# HOME DAMPNESS, FUNGI AND HOUSE DUST MITES, AND RESPIRATORY SYMPTOMS IN CHILDREN

Cover: *Penicillium chrysogenum*, 52.000x
(provided by Rob Samson, Centraalbureau voor Schimmelcultures, Baarn)
Photography: René Gonkel
Lay-out: Louk Smulders
Printer: Ponsen & Looijen b.v., Wageningen
Lay-out: Lenneke Brachel

ISBN 90-9007164/CIP

*Publication of this thesis was made possible by a grant of ``Stichting Sarphati''*

# HOME DAMPNESS, FUNGI AND HOUSE DUST MITES, AND RESPIRATORY SYMPTOMS IN CHILDREN

Vochtige woningen, schimmels en huisstofmijten,
en luchtwegsymptomen bij kinderen

**PROEFSCHRIFT**

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam

op gezag van de rector magnificus
Prof.Dr. P.W.C. Akkermans M.Lit.
en volgens besluit van het College van Dekanen

De openbare verdediging zal plaatsvinden op
woensdag 1 juni 1994 om 13.45 uur

door

**Arnoud Paul Verhoeff**

geboren te 's-Gravenzande

**Promotiecommissie**

| | | |
|---|---|---|
| Promotores | : | Prof.Dr. P.J. van der Maas |
| | | Prof.Dr. Ir. B. Brunekreef |
| Co-promotor | : | Dr. J.H. van Wijnen |
| Overige leden | : | Prof.Dr. A. Hofman |
| | | Prof.Dr. H.J. Neijens |

*Ter nagedachtenis aan mijn grootvader*
*Dr. Paul de Fremery,*
*bioloog*
*1900-1941*

# Index


1. General introduction 1

2. Epidemiological studies on the relation between home dampness and respiratory morbidity 7
   - 2.1 Introduction 8
   - 2.2 Home dampness and respiratory morbidity in children 8
   - 2.3 Home dampness and respiratory morbidity in adults 17
   - 2.4 Discussion and conclusions 19

3. Fungi and house dust mites: ecology and health effects 29
   - 3.1 Introduction 30
   - 3.2 Fungi: ecology 30
   - 3.3 Fungi: health effects 31
   - 3.4 House dust mites: ecology 35
   - 3.5 House dust mites: health effects 36
   - 3.6 Concluding remarks 38

4. Assessment of the exposure to fungi and house dust mites 39
   - 4.1 Introduction 40
   - 4.2 Assessment of the exposure to house dust mites 41
     - 4.2.1 Air sampling of house dust mites 41
     - 4.2.2 Dust sampling of house dust mites 42
   - 4.3 Assessment of the exposure to fungi 44
   - 4.4 Enumeration and identification of airborne viable mould propagules in houses; a field comparison of selected techniques 47
     - 4.4.1 Abstract 47
     - 4.4.2 Introduction 47
     - 4.4.3 Material and methods 48
     - 4.4.4 Results 50
     - 4.4.5 Discussion 56
   - 4.5 Fungal propagules in house dust; comparison of analytic methods and their value as estimators of potential exposure 59
     - 4.5.1 Abstract 59
     - 4.5.2 Introduction 59
     - 4.5.3 Material and methods 60
     - 4.5.4 Results 62
     - 4.5.5 Discussion 66

5. The presence of fungi and house dust mites in houses in relation to building characteristics 69
   - 5.1 Introduction 70
   - 5.2 Presence of viable mould propagules in indoor air in relation to house damp and outdoor air 71
     - 5.2.1 Abstract 71
     - 5.2.2 Introduction 71
     - 5.2.3 Material and methods 72
     - 5.2.4 Results 74
     - 5.2.5 Discussion 84

5.3 Fungal propagules in house dust; relation with residential characteristics, and respiratory symptoms 87
5.3.1 Abstract 87
5.3.2 Introduction 88
5.3.3 Material and methods 89
5.3.4 Results 91
5.3.5 Discussion 96
5.4 Mite antigen in house dust: relationship with different housing characteristics in the Netherlands 99
5.4.1 Abstract 99
5.4.2 Introduction 99
5.4.3 Materials and methods 100
5.4.4 Results 103
5.4.5 Discussion 113

6. Home dampness and respiratory symptoms in children: a case-control study 117
6.1 Introduction 118
6.2 Damp housing and childhood respiratory symptoms: the role of sensitization to dust mites and moulds 119
6.2.1 Abstract 119
6.2.2 Introduction 119
6.2.3 Materials and methods 120
6.2.4 Results 121
6.2.5 Discussion 127
6.3 House dust mite allergen (*Der p* I) and respiratory symptoms in children; a case control study 130
6.3.1 Abstract 130
6.3.2 Introduction 130
6.3.3 Materials and methods 131
6.3.4 Results 133
6.3.5 Discussion 138

7. General discussion 141
7.1 Introduction 142
7.2 Epidemiology 143
7.3 Suspected causal factors: fungi and house dust mites 146
7.4 Assessment of the exposure to fungi and house dust mites 147
7.5 The presence of fungi and house dust mites in relation to home dampness and other residential characteristics 151
7.6 The role of sensitization to fungal and house dust mite allergens in explaining the relation between home dampness and respiratory symptoms 154
7.7 The relation between exposure to fungi and house dust mites, and health effects 156
7.8 General conclusions 158

References 161
Summary 175
Samenvatting 181
List of abbreviations 187
Acknowledgements 189
Curriculum vitae 191

# General introduction

Damp houses are common in the Netherlands. A study conducted in 1985 at the request of the Ministry of Housing, Physical Planning and Environment, among housing corporations that managed 1.6 million houses (almost one third of the total housing stock in the Netherlands at that time) showed that approximately 18% of these houses were affected by dampness to at least some degree (Tammes *et al.* 1985). More recently, the prevalence of home dampness in the Netherlands was investigated in a representative sample of the total housing stock (approximately 6 million houses) within the framework of the 'Qualitative Housing Registration 1989-1991' (Ministry of Housing, Physical Planning and Environment 1993). Approximately 20% of the total housing stock was affected by home dampness. The highest rate of home dampness reported for rented houses was in the private sector (26.3%), with slightly fewer (21.7%) in public sector housing, and 16.8% of private houses. Home dampness was more common in older houses (build before 1946) than in relatively new houses (build after 1968).

Damp houses are not only common in the Netherlands, but are also found in other countries with a temperate climate. For example, in the UK it has been estimated that 2.5 million houses are seriously affected by dampness (Flannigan *et al.* 1991). In the Netherlands as well as in several other countries, it has been recognized that energy saving programmes involving insulation and sealing of homes, have reduced the exchange of air between the indoors and outdoors, which has led to 'trapping' of moisture and air pollutants generated indoors.

At the Housing Inspectorate of the Public Health Service in Amsterdam, more than 200 complaints are received annually concerning the indoor air quality of houses in relation to health problems of its occupants. Approximately half of these complaints are about dampness in homes, and approximately 25% of them concern mould problems. The author of the present thesis was involved in dealing with these complaints and confronted with gaps in knowledge about the relation between home dampness and health complaints.

