necessary as mould reappeared at new sites within 12 months.

Future research would benefit from the following considerations:

1. Any means by which the subject may be blinded to their treatment status. This is difficult to achieve in practice but a placebo fungicidal treatment indistinguishable to the real one could be applied. A cross-over design could be applied by operation of a PIV fan which is switched on for a period and then switched off for another for the entire group of subjects.
2. An objective measurement of medication usage could be considered whereby asthma inhalers are collected and weighed, or a counter is added to them to indicate the number of uses.
3. Clearly if resources permit a larger sample of people and homes would increase the probability of observing a result that is statistically significant.
4. More complete environmental sampling of the home including ventilation rates, air leakage and air movement measurements would be beneficial.
5. Development of equipment to improve the measurement of bioaerosols is necessary.

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Appendix 1

Date_____ PID _____

CONFIDENTIAL CHEST HEALTH QUESTIONNAIRE 1

Name: _____

Address:

_____

_____

Male ☐ Female ☐ **Date of Birth:** _____

Do you have any pets? Yes ☐
If yes, what and how many pets do you have? No ☐

_____

1. Have you had wheezing or whistling in the chest in the last 4 weeks? Yes ☐ No ☐

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6

2. In the last 4 weeks, how often, on average, has your sleep been disturbed due to wheezing?

Never woken with wheezing ☐
Less than one night per week ☐
One or more nights per week ☐

3. In the last 4 weeks, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths? Yes ☐ No ☐

4. In the last 4 weeks, how much did this wheezing interfere with your daily activities?

Not at all ☐
A little ☐
A moderate amount ☐
A lot ☐

5. In the last 4 weeks, have you had wheezing or whistling in the chest when you did not have a cold or flu? Yes ☐ No ☐

---

6. Have you ever had asthma? Yes ☐ No ☐

7. In the last 4 weeks, have you taken any treatment (medicines, tablets, inhalers) for wheezing or asthma? Yes ☐ No ☐

If yes what is the name of the inhaler?

_____

What is the name of the medicine/tablet?

_____

8. Have you had a cold or the flu during the last 4 weeks? Yes ☐ No ☐

9. During the last 4 weeks, have you had a cough on most days? Yes ☐ No ☐

10. During the last 4 weeks, have you brought up phlegm (spit) from your chest on most days? Yes ☐ No ☐

The following questions are about nose problems
which occur when you do not have a cold or the flu

11. Have you ever had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 15

---

12. During the last 4 weeks, have you had a problem with sneezing or a runny, or blocked nose when you did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 15

13. During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? Yes ☐ No ☐

14. During the last 4 weeks, how much did this nose problem interfere with your daily activities?

- Not at all ☐
- A little ☐
- A moderate amount ☐
- A lot ☐

15. Have you ever had hay fever? Yes ☐ No ☐

16. In the last 4 weeks, have you taken any treatment (medicines or sprays) for a runny or blocked nose problem or hay fever? Yes ☐ No ☐

17. Have you ever had eczema? Yes ☐ No ☐

18. In the past 4 weeks, have you taken any treatment (medicines, creams, ointments) for an itchy rash or eczema? Yes ☐ No ☐

Smoking

19. Have you ever smoked cigarettes every day for as long as a year? Yes ☐ No ☐

If YES, how many do you smoke per day at present?

- None ☐
- 1 – 4 ☐
- 5 – 14 ☐
- 15 – 24 ☐
- 25 or more ☐

If NONE, how long ago did you stop smoking cigarettes?

Less than a year ago ☐

1 – 4 years ago ☐

5 – 9 years ago ☐

10 or more years ago ☐

20. Do you smoke a pipe? Yes ☐ No ☐

21. Do you smoke cigars (one or more per week)? Yes ☐ No ☐

22. Does anyone else living in the house smoke? Yes ☐ No ☐

THANK YOU FOR YOUR HELP WITH THIS STUDY

Date_____ PID _____

CONFIDENTIAL CHEST HEALTH QUESTIONNAIRE 1C

FOR CHILDREN UNDER 12 YEARS OLD AT FIRST CONTACT

Name: _____

Address:

_____

_____

Male ☐ Female ☐ **Date of Birth:** _____

Do you have any pets? Yes ☐

If yes, what and how many pets do you have? No ☐

_____

---

1. Has your child had wheezing or whistling in the chest Yes ☐

   in the last 4 weeks? No ☐

IF YOU HAVE ANSWERED "No" PLEASE SKIP TO QUESTION 6

---

3. In the last 4 weeks, how often, on average, has sleep been disturbed due to wheezing? ☐

   Never woken with wheezing ☐

   Less than one night per week ☐

   One or more nights per week ☐

3. In the last 4 weeks, has wheezing ever been severe enough to limit Yes ☐

   speech to only one or two words at a time between breaths? No ☐

4. In the last 4 weeks, how much did this wheezing interfere with daily activities?

   Not at all ☐

   A little ☐

   A moderate amount ☐

   A lot ☐

5. In the last 4 weeks, has he/she had wheezing or whistling in the chest when he/she did not have a cold or flu? Yes ☐ No ☐

---

6. Has he/she ever had asthma? Yes ☐ No ☐

7. In the last 4 weeks, has he/she taken any treatment (medicines, tablets, inhalers) for wheezing or asthma? Yes ☐ No ☐

If yes, what is the name of the inhaler? _____

What is the name of the medicine/tablet? _____

8. Has he/she had a cold or the flu during the last 4 weeks? Yes ☐ No ☐

9. During the last 4 weeks, has he/she had a cough on most days? Yes ☐ No ☐

10. During the last 4 weeks, has he/she brought up phlegm (spit) from his/her chest on most days? Yes ☐ No ☐

The following questions are about nose problems
which occur when you do not have a cold or the flu

11. Has he/she ever had a problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "No" PLEASE SKIP TO QUESTION 15

---

12. During the last 4 weeks, has he/she had a problem with sneezing or a runny, or blocked nose when he/she did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "No" PLEASE SKIP TO QUESTION 15

---

13. During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? Yes ☐ No ☐

15. During the last 4 weeks, how much did this nose problem interfere with daily activities?

Not at all ☐
A little ☐
A moderate amount ☐
A lot ☐

---

15. Have he/she ever had hay fever? Yes ☐ No ☐

16. In the last 4 weeks, has he/she taken any treatment (medicines or sprays) for a runny or blocked nose problem or hay fever? Yes ☐ No ☐

17. Has he/she ever had eczema? Yes ☐ No ☐

18. In the past 4 weeks, has he/she taken any treatment (medicines, creams, ointments) for an itchy rash or eczema? Yes ☐ No ☐

19. Does anyone living in the house smoke? Yes ☐ No ☐

THANK YOU FOR YOUR HELP WITH THIS STUDY

Date _____ PID _____

CONFIDENTIAL CHEST HEALTH QUESTIONNAIRE 2

Name: _____

Address: _____

_____ What is your height? _____

1. Do you have any pets? _____ Yes ☐
   If yes, what and how many pets do you have? _____ No ☐

2. Have you had wheezing or whistling in the chest in the last 4 weeks? Yes ☐
   No ☐

IF YOU HAVE ANSWERED "No" PLEASE SKIP TO QUESTION 9

3. If yes, how much did you have? A little ☐
   A moderate amount ☐
   A lot ☐

4. In the last 4 weeks, how often, on average, has your sleep been disturbed due to wheezing?
   Never woken with wheezing ☐
   Less than one night per week ☐
   One or more nights per week ☐

5. In the last 4 weeks, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths? Yes ☐ No ☐

6. In the last 4 weeks, have you been at all breathless when the wheezing noise was present? Yes ☐ No ☐

7. In the last 4 weeks, how much did this wheezing interfere with your daily activities?
   Not at all ☐
   A little ☐
   A moderate amount ☐
   A lot ☐

8. In the last 4 weeks, have you had wheezing or whistling in the chest when you did not have a cold or flu? Yes ☐ No ☐

---

9. How is your breathing compared with how it was 6 months ago? Better ☐
   Same ☐
   Worse ☐

Date _____ PID _____

10. Have you ever had asthma? Yes ☐ No ☐

11a. In the last 12 months, have you taken any medication to help your breathing? Yes ☐ No ☐

IF YOU HAVE ANSWERED "No" PLEASE SKIP TO QUESTION 12

11b. If yes, in the last 6 months have you used your medication : Less, ☐ the Same ☐ or More ☐ than/as previously?

11c. And in the last 4 weeks, have you taken any treatment (medicines, tablets, inhalers) for wheezing or asthma? Yes ☐ No ☐

If yes, what is the name of the inhaler? _____

What is the name of the medicine/tablet? _____

12. Have you had a cold or the flu during the last 4 weeks? Yes ☐ No ☐

13. During the last 4 weeks, have you had a cough on most days? Yes ☐ No ☐

14. During the last 4 weeks, have you brought up phlegm (spit) from your chest on most days? Yes ☐ No ☐

The following questions are about nose problems which occur when you do not have a cold or the flu

15. Have you ever had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "No" PLEASE SKIP TO QUESTION 19

16. During the last 4 weeks, have you had a problem with sneezing or a runny, or blocked nose when you did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "No" PLEASE SKIP TO QUESTION 19

17. During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? Yes ☐ No ☐

18. During the last 4 weeks, how much did this nose problem interfere with your daily activities? Not at all ☐ A little ☐ A moderate amount ☐ A lot ☐

Date _____ PID _____

19. Have you ever had hay fever? Yes ☐ No ☐

20. In the last 4 weeks, have you taken any treatment (medicines or sprays) for a runny or blocked nose problem or hay fever? Yes ☐ No ☐
Please name the medications. _____

21. Have you ever had eczema? Yes ☐ No ☐

22. In the past 4 weeks, have you taken any treatment (medicines, creams, ointments) for an itchy rash or eczema? Yes ☐ No ☐
Please name the medications. _____

Smoking

23. How many cigarettes do you smoke per day? None ☐ 1 – 4 ☐ 5 – 14 ☐ 15 – 24 ☐ 25 or more ☐

24. If you have stopped smoking cigarettes, when was that? _____

25. Does anyone else living in the house smoke? Yes ☐ No ☐

26. Have you used an allergy-proof mattress cover in the last 6 months? Yes ☐ No ☐

27. Have you replaced any carpets in the last 6 months ? Yes ☐ No ☐

28. Have you redecorated or renovated in the last 6 months? Yes ☐ No ☐
For questions 27 & 28 please specify including which rooms

_____

29. Approximately how many years old is your mattress? _____

30. Do you routinely fold back the bedding when the bed is unoccupied to air it? Yes ☐ No ☐

30. Is there any mould on surfaces inside the house? Yes ☐ No ☐
If yes, where? _____

THANK YOU FOR YOUR HELP WITH THIS STUDY

Date _____ PID _____

CONFIDENTIAL CHEST HEALTH QUESTIONNAIRE 2C

FOR CHILDREN UNDER 12 YEARS OLD AT FIRST CONTACT

Name: _____

Address: _____

_____What is your height? _____

1. Do you have any pets? _____ Yes ☐
   If yes, what and how many pets do you have? _____ No ☐

2. Has your child had wheezing or whistling in the chest in the last 4 weeks? Yes ☐
   No ☐

IF YOU HAVE ANSWERED "No" PLEASE SKIP TO QUESTION 9

3. If yes, how much did he/she have?
   A little ☐
   A moderate amount ☐
   A lot ☐

4. In the last 4 weeks, how often, on average, has sleep been disturbed due to wheezing?
   Never woken with wheezing ☐
   Less than one night per week ☐
   One or more nights per week ☐

5. In the last 4 weeks, has wheezing ever been severe enough to limit speech to only one or two words at a time between breaths? Yes ☐ No ☐

6. In the last 4 weeks as your child been at all breathless when the wheezing noise was present? Yes ☐ No ☐

7. In the last 4 weeks, how much did wheezing interfere with his/her daily activities?
   Not at all ☐
   A little ☐
   A moderate amount ☐
   A lot ☐

8. In the last 4 weeks, has he/she had wheezing or whistling in the chest when he/she did not have a cold or flu? Yes ☐ No ☐

---

9. How is his/her breathing compared with how it was 6 months ago?
   Better ☐
   Same ☐
   Worse ☐

Date _____ PID _____

10. Has he/she ever had asthma? Yes ☐ No ☐

11a. In the last 12 months, has he/she taken any medication to help his/her breathing? Yes ☐ No ☐

IF YOU HAVE ANSWERED "No" PLEASE SKIP TO QUESTION 12

11b. If yes, in the last 6 months has he/she used his/her medication : Less, ☐ the Same ☐ or More ☐ than/as previously?

11c. And in the last 4 weeks, has he/she taken any treatment (medicines, tablets, inhalers) for wheezing or asthma? Yes ☐ No ☐

If yes, what is the name of the inhaler? _____

What is the name of the medicine/tablet? _____

12. Has he/she had a cold or the flu during the last 4 weeks? Yes ☐ No ☐

13. During the last 4 weeks, has he/she had a cough on most days? Yes ☐ No ☐

14. During the last 4 weeks, has he/she brought up phlegm (spit) from his/her chest on most days? Yes ☐ No ☐

The following questions are about nose problems which occur when your child does not have a cold or the flu

15. Has he/she ever had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "No" PLEASE SKIP TO QUESTION 19

16. During the last 4 weeks, has he/she had a problem with sneezing or a runny, or blocked nose when he/she did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "No" PLEASE SKIP TO QUESTION 19

17. During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? Yes ☐ No ☐

18. During the last 4 weeks, how much did this nose problem interfere with his/her daily activities?

Not at all ☐
A little ☐
A moderate amount ☐
A lot ☐

Date _____ PID _____

19. Has he/she ever had hay fever? Yes ☐ No ☐

20. In the last 4 weeks, has he/she taken any treatment (medicines or sprays) for a runny or blocked nose problem or hay fever? Yes ☐ No ☐
Please name the medications. _____

21. Has he/she ever had eczema? Yes ☐ No ☐

22. In the past 4 weeks, has he/she taken any treatment (medicines, creams, ointments) for an itchy rash or eczema? Yes ☐ No ☐
Please name the medications. _____

23. Does anyone living in the house smoke? Yes ☐ No ☐

24. Has he/she used an allergy-proof mattress cover in the last 6 months? Yes ☐ No ☐

25. Have any carpets been replaced in the last 6 months? Yes ☐ No ☐

26. Has the home been redecorated or renovated in the last 6 months? Yes ☐ No ☐
For questions 25 & 26 please specify including which rooms

_____

27. Approximately how many years old is his/her mattress? _____

28. Does he/she routinely fold back the bedding when the bed is unoccupied to air it? Yes ☐ No ☐

29. Is there any mould on surfaces inside the house? Yes ☐
If yes, where? _____ No ☐

THANK YOU FOR YOUR HELP WITH THIS STUDY

Date _____ PID _____

CONFIDENTIAL CHEST HEALTH QUESTIONNAIRE 3

Name: _____

Address: _____

_____

1. Do you have any pets? _____ Yes ☐
   If yes, what and how many pets do you have? _____ No ☐

2. Have you had wheezing or whistling in the chest in the last 4 weeks? Yes ☐
   No ☐

IF YOU HAVE ANSWERED "No" PLEASE SKIP TO QUESTION 9

3. If yes, how much did you have?
   A little ☐
   A moderate amount ☐
   A lot ☐

4. In the last 4 weeks, how often, on average, has your sleep been disturbed due to wheezing?
   Never woken with wheezing ☐
   Less than one night per week ☐
   One or more nights per week ☐

5. In the last 4 weeks, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths? Yes ☐ No ☐

6. In the last 4 weeks, have you been at all breathless when the wheezing noise was present? Yes ☐ No ☐

7. In the last 4 weeks, how much did this wheezing interfere with your daily activities?
   Not at all ☐
   A little ☐
   A moderate amount ☐
   A lot ☐

8. In the last 4 weeks, have you had wheezing or whistling in the chest when you did not have a cold or flu? Yes ☐ No ☐

---

9. How is your breathing compared with how it was 12 months ago? Better ☐
   Same ☐
   Worse ☐

Date _____ PID _____

10. Have you ever had asthma? Yes ☐ No ☐

11a. In the last 12 months, have you taken any medication to help your breathing? Yes ☐ No ☐

IF YOU HAVE ANSWERED "No" PLEASE SKIP TO QUESTION 12

11b. If yes, in the last 6 months have you used your medication :
Less, ☐ the Same ☐ or More ☐ than/as previously?

11c. And in the last 4 weeks, have you taken any treatment (medicines, tablets, inhalers) for wheezing or asthma? Yes ☐ No ☐

If yes, what is the name of the inhaler? _____

What is the name of the medicine/tablet? _____

12. Have you had a cold or the flu during the last 4 weeks? Yes ☐ No ☐

13. During the last 4 weeks, have you had a cough on most days? Yes ☐ No ☐

14. During the last 4 weeks, have you brought up phlegm (spit) from your chest on most days? Yes ☐ No ☐

The following questions are about nose problems which occur when you do not have a cold or the flu

15. Have you ever had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "No" PLEASE SKIP TO QUESTION 19

16. During the last 4 weeks, have you had a problem with sneezing or a runny, or blocked nose when you did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "No" PLEASE SKIP TO QUESTION 19

17. During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? Yes ☐ No ☐

18. During the last 4 weeks, how much did this nose problem interfere with your daily activities? Not at all ☐ A little ☐ A moderate amount ☐ A lot ☐

Date _____ PID _____

19. Have you ever had hay fever? Yes ☐ No ☐

20. In the last 4 weeks, have you taken any treatment (medicines or sprays) for a runny or blocked nose problem or hay fever? Yes ☐ No ☐
Please name the medications. _____

21. Have you ever had eczema? Yes ☐ No ☐

22. In the past 4 weeks, have you taken any treatment (medicines, creams, ointments) for an itchy rash or eczema? Yes ☐ No ☐
Please name the medications. _____

Smoking

23. How many cigarettes do you smoke per day?
None ☐
1 – 4 ☐
5 – 14 ☐
15 – 24 ☐
25 or more ☐

24. If you have stopped smoking cigarettes, when was that? _____

25. Does anyone else living in the house smoke? Yes ☐ No ☐

26. Have you used an allergy-proof mattress cover in the last 6 months? Yes ☐ No ☐

27. Have you replaced any carpets in the last 6 months ? Yes ☐ No ☐

28. Have you renovated or redecorated in the last 6 months? Yes ☐ No ☐
For questions 27 & 28 please specify including which rooms.