As far back as the 1920's, Storm van Leeuwen and others (Storm van Leeuwen *et al.* 1923; Varekamp 1925) drew attention to the possible influence of dampness in the home on the prevalence of asthma. Later, Varekamp and Voorhorst (1961) reported a strong positive relation between housing conditions, especially the presence of wood rot, and asthma in atopic patients. Wood rot can be considered as an indicator of serious dampness problems in homes. Since then no major studies on this subject have been published till the 1980's. In recent years, epidemiological studies carried out in Western-Europe and North-America again showed increased respiratory (and other) complaints in children and adults, associated with the presence of home dampness in modern homes. However, the causal relationship between home dampness and respiratory illnesses had not been fully elucidated.

The hypothesis is that home dampness promotes the development of fungi and house dust mites. Fungi and house dust mites both produce inhalant allergens and susceptible individuals may develop allergic reactions against these (Platts-Mills *et al.* 1992; Commission of the European Communities 1993). This could lead to an increased prevalence of respiratory symptoms among occupants of damp houses.

It is also suggested that non-allergic mechanisms play an etiological role in the relation between damp houses and health effects. Fungal products, like volatile organic compounds, mycotoxins and glucans, might be responsible for non-allergic health symptoms, such as headaches and mucous membrane irritation, in occupants of damp homes. Products of other microorganisms, such as Gram-negative bacteria, might also be

involved (Flannigan *et al.* 1991; Commission of the European Communities 1993). Home dampness may reflect poor overall ventilation and therefore increased concentrations of other indoor air pollutants.

However, house dust mites and fungi, which both encounter favourable conditions in damp houses, are thought to be the major causes of respiratory symptoms in people living in damp houses. Figure 1.1 outlines the theoretical causal relationships between home dampness and respiratory symptoms, and the factors involved in this relationship.

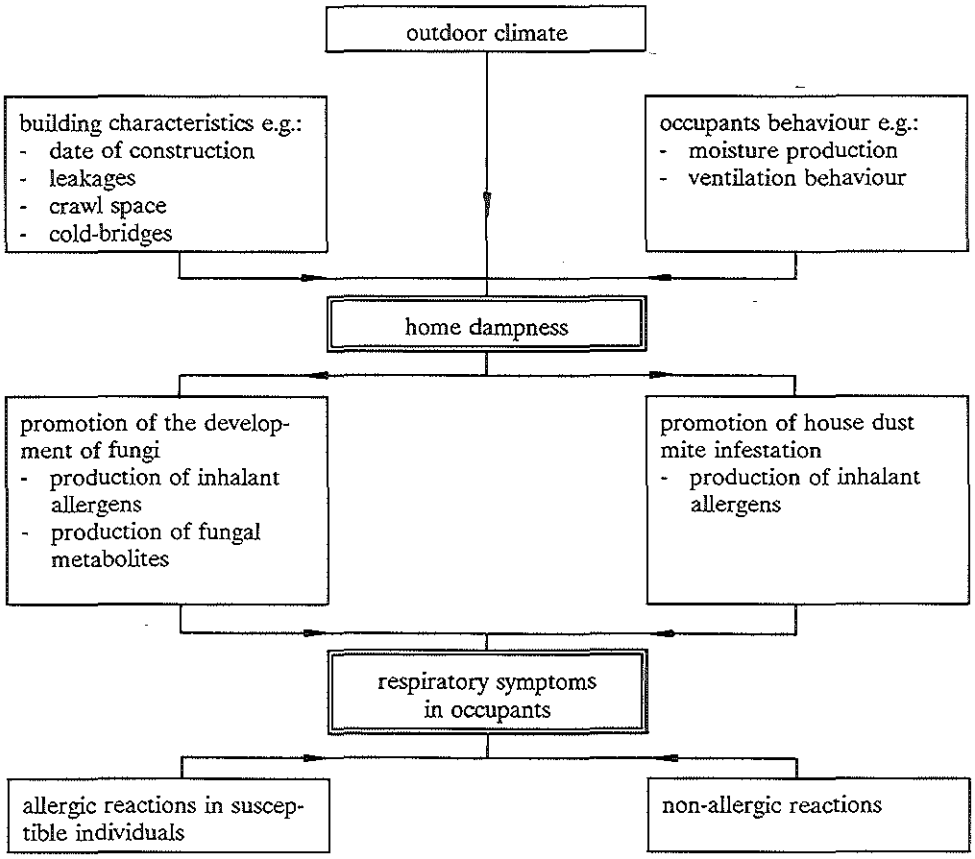


Figure 1.1 Outline of the theoretical causal relationship between home dampness and respiratory symptoms of occupants

To elucidate the relation between home dampness and respiratory symptoms of occupants, information on the following aspects is needed.

1. *Home dampness*
   Home dampness should be well defined and a reliable method should be available to assess the dampness of a house.
2. *Fungi*
   a The health effects on human beings of fungi and fungal products should be known.
   b A positive correlation between the presence of fungi in homes and home dampness should be demonstrated.
   c This requires an accurate and precise method of measuring the presence of fungi and their relevant products in homes. Ideally, personal exposure to fungi and their relevant products should also be assessed.
   d Then an exposure dependent relation between fungi and health effects (including respiratory symptoms) should be demonstrated.
3. *House dust mites*
   a The health effects of house dust mites on human beings should be known.
   b A positive correlation between the presence of house dust mites in homes and home dampness should be demonstrated.
   c This requires an accurate and precise method of measuring the presence of house dust mites and/or their allergens in homes. Ideally, personal exposure to house dust mites and/or their allergens should also be assessed.
   d Then an exposure dependent relation between house dust mites and health effects (including respiratory symptoms) should be demonstrated.

For some of the topics mentioned above, e.g. the possible health effects of house dust mites, extensive data were available in the literature. However, at the start of the studies presented in this thesis, knowledge about the validity of measurements of the presence of, and the exposure to house dust mites and fungi was only limited, especially for fungi. The relation between home dampness and other home characteristics in (modern) homes and the presence of fungi and house dust mites was then largely unknown. Also the dose-response relationships between exposure to these biological agents and health effects were poorly documented.

The studies presented in this thesis focus on 1) the measurement of the presence of, and the exposure to, fungi in homes, 2) the relation between the presence of fungi and house dust mite allergens in dwellings and home dampness as well as other home characteristics, and 3) the relation between home dampness, the presence of mite allergens and fungi, and respiratory symptoms in children.

In Chapter 2 a review is given of recent epidemiological studies on the relation between home dampness and respiratory symptoms in children and adults, conducted in the Netherlands and elsewhere.

Chapter 3 provides an overview of the ecology and health effects of both suspected causal factors, i.e. fungi and house dust mites.

Chapter 4 deals with the measurement of the presence of, and the exposure to, fungi and house dust mites. For house dust mites, a brief literature review of the available methods will be presented. The results for fungi are presented as studies of the reliability of the results of air sampling and dust sampling of these organisms in homes. The use

of the measurement results as estimators of the (potential) exposure in epidemiological studies is discussed. The main hypotheses tested in this Chapter are: 1) that there are no differences in the results obtained with different techniques for the enumeration and identification of viable fungal particles in the indoor air of houses, 2) that there are no differences in the results obtained with different analytical methods for the enumeration and identification of viable fungal particles in settled house dust, and 3) that measurement of fungi in single samples of indoor air or settled house dust does not provide a reliable estimation of the potential exposure.

Chapter 5 presents three studies of the relationships between the presence of fungi in indoor air and settled house dust, the presence of house dust mite allergen in settled house dust, and home dampness and other residential characteristics. The main hypotheses tested in this Chapter are: 1) that there is no relation between the presence of fungi in indoor air and home dampness, 2) that there is no relation between the presence of fungi in settled house dust and home dampness, and 3) that there is no relation between house dust mite allergen in settled house dust and home dampness.