_____

29. Approximately how many years old is your mattress? _____

30. Do you routinely fold back the bedding when the bed is unoccupied to air it? Yes ☐ No ☐

30. Is there any mould on surfaces inside the house? Yes ☐ No ☐
If yes, where? _____

THANK YOU FOR YOUR HELP WITH THIS STUDY

Date _____ PID _____

CONFIDENTIAL CHEST HEALTH QUESTIONNAIRE 3C

FOR CHILDREN UNDER 12 YEARS OLD AT FIRST CONTACT

Name: _____

Address: _____

_____

1. Do you have any pets? _____ Yes ☐
   If yes, what and how many pets do you have? _____ No ☐

2. Has your child had wheezing or whistling in the chest in the last 4 weeks? Yes ☐
   No ☐

IF YOU HAVE ANSWERED "No" PLEASE SKIP TO QUESTION 9

3. If yes, how much did he/she have?
   A little ☐
   A moderate amount ☐
   A lot ☐

4. In the last 4 weeks, how often, on average, has sleep been disturbed due to wheezing?
   Never woken with wheezing ☐
   Less than one night per week ☐
   One or more nights per week ☐

5. In the last 4 weeks, has wheezing ever been severe enough to limit speech to only one or two words at a time between breaths? Yes ☐ No ☐

6. In the last 4 weeks as your child been at all breathless when the wheezing noise was present? Yes ☐ No ☐

7. In the last 4 weeks, how much did wheezing interfere with his/her daily activities?
   Not at all ☐
   A little ☐
   A moderate amount ☐
   A lot ☐

8. In the last 4 weeks, has he/she had wheezing or whistling in the chest when he/she did not have a cold or flu? Yes ☐ No ☐

---

9. How is his/her breathing compared with how it was 12 months ago? Better ☐
   Same ☐
   Worse ☐

Date _____ PID _____

10. Has he/she ever had asthma? Yes ☐ No ☐

11a. In the last 12 months, has he/she taken any medication to help his/her breathing? Yes ☐ No ☐

IF YOU HAVE ANSWERED "No" PLEASE SKIP TO QUESTION 12

11b. If yes, in the last 6 months has he/she used his/her medication :
Less, ☐ the Same ☐ or More ☐ than/as previously?

11c. And in the last 4 weeks, has he/she taken any treatment (medicines, tablets, inhalers) for wheezing or asthma? Yes ☐ No ☐

If yes, what is the name of the inhaler? _____

What is the name of the medicine/tablet? _____

12. Has he/she had a cold or the flu during the last 4 weeks? Yes ☐ No ☐

13. During the last 4 weeks, has he/she had a cough on most days? Yes ☐ No ☐

14. During the last 4 weeks, has he/she brought up phlegm (spit) from his/her chest on most days? Yes ☐ No ☐

The following questions are about nose problems which occur when your child does not have a cold or the flu

15. Has he/she ever had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "No" PLEASE SKIP TO QUESTION 19

16. During the last 4 weeks, has he/she had a problem with sneezing or a runny, or blocked nose when he/she did not have a cold or the flu? Yes ☐ No ☐

IF YOU ANSWERED "No" PLEASE SKIP TO QUESTION 19

17. During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? Yes ☐ No ☐

18. During the last 4 weeks, how much did this nose problem interfere with his/her daily activities?

Not at all ☐
A little ☐
A moderate amount ☐
A lot ☐

Date _____ PID _____

19. Has he/she ever had hay fever? Yes ☐ No ☐

20. In the last 4 weeks, has he/she taken any treatment (medicines or sprays) for a runny or blocked nose problem or hay fever? Yes ☐ No ☐
Please name the medications. _____

21. Has he/she ever had eczema? Yes ☐ No ☐

22. In the past 4 weeks, has he/she taken any treatment (medicines, creams, ointments) for an itchy rash or eczema? Yes ☐ No ☐
Please name the medications. _____

23. Does anyone living in the house smoke? Yes ☐ No ☐

24. Has he/she used an allergy-proof mattress cover in the last 6 months? Yes ☐ No ☐

25. Have any carpets been replaced in the last 6 months? Yes ☐ No ☐

26. Has the home been redecorated or renovated in the last 6 months? Yes ☐ No ☐
For questions 25 & 26 please specify including which rooms

_____

27. Approximately how many years old is his/her mattress? _____

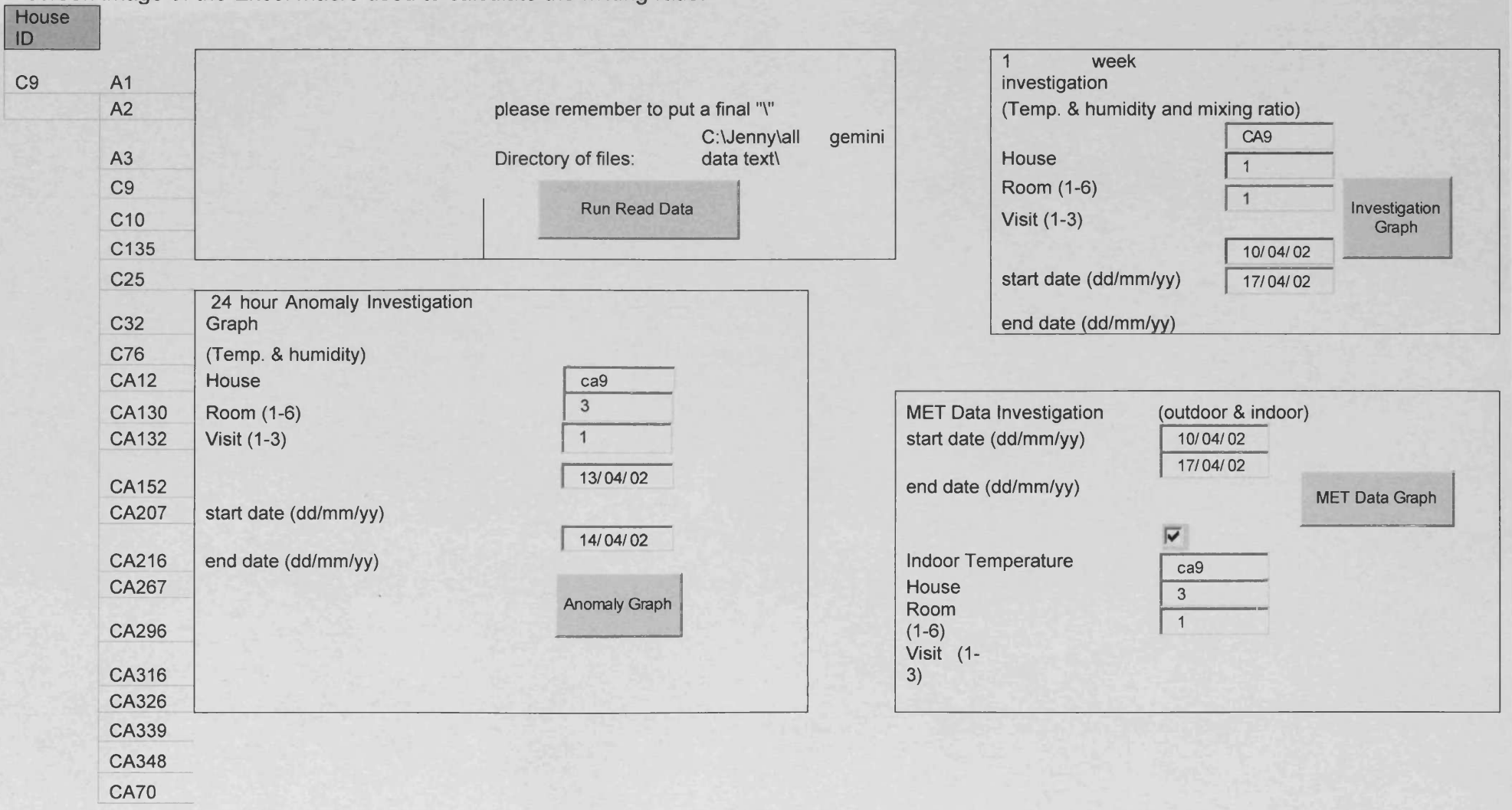
28. Does he/she routinely fold back the bedding when the bed is unoccupied to air it? Yes ☐ No ☐

29. Is there any mould on surfaces inside the house? Yes ☐
If yes, where? _____ No ☐

THANK YOU FOR YOUR HELP WITH THIS STUDY

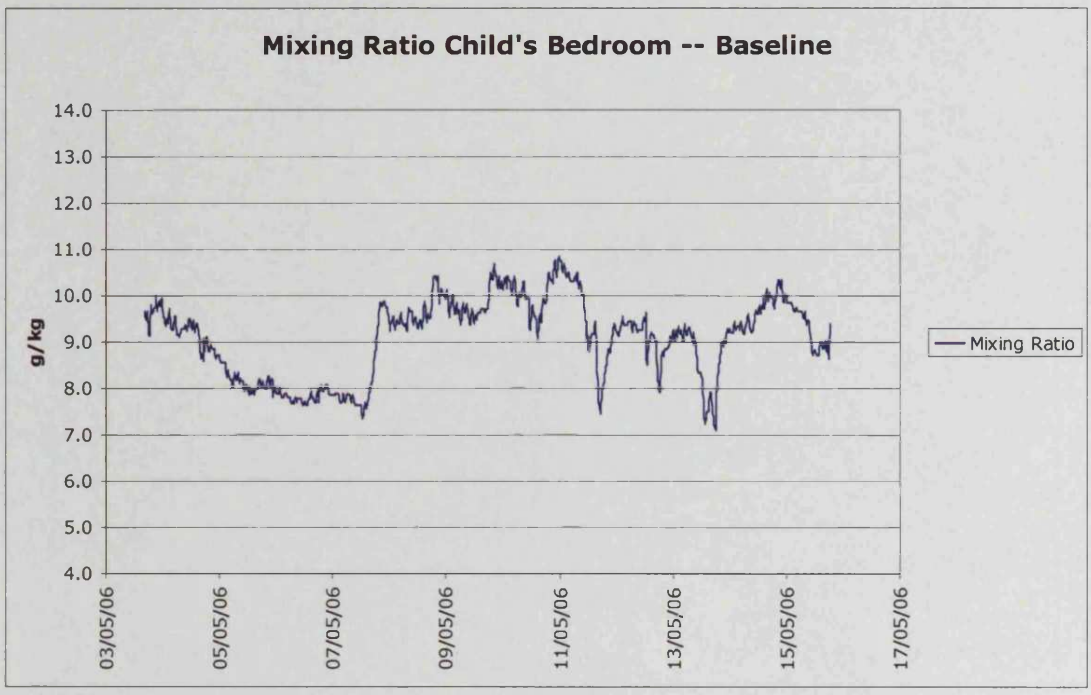
Appendix 2

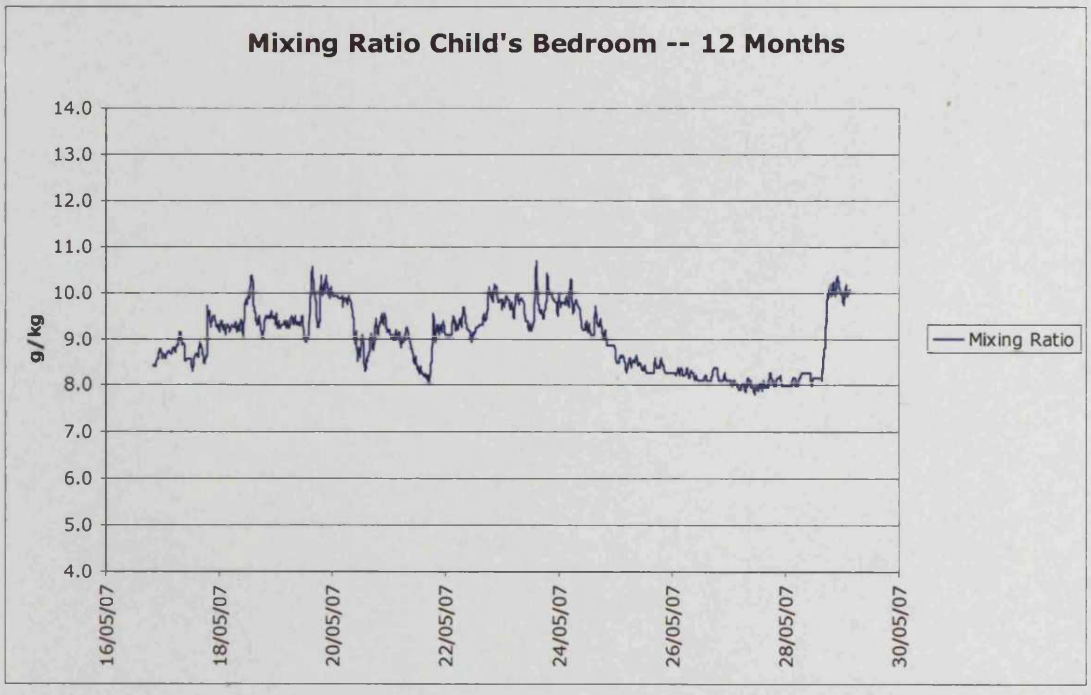
A screen image of the Excel macro used to calculate the mixing ratio.



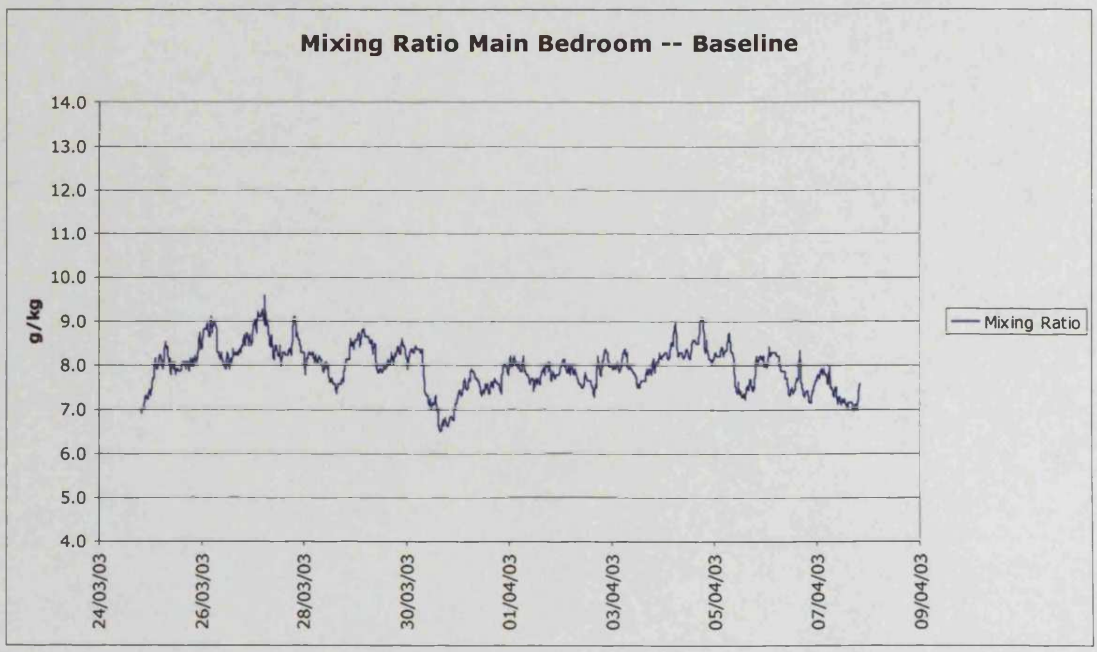
Mixing Ratio Examples in Control Homes

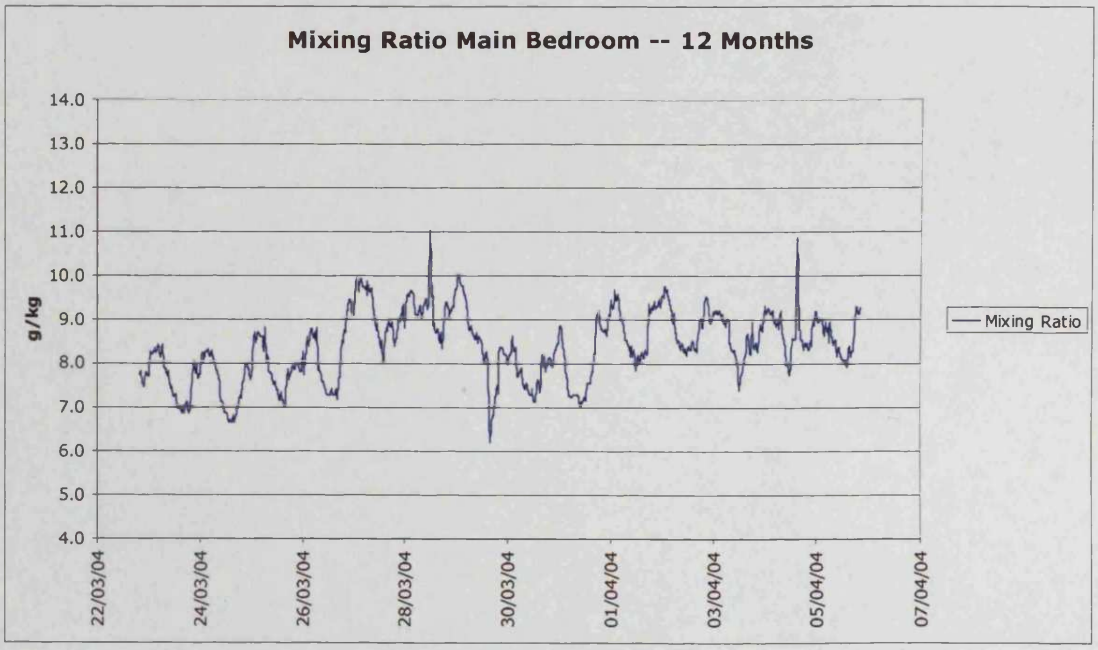
An example of a semi-detached home.



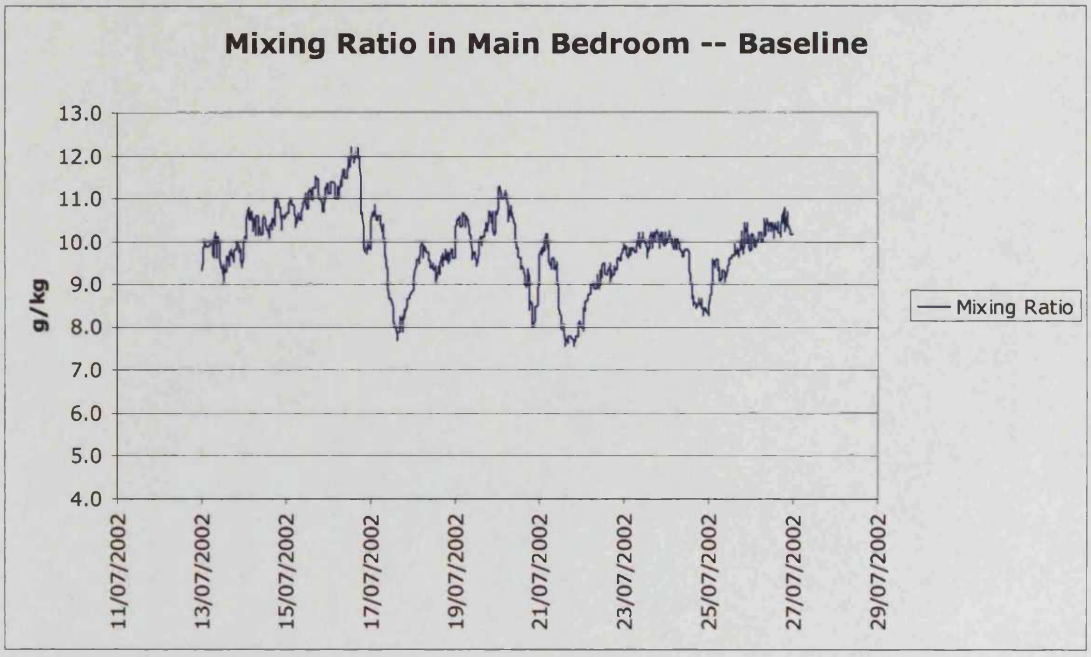


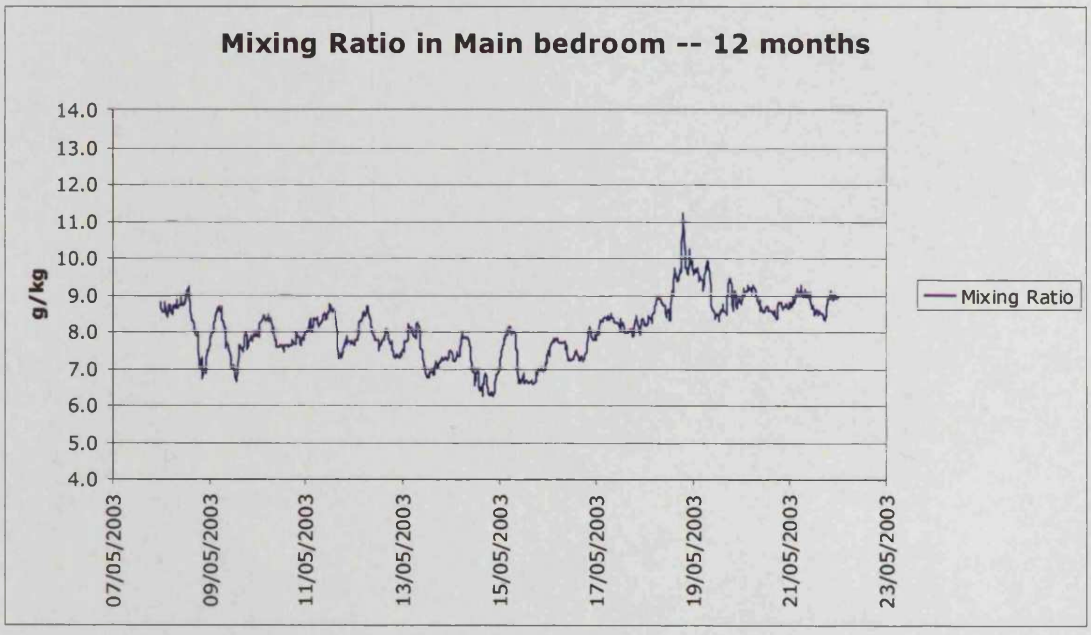
An example of a mid-terrace (2 storey) from the control group.



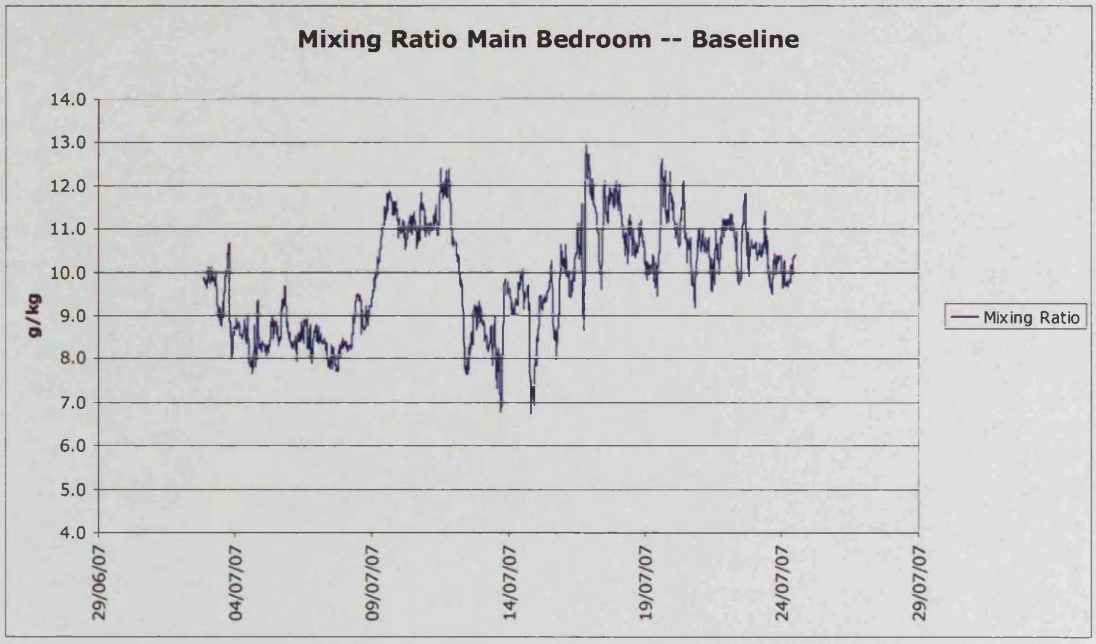


An example of a three storey mid-terrace from the control group.

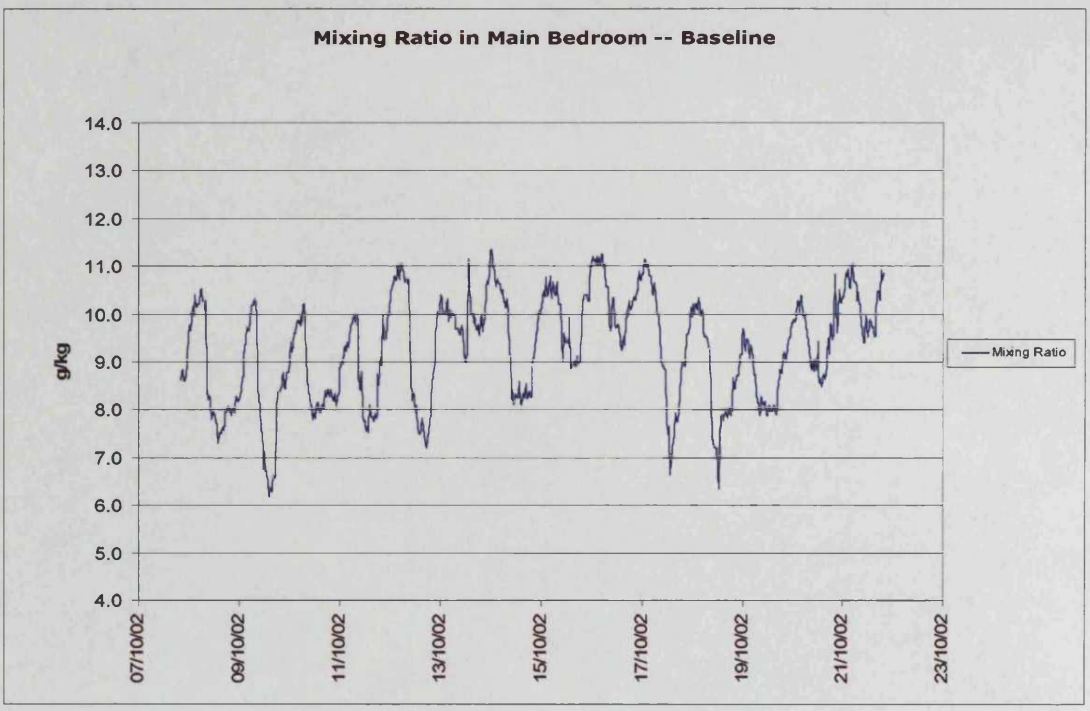


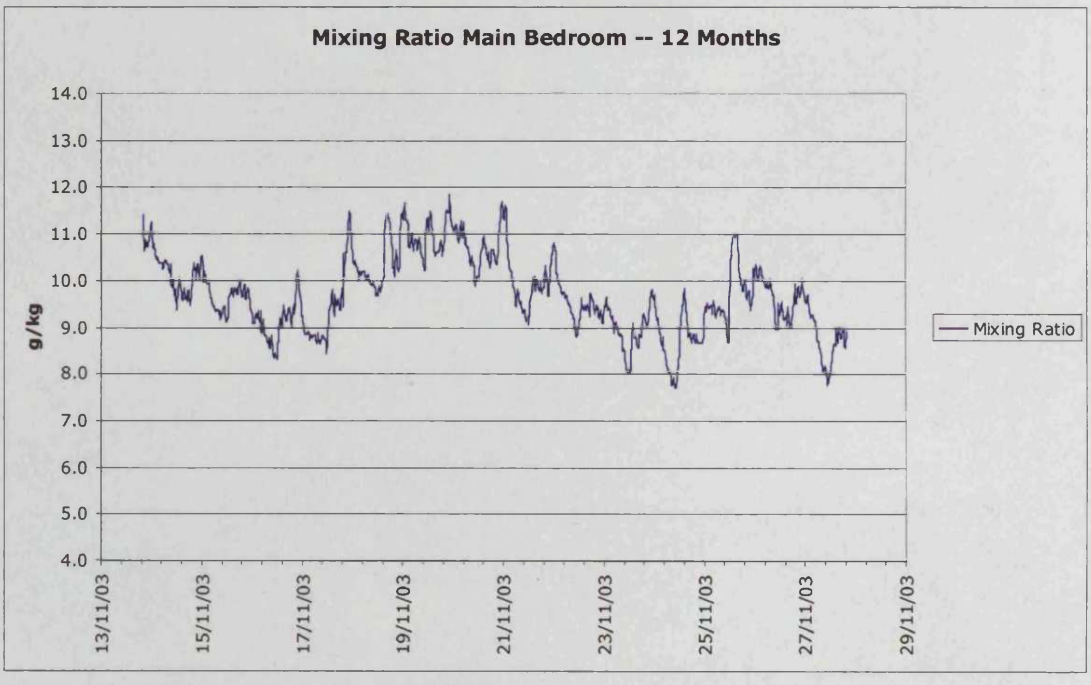


An example of a flat from the control group.



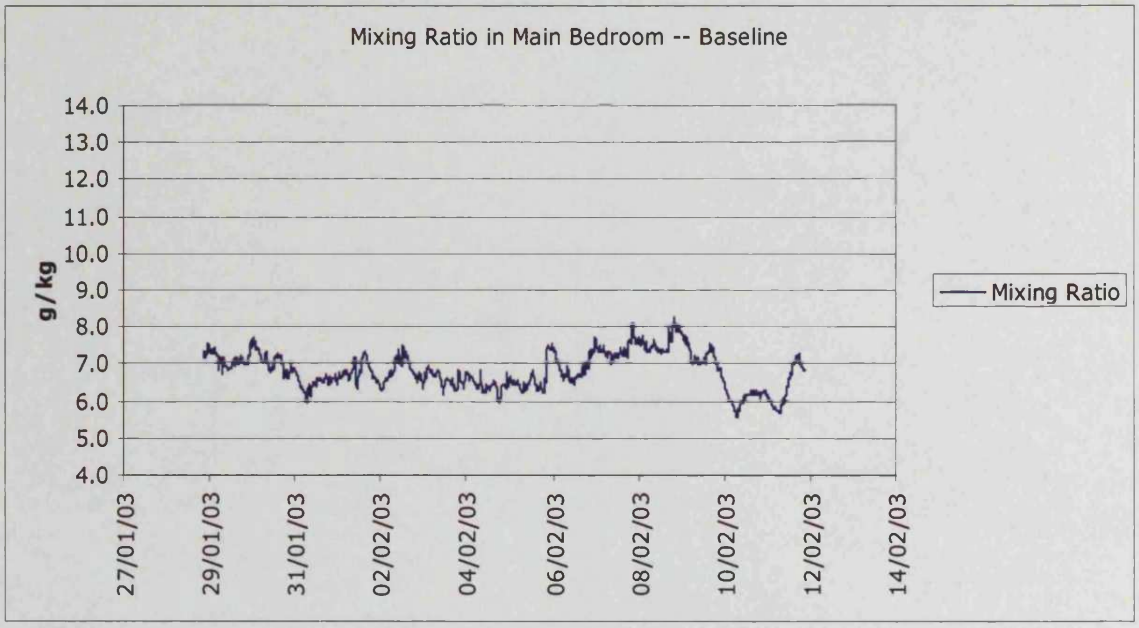
An example of a bungalow from the control group.

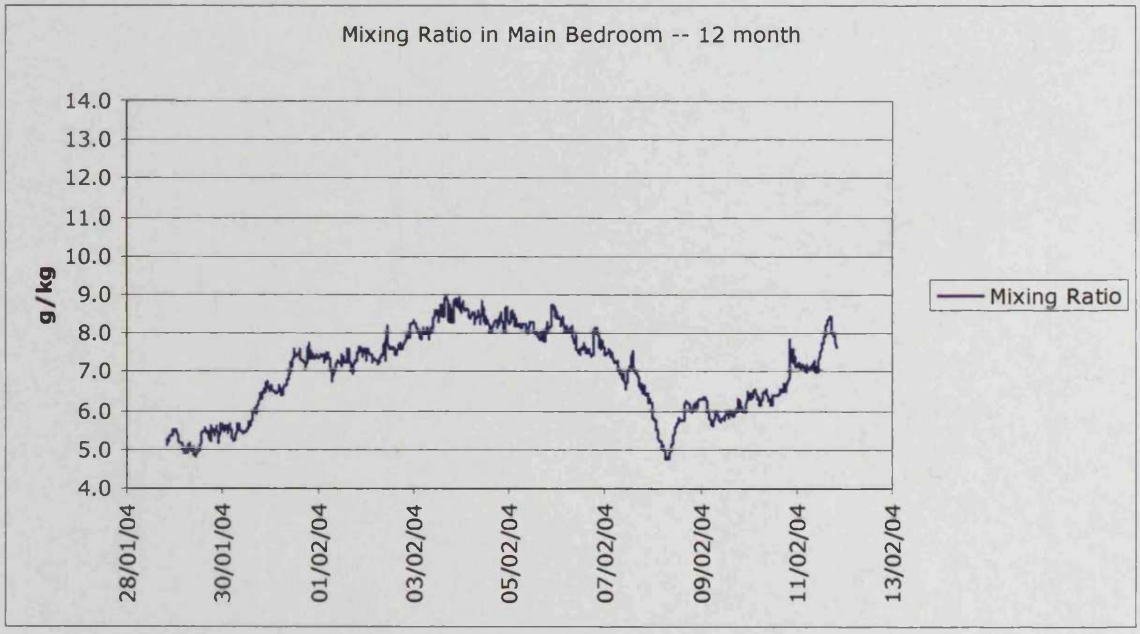




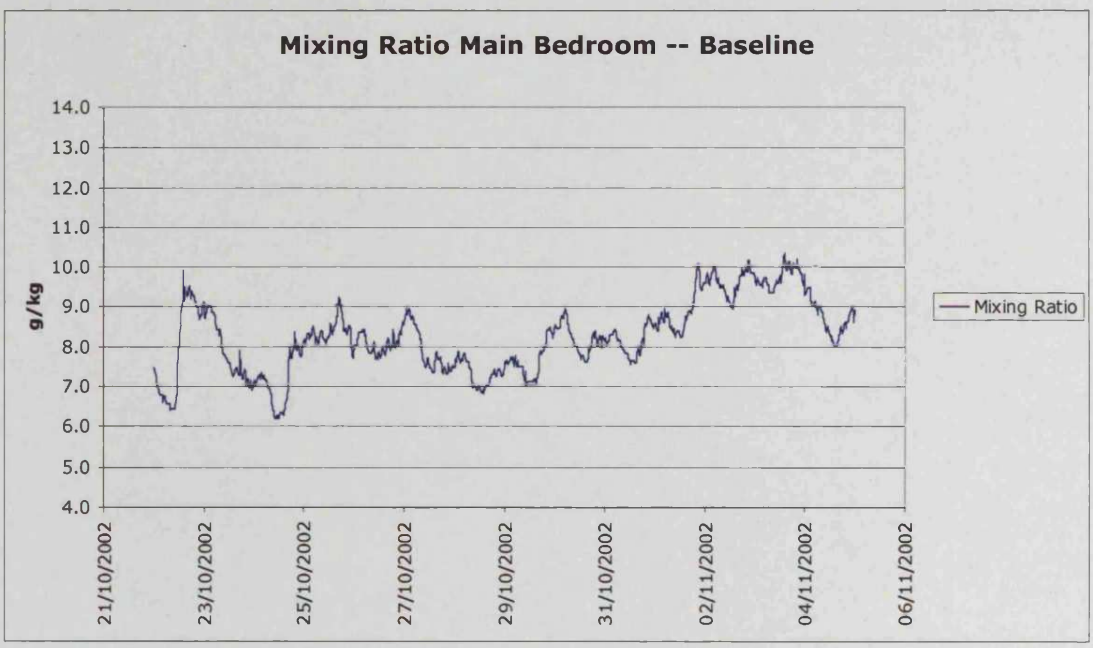
Mixing Ratio Examples in the Intervention Homes

An example of a bungalow in the intervention group.