In Chapter 6 the results are presented of a case-control study on the relation between home dampness and respiratory symptoms in children. The main hypotheses tested in the research presented in this Chapter are: 1) that there is no relation between reported home dampness and reported respiratory symptoms in children, taking into account sensitization against fungal and house dust mite allergens, and 2) that there is no relation between the levels of house dust mite allergen in settled house dust and reported respiratory symptoms in children, taking into account sensitization against house dust mite allergens.

Finally, Chapter 7 provides a general discussion and draws conclusions on the relation between home dampness and respiratory symptoms. Attention is given to the significance of the results for the design of epidemiological studies on the relation between home dampness and respiratory symptoms, and for the daily practice in the treatment of health complaints related to home dampness.

Figure 1.2 summarizes the contributions of the studies presented in this thesis to the further clarification of the relationship between home dampness and respiratory symptoms, as outlined in Figure 1.1.

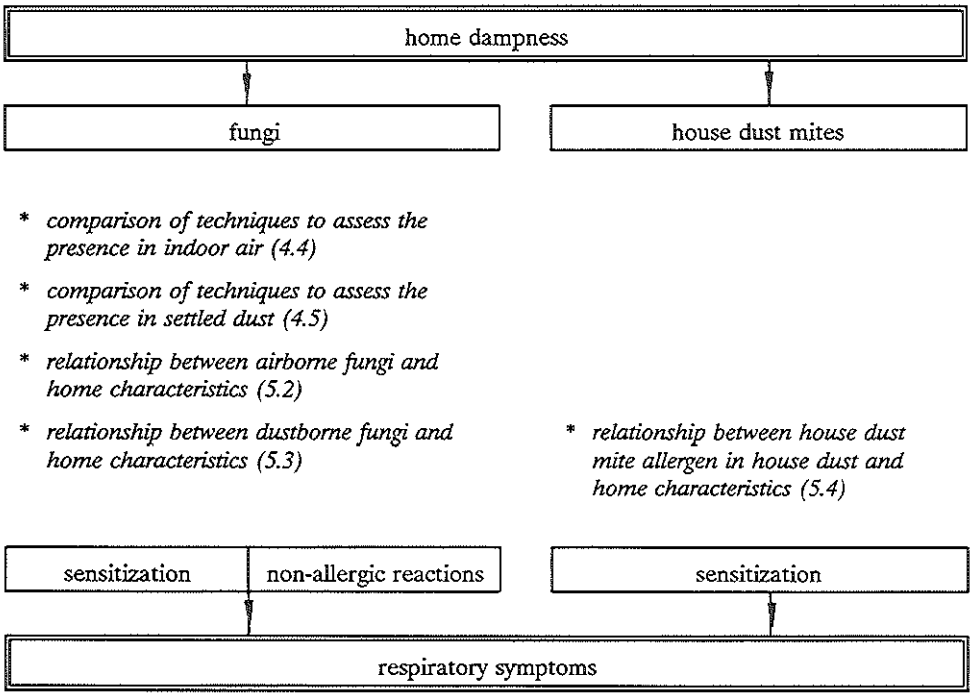


Figure 1.2 Overview of the contributions of the studies presented in this thesis to the further clarification of the relationship between home dampness and respiratory symptoms

# Epidemiological studies on the relation between home dampness and respiratory morbidity

## 2.1 Introduction

In this Chapter a review will be presented of the results of recent epidemiological studies on the relation between home dampness and respiratory morbidity of the occupants. Most of the studies were conducted in Europe, especially in the United Kingdom and in the Netherlands, and in North America. The results of studies on children and adults will be described separately. Table 2.1 presents an overview of the main results of the major studies conducted among children, whereas Table 2.2 summarizes the results of the studies carried out among adults. A general discussion, and conclusions on the design and the results of the epidemiological studies, concludes this Chapter.

## 2.2 Home dampness and respiratory morbidity in children

One of the first larger studies on the relation between home dampness and respiratory symptoms in children was conducted in Leiden, the Netherlands, by Varekamp and Leupen (1970). This was carried out in approximately 450 randomly selected homes, in which a total of 1,001 children lived. A questionnaire was used to obtain information about chronic respiratory symptoms, including asthma and bronchitis during the past year. A checklist was used to scale several building characteristics related to 1) the likelihood of home dampness (e.g. date of construction, building materials, the presence of a crawl space), 2) the possibilities of moisture elimination (e.g. ventilation and heating facilities), and 3) visible signs of dampness like damp patches, mould growth, wet crawl space and wood rot. For each room in a house, a "dampness score" was calculated. The average score for all rooms was thought to represent the dampness of the entire home; the higher the score, the more damp the house was considered to be. The checklist was completed by a trained investigator.

Out of the total group of children, 82 (8.2%) reported asthma and/or bronchitis in the past year. The prevalence of both asthma and bronchitis was positively associated with the dampness score. The percentage of children with reported asthma and/or bronchitis in the past year ranged from 4% in dwellings with a dampness score below 9, to more than 20% in dwellings with a dampness score higher than 36.

The checklist, described above, was also used in a study on chronic non-specific respiratory symptoms among children in relation to ambient air pollution and other factors, conducted in a total of 190 homes in Amsterdam and Vlaardingen, the Netherlands (Van der Maas, 1979). Children with reported chronic non-specific respiratory symptoms lived in half of these houses, whereas the other half was inhabited by symptom-free children. Children with chronic non-specific respiratory symptoms were more frequently found living in houses classified as damp (22 out of 95, 23%), compared with symptom free children (10 out of 95, 11%).

More recently, Waegemaekers *et al.* (1989) conducted a study on home dampness and respiratory symptoms among adults and children living in Katwijk, the Netherlands. Many people in the study area complained about home dampness and were concerned about the possible adverse health effects. Questionnaires were used to obtain information on respiratory symptoms. Home dampness was assessed in three different ways: 1) the presence of two or more of the following reported characteristics: mould growth, damp patches, silver-fish or sow-bugs, a stale-odour or a wet crawl space; 2) whether the home was considered dry or damp by the residents; and 3) the results of measurements

of airborne fungal propagules in 36 homes, selected on the basis of reported dampness. Fungal propagules were sampled with the N6-Andersen sampler with Malt Extract Agar as collection medium (see Chapter 4).

Of the homes included in the study, approximately 42% were classified as damp, whereas approximately 25% of the residents considered their home to be damp. The geometric mean (GM) number of Colony Forming Units (CFU)/$m^3$ was higher in dwellings classified as damp (GM 192 CFU/$m^3$) than in dwellings classified as dry (GM 107 CFU/$m^3$). The numbers of CFU/$m^3$ were also positively associated with reported mould growth and the residents' own perception of home dampness.

For the children, crude odds ratios for the association between reported dampness and reported respiratory conditions ranged from 1.02 (95% CI 0.41-2.51) for shortness of breath, to 3.20 (95% CI 1.26-8.17) for pneumonia. For non-respiratory symptoms (e.g. headache, tiredness, eye and skin irritation, nausea and vomiting) they were also greater than one. Adjustment for age, gender, parental smoking, heating system, closed or open kitchen, presence of pets and type of floor cover in the living room and bedroom increased most of the odds ratios. Furthermore, a positive association was found between the number of CFU/$m^3$ and the prevalences of reported respiratory symptoms. Responder bias could have been an important factor in this study since home dampness was considered a major problem by the study group. However, the authors indicated that the associations found could not be caused only by responder bias, because home dampness and respiratory symptoms were both associated with fungal spore counts.