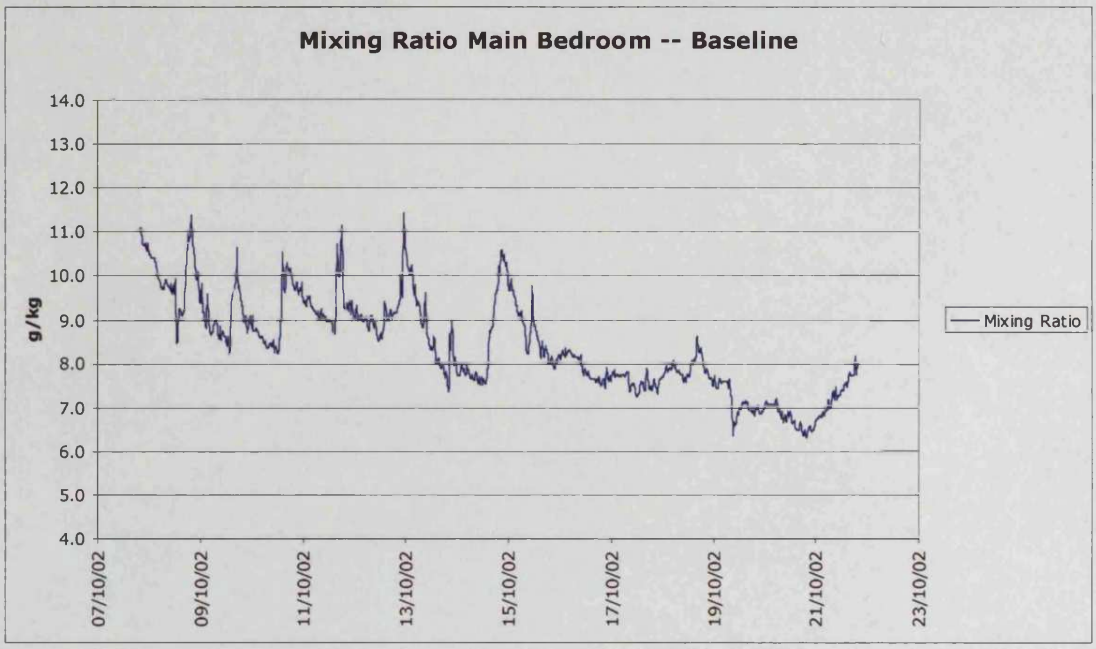


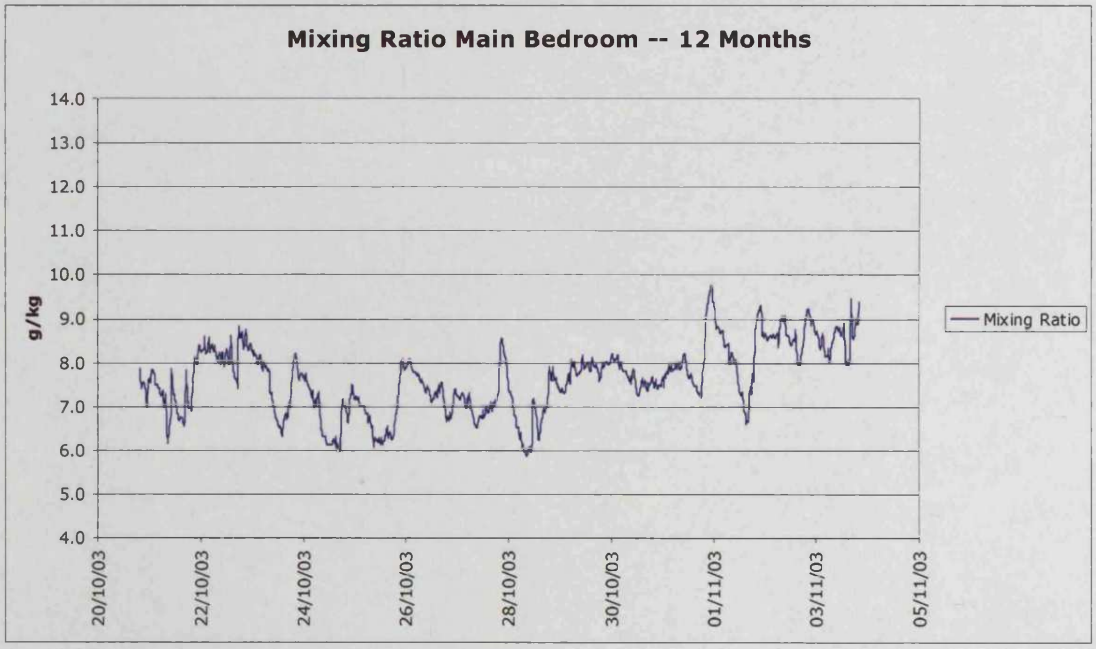


An example of a mid terrace in the intervention group.

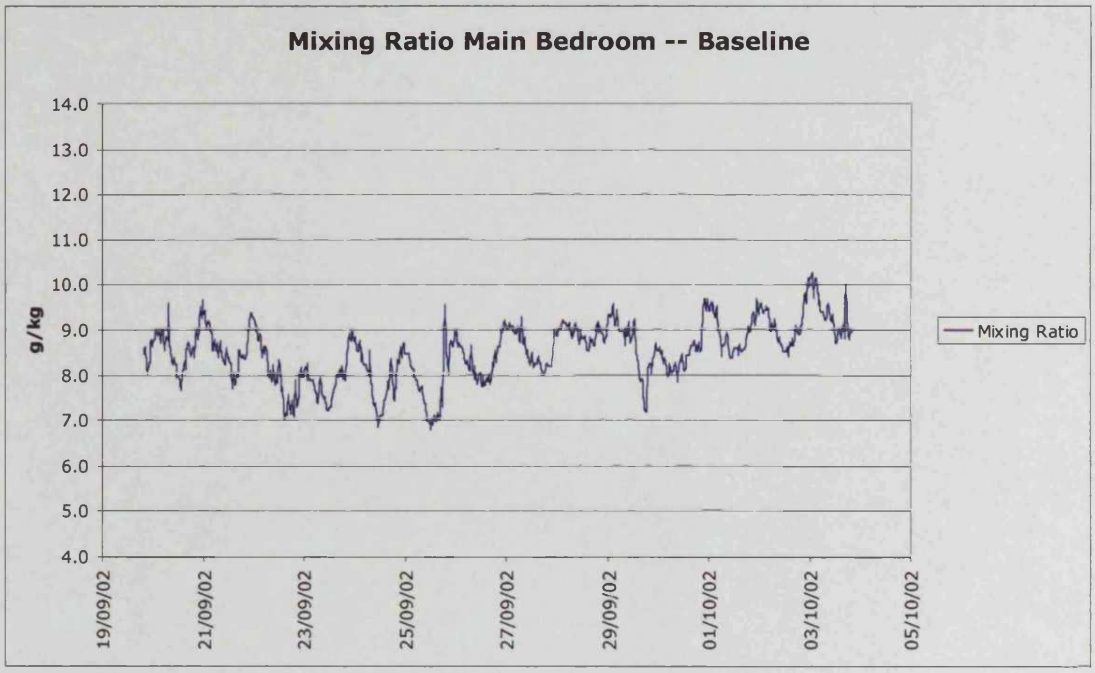


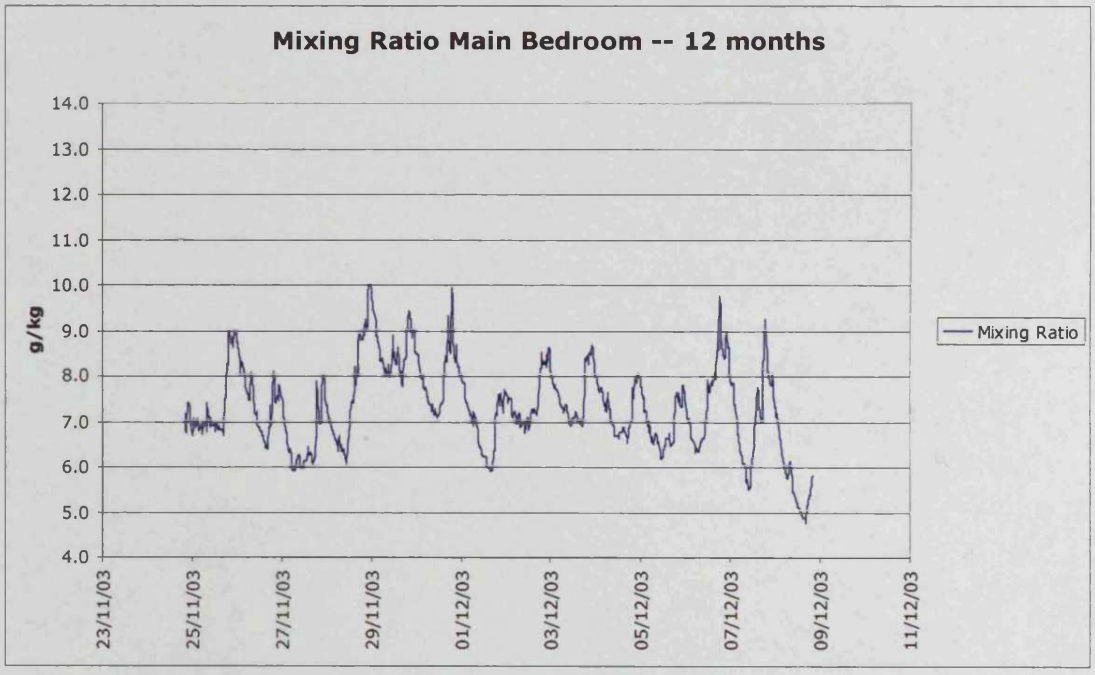
An example of a semi-detached home from the intervention group.





An example of a detached home from the intervention group.





Macro used to calculate the mixing ratio and plot graphs.

```
Sub Rob()
'
' Rob Macro
' Macro recorded 08/07/02 by INFOS
Dim roomvisit(150) As Integer


'set the directory to work with
 direct$ = Sheets("House IDs").Range("E4").Value

For housenum% = 1 To 150
'choose the house to work with
If Sheets("House IDs").Range("A" & housenum% + 1).Value = "" Then
'there are no more houses
   Exit Sub
Else
'read in the house name
   House = Sheets("House IDs").Range("A" & housenum% + 1).Value
End If


'check to see if the workbook exists
Set fs = Application.FileSearch
With fs
   .NewSearch
   .LookIn = direct$
'   .MatchAllWordForms = True
   .MatchTextExactly = True
   .FileName = House & ".xls"
   .FileType = msoFileTypeExcelWorkbooks
   If  .Execute(SortBy:=msoSortByFileName,  SortOrder:=msoSortOrderAscending)
> 0 Then
   'file was found so append to it
      Workbooks.Open (direct$ & House & ".xls")
   Else
   'add a new workbook for that house
      Workbooks.Add
      ActiveWorkbook.SaveAs    FileName:=direct$    &    House    &    ".xls",
FileFormat:=xlNormal, _
         Password:="", WriteResPassword:="", ReadOnlyRecommended:=False, _
         CreateBackup:=False
   End If
End With

   'start the loop for visit
   For i% = 1 To 3 Step 1
   'start the loop for room
      For j% = 1 To 6 Step 1
      'look to see if the file exists
      Set fs = Application.FileSearch
```

```
        With fs
        'look for the humidity file
        .LookIn = direct$
        .FileName = House & "H" & j% & i% & ".txt"
          If                                    .Execute(SortBy:=msoSortByFileName,
SortOrder:=msoSortOrderAscending) > 0 Then
          'file does exist
          'check that it has not already been read in
          exist = False
            For k% = 1 To Worksheets.Count
              If Workbooks(House & ".xls").Worksheets(k%).Name = House & "H"
& j% & i% Then
              'sheet exists
              exist = True
              End If
            Next k%
            If exist = False Then
            'sheet does not exist so create it
'             exist1 = False
              Workbooks.OpenText FileName:=direct$ & House & "H" & j% & i%
& ".TXT", Origin:=xlWindows, startrow:= _
              1, DataType:=xlDelimited, TextQualifier:=xlDoubleQuote, _
              ConsecutiveDelimiter:=False,     Tab:=True,     Semicolon:=False,
Comma:=False _
              , Space:=False, Other:=False, FieldInfo:=Array(1, 1)
              Windows(House & ".xls").Activate
                Workbooks(House & "H" & j% & i% & ".txt").Sheets(House & "H"
& j% & i%).Move After:=Workbooks(House & ".xls").Sheets(Worksheets.Count)
              Columns("A:B").ColumnWidth = 15
              Columns("D:D").Select
              Selection.NumberFormat = "dd/mm/yy"
              Columns("A:A").Select
              Selection.NumberFormat = "dd/mm/yy"
              Columns("E:E").Select
              Selection.NumberFormat = "hh:mm"
            numreadings% = 0
            'count how many readings have been taken
            For l% = 1 To 10000
            If Sheets(House & "H" & j% & i%).Range("A" & 34 + l%).Value = ""
Then
            'there are no more readings
              Exit For
            Else
            'count the number of readings
              numreadings% = numreadings% + 1
            End If
            Next l%
             Sheets(House & "H" & j% & i%).Select
              For k% = 1 To numreadings%
                  Range("D" & k% + 34).Select
                  ActiveCell.FormulaR1C1 = "=RC[-3]"
                  Range("E" & k% + 34).Select
```

```
                    ActiveCell.FormulaR1C1 = "=RC[-4]"
                Next k%
              End If
            End If
          'look for the temperature file
          .LookIn = direct$
          .FileName = House & "T" & j% & i% & ".txt"
            If                                    .Execute(SortBy:=msoSortByFileName,
SortOrder:=msoSortOrderAscending) > 0 Then
            'file does exist
            'check that it has not already been read in
            exist = False
              For k% = 1 To Worksheets.Count
                If Workbooks(House & ".xls").Worksheets(k%).Name = House & "T"
& j% & i% Then
                'sheet exists
                exist = True
                End If
              Next k%
              If exist = False Then
              'sheet does not exist so create it
                Workbooks.OpenText FileName:=direct$ & House & "T" & j% & i%
& ".TXT", Origin:=xlWindows, startrow:= _
                1, DataType:=xlDelimited, TextQualifier:=xlDoubleQuote, _
                ConsecutiveDelimiter:=False,      Tab:=True,      Semicolon:=False,
Comma:=False _
                , Space:=False, Other:=False, FieldInfo:=Array(1, 1)
                Windows(House & ".xls").Activate
                  Workbooks(House & "T" & j% & i% & ".txt").Sheets(House & "T" &
j% & i%).Move After:=Workbooks(House & ".xls").Sheets(Worksheets.Count)
                Columns("A:B").ColumnWidth = 15
                 Columns("D:D").Select
                Selection.NumberFormat = "dd/mm/yy"
                 Columns("A:A").Select
                Selection.NumberFormat = "dd/mm/yy"
                Columns("E:E").Select
                Selection.NumberFormat = "hh:mm"
              numreadings% = 0
              'count how many readings have been taken
              For l% = 1 To 10000
              If Sheets(House & "T" & j% & i%).Range("A" & 34 + l%).Value = ""
Then
              'there are no more readings
                Exit For
              Else
              'count the number of readings
                numreadings% = numreadings% + 1
              End If
              Next l%
              Sheets(House & "T" & j% & i%).Select
                For k% = 1 To numreadings%
                      Range("D" & k% + 34).Select
```

```
                ActiveCell.FormulaR1C1 = "=RC[-3]"
                Range("E" & k% + 34).Select
                ActiveCell.FormulaR1C1 = "=RC[-4]"
            Next k%
        'now caclulate the  Mixing Ratio
        pressure = 101.3
        For k% = 0 To (numreadings% - 1)
            Workbooks(House & ".xls").Sheets(House & "T" & j% & i%).Activate
            temp = Cells(k% + 35, 2).Value
            Workbooks(House & ".xls").Sheets(House & "H" & j% & i%).Activate
            humidity = Cells(k% + 35, 2).Value
            humidity = humidity / 100
            sp = 610.78 * 2.718282 ^ ((temp / (temp + 238.3)) * 17.2694)
            awvp = (sp / 1000) * humidity
            sh = 0.62197 * (awvp / (pressure + (awvp * (0.62197 - 1))))
            'write the  Mixing Ratio out to sheet 2
            Workbooks(House & ".xls").Sheets("Sheet2").Activate
            Cells(k% + 1, (i% - 1) * 7 + (j% + i%)).Value = sh * 1000
            Columns("A:Z").NumberFormat = "0.00"
            Columns("A:A").NumberFormat = "dd/mm/yy"
            Columns("I:I").NumberFormat = "dd/mm/yy"
            Columns("Q:Q").NumberFormat = "dd/mm/yy"
        Next k%
        'draw the chart to go with it
        'copy the axis values first
            Sheets(House & "T" & j% & i%).Select
            Range("A35:A" & numreadings% + 35).Select
            Selection.Copy
            Sheets("Sheet2").Select
            Range("A1").Select
            Cells(1, (i% - 1) * 7 + i%).Select
            ActiveSheet.Paste
            Columns("A:A").ColumnWidth = 15#
            Columns("I:I").ColumnWidth = 15#
            Columns("Q:Q").ColumnWidth = 15#
            Columns("A:A").NumberFormat = "dd/mm/yy"
            Columns("I:I").NumberFormat = "dd/mm/yy"
            Columns("Q:Q").NumberFormat = "dd/mm/yy"
        'draw the chart for  Mixing Ratio
            ActiveWindow.Zoom = 100
            Charts.Add
            ActiveChart.ChartType = xlLine
            ActiveChart.SetSourceData Source:=Sheets("Sheet2").Range("E30")
            ActiveChart.SeriesCollection.NewSeries
            ActiveChart.SeriesCollection(1).XValues = "=Sheet2!R1C" & ((i% - 1) *
7 + i%) & ":R" & (numreadings%) & "C" & ((i% - 1) * 7 + i%)
            ActiveChart.SeriesCollection(1).Values = "=Sheet2!R1C" & ((i% - 1) * 7
+ (i% + j%)) & ":R" & (numreadings%) & "C" & ((i% - 1) * 7 + (i% + j%))
            ActiveChart.SeriesCollection(1).Name = "="" Mixing Ratio""""
            ActiveChart.Location Where:=xlLocationAsObject, Name:="Sheet2"
            With ActiveChart
                .HasTitle = True
```

```
                .ChartTitle.Characters.Text = "House " & House & " Room " & j% & "
visit " & i%
                .Axes(xlCategory, xlPrimary).HasTitle = False
                .Axes(xlValue, xlPrimary).HasTitle = False
            End With
            ActiveChart.Axes(xlCategory,        xlPrimary).CategoryType        =
xlCategoryScale
        'scale the chart and move it
            'size the window
            ActiveWindow.Visible = False
            With ActiveChart.Legend
                .Left = 279
                .Top = 1
            End With
            With ActiveChart.PlotArea
                .Left = 1
                .Top = 33
                .Width = 338
                .Height = 155
            End With
            With ActiveChart.Axes(xlCategory)
                .CrossesAt = 1
                .TickLabelSpacing = 48
                .TickMarkSpacing = 24
                .AxisBetweenCategories = True
                .ReversePlotOrder = False
'               .Font = 10
            End With
            With ActiveChart.PlotArea.Border
                .ColorIndex = 16
                .Weight = xlThin
                .LineStyle = xlContinuous
            End With
            With ActiveChart.PlotArea
                .Interior.ColorIndex = xlNone
            End With
            With Selection.Interior
                .ColorIndex = 2
                .PatternColorIndex = 1
                .Pattern = xlSolid
            End With
            With ActiveChart.Legend
                .Delete
            End With
            With ActiveChart.Axes(xlValue)
                .TickLabels.NumberFormat = "0.000"
            End With
            With ActiveChart
                .Axes(xlValue, xlPrimary).HasTitle = True
                .Axes(xlValue, xlPrimary).AxisTitle.Characters.Text = " Mixing Ratio
(g/kg)"
            End With
```

```
            With ActiveChart
               .Axes(xlValue).TickLabels.NumberFormat = "0.0"
            End With
            'move it to the correct location
            Selection.Name = "Chart " & j% + ((i% - 1) * 6)
            With ActiveChart.ChartArea
               ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).IncrementLeft -
191.25
               ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).IncrementTop -
98.25
               ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).ScaleWidth 1.04,
msoFalse, msoScaleFromTopLeft
               ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).ScaleHeight 1.32,
msoFalse, msoScaleFromTopLeft
            End With
            With ActiveChart.ChartArea
               ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).IncrementLeft
(384.75 * (j% - 1))
               ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).IncrementTop
(260.25 * (i% - 1))
            End With
            ActiveWindow.Zoom = 50
          'draw the temperature and humidity chart
            Sheets("sheet1").Select
            ActiveWindow.Zoom = 100
            Charts.Add
            ActiveChart.ChartType = xlLine
            ActiveChart.SeriesCollection.NewSeries
            ActiveChart.SeriesCollection.NewSeries
            ActiveChart.SeriesCollection(1).XValues = "=" & House & "H" & j% &
i% & "!R35C4:R" & (numreadings% + 1) & "C4"
            ActiveChart.SeriesCollection(1).Values = "=" & House & "H" & j% & i%
& "!R35C2:R" & (numreadings% + 1) & "C2"
            ActiveChart.SeriesCollection(1).Name = "=""Humidity"""
            ActiveChart.SeriesCollection(2).Values = "=" & House & "T" & j% & i%
& "!R35C2:R" & (numreadings% + 1) & "C2"
            ActiveChart.SeriesCollection(2).Name = "=""Temperature"""
            ActiveChart.Location Where:=xlLocationAsObject, Name:="Sheet1"
            With ActiveChart.Axes(xlCategory)
               .HasMajorGridlines = False
               .HasMinorGridlines = False
            End With
            With ActiveChart.Axes(xlValue)
               .HasMajorGridlines = False
               .HasMinorGridlines = False
            End With
            'make it a chart with two y axes
            ActiveChart.Axes(xlCategory,          xlPrimary).CategoryType          =
xlCategoryScale
            ActiveChart.ApplyCustomType ChartType:=xlBuiltIn, TypeName:= _
               "Lines on 2 Axes"
            ActiveChart.SeriesCollection(2).Select
```

```
With Selection.Border
    .Weight = xlThin
    .LineStyle = xlAutomatic
End With
'remove the grey background to white
ActiveChart.SeriesCollection(1).Select
With Selection.Border
    .Weight = xlThin
    .LineStyle = xlAutomatic
End With
With Selection
    .MarkerBackgroundColorIndex = xlAutomatic
    .MarkerForegroundColorIndex = xlAutomatic
    .MarkerStyle = xlNone
    .Smooth = False
    .MarkerSize = 5
    .Shadow = False
End With
ActiveChart.SeriesCollection(2).Select
With Selection.Border
    .Weight = xlThin
    .LineStyle = xlAutomatic
End With
With Selection
    .MarkerBackgroundColorIndex = xlAutomatic
    .MarkerForegroundColorIndex = xlAutomatic
    .MarkerStyle = xlNone
    .Smooth = False
    .MarkerSize = 5
    .Shadow = False
End With
'scale the axes
ActiveChart.Axes(xlValue).Select
With ActiveChart.Axes(xlValue)
    .MinimumScale = 30
    .MaximumScale = 100
    .MinorUnitIsAuto = True
    .MajorUnitIsAuto = True
    .Crosses = xlAutomatic
    .ReversePlotOrder = False
    .ScaleType = xlLinear
End With
ActiveChart.Axes(xlValue, xlSecondary).Select
With ActiveChart.Axes(xlValue, xlSecondary)
    .MinimumScale = 10
    .MaximumScale = 30
    .MinorUnitIsAuto = True
    .MajorUnitIsAuto = True
    .Crosses = xlAutomatic
    .ReversePlotOrder = False
    .ScaleType = xlLinear
End With
```

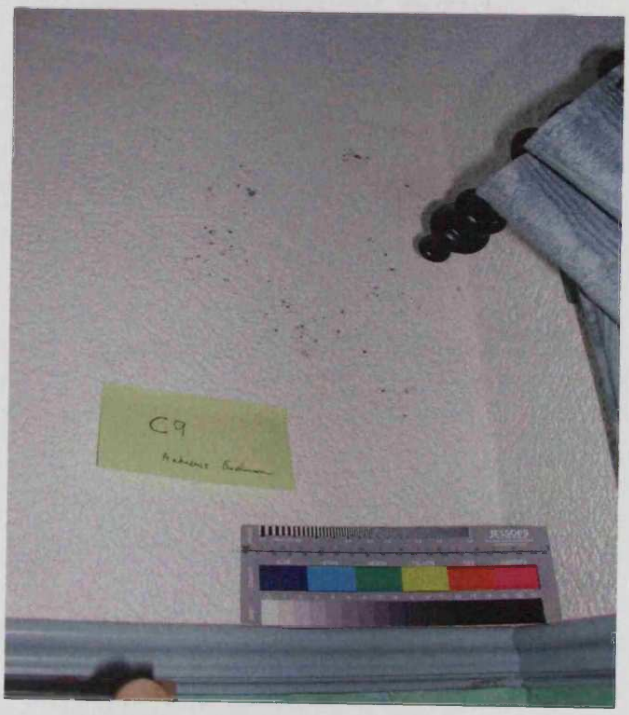
```
            'change the titles
            With ActiveChart
               .HasTitle = True
               .ChartTitle.Characters.Text = "House " & House & " Room " & j% & "
Visit " & i%
               .Axes(xlCategory, xlPrimary).HasTitle = True
               .Axes(xlCategory, xlPrimary).AxisTitle.Characters.Text = "Date"
            End With
            ActiveChart.PlotArea.Select
            With Selection.Border
               .ColorIndex = 16
               .Weight = xlThin
               .LineStyle = xlContinuous
            End With
            With Selection.Interior
               .ColorIndex = 2
               .PatternColorIndex = 1
               .Pattern = xlSolid
            End With
            'change the x axis scale
            With ActiveChart.Axes(xlCategory)
               .CrossesAt = 1
               .TickLabelSpacing = 48
               .TickMarkSpacing = 24
               .AxisBetweenCategories = True
               .ReversePlotOrder = False
            End With
            'add axes labels
            With ActiveChart
               .Axes(xlValue, xlPrimary).HasTitle = True
               .Axes(xlValue,   xlPrimary).AxisTitle.Characters.Text   =   "Relative
Humidity"
               .Axes(xlValue, xlSecondary).HasTitle = True
               .Axes(xlValue,         xlSecondary).AxisTitle.Characters.Text         =
"Temperature"
            End With
            'size the window
            ActiveWindow.Visible = False
            With ActiveChart.Legend
               .Left = 279
               .Top = 1
            End With
            With ActiveChart.PlotArea
               .Left = 1
               .Top = 33
               .Width = 338
               .Height = 155
            End With
            'move it to the correct location
            Selection.Name = "Chart " & j% + ((i% - 1) * 6)
            With ActiveChart.ChartArea
```

```
                ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).IncrementLeft -
191.25
                ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).IncrementTop -
98.25
                ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).ScaleWidth 1.04,
msoFalse, msoScaleFromTopLeft
                ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).ScaleHeight 1.32,
msoFalse, msoScaleFromTopLeft
              End With
              With ActiveChart.Axes(xlCategory).AxisTitle
                .Left = 335
                .Top = 252
              End With
              With ActiveChart.ChartArea
                ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).IncrementLeft
(384.75 * (j% - 1))
                ActiveSheet.Shapes("Chart " & j% + ((i% - 1) * 6)).IncrementTop
(260.25 * (i% - 1))
              End With
              ActiveWindow.Zoom = 50
             'end the creating temperature sheet
              End If
            'end the temperature file
            End If
          End With
        Next j%
      Next i%

'save the workbook with changes
ActiveWorkbook.Save
ActiveWorkbook.Close

'end the house loop
Next housenum%

End Sub
```

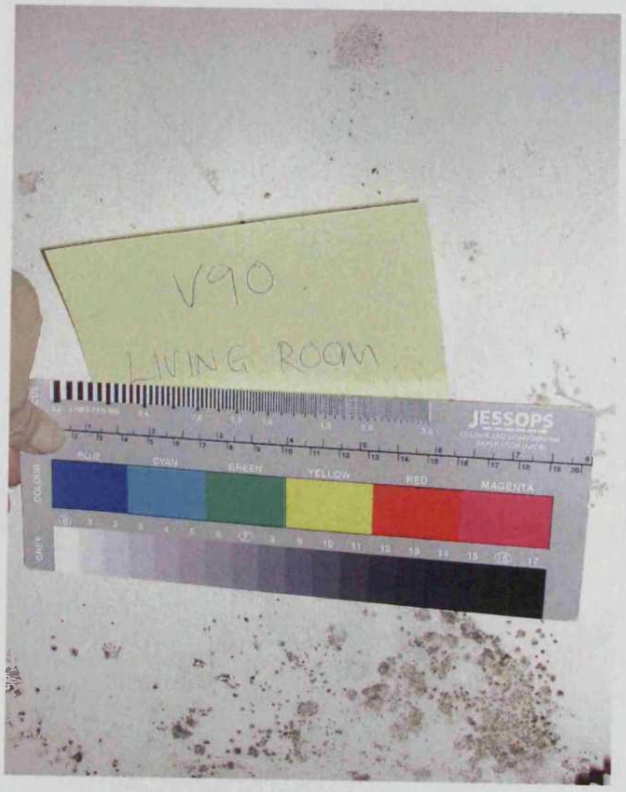
Appendix 3

Examples of the six mould density photographs.

Trace

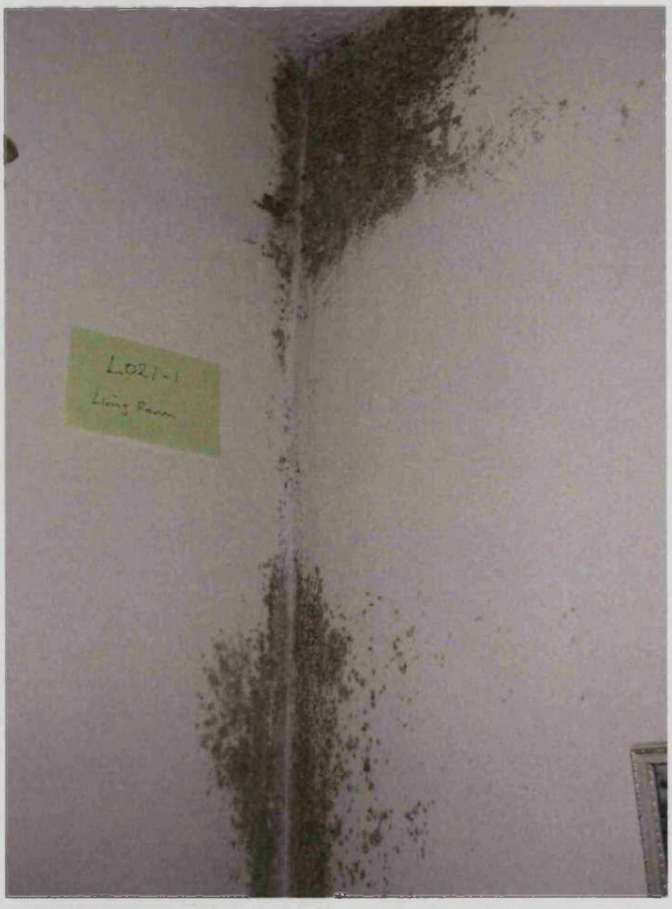


Spotty

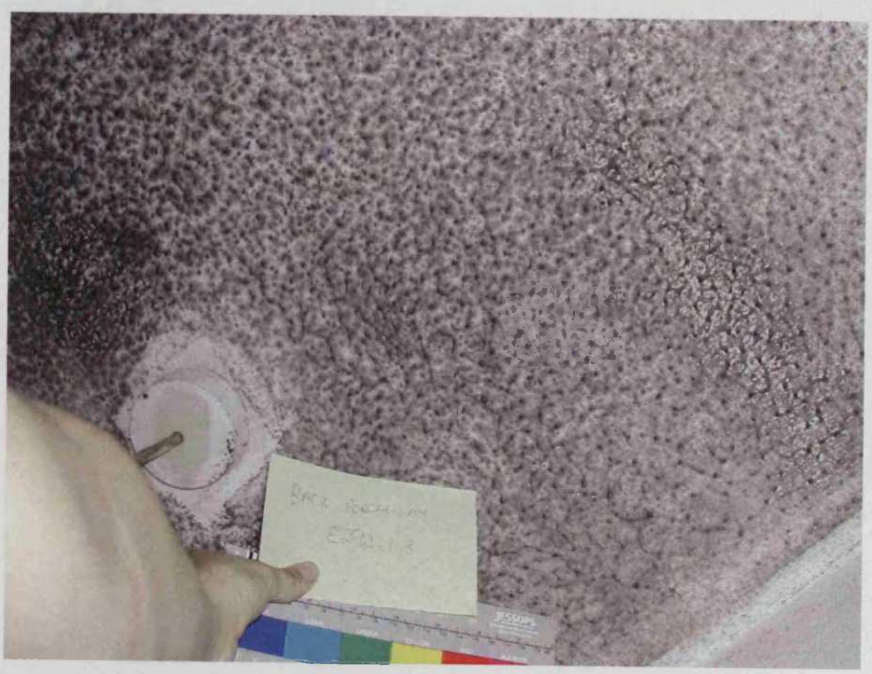


Obvious and discrete

Obvious and patchy



Obvious and widespread



Appendix 4

Asthma and Mould in the Home

Adult Consent Form

Name:.....