The association between home dampness and respiratory symptoms was further investigated in two groups of 1,051 and 3,344 children respectively, aged 6 to 12 years in the south-east of the Netherlands (Dijkstra *et al.* 1990; Brunekreef 1992a), where home dampness was not considered to be a widespread problem. Information about home dampness and respiratory symptoms was gathered by questionnaires. Home dampness was assessed by questions on the presence of damp patches and visible mould growth in the home for the past two years. Cough, wheeze and asthma or the presence of any of these symptoms were used as outcome variables. Pulmonary function (FVC, $FEV_1$, PEF and MMEF) was measured only in the children belonging to the first group. Data on potential confounders such as smoking in the home, indoor sources of nitrogen dioxide, socio-economic status, were also collected by questionnaires.

In the first group, damp patches were reported in 14.8% of the dwellings, mould growth in 9.1%, and both in 7.6%, however, in the second, these figures were 23.6%, 15.0% and 13.3% respectively. The crude odds ratios in the first group using damp patches as exposure variable ranged from 1.43 (95% CI 0.80-2.56) for wheeze, to 2.34 (95% CI 1.24-4.42) for cough. Using visible mould as exposure variable, the crude odds ratios varied from 1.34 (95% CI 0.54-3.33) for asthma, to 3.13 (95% CI 1.62-6.05) for cough. In the second study, the crude odds ratios were also all greater than one, and statistically significant except for asthma in relation to damp patches (OR 1.20, 95% CI 0.88-1.64). After adjustment for potential confounders, the odds ratios remained greater than one, however, the confidence intervals became larger.

There was no clear association between pulmonary function and home dampness. Only MMEF was marginally ($0.10>p>0.05$) lower when mould growth was reported. The associations between reported respiratory symptoms and pulmonary function for children in homes with visible mould were comparable to those for the children in homes without visible moulds. This indicated that in this study parents living in damp and mouldy homes were not over-reporting respiratory symptoms. Furthermore, there was no relation between socio-economic status and reporting of visible mould growth,

but parents who reported that damp patches and mould growth were 'always' present in their homes, reported less respiratory symptoms in their children, than parents reporting that damp patches and mould growth were 'often' present. This finding suggested that parents of symptomatic children selectively avoid home dampness by moving or taking remedial action. Therefore, the author concluded that a selection process might influence the relation between reported dampness and reported respiratory symptoms.

In the United Kingdom, Strachan and Elton (1986) conducted a study, in one of the most socially deprived areas of Edinburgh on the association between respiratory morbidity and the home environment among 198 children, aged 7 to 8 years. The records of these children were obtained from the general practice that served this area and screened for wheezing episodes and lower respiratory tract illnesses. Questionnaires, given to the parents, were used to obtain information about current respiratory symptoms (absence from school due to chest trouble, nocturnal cough and wheezing) and twelve different home characteristics, including home dampness and visible moulds. The response rate was 83%. The response rates were similar for children with and children without a history of wheeze in their general practice record. Therefore, the authors indicated that there was only minimal bias in the study, at least with respect to wheezing.

The prevalence rate for wheezing at some time was 20%. For school absence owing to chest trouble, the prevalence was 25% and for nocturnal cough 49%. Thirty percent of the homes were reported to be affected by dampness, whereas moulds were reported in only 21% of the homes. There was a considerable overlap: 66% of the 50 homes in which dampness was reported, were also affected by mould growth. Wheezing, school absence and nocturnal cough were all significantly related to a family history of wheeze, reported damp and reported mould growth. Wheezing was also associated with a window being open at night, while school absence was related to parental smoking. The relative odds of morbidity varied from 2.6 ($p<0.05$) for both wheezing and school absence in relation to a family history of wheeze, to 4.8 ($p<0.001$) for nocturnal cough in relation to moulds. Multiple regression analyses revealed that wheezing was related to a family history of wheezing, an open bedroom window and visible moulds. School absence was related to a family history of wheeze and moulds, and nocturnal cough was related to a family history of wheeze, the presence of a coal-fired appliance in the home and moulds.

The association between the home characteristics and general practice consultations was also investigated. In contrast to reported respiratory morbidity, no associations were found between consultations for wheeze or lower respiratory tract illnesses and the home characteristics. The association between parental reports of respiratory symptoms and general practice consultations was not very strong either. According to the authors, these findings suggest that reporting bias may account for at least part of the associations found between reported respiratory morbidity and home characteristics. However, they also pointed out that the two questionnaires with regard to respiratory morbidity and the home environment were completed by the parents at two different points in time. The first questionnaire focused on respiratory symptoms and did not mention the interest in the home environment. Therefore, they concluded that the associations found were not entirely caused by reporting bias.

To address the possible influence of reporting bias, a second study was conducted in a random sample of 873 children, aged 7 years, in Edinburgh, UK (Strachan 1988). A questionnaire, given to the parents, was used to elicit information about respiratory and non-respiratory symptoms, including wheeze, sore throat, pain or discharge in the ear, hay fever, sneezing attacks, cough and blocked or running nose. Furthermore, informa-

tion about home characteristics, including condensation or damp patches on walls and the presence of visible mould growth was obtained at the same time. To validate reported respiratory symptoms, pulmonary function was measured before, and five and ten minutes after six minutes of free running.

Visible mould growth was reported in 10.2% (89 out of 873) of the homes and damp patches in 16.8% (147 out of 873). The two characteristics were again closely related: 48% of the homes affected by dampness were also reported to be mouldy, and 80% of the homes with reported mould growth were also reported to be damp. The prevalences of wheeze and chest colds during the past year were approximately two to three times higher among children living in homes with reported damp patches and/or moulds. The highest prevalences were found for those children with reported damp patches and/or mould in their own bedroom. Cough at night was strongly associated with smoking in the home, and to a lesser extent with damp patches in the child's bedroom. Cough during the day was also significantly more common among the children with damp patches in their bedrooms. Wheeze during the past year was only associated with home dampness and not with other environmental factors in the home. The crude odds ratio for wheeze in the past year and mould anywhere in the home was 3.70 (95% CI 2.22-6.15). After adjustment for housing tenure, number of people per room, number of smokers in the home, and gas cooking, the odds ratio was 3.00 (95% CI 1.72-5.25) and the adjusted odds ratio for chest colds was 2.08 (95% CI 1.22-3.51). However, including wheeze during the past year as explanatory variable reduced this odds ratio (1.43, 95% CI 0.77-2.70), indicating that part of the latter association was related to recent wheeze. The adjusted odds ratios for cough during the night and during the day, and reported mould growth, were 0.92 and 0.95 respectively.

Wheeze during the past year was more frequently reported for children experiencing bronchospasm after exercise. If no reporting bias exists, this relation will be independent of housing conditions. However, for any degree of bronchial lability - expressed as the ratio between the $FEV_1$ after exercise and the $FEV_1$ before exercise - wheeze was more commonly reported by parents also reporting visible mould growth in the home. This finding suggested that reporting bias explained a substantial part of the association found between reported wheeze and home dampness.

In 778 of the homes included in the study described above, the relative humidity in the children's bedroom was assessed semi-quantitatively (Strachan and Sanders 1989). Relative humidity was measured using a block of ramin hardwood which was placed in the child's bedroom by their parents for one week. The moisture content of each block was measured by an electrical conductance meter within 48 h after collection from the home and they were then allowed to equilibrate for 10 days at a relative humidity of 55%. Then their moisture content was reassessed and the difference between the two measurements was used as the estimate of the relative humidity in the bedroom. Furthermore, in 317 bedrooms (including the top quintile of humidity, as assessed with the wood blocks) both humidity and temperature were measured with a thermohygrograph for a period of one week. As the measurements were carried out during a period of four months, the results obtained were adjusted for outside climatic variations during this period, using continuous recordings in five homes.

The range of relative humidity assessed with the ramin blocks was from less than 45% to over 80%. The mean weekly relative humidity measured with the thermohygrographs varied from 28% to 77%. Multiple regression analysis revealed that the number of children in the bedroom, heating during the day and dampness on walls, all significantly contributed to the variance of relative humidity as assessed with the thermo-

hygrographs. However, the model explained only approximately 10% of the total variance. The mean relative humidity - assessed with the thermohygrographs - tended to be higher in the bedrooms with reported damp or mould patches compared with bedrooms without reported signs of damp (dampness: 56.7% vs 54.3%; mould: 56.7% vs 52.5%). However, the prevalences of the different respiratory symptoms (wheeze, chest colds, cough during the day and during the night) showed no significant relationship with the bedroom relative humidity as assessed with the ramin blocks. Furthermore, the mean relative humidity as measured with the thermohygrographs did not differ between children with or without respiratory symptoms. There was also no association between the relative humidity and $FEV_1$ before exercise or bronchial lability. The authors did not report on the relation between the relative humidity assessed by the ramin blocks and assessed by the thermohygrographs.

In a second follow-up study (Strachan *et al.* 1990), airborne fungal propagules were measured in 34 homes of children with wheeze in the past year and in 44 homes of children without wheeze. In 11 and 29 homes respectively mould had been reported. The authors postulated that if substantial reporting bias had occurred in the initial study (Strachan 1988), the reports of mould growth by parents of wheezy children should be associated with lower levels of airborne fungi than similar reports of children without wheeze. Sampling was performed four times at approximately monthly intervals with an Andersen 6-stage impactor with Malt Extract Agar as collection medium, provided with inhibitors for bacterial growth. In each house samples were taken in the living room and the child's bedroom. If visible mould growth was a problem elsewhere in the home, sampling was also performed in that specific room, otherwise a third sample was taken in the kitchen. Sampling was carried out during October 1987 to March 1988.

The fieldworkers observed mould growth in only 10 of the 40 homes where moulds were reported at the beginning of the study (1986), but in three of the homes without reported mould growth, moulds were observed at the time of sampling. The median numbers of CFU/m$^3$ in the living room were very similar for the four different groups: 294 CFU/m$^3$ in mouldy living rooms of wheezy children, 271 CFU/m$^3$ in non-mouldy living rooms of wheezy children, 283 CFU/m$^3$ in mouldy living rooms of the controls and 271 CFU/m$^3$ in non-mouldy living rooms of the controls. Comparable results were obtained for the child's bedroom and the other room in the house. The geometric mean numbers of CFU/m$^3$ found in the homes of wheezy and non-wheezy children were not different. This was explained by the authors partly from bias in the reporting of symptoms and partly because mild to moderate visible mould growth in the home might have had only a small impact on the numbers of CFU/m$^3$. They therefore concluded that the absence of clear differences in the numbers between the homes with reported mould growth by parents of children with or without wheeze might not be remarkable. Furthermore, it should be pointed out that the initial reports on mould growth and wheezing were obtained in 1986, whereas sampling for fungal propagules was carried out approximately one year later.

Martin *et al.* (1987) also carried out a study in a deprived area of Edinburgh, UK. This was conducted in response to the concern of residents about the possible adverse health effects of home dampness. Occupants of a random sample of 500 homes out of 2,000 were asked to participate in the study and the response rate was 73%. A questionnaire was used to obtain information about children's health in the previous two months, possible confounding variables (among others socio-economic status, smoking), and whether the residents considered their home damp or not. Environmental health officers inspected 300 homes for signs of dampness and mould growth and according to these

officers, 24% of the dwellings were damp, and mould growth was observed in 17% of the homes. Damp houses were frequently overcrowded, their residents significantly younger and more likely to have children, than those in dry houses.

The prevalence of health symptoms among adults was high; 85% of the respondents reported at least one symptom. For adults no significant differences in reported symptom prevalences were found between those living in damp houses and those in dry houses, but for children living in damp houses (n=33) aches and pains, diarrhoea, nerves, headache and any respiratory symptom were significantly more reported than for children living in dry homes (n=68). Controlling for smoking and number of children in the home did not change these differences. A comparison was also made between children living in mouldy homes (n=24) and in non-mouldy homes (n=77). With the addition of vomiting and sore throat, the same differences were found as for dampness. Using reported home dampness, no significant differences were found between the reported symptom prevalences of children living in damp and dry houses. Therefore the authors concluded that over-reporting of symptoms by respondents who claimed that their homes were damp, was not a likely explanation of the associations found in the comparison of observed damp and dry homes.

In a follow-up of this work, a cross-sectional study of a random sample of 597 households in specific areas of Glasgow, Edinburgh and London, UK, including 1,169 children, was conducted (Platt *et al.* 1989). A questionnaire was used to obtain information about the health status (including some respiratory symptoms) of the children in the past two weeks. Questions on a number of possible confounders, e.g. smoking in the home, type of heating, socio-economic status and the presence of pets, were also included. Then, the house was inspected by trained investigators to assess home dampness, visible mould growth, and details of the construction of the home. For each room in the home a score was given for dampness and mould on a four point scale of severity (0=none; 3=severe). The average score for the entire home was considered to be the overall dampness or mouldiness. Furthermore, air samples, taken with a Surface Air System sampler (see Chapter 4), were carried out in the kitchen, living room, and bedrooms of the houses in Glasgow and Edinburgh, to investigate the presence of fungal propagules. The number of CFU/m$^3$ were divided into five groups (low, <100 CFU/m$^3$; medium, 101-300 CFU/m$^3$; high, 301-1,000 CFU/m$^3$; very high, 1,001-5,000 CFU/m$^3$; and extremely high, >5,000 CFU/m$^3$).

In approximately 30% of the homes no dampness or mould was observed by the investigators. Evidence of dampness was observed in approximately 23% of the dwellings, whereas in 45.9% of the homes visible mould growth was observed. There were no clear differences in the other home characteristics investigated between the three different groups of houses. The observations made by the investigators were significantly and positively related to self reported dampness and mould growth (kappa=0.26, $p<0.001$). However, there was disagreement about the damp and mould state in approximately 31% of the homes. A significant positive association was found between the number of CFU/m$^3$ and self reported dampness and mould growth ($r=0.14$, $p<0.001$).