Address:.....

I have read the information sheet about the Asthma and Indoor Mould Study.
I understand that my participation is entirely voluntary and that I may decline to take part in any aspect of the study or withdraw from it at any time without my decision affecting my current or future care.

I understand that all information will be treated in strict confidence.

I agree to the following procedures: (delete any that you do not agree to)

- Answering a questionnaire;
- Skin prick tests to see what I am allergic to;
- Peak flow breathing tests;
- The removal of mould;
- The siting of an air pump in my home for 24hrs to measure mould spores in the air;
- The placing of two instruments (TV remote-control size) in my home for two weeks to measure humidity and temperature.

If my home is randomly chosen for treatment of the conditions causing mould then I agree that a fan may be fitted free of charge in my home to improve ventilation.

Signed:.....

Dated:.....

Asthma and Mould in the Home

Consent Form for Children

Name:.....

Address:.....

I have read the information sheet about the Asthma and Indoor Mould Study.
I understand that it is entirely up to me whether I want to take part in this study or not. If I do decide to take part then I can still change my mind at any time and stop taking part without affecting my current or future care.

I agree to the following things I have ticked:

- Answer some questions on a form about my asthma symptoms ☐
- Let a person from the hospital test the skin on the back of my arm by giving it a tiny scratch to see if I am allergic to moulds or house dust mites ☐
- Breath into a peak flow meter on a number of occasions to get a measurement of how fast I can breath out ☐
- Let somebody from the study remove any mould in the home and put a box in the home for 24 hours to measure any mould in the air ☐
- Have two instruments (the size of a TV remote control) in my home for two weeks to measure temperature and dampness in the air ☐

Signed:.....

Dated:.....

«Title» «Forename» «Surname»
«house_number» «subnumber» «street»
«area»
«town»
«postcode»

Date as postmark
«ID»

Dear «Title» «Surname»

RE: HOUSING AND HEALTH

With the aid of a grant from the National Asthma Campaign, some Colleagues from the University of Wales College of Medicine are looking into possible effects of housing conditions on breathing problems including asthma. We in this practice would like our asthmatic patients to take part in this study and I am therefore asking if you would complete the enclosed questionnaire and send it back in the envelope provided.

Your decision whether to take part or not is entirely up to you and will not effect your treatment in any way, but we believe this is a worthwhile study and we hope that as many as possible will fill in this questionnaire.

In some cases a research worker from the College of Medicine would like to visit the house to obtain more details. Please indicate on the questionnaire whether you would be willing to have such a visit.

If you have any queries you can telephone the Research Officer on 02920 742435.

Yours sincerely,

«Title» «Initial» «Surname»
«HOUSENO» «STREET1»
«STREET2»
«TOWN»
«POSTCODE»

Date as postmark

«ID»

Dear «Title» «Surname»

There is growing evidence to suggest that housing interiors can affect asthma sufferers. The HANAH team has recently obtained funding from the National Asthma Campaign to carry out surveys of condensation in homes of asthmatics. The results of these surveys will suggest ways of treating condensation problems to reduce moulds and thereby reduce symptoms.

The HANAH team is currently looking for households to take part in the study. This will involve the siting of an air sampler in your home for 24 hours to measure mould and placing two instruments (the size of a TV remote control) in your home for a week to measure the temperature and water content of the air.

We are asking you to please complete the enclosed short questionnaire and return it to the College of Medicine in the pre-paid envelope provided.

If you have any queries or require further information about the project please ring Truda Bell on 029 2074 2435.

Thank you for your help.

Prof. Ian Matthews

'HANAH' Green Card

«ID»
«number» «street»
«area»
«town»
«postcode»

Please answer the following questions by ticking ONE box on each line.
If the person affected is a child, please complete the questionnaire on the child's behalf.
Your answers will be treated as confidential by the research team.

In the past 12 months has anyone in your house had wheezing, whistling in the chest, or asthma?	Yes ☐ No ☐
In the past 12 months have you seen any damp patches on the walls of any room in your house?	Yes ☐ No ☐
Do you currently have any mould, mildew or black growth (often refered to as condensation or damp) on any surfaces (other than food) in your house?	Yes ☐ No ☐
Which fuel is used for heating your house? Do you have central heating?	electricity ☐ gas ☐ oil ☐ other ☐ Yes ☐ No ☐
I am willing to take part in the study and for a researcher from the University of Wales College of Medicine to visit my home to obtain more details.	Yes ☐ No ☐
Please provide us with your telephone number:	☎----------

Please give the names and dates of birth of all household members who have had wheezing, whistling of the chest or asthma in the past 12 months.

Name	Date of Birth

Please tick a box to show when it would be most convenient for a researcher to contact you

	Weekday Morning	Weekday Afternoon	Weekday Evening	Weekends	Anytime
The best time to contact me is:					

Please post the questionnaire in the pre-paid envelope provided.
Thank you for your time.

«Title» «Forename» «Surname»
«number» «street»
«area»
«town»
«postcode»

Date as postmark

Dear «Title» «Surname»

Thank you for taking part in the HANAH study which is looking into the effects of housing interiors on asthma sufferers.

Hannah Davies or Rob Arthur called on you recently to conduct some tests. Your household has been randomised to the intervention arm of the study which means that you will be receiving the intervention fan and mould eradication. We will contact you again when you have completed your peak flow diary and make arrangements to carry out the mould eradication.

If you have any queries or require further information about the project please ring Rob Arthur or Hannah Davies on ☎ 029 2074 2831

Thank you for your help.

«Title» «Forename» «Surname»
«number» «street»
«area»
«town»
«postcode»

Date as postmark

Dear «Title» «Surname»

Thank you for taking part in the HANAH study which is looking into the effects of housing interiors on asthma sufferers.

Hannah Davies or Rob Arthur called on you recently to conduct some tests. As you are aware this is a randomised controlled trial where half of the properties visited will be randomised to receive an intervention aimed at improving the ventilation within the household. At the end of the study the two halves will be compared

Your household has been randomised to the control arm of the study which means that you will not be receiving the intervention at this time.

One of us will contact you in a months time to make arrangements for Rob or Hannah to visit and leave the pump with you once again to sample mould levels

If you have any queries or require further information about the project please ring Rob Arthur ☎ 029 2074 2831

Thank you for your help.

Dear

Thank you for all the help you gave us in our work on housing and asthma. The study has now finished and we can tell you what we have found.

It has often been noticed that people who have mould growing inside their homes seem to have more chest trouble than other people, but it wasn't clear whether the mould contributed to this. We wanted to find out whether getting rid of indoor mould leads to any improvement in chest trouble, particularly asthma. The charity Asthma UK (previously The National Asthma Campaign) gave us some money to look into this possibility.

We recruited 207 people with asthma whose homes contained some mould. In half of these homes we arranged for the mould to be removed and a fan to be placed in the loft if possible to improve ventilation. In the other half, no action was taken for a year. All of you were asked about your symptoms at intervals and you made measurements of your breathing.

On average, those of you whose homes the mould had been removed from showed improvement in chest symptoms in the first 6 months and a reduction in your use of anti-asthma medication. By 12 months you also had less irritation of your noses and eyes. These improvements were not seen in the other group, so we think they were due to removal of the mould. We didn't find any obvious effect on your breathing measurements, but that may be because people with asthma use enough medication to keep their breathing reasonably normal.

We believe that these findings are important because they show how asthma can be improved by dealing with conditions inside the home, so that patients can reduce the amount of medication they use. We intend to put these results in a widely-read medical journal so that doctors all over the world can advise their asthmatic patients to get rid of any mould in their homes.

Thank you again for all your help in making this work possible. Those of you who put up with the mould during the study should have been given an anti-mould kit which we hope will have got rid of the problem in your homes too.

Please contact us if you would like further details.

Yours sincerely,

Professor Ian Matthews

Date _____ PID _____

Peak Flow Diary Card

Name	**What is your height?**

Month and Year

Date																					
Day	Sun	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat
Peak Flow Rates l/min																					
After getting up 1																					
2																					
3																					
Late Evening 1																					
2																					
3																					
Symptoms																					
Cold																					
Cough																					
Wheeze																					

Peak flow measurement

Please measure your peak flow rate for two weeks on getting up every morning before taking any medication, and again just before you go to bed.

How to measure your peak flow rate

Set marker to zero.
Stand, holding the meter horizontally in front of mouth.
Breathe in as fully as possible.
Put your lips firmly around the mouthpiece.
Breathe out as hard and as fast as possible.
Write the reading shown by the marker.
Repeat this sequence twice more.

Symptoms

If you have a cold, cough or a wheeze in your chest on any day, please put a tick in the appropriate symptom box.

Appendix 5

Date _____ PID _____

Mould and Asthma Site Sheet

Initial site visit	House type *Semi detached, flat, mid terrace, bung*		Intervention: Yes / No
Surveyor Name			
Occupant details	Administer the Consent Form		
Name(s)			
Address			
Postcode			
Telephone number			
How many smokers are there in the house?		Tenure? Private/Council home	

Rate your house on the following	Very poor				Excellent
Tick one box only	1	2	3	4	5
Heating costs	High				Low
The quality of the air indoors					
The uniformity of comfort throughout the house					
Overall					

How often are these a problem	Never				Very often
Tick one box only	1	2	3	4	5
House is too hot in summer					
House is too cold in summer					
House is too hot in winter					
House is too cold in winter					
Stuffiness					
Draughts					
Humidity (dose)					
Are the walls damp					
Condensation on walls or windows					
Noise					

Comments

Do you use a dehumidifier?	Yes	No	**Is** any dust mite protection used?	Yes No
What is the age of your mattress? (years)	_____			
How often do you vacuum your mattress?				

Questions for adults if lived elsewhere			
Where did you grow up?	Urban (city)		Pen & paper
	Suburban		Peak flow meters and log sheet
	Rural		Asthma questionnaires
Type of house?			RH & temperature loggers -- set
			Sample bags and swabs for mould
			Camera
Any history of asthma in the family?	Yes	No	Labels
Relationship?			Logbook
			Tape measure / ultrasonic tape
Any recollection of mould/condensation?	Yes	No	Consent Forms
			Toolbox

Date _____ PID _____

House Details

House type *Semi detached, flat, mid-terrace, bung*			Number of storeys	Storey height (m)	
Rooms with mould *indicate all rooms, severity % cover*					
Window type *single or double glazed – vented*				Front window area (m)	
Heating system *Gas, elec, wood, solid*	Type		How old is the system	When is the system on	
	How many rooms heated		What is the room stat temp		
Number of mould samples			Comments		
Carpet in living room?		Cleaning interval?		Last Cleaned?	
Carpet in bedroom?		Cleaning interval?		Last Cleaned?	
Ensure the carpets are not cleaned 24hours before second visit					
Bed clothes changing interval?					

Loggers

Position on room plan *Show power socket for pump*		Position on room plan *Show power socket for pump*	
Living space room plan		Bedroom room plan	
Logger Serial Number(s)		Logger Serial Number(s)	

Position on room plan *Show power socket for pump*		Position on room plan *Show power socket for pump*	
Bedroom room plan			
Logger Serial Number(s)		Logger Serial Number(s)	

Check list

Show occupant how to use peak flow meters and how to log results	
Choose the bed to vacuum *(Bed of the worst asthma victim)*	
Find power for vacuum cleaner in bedroom	
Find mould in house	
Photograph mould and use grey scale in photo	
Dry swab sample of mould into bag and label	
Select area to shield with 30cm square plastic sheet *select type of fixing tape to be used*	
Administer the asthma questionnaires *label them for later identification*	

Date _____ PID _____

Second day, 1st visit

Surveyor Name	

Mould sampler

Set up mould sample pump, tube on tripod and mark position on plan		Sample rate 12L/min	
Room plan -- Visit 1	Pump number	Room plan -- Revisit 1	Pump number
Pump run time	Finished sample rate	Pump run time	Finished sample rate

Checklist

Vacuum the bed and floor using 1m square jig for 2 minutes in diagonal pattern, bag the bags and label.	
Tape 30cm square sheet of plastic on to wall where the mould occurs	
Ventilation state *doors/windows open or fans on*	
Outside air temperature	
Wind speed	
Wind Direction	
Description of weather conditions	

Comments	Things to take
	Set Loggers for the day Pen & paper 30cm square plastic sheet and mounting tape Mould sampling Pumps and tubes Tripod Polycarbonate filters -- loaded Sample bags and swabs for mould Vacuum cleaner cleaner bags sample bags 1 m square jig Stop watch Labels sharps box Logbook Tape measure / ultrasonic tape Toolbox

Date _____ PID _____

Removal Checklist					
Disconnect the mould sampler, remove sample place in tin, label including date.					
Protimeter readings			Reading 1	Reading 2	Reading 3
Calibrate before each set of readings	Location				
	Location				
Logger name convention			1- Living room	2 - Kitchen	3 - Main Bed
			4 - Bed 2	5 - Bed 3	6 - Bed 4/Attic
i e. GN807H21 – GN807, PID code; H - humidity or T - temperature; 2 - room number - kitchen; 1 - visit number, first site visit.			7 - Other	8 - Basement	

Do Allergen prick test, dispose of equipment in sharps box	Size mm	Size mm	Size mm
Asthmatic's name			
A (Negative Control)			
B (Penicillium Notatum)			
C (Cat Fur)			
D (Der Pteronyssinus)			
E (Cladosporium cladosporiodes)			
F (Alternaria Alternata)			
G (Grass Pollens)			
H (Aspergillus Fumigatus)			
I (Histamine–Positive Control)			
No. of people in the bed			
Height of asthmatic (m)			

Things to take

Set Loggers for the day
Pen & paper
Sample tin for mould
Labels
Logbook
Protimeter
Tape measure / ultrasonic tape
Prick test kit -- samples, lancets and **scale**
sharps box
Toolbox

Date _____ PID _____

12 Month Mould and Asthma Site Sheet

Third site visit	House type *Semi detached, flat, mid-terrace, bung*	Intervention: Yes / No
Surveyor Name		
Occupant details	**Number of occupants**	
Name(s)		
Address		
Postcode		
Telephone number		
How many smokers are there in the house?	Tenure? Private/Council home	

Rate your house on the following	Very poor				Excellent
Tick one box only	1	2	3	4	5
Heating costs	High				Low
The quality of the air indoors					
The uniformity of comfort throughout the house					
Overall					

How often are these a problem	Never				Very often
Tick one box only	1	2	3	4	5
House is too hot in summer					
House is too cold in summer					
House is too hot in winter					
House is too cold in winter					
Stuffiness					
Draughts					
Humidity (dose)					
Are the walls damp					
Condensation on walls or windows					
Noise					
How often do you use a dehumidifier?					

Questions for adults if lived elsewhere			
Where did you grow up?	Urban (city)		
	Suburban		
	Rural		
Type of house?			
Any history of asthma in the family?		**Y**	**N**
Relationship?			
Type of heating system used when a child?			
Any recollection of mould/condensation?		**Y**	**N**
Did anyone smoke in the home where you grew up?			
Second set on page 3			

- Pen & paper
- Peak flow meters and log sheet
- Asthma questionnaires
- RH & temperature loggers -- set
- Sample bags and swabs for mould
- Camera
- Labels
- Logbook
- Tape measure / ultrasonic tape
- Consent Forms
- Toolbox

Date _____ PID _____

House Details

Where do you dry your clothes? Give summer/winter values if differ	On radiators or clothes horse		Rate from 1 to 3 in order as necessary with 1 being the most used and 3 the least. Enter 0 for those not used.
	In a dryer		
	On the line		
Rooms with mould *indicate all rooms and severity*			
Window type & material *single or double glazed – vented*			
Heating system *Gas, elec, wood, solid*	Type	Have any changes been made in the last year?	

Carpet in living room?		Cleaning interval?		Last Cleaned?	
Carpet in bedroom?		Cleaning interval?		Last Cleaned?	

Ensure the carpets are not cleaned 24hours before second visit	
Bed clothes changing interval?	

Loggers

Position on room plan	Position on room plan
Living space room plan	Bedroom 3 plan
Logger Serial Number(s)	Logger Serial Number(s)

Position on room plan	Position on room plan
Bedroom 4 plan	
Logger Serial Number(s)	Logger Serial Number(s)

Show occupant how to use peak flow meters and how to log results	
Choose the bed to vacuum *(Bed of the worst asthma victim)*	
Find power for vacuum cleaner in bedroom	
Find mould in house	
Photograph mould and use grey scale in photo	
Dry swab sample of mould into bag and label	
Administer the asthma questionnaires *label them for later identification*	

Date _____ PID _____

Forth site visit

Surveyor Name	

Mould sampler

Set up mould sample pump, tube on tripod and mark position on plan		Sample rate 12L/min	
	Run time		Pump number

Questions for second adult if lived elsewhere			Finished sample rate
Where did you grow up?	Urban (city) Suburban Rural		Sketch location
Type of house?			
Any history of asthma in the family?	Y	N	
Relationship?			
Type of heating system used when a child?			
Any recollection of mould/condensation?	Y	N	
Did anyone smoke in the home where you grew up?			

Number of mould samples	

Checklist

Vacuum the bed and floor using 1m square jig for 2 minutes in diagonal pattern, bag the bags and label.	
Tape 30cm square sheet of plastic on to wall where the mould occurs	
Ventilation state *doors/windows open or fans on*	
Outside air temperature	
Wind speed	
Wind Direction	
Description of weather conditions	

Note Infra-Red temperature readings from internal and external walls of mouldy rooms.

Room	Temp. °C Int	External

Comments

Date _____ PID _____

Asthmatic's Mattress	Name		Name		Name	
Do you use a mattress cover?	Y	N	Y	N	Y	N
Do you encase the mattress?	Y	N	Y	N	Y	N
How often do you vacuum the bed?						
Height of asthmatic (m)						
Employment	Name		Name		Name	
Occupation?						
Have you been employed in a job for 3 continuous months or longer since the last survey?	Y	N	Y	N	Y	N
Is there smoking where you work?	Y	N	Y	N	Y	N
Have any of your jobs ever made your chest tight or wheezy?	Y	N	Y	N	Y	N
Which ones?						

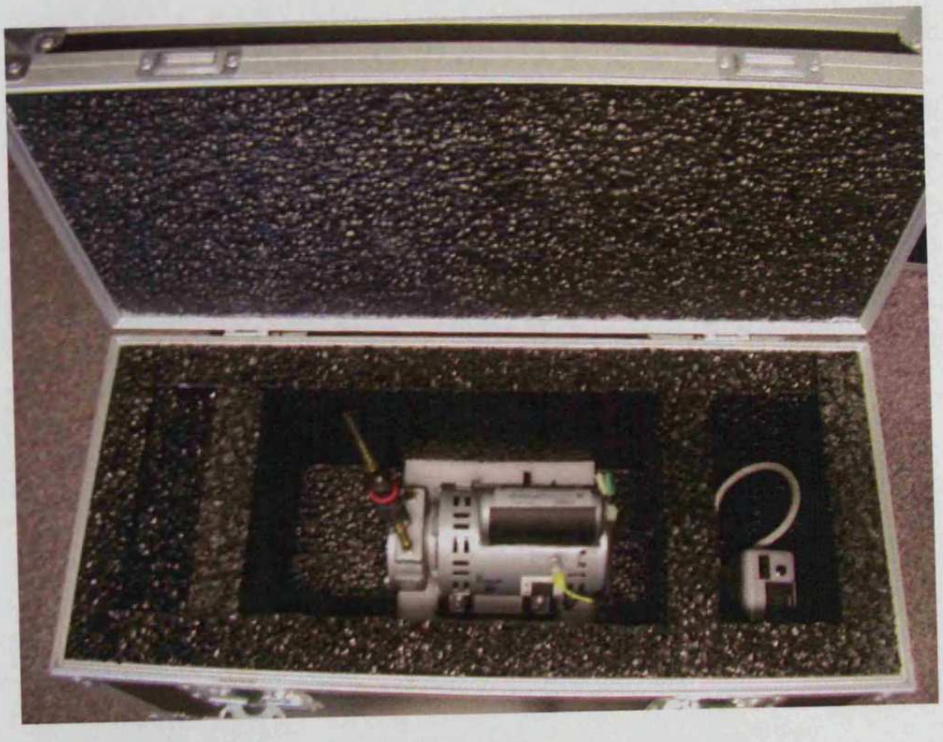
Please rate the following from:	Never		to			Very often
Circle one number only in each box	1		2	3	4	5
Name						
Does your workplace affect your asthma?	1 2 3 4 5		1 2 3 4 5		1 2 3 4 5	
How often are you annoyed by outdoor air pollution if you keep the windows open?	1 2 3 4 5		1 2 3 4 5		1 2 3 4 5	
How often have you been exposed to tobacco smoke regularly in the past 12 months? i.e. pubs, work	1 2 3 4 5		1 2 3 4 5		1 2 3 4 5	
Have you used anti-dust mite sprays on the furniture, carpets or mattress?	1 2 3 4 5		1 2 3 4 5		1 2 3 4 5	
Note as to where						

Logger name convention	1- Living room	2 - Kitchen	3 - Main Bed
	4 - Bed 2	5 - Bed 3	6 - Bed 4/Attic
i.e. GN807H21 – GN807, PID code; H - humidity or T - temperature;	7 - Other		
2 - room number - kitchen; 1 - visit number, first site visit.			

Removal Checklist				
Disconnect the mould sampler, remove sample place in tin, label including date.				
Protimeter readings		Reading 1	Reading 2	Reading 3
Calibrate before each set of readings	Location			
Number of dust mite samples taken	Location			
	Location			

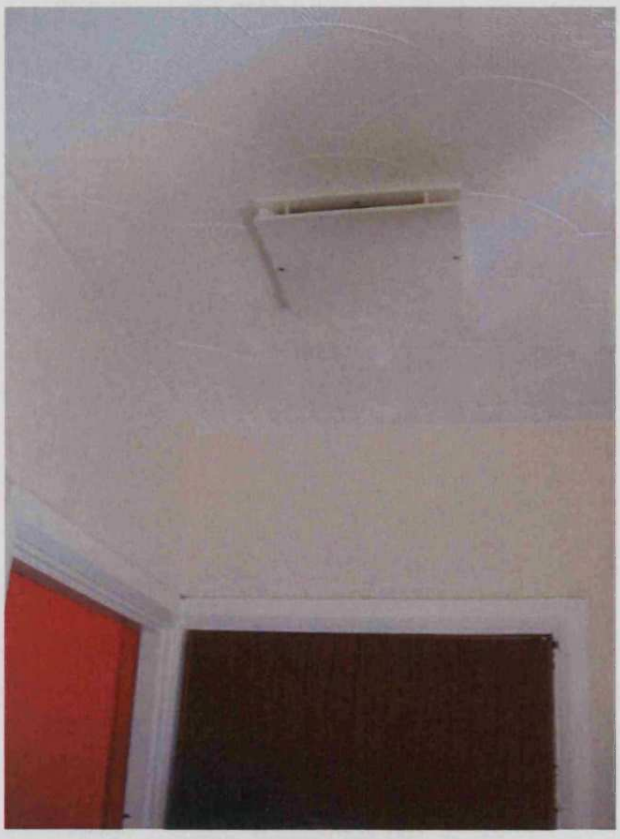
Appendix 6

Photos of the Gast pump and soundproof box used for collecting air samples.



Positive Input Ventilation Unit. A typical attic installation.

Outlet usually located in the hall ceiling.



Appendix 7

Confidence Intervals for the difference between net differences

		Probability	No. Observations
3 outcomes -	Better	P_1	$\frac{X_1}{n}$
	Same	P_2	$\frac{X_2}{n}$
	Worse	$P_3 = 1 - P_1 - P_2$	$\frac{X_3}{n}$

Confidence interval for $P_1 - P_3$ estimated by $\frac{X_1 - X_3}{n}$

X_1, X_3 dependent

$$\text{Var}(X_1 - X_3) = \text{Var}(X_1) + \text{Var}(X_3) - 2\text{Covar}(X_1, X_3)$$

$$= np_1(1-p_1) + np_3(1-p_3) + 2np_1p_3$$

$$\text{So Var}(\hat{p}_1 - \hat{p}_3) = \frac{p_1(1-p_1) + p_3(1-p_3) + 2p_1p_3}{n} = \frac{\sigma_1^2}{n_1}$$

The following assumption is the made:

$$\theta = \hat{p}_1 - \hat{p}_3 \sim N(p_1 - p_3, \frac{\sigma^2}{n})$$

Then a CI is $\hat{p}_1 - \hat{p}_3 \pm 1.96 \frac{\sigma^2}{n}$

To estimate σ replace p_1p_3 by $\hat{p}_1\hat{p}_3$

Then standard methods are used for combining the standard errors and thus obtain the confidence intervals:-

$$\theta_1 - \theta_2 \pm 1.96 \sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}$$

Appendix 8

Psychrometric Chart and description

It is possible to use only calculations and tables to relate the moisture content of air to the wet and dry bulb temperatures but it is easier to understand visually using the psychrometric chart, see below (giving the same information as the Mollier chart used in Europe). The following is a hypothetical example to show how the chart may be used:

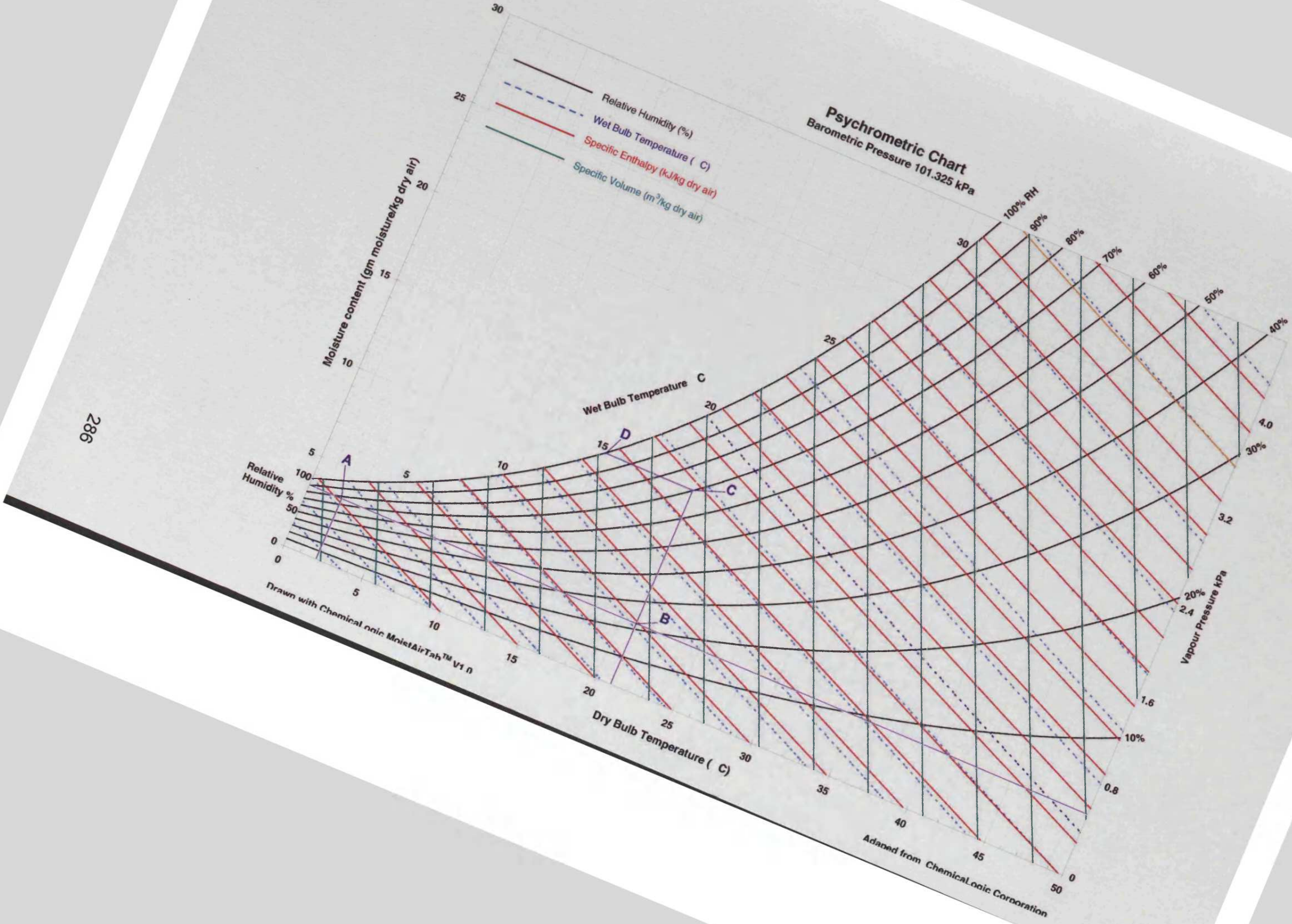
Point A on the chart refers to an air condition of 2°C and 78% RH, average winter conditions in Cardiff, Wales. Reading horizontally to the left, the moisture content shows that the air contains 3.5 grams of moisture per kilogram of air and to the right, the vapour pressure is ~0.55 kPa. If the outside air is drawn into the house and heated to 21°C without the addition of more moisture then the relative humidity would drop to 22%, point B, the moisture content and pressure values would remain the same.

Point C indicates the condition when the air is still at 21°C but the air has absorbed moisture from the home through cooking, bathing, breathing etc. The vapour pressure increases to 1.76 kPa, the moisture content is now 11g/kg and the relative humidity has risen to 70%, a condition suitable for moulds to begin growing.

Reading horizontally to the left from point C to point D shows when saturation will occur, this is the dewpoint. Point D represents the point when the water vapour has cooled to the point that condensation occurs, the temperature is 15.4°C.

Psychrometric Chart

Barometric Pressure 101.325 kPa



Appendix 9

Procedure for mounting slides for the Hirst Spore Trap

Glycerine Jelly Recipe

Gelatine	1g
Glycerol	7g
Water	6ml

With the addition of 1% phenol as a preservative

From: Plant Pathologist's Pocketbook
Commonwealth Agricultural Bureau, 1968.

Preparation of slides:

Glycerine jelly is dissolved in a water bath. A drop is placed onto the clean warmed glass microscope slide and quickly spread evenly over the surface of the slide. The slide is then heated to allow the glycerine jelly to spread into a smooth surface.

Mounting of slides:

A few drops of glycerine jelly are spread onto a cover slip of appropriate size and the slide is lowered on to the cover slip. The whole is turned over and warmed to allow the glycerine jelly to melt sufficiently for the mounting material to spread onto the cover slip. As the adhesive surface on the slide and the mounting material are the same they have the same refractive index.

Appendix 10

P-values.

Results of comparisons between mould quantities and the data recorded from the RCT site sheets, as p values from Chi-square test.

	mould density	mould area	visual mould
3 band occupants	0.699	0.021	0.005
Fish or not	0.739	0.088	0.034
Always open or sometimes open and closed windows	0.015	0.035	0.064
Fish	0.907	0.169	0.073
Cats	0.236	0.287	0.079
Openings	0.070	0.097	0.081
3 band dry on line	0.309	0.184	0.090
3 band openings	0.025	0.107	0.179
Dehumidifier use	0.295	0.308	0.199
House type	0.414	0.492	0.273
Glazing type	0.092	0.139	0.275
3 band tumble drying	0.183	0.926	0.388
Carpet in living room	0.164	0.228	0.406
Smokers or not	0.251	0.243	0.475
Cat or not	0.824	0.358	0.500
2 band heating system age	0.190	0.472	0.500
2 band window area	0.797	0.666	0.507
Smokers	0.669	0.437	0.543
Heating type	0.843	0.833	0.655
Tenure	0.615	0.591	0.727
Dog or not	0.511	0.328	0.733
2 band floor area	0.419	0.573	0.768
3 band drying on radiators	0.704	0.800	0.937
2 band storey height	0.040	0.908	0.943
Dogs	0.592	0.822	0.943
Window type	0.116	0.944	0.987

Values in yellow show significance at $p<0.05$ level.

Appendix 11

Table A11.1. Net improvement of symptoms in intervention group (without mould) and the control group when comparing baseline to 6 months results

	Intervention group (without mould)					Control group					
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)
(2) Have you had wheezing or whistling in the chest in the last 4 weeks?	36	10	4	22	16.7	59	7	5	47	3.4	13.3 (-9.5,36.0)
(4) In the last 4 weeks, how often, on average, has your sleep been disturbed due to wheezing?	36	13	3	20	27.8	59	17	11	31	10.2	17.6 (-8.7, 44.0)
(5) In the last 4 weeks, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths?	34	13	3	20	29.4	59	7	3	49	6.8	22.6 (-0.6, 45.9)

Table A11.1. Net improvement of symptoms in intervention (without mould) and control groups at 6 months when compared to baseline results

	Intervention group (without mould)					Control group					
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)
(7) In the last 4 weeks, how much did this wheeze interfere with your daily activities?	36	17	2	17	41.7	59	14	11	34	5.1	36.6 (11.0, 62.1)
(8) In the last 4 weeks, have you had wheezing or whistling in the chest when you did not have a cold or flu?	35	15	2	18	37.1	58	14	4	41	17.2	19.9 (-3.9, 43.7)
(9) How is your breathing compared with how it was 6 months ago?	36	26	0	10	72.2	56	12	12	32	0	72.2 (49.7, 94.8)
(11b) In the last 6 months have you used your medication less, the same or more than/as previously?	34	24	2	8	64.7	57	7	17	23	-17.5	82.2 (56.7, 107.8)
(11c) In the last 4 weeks, have you taken any treatment for wheezing or asthma?	35	6	2	27	11.4	59	7	2	50	8.5	3.0 (-15.2, 21.2)

Table A11.1. Net improvement of symptoms in intervention (without mould) and control groups at 6 months when compared to baseline results

	Intervention group (without mould)					Control group					
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)
(13) During the last 4 weeks, have you had a cough on most days?	36	5	4	27	2.8	59	11	7	41	6.8	-4.0 (-25.5, 17.5)
(14) During the last 4 weeks, have you brought up phlegm from your chest on most days?	36	6	2	28	11.1	59	9	10	40	-1.7	12.8 (-8.0, 33.6)
(16) During the last 4 weeks, have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu? (Rhinitis)	34	10	3	21	20.6	59	9	6	44	5.1	15.5 (-7.9, 38.9)
(17) During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? (Rhinoconjunctivitis)	34	10	3	21	20.6	59	6	9	44	-5.1	25.7 (2.3, 49.1)

Table A11.1. Net improvement of symptoms in intervention (without mould) and control groups at 6 months when compared to baseline results

	Intervention group (without mould)					Control group					
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)
(18) During the last 4 weeks, how much did this nose problem interfere with your daily activities?	34	12	2	20	29.4	59	18	12	29	10.2	19.2 (-7.1, 45.5)
(20) In the last 4 weeks, have you taken any treatment for a runny or blocked nose problem or hay fever?	35	3	5	27	-5.7	57	9	4	44	8.8	-14.5 (-34.4, 5.4)
(22) In the past 4 weeks, have you taken any treatment for an itchy rash or eczema?	35	3	2	30	2.9	57	7	5	45	3.5	-0.7 (-17.9, 16.6)

Note: ***Questions 9 and 11 b above are a comparison between baseline and six months.***

*Difference = value in intervention group minus value in control group.