The prevalence of reported health symptoms was highest for children living in homes with visible mould growth, followed by the children living in homes with dampness only. These differences were significant for wheezing, sore throat, persistent headaches, fever and high temperature, persistent cough and runny nose. After controlling for overcrowding, smoking in the home and socio-economic status, these differences remained significant, except for persistent cough. Dose-response relationships were calculated

between the severity of dampness, mould growth, and numbers of CFU/m$^3$, and the reported prevalences (Kendall's tau c). Significant relations were found between the severity of dampness and wheezing, vomiting, sore throat, persistent headaches, fever and high temperature, persistent cough and runny nose. The severity of visible mould growth correlated significantly with wheezing, sore throat, persistent headaches, fever and high temperature, and runny nose. Higher numbers of CFU/m$^3$ were associated with a higher prevalence of wheeze and fever and high temperature.

According to the authors, the design of the study ruled out the influence of investigator and respondent bias on the associations found. They also stated that selection bias was unlikely to account for the findings. Therefore, the authors concluded that home dampness and mould growth have adverse effects on the physical and psychological well-being of children (and adults, see section 2.2).

Ross and co-workers (1990) investigated the relation between upper respiratory tract infection in 297 children aged 24 to 59 months and indoor humidity in Staffordshire, UK. Indoor temperature and humidity were measured during six days in the living room and the child's bedroom, using thermohygrographs. These measurements were carried out in parallel in approximately 20 homes and the results of the inside measurements were corrected for the mean outside conditions during the study period (December 1984 to May 1985). Information about upper respiratory tract infection was obtained by questionnaire and medical records.

There were no differences between mean relative humidity in the living room or bedroom for children with or without reported upper respiratory tract infections. Also, no association was found with recorded upper respiratory infection. Furthermore, there was no relation between relative indoor humidity and reported respiratory symptoms, e.g. cough, attacks of asthma and bronchitis. However, cooler bedroom temperatures were associated with wheezing. In this study, no information was gathered about the presence of damp patches or visible mould growth in the homes.

Two studies were reported from Scandinavia. Andrae *et al.* (1988) investigated the development of bronchial hyperreactivity and allergic disorders of the respiratory tract in children, in relation to a number of environmental factors, including home dampness. In total 4,990 children, aged 6 months to 16 years, were included in the study, which was conducted in the suburbs of Norrköping, Sweden. A questionnaire was used to obtain data on respiratory symptoms (prolonged cough, exercise-induced cough, heavy breathing and wheezing, allergic asthma and allergic rhinitis) and indications of damage in the home due to dampness. Skin prick tests were performed with, amongst others, extracts of house dust mites and moulds in a random sample of the study population. Also, total IgE and specific IgE was measured by PRIST and RAST respectively. A random sample of 34 homes was visited by health inspectors to evaluate damage in the home due to dampness.

Damage in the home due to dampness was reported for approximately 8% of the homes. There was a good agreement between the reports of the parents and the observations made by the health inspectors. The odds ratios using damage due to dampness as exposure variable, restricted to children not exposed to environmental tobacco smoke, ranged from 1.0 (95% CI not presented) for exercise induced cough to 1.9 (95% CI 1.3-2.9) for prolonged cough after respiratory tract infection. Only the last one was statistically significant. A similar comparison for children whose parents smoked revealed higher odds ratios for all respiratory symptoms investigated, ranging from 1.8

(95% CI 1.1-2.8) for allergic rhinitis to 2.9 (95% CI 1.5-5.5) for birch pollen induced asthma, suggesting synergistic effects. The analyses were also stratified according to the type of dwelling and residential area. The associations were stronger for those children with a family history of atopic disease, however, no results were presented regarding the skin prick tests and total and specific IgE determinations.

Jaakkola and co-workers (1993) conducted a population-based cross-sectional study among 2,568 children aged 1 to 6 years in Espoo, Finland. Questionnaires were used to obtain information on respiratory symptoms during the previous year (nasal congestion, nasal excretion, persistent cough, persistent phlegm, persistent wheeze and current asthma) and home dampness (mould odour, visible mould, water damage and damp stains). For the last three of these, information was obtained for the previous year and earlier, separately.

In almost 20% of the homes dampness or mould problems were reported to have occurred some time in the past and in 12% of the homes during the past year. Visible mould and mould odour during the past year were both reported in approximately 3% of the homes. There were no essential differences in the distribution of personal characteristics (e.g. age, gender, socio-economic status) and other potential confounders like passive smoking, the presence of a gas stove, the presence of furred or feathered pets, between the exposed and reference group. The prevalences of the respiratory symptoms considered in the study - except from current asthma - were higher among the exposed children. The estimated odds ratios, adjusted for potential confounders, using visible mould in the past year as exposure variable, ranged from 0.63 (95% CI 0.08-4.76) for current asthma to 4.71 (95% CI 1.91-11.6) for persistent phlegm. Using moisture during the past year as exposure variable, significant relations were found for weekly nasal congestion (OR 2.01. 95% 1.18-3.42), persistent cough (OR 2.29, 95% CI 1.25-4.19) and persistent phlegm (OR 2.93, 95% CI 1.47-5.82). The adjusted odds ratios were lower, using visible mould or moisture at earlier periods as indicators of exposure. However, water damage during earlier periods was associated more strongly with respiratory symptoms than water damage during the past year.

The presence of mould odour during the past year was strongly associated with reported respiratory symptoms. The adjusted odds ratios were highest for persistent phlegm (6.87, 95% CI 3.17-14.9), persistent wheezing (4.31, 95% CI 1.61-11.6) and persistent cough (3.88, 95% CI 1.88-8.01). The association was also significant for weekly nasal congestion and weekly nasal excretion (2.39, 95% CI 1.15-4.98 and 2.38, 95% CI 1.13-4.99 respectively), but not for current asthma (1.46, 95% CI 0.34-6.29). Furthermore, a dose-response relationship was found with the frequency of reported mould odour.

The role of atopy was investigated by estimating adjusted odds ratios for atopic and non-atopic children separately; these were similar for both groups.

The authors stated that in their study, information bias was not likely to have had a major impact on the associations found as the work was part of a general study on the relation between indoor environmental factors and childhood respiratory disease with no special attention to dampness and mould questions. Furthermore, the study population was not generally aware of the potentially adverse health effects of home dampness. Therefore, the authors concluded that their investigations provided further evidence for the relation between home dampness and moulds in the home and respiratory morbidity in children. They stressed the possible importance of mould odour as indicator of exposure.

In the United States, the relationship between measures of home dampness and respiratory morbidity in children was investigated as part of a large epidemiological study on air pollution and respiratory health (Brunekreef *et al.* 1989). A cohort of 4,625 white children from 7 to 11 years of age, living in six US communities was included in the study. Information about home dampness, which included visible mould growth, water damage and flooding of the basement floor, was obtained using a questionnaire, completed by the parents. A home was considered damp if any of the above features was present. A standardized questionnaire for respiratory symptoms was used and information about the prevalence of nonchest illnesses was also obtained. Furthermore, pulmonary function was measured by $FEV_1$, FVC and $FEF_{25-75}$.

Overall, approximately 55% of the homes were considered to be damp, with visible mould growth reported in approximately 30% of the homes and water damage and flooding of the basement floor in approximately 17% and 32% respectively. The prevalence of reported respiratory symptoms of children living in damp homes was consistently higher than the prevalence for children living in homes without reported dampness. Using visible mould growth as indicator of exposure, the adjusted odds ratios varied from 1.27 (95% CI 0.93-1.74) for doctor-diagnosed asthma, to 2.12 (95% CI 1.64-2.32) for cough. The odds ratios were adjusted for age, sex, city of residence, parental education and maternal smoking. Using home dampness as measure of exposure the adjusted odds ratios varied from 1.23 for wheeze (95% CI 1.10-1.39) to 2.16 (95% CI 1.64-2.84) for cough. Similar results were obtained using water damage or flooding of the basement floor as indicators of exposure. Using nonchest illness as outcome variable, the adjusted odds ratios were 1.40 (95% CI 1.13-1.74) for visible mould growth and 1.55 (95% CI 1.25-1.93) for home dampness.