Table A11.2. Net improvement of symptoms in intervention group (with mould) and the control group when comparing baseline to 6 months results

	Intervention group (with mould)					Control group					
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)
(2) Have you had wheezing or whistling in the chest in the last 4 weeks?	29	3	3	23	0	59	7	5	47	3.4	-3.4 (-23.5, 16.8)
(4) In the last 4 weeks, how often, on average, has your sleep been disturbed due to wheezing?	29	10	3	16	24.1	59	17	11	31	10.2	14.0 (-14.6, 42.6)
(5) In the last 4 weeks, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths?	29	4	1	24	10.3	59	7	3	49	6.8	3.6 (-14.4, 21.5)

Table A11.2. Net improvement of symptoms in intervention (with mould) and control groups at 6 months when compared to baseline results

	Intervention group (with mould)					Control group					
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)
(7) In the last 4 weeks, how much did this wheeze interfere with your daily activities?	29	8	4	17	13.8	59	14	11	34	5.1	8.7 (-19.5, 36.9)
(8) In the last 4 weeks, have you had wheezing or whistling in the chest when you did not have a cold or flu?	29	7	2	20	17.2	58	14	4	41	17.2	0.0 (-23.6, 23.6)
(9) How is your breathing compared with how it was 6 months ago?	29	11	4	14	24.1	56	12	12	32	0	24.1 (-5.8, 54.1)
(11b) In the last 6 months have you used your medication less, the same or more than/as previously?	29	12	7	10	17.2	57	7	17	23	-17.5	34.8 (1.7, 67.8)
(11c) In the last 4 weeks, have you taken any treatment for wheezing or asthma?	29	3	0	26	10.3	59	7	2	50	8.5	1.9 (-12.9, 16.6)

Table A11.2. Net improvement of symptoms in intervention (with mould) and control groups at 6 months when compared to baseline results

	Intervention group (with mould)					Control group					
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)
(13) During the last 4 weeks, have you had a cough on most days?	29	6	3	20	10.3	59	11	7	41	6.8	3.6 (-20.8, 27.8)
(14) During the last 4 weeks, have you brought up phlegm from your chest on most days?	28	3	4	21	-3.6	59	9	10	40	-1.7	-1.8 (-24.7, 21.2)
(16) During the last 4 weeks, have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu? (Rhinitis)	29	5	4	20	3.4	59	9	6	44	5.1	-1.6 (-25.6, 22.3)
(17) During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? (Rhinoconjunctivitis)	29	5	5	19	0	59	6	9	44	-5.1	5.1 (-19.8, 30.0)

Table A11.2. Net improvement of symptoms in intervention (with mould) and control groups at 6 months when compared to baseline results

	Intervention group (with mould)					Control group					
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Difference in net % better* (95% CI)
(18) During the last 4 weeks, how much did this nose problem interfere with your daily activities?	29	4	8	17	-13.8	59	18	12	29	10.2	-24.0 (-53.1, 5.1)
(20) In the last 4 weeks, have you taken any treatment for a runny or blocked nose problem or hay fever?	29	1	4	24	-10.3	57	9	4	44	8.8	-19.1 (-38.2, -0.1)
(22) In the past 4 weeks, have you taken any treatment for an itchy rash or eczema?	29	4	2	23	6.9	57	7	5	45	3.5	3.4 (-16.8, 23.6)

Note: ***Questions 9 and 11 b above are a comparison between baseline and six months.***

**Difference = value in intervention group minus value in control group.*

Table A11.3. Net improvement of symptoms in intervention group (without mould) and the control group when comparing baseline to 12 months results

	Intervention group (without mould)					Control group					Difference in net % better * (95% CI)
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	
(2) Have you had wheezing or whistling in the chest in the last 4 weeks?	51	13	4	34	17.6	80	20	4	56	20.0	-2.4 (-21.1, 16.4)
(4) In the last 4 weeks, how often, on average, has your sleep been disturbed due to wheezing?	51	18	5	28	25.5	80	35	4	41	38.8	-13.3 (-34.5, 8.0)
(5) In the last 4 weeks, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths?	49	6	0	43	12.2	80	5	3	72	2.5	9.74 (-1.7, 21.2)

Table A11.3. Net improvement of symptoms in intervention (without mould) and control groups at 12 months when compared to baseline results

	Intervention group (without mould)					Control group					Difference in net % better * (95% CI)
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	
(7) In the last 4 weeks, how much did this wheeze interfere with your daily activities?	52	20	7	25	25.0	80	29	12	39	21.3	3.8 (-20.0, 27.5)
(8) In the last 4 weeks, have you had wheezing or whistling in the chest when you did not have a cold or flu?	52	16	3	33	25.0	80	20	4	56	20.0	5.0 (-13.7, 23.7)
(9) How is your breathing compared with how it was 12 months ago?	52	30	3	19	51.9	79	25	8	46	21.5	30.4 (-9.2, 51.6)
(11b) In the last 6 months have you used your medication less, the same or more than/as previously?	39	18	6	15	30.8	67	29	15	23	20.9	9.9 (-19.5, 39.3)
(11c) In the last 4 weeks, have you taken any treatment for wheezing or asthma?	51	14	4	33	19.6	80	8	4	68	5.0	14.6 (-2.9, 32.1)

Table A11.3. Net improvement of symptoms in intervention (without mould) and control groups at 12 months when compared to baseline results

	Intervention group (without mould)					Control group					Difference in net % better * (95% CI)
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	
(13) During the last 4 weeks, have you had a cough on most days?	52	5	9	38	-7.7	80	15	8	57	8.8	-16.4 (-34.6, 1.7)
(14) During the last 4 weeks, have you brought up phlegm from your chest on most days?	52	8	7	37	1.9	80	11	8	61	3.8	-1.8 (-19.9, 16.2)
(16) During the last 4 weeks, have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu? (Rhinitis)	52	17	5	30	23.1	80	7	11	62	-5.0	28.1 (8.6, 47.6)
(17) During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? (Rhinoconjunctivitis)	52	14	4	34	19.2	80	8	12	60	-5.0	24.2 (5.6, 42.9)

Table A11.3. Net improvement of symptoms in intervention (without mould) and control groups at 12 months when compared to baseline results

	Intervention group (without mould)					Control group					Difference in net % better * (95% CI)
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	
(18) During the last 4 weeks, how much did this nose problem interfere with your daily activities?	52	19	10	23	17.3	80	17	17	46	0	17.3 (-7.1, 41.7)
(20) In the last 4 weeks, have you taken any treatment for a runny or blocked nose problem or hay fever?	52	12	6	34	11.5	79	9	11	59	-2.5	14.1 (-5.1, 33.3)
(22) In the past 4 weeks, have you taken any treatment for an itchy rash or eczema?	52	5	4	43	1.9	79	20	4	55	20.3	-18.3 (-34.3, -2.3)

Note: ***Question 9 above is a comparison between baseline and 12 months.***

Question 11b above is a comparison between six months and 12 months.

**Difference = value in intervention group minus value in control group.*

Table A11.4. Net improvement of symptoms in intervention group (with mould) and the control group (both visited at 12 months) when comparing baseline to 12 months results

	Intervention group (with mould)					Control group					Difference in net % better * (95% CI)
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	
(2) Have you had wheezing or whistling in the chest in the last 4 weeks?	38	9	3	26	15.8	80	20	4	56	20.0	-4.2 (-24.7, 16.3)
(4) In the last 4 weeks, how often, on average, has your sleep been disturbed due to wheezing?	38	12	5	21	18.4	80	35	4	41	38.8	-20.3 (-44.4, 3.8)
(5) In the last 4 weeks, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths?	38	5	1	32	10.5	80	5	3	72	2.5	8.0 (-6.0, 22.0)

Table A11.4. Net improvement of symptoms in intervention (with mould) and control groups at 12 months when compared to baseline results

	Intervention group (with mould)					Control group					Difference in net % better * (95% CI)
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	
(7) In the last 4 weeks, how much did this wheeze interfere with your daily activities?	38	16	4	18	31.6	80	29	12	39	21.3	10.3 (-15.3, 35.9)
(8) In the last 4 weeks, have you had wheezing or whistling in the chest when you did not have a cold or flu?	38	12	3	23	23.7	80	20	4	56	20.0	3.6 (-18.0, 25.3)
(9) How is your breathing compared with how it was 12 months ago?	38	21	2	15	50.0	79	25	8	46	21.5	28.5 (-5.2, 51.7)
(11b) In the last 6 months have you used your medication less, the same or more than/as previously?	32	15	7	10	25.0	67	29	15	23	20.9	4.1 (-29.0, 37.3)
(11c) In the last 4 weeks, have you taken any treatment for wheezing or asthma?	38	11	1	26	26.3	80	8	4	68	5.0	21.3 (3.4, 39.2)

Table A11.4. Net improvement of symptoms in intervention (with mould) and control groups at 12 months when compared to baseline results

	Intervention group (with mould)					Control group					Difference in net % better * (95% CI)
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	
(13) During the last 4 weeks, have you had a cough on most days?	38	11	4	23	18.4	80	15	8	57	8.8	9.7 (-12.7, 32.0)
(14) During the last 4 weeks, have you brought up phlegm from your chest on most days?	37	7	2	28	13.5	80	11	8	61	3.8	9.8 (-8.9, 28.4)
(16) During the last 4 weeks, have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu? (Rhinitis)	38	5	1	32	10.5	80	7	11	62	-5.0	15.5 (-0.5, 31.5)
(17) During the last 4 weeks, has this nose problem been accompanied by itchy-watery eyes? (Rhinoconjunctivitis)	38	7	3	28	10.5	80	8	12	60	-5.0	15.5 (-3.8, 34.9)

Table A11.4. Net improvement of symptoms in intervention (with mould) and control groups at 12 months when compared to baseline results

	Intervention group (with mould)					Control group					Difference in net % better * (95% CI)
	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	Total no.	Number improvement	Number deterioration	Number no change	Net % improvement	
(18) During the last 4 weeks, how much did this nose problem interfere with your daily activities?	38	7	9	22	-5.3	80	17	17	46	0.0	-5.3 (-30.3, 19.8)
(20) In the last 4 weeks, have you taken any treatment for a runny or blocked nose problem or hay fever?	38	13	3	32	26.3	79	9	11	59	2.5	28.8 (-7.0, 50.7)
(22) In the past 4 weeks, have you taken any treatment for an itchy rash or eczema?	38	5	0	33	13.2	79	20	4	55	20.3	-7.1 (-22.7, 8.5)

Note: ***Question 9 above is a comparison between baseline and 12 months.***

Question 11b above is a comparison between six months and 12 months.

**Difference = value in intervention group minus value in control group.*

Appendix 12

Abbreviations / Acronyms

ACH	Air changes per hour
AD	Atopic dermatitis
AH	Absolute Humidity (ρ, density, g/m^3)
ALA	American Lung Association
AM	Alveolar macrophages
ANOVA	Analysis of variance
ATS	American Thoracic Society
Bla g1	Cockroach allergen
Can f1	Dog allergen
CAPS	Childhood Asthma Prevention Study
CD	Cluster of differentiation
CFU	Colony-forming units
CI	Confidence interval
CMR	Corrected missing ratio
COPD	Chronic obstructive pulmonary disease
DEPs	Diesel exhaust particulates
Der f1	Dermatophagoides farinae
Der p1	Dermatophagoides pteronyssinus
df	Degrees of freedom
DNA	Deoxyribonucleic acid
e	Water vapour pressure
e_s	Saturation water vapour pressure
ECRHS	European Community Respiratory Health Survey
ELISA	Enzyme-Linked Immunosorbent Assay
ETS	Environmental Tobacco Smoke
Fel d1	Feline antigen d1
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GC	Gas Chromatograph
GINA	Global Initiative for Asthma
HDM	House Dust Mite
HPLC	High Performance Liquid Chromatography
ICS	Inhaled corticosteroids
IgE	Immunoglobulin E

IRR	Incidence rate ratios
ISAAC	International Study of Asthma and Allergies in Childhood
MAS	Multicentre Allergy Study
MR	Mixing Ratio (g/kg)
MRC	Medical Research Council
MS	Mass Spectrometer
MVOC	Microbial volatile organic compounds
NAC	National Asthma Campaign
NACMAAS	NAC Manchester Asthma and Allergy Study
OR	Odds ratio
P	Atmospheric pressure (kPa or mb)
p	Probability
ρ	Absolute humidity (g/m^3)
PCR	Polymerase Chain Reaction
PD_{20} FEV_1	Provocative dose of methacholine required to cause a 20% fall in FEV_1 from baseline
PEFR	Peak expiratory flow rate
PFT	Perfluorocarbon tracer gas
PFV	Peak Flow Variability (daily)
PM	Particle Matter (usually denoted $PM_{2.5}$ or PM_{10})
q	Specific humidity (g/kg)
RCT	Randomised Controlled Trial
RH	Relative Humidity (%)
RSV	Respiratory syncytial virus (a common cold virus)
RR	Relative risk or rate ratio
SCARPOL	Swiss Study on Childhood Allergy & Respiratory symptoms; Air Pollution team
SD	Standard deviation
SNP	Single nucleotide polymorphism
SOB	Shortness of breath
SPT	Skin Prick Test
Th1	T helper 1 cytokines
Th2	T helper 2 cytokines
VOCs	Volatile organic compounds
ω	Mixing ratio (g/kg)
WHO	World Health Organisation

Definitions

Absolute humidity: The mass of water vapour contained in one cubic metre of air; a density measure in g/m^3.

Adjuvant: an additive that enhances the effectiveness of a medical treatment or it acts to increase a response to an allergen without having direct affects itself.

Aflatoxin: any of a group of toxic compounds (mycotoxins) produced from moulds, especially *Aspergillus flavus*, often found in animal feed, maize and peanuts.

Allele: any one of a number of alternative forms of the same gene occupying a given locus (position) on a chromosome.

Allergen: any substance that causes an allergic reaction (an IgE response).

Antibody: a specialised immune protein (immunoglobulin) molecule produced in response to an antigen in the body. It is able to combine with the antigen that triggered its production.

Antigen: a substance that is capable of causing the production of an antibody but does not necessarily lead to an allergic reaction.

Chromosome: is a very long, continuous piece of DNA, which contains many genes, regulatory elements and other intervening sequences, usually in pairs.

Conjunctivitis: it is the inflammation of the membrane that covers the eyelid's inner surface, often called pinkeye and can result from a bacterial or viral infection.

Cytokines: are proteins produced by white blood cells that act as chemical messengers between cells. They can stimulate or inhibit the growth and activity of various immune cells.

Dermographism: a form of hives caused by stroking the skin with an object.

Dyspnoea: shortness of breath.

Endotoxin: part of the outer membrane of the cell wall of Gram-negative bacteria and is only released upon destruction of the bacterial cell.

Eosinophil: A type of leukocyte (white blood cell) with coarse round granules of uniform size within its cytoplasm and typically a two-lobed nucleus.

Extrinsic asthma: referring to an allergic form of asthma reacting to identifiable allergens usually in childhood.

Helminth: a parasitic worm that can live in humans, (i.e. roundworms, pinworms, Trichina spiralis, tapeworms, flukes).

IgE: Immunoglobulin E, are a class of antibodies elicited by an allergic substance or allergen. Elevated IgE can be detected in the blood of susceptible individuals. The E in IgE means erythema or redness.

Intrinsic asthma: non-atopic asthma usually developing in adulthood.

Irritant: respiratory irritants do not provoke a response from the immune system but can exacerbate an asthmatic's condition.

Leukocyte: a type of white blood cell filled with microscopic granules (tiny sacs) containing enzymes that digest microorganisms.

Macrophage: any of the large phagocytic cells located in reticular connective tissue (i.e. spleen) that are responsible for engulfing (phagocytosis) and removing cellular debris, old cells, pathogens, and foreign bodies from the bloodstream.

Methacholine: a parasympathomimetic drug that stimulates secretions and smooth muscle activity used in airways challenge tests for asthma.

Mixing Ratio: the mass of water vapour (g) contained in 1 kilogram of dry air.

Mycotoxin: any of a number of poisonous toxins produced by certain moulds during metabolic cycles under particular environmental conditions.

Neutrophils: (neutrophil granulocytes) are a class of granular white blood cells, which are part of the immune system.

Otitis media: an inflammation and/or infection of the middle ear.

Parasite: a disease-causing organism that lives on or in human or other animals and derives its nourishment from its host, (i.e. bacteria, viruses, lice).

Patulin: a toxic antibiotic derived from metabolites of certain fungi (i.e. *Aspergillus*, *Penicillium* and *Gymnoascus*) and having carcinogenic properties.

Phagocytosis: the engulfing and ingestion of bacteria or other foreign substances by phagocytes (i.e. neutrophils, monocytes & macrophages).

Polymorphism: a genetic variant that appears in at least 1% of a population.

Relative Humidity: The ratio of the actual quantity of water vapour in the air compared with the maximum amount the air can hold at a given temperature, usually expressed as a percent (%).

Rhinitis: inflammation of the mucous membrane of the nose marked especially by rhinorrhea, nasal congestion, itching, and sneezing.

Rhinoconjunctivis: a combination of (the previously defined terms) rhinitis and conjunctivitis commonly occurring during the pollen season.

Specific Humidity: very similar to the mixing ratio, it is the mass of water vapour contained in the total mass of air including the water vapour in g/kg.