Associations between reported moulds and respiratory symptoms were also expressed as prevalence ratios for three different groups of children: doctor-diagnosed asthmatics (n=214), children with wheeze but without doctor-diagnosed asthma (n=291) and children with neither asthma or wheeze (n=3,799). For the first group the prevalence ratios varied from 0.88 for bronchitis to 1.50 for cough, and in the second, the prevalence ratios varied from 1.13 for respiratory illness before the age of 2 years to 1.73 for cough. For the non-asthmatic non-wheezers the prevalence ratios varied from 1.13 for chest illness to 1.74 for bronchitis. There were no significant associations between the different home dampness variables and FVC or $FEV_1$, after adjustment for age, height, weight, gender, city of residence, parental education and maternal smoking. $FEF_{25-75}$ was negatively related to visible mould growth.

To address the issue of possible over-reporting of symptoms by parents living in damp homes, the relation between persistent wheeze and pulmonary function was assessed separately for children living in homes with and without reported mould growth. $FEV_1$ and $FEF_{25-75}$ were similarly reduced in both groups, irrespective of the presence of visible mould. Therefore, the authors concluded that the associations found were not caused by over-reporting of symptoms by parents living in damp homes. However, the results did show a consistent and strong association between reported home dampness and childhood respiratory symptoms, apart from reported doctor-diagnosed asthma. The authors suggested that parents of this latter group tend to take remedial action (move or modify the home environment to reduce the exposure to inhalant allergens). They also stressed the association found between home dampness and nonchest illnesses, because it has been suggested that moulds can also cause systemic effects.

Dales *et al.* (1991a) conducted a questionnaire-based study on the association between respiratory symptoms of approximately 14,500 children aged 5 to 8 years from

30 different Canadian communities and home dampness and mould. The exposure variables used were mould sites (number of sites with visible mould during the past year), moisture (appearance of wet or damp spots in the past year, excluding the basement), flooding (flooding, water damage or leaking in the basement during the past year), and dampness/mould, in which the previous variables were combined. The outcome variables were persistent cough, persistent wheeze, wheeze with dyspnoea, current asthma, chest illness, upper respiratory symptoms, nonrespiratory symptoms and eye irritation. Information was also obtained about possible confounders. Stratification by the presence or absence of a report of a physician-confirmed allergy to mould or dust occurring in the past year was performed in the statistical analyses. Furthermore, the data were also stratified by the presence or absence of "accidents or illnesses", to investigate the possible impact of reporting bias.

Visible moulds were reported in 32.4% of the homes, moisture in 14.1% and flooding in 24.1% of the homes with a basement. The reported prevalences of cough, wheeze, asthma, bronchitis and chest illness were approximately 50% higher in damp houses and those of upper respiratory symptoms and nonrespiratory symptoms were increased by 20% to 25%. The unadjusted odds ratios were as follows: cough 1.89 (95% CI 1.63-2.20), wheeze 1.58 (95% CI 1.42-1.76), asthma 1.45 (95% CI 1.23-1.71), bronchitis 1.32 (95% CI 1.18-1.48) and chest illness 1.52 (95% CI 1.37-1.70). For nonrespiratory symptoms and eye irritation the crude odds ratios were 1.43 (95% CI 1.33-1.55) and 1.53 (95% CI 1.33-1.76) respectively. A positive association was found with the number of mould sites in the home. The crude odds ratios were higher comparing two versus zero sites, than one versus zero. The odds ratios were adjusted for age, gender, race, education of parent/guardian, gas cooking, number of smokers in the home and region of residence. The adjusted odds ratios ranged from 1.08 for wheeze with dyspnoea in relation to flooding, to 2.55 for cough in relation to the number of mould patches. Overall, the adjusted odds ratios were similar to the crude odds ratios. When the analysis was stratified according to the presence or absence of physician-diagnosed mould allergy, lower odds ratios were found for children with mould allergy. The odds ratios ranged from 0.64 for asthma to 1.75 for upper respiratory symptoms. The same pattern was found if the analysis was stratified according to the presence of dust allergy. Lower odds ratios were found for those with reported dust allergy. Stratification by reported accidents or illnesses not of the chest (as crude indicators of over-reporting), revealed no differences.

### 2.3 Home dampness and respiratory morbidity in adults

In the study reported by Waegemaekers *et al.* (1989), the association between adult respiratory symptoms and home dampness was also assessed. For men, the crude odds ratios ranged from 0.74 (95% CI 0.19-2.86) for asthma, to 4.45 (95% CI 1.50-13.22) for persistent wheeze. For women, the crude odds ratios varied from 2.23 (95% CI 0.27-32.09) for shortness of breath, to 5.94 for phlegm (95% CI 0.67-51.96) and persistent wheeze (95% CI 1.30-27.10). Adjustment for age, smoking, heating system, closed or open kitchen, presence of pets and type of floor cover in the living room and bedroom increased most of the odds ratios for both men and women, several becoming statistically significant at a p-value of 0.01. As indicated before, these results may partly have been caused by responder bias since home dampness was a known problem in this study population.

Brunekreef (1992b) reported on the relationship between home dampness and adult respiratory symptoms among a group of adults in Helmond, the Netherlands (parents or guardians of the 3,344 children studied by Brunekreef 1992a). The data were obtained as part of a study on childhood respiratory symptoms and was carried out for reasons not related to parental anxiety about home dampness. A questionnaire was used to obtain information about the presence of damp stains and/or mould patches in the home during the previous two years. The outcome variables included cough, phlegm, wheeze, asthma, inhalation allergy, and an index for lower respiratory symptoms.

Damp stains were reported in approximately 24% of the homes, and visible mould growth in 15% of the homes. For women, cough, wheeze and lower respiratory symptoms were statistically significantly related to both damp stains and mould patches. The crude prevalence ratios varied from 1.30 (95% CI 1.11-1.30) for wheeze, to 1.52 (95% CI 1.19-1.95) for cough. For men, significant associations were found for cough, phlegm, wheeze and lower respiratory symptoms. The crude prevalence ratios varied from 1.44 (95% CI 1.15-1.79) for wheeze, to 2.58 (95% CI 1.80-2.98) for phlegm. Adjustment for active and passive smoking, socio-economic status and indoor nitrogen dioxide sources did not change these associations substantially. The adjusted odds ratios were highest for cough and phlegm and lowest for asthma and allergy. Furthermore, stratification by reported allergy for house dust or pollen indicated comparable odds ratios for allergic and non-allergic subjects. Therefore, the author concluded that mechanisms other than immediate hypersensitivity type I reactions might be of importance in the relation between home dampness and respiratory symptoms. This is supported by the fact that the strongest associations were found for cough and phlegm, instead of wheeze and asthma. However, as it was a cross-sectional study, control for potential selection bias caused by allergen avoidance measures taken in the past by allergic and asthmatic subjects, was not possible.

In the study by Platt and co-workers (1989), a health questionnaire was also administered to the principal occupant (usually the female in the household). The prevalence of reported health symptoms was highest in adults living in homes with visible mould growth, followed by those living in homes with dampness only. These differences were significant for bad nerves, aching joints, nausea-vomiting, backache, blocked nose, fainting spells, constipation and breathlessness. After controlling for socio-economic status and smoking in the home, using logistic regression analysis, these differences remained significant. Persistent cough and wheezing were also reported more frequently in adults living in mouldy homes, however, these differences were not significant. Dose-response relationships were calculated between the severity of dampness, mould growth, and numbers of CFU/m$^3$, and the reported prevalences (Kendall's tau c). Significant relations were found between the severity of dampness and tiredness, high blood pressure, persistent cough, bad nerves, wheezing, nausea-vomiting, blocked nose, fainting spells and breathlessness. The severity of visible mould growth was significantly correlated with bad nerves, aching joints, nausea-vomiting, blocked nose and feeling depressed. Higher numbers of CFU/m$^3$ were associated with a higher prevalence of high blood pressure, bad nerves, backache, palpitations, and breathlessness.

Dales and co-workers (1991b) also investigated the relation between home dampness and respiratory symptoms among adults (over 21 years of age), as part of their Canadian study of young children. The exposure variables used were mould sites (number of sites with visible mould during the past year), moisture (appearance of wet or damp spots in

the past year, excluding the basement), flooding (flooding, water damage or leaking in the basement during the past year), and dampness/mould, in which the previous variables were combined. The outcome variables were persistent cough, persistent phlegm, wheeze, wheeze with dyspnoea, asthma, chronic respiratory disease, upper respiratory symptoms, eye irritation and other symptoms. Information was also obtained about possible confounders. Completed questionnaires were available for 14,799 respondents, of whom approximately 80% were female. Dampness and mould were reported for approximately 36% of the homes. The odds ratios, which were adjusted for age, gender, education, ethnic group, smoking and presence of pets, ranged from 1.45 (95% CI 1.29-1.64) for chronic respiratory disease, to 1.62 (95% CI 1.48-1.78) for lower respiratory symptoms. A positive relation was found with the number of mould sites in the home and this was comparable with the association found for children (Dales *et al.* 1991a). The analysis was also stratified according to the presence or absence of physician-diagnosed allergy (mould, dust, pollen or animals) and physician-diagnosed asthma. The adjusted odds ratios were comparable, irrespective of the presence or absence of allergy and asthma. To investigate whether the associations found were caused by subjects over-reporting both symptoms and home dampness, reported accidents or illnesses not related to dampness and moulds, were used as crude indicators of over-reporting. For those groups not reporting accidents or other illnesses, the association between home dampness and respiratory symptoms remained statistically significant.

## 2.4 Discussion and conclusions

In this Chapter a review has been presented of recent epidemiological studies on the relation between home dampness and respiratory symptoms in both children and adults. These show several common features. Firstly, the design of all studies was cross-sectional, and secondly, in most studies information about the exposure variables (home dampness and mould) and the outcome variables (respiratory symptoms) was obtained by questionnaires, completed by one of the adult occupants. In addition, in four of the studies home dampness was assessed using a checklist (Varekamp and Leupen 1970; Van der Maas 1979) or by trained housing inspectors (Martin *et al.* 1987; Platt *et al.* 1989), in order to obtain more objective information about the potential exposure. In three studies, further information about the exposure was obtained by measuring the numbers of airborne fungal propagules in (part of) the homes (Waegemaekers *et al.* 1989; Platt *et al.* 1989; Strachan *et al.* 1990). Reported respiratory symptoms in children were compared in three studies (Strachan 1988; Brunekreef *et al.* 1989; Brunekreef 1992a), to measures of pulmonary function of the children.

The results of all these epidemiological studies indicate that *reported* home dampness and mould are associated with increased prevalences of reported respiratory symptoms in both children and adults. The magnitude of the associations found in the different population based studies, are comparable (see Tables 2.1 and 2.2). For example, the adjusted odds ratios for reported dampness and cough in children range from 1.57 (95% CI 1.06-2.32, Brunekreef 1992a) to 2.29 (95% CI 2.29-4.19, Jaakkola *et al.* 1993). For wheeze these figures are 1.23 (95% CI 1.10-1.39, Brunekreef *et al.* 1989) and 1.96 (95% CI 0.80-4.78, Jaakkola *et al.* 1993). The largest variation is found for asthma: from 0.24 (95% CI 0.03-1.24, Jaakkola *et al.* 1993) to 1.70 (95% CI 0.75-3.87, Brunekreef 1992a). Using reported mould as exposure variable, the adjusted odds ratios for cough range from 1.89 (95% CI 1.63-2.20, Dales *et al.* 1991a) to 3.06 (95% CI 1.29-7.26, Brunekreef

1992a). For wheeze these figures are 1.37 (95% CI 0.58-3.26, Brunekreef 1992a) and 3.00 (95% CI 1.72-5.25, Strachan 1988), and for asthma 0.63 (95% CI 0.08-4.76, Jaakkola *et al.* 1993) and 1.96 (95% CI 1.50-2.60, Brunekreef 1992a). In general, the associations were stronger using reported moulds as exposure variable than reported dampness. Overall, the highest odds ratios were found for reported cough, followed by reported wheeze. The lowest odds ratios were found for reported asthma. This might be caused by frequent remedial action taken by parents of asthmatic children compared with the parents of children with reported wheeze or cough.

Using *observed* home dampness as exposure variable, the prevalence ratios for children with reported cough range from 1.37 to 1.38, and for reported wheeze from 1.61 to 1.66 (Martin *et al.* 1987; Platt *et al.* 1989). Reported asthma was not included in these studies.

A number of potential confounders might have resulted in an artificial association between reported or observed home dampness and reported respiratory symptoms. Firstly, respiratory health and home dampness might be indirectly related through socio-economic status or through other indoor environmental factors such as type of heating and cooking fuels used, the presence of pets, or smoking in the home. However, in most of the studies the crude associations between reported home dampness and reported respiratory health did not alter substantially after adjustment for these potential confounders. A detailed breakdown of reported mould by paternal and maternal education level performed by Brunekreef (1992a), showed no clear relationship between educational level and the reporting of mould in the home.

Secondly, the outdoor environment might have affected the prevalence of reported respiratory symptoms and reported dampness indoors. The reviewed epidemiological studies have been carried out in a range of different climatic conditions, from areas with prolonged periods of cold and dry weather (Finland, Sweden, Canada), to areas with a more temperate climate (UK, the Netherlands). Even within one country (Canada, US), the studies have been carried out in different regions, where there can be large differences in average temperature, humidity and ambient pollution. In all these studies, connections between reported or observed home dampness and reported respiratory symptoms were found, indicating that these associations are relatively unaffected by outdoor environmental conditions.

Because most of the studies used questionnaires to obtain information about both the exposure variables and the outcome variables, the associations found might have been (partly) caused by two different types of information bias. The first type of bias is the fact that some respondents have a tendency toward over-reporting and others toward under-reporting, which results in those reporting more respiratory symptoms to report more dampness, and vice versa. Information about the presence and possible impact of this type of bias can be obtained by including questions assessing this type of information bias. In the studies of Dales *et al.* (1991a, 1991b) the possible impact of this type of bias was investigated using the question "Within the past year have you had any other major illnesses or accident?" as indication of general over-reporting. After stratification of the analyses by the responses to this question, the observed associations persisted.

The second type of information bias is introduced if symptomatic subjects or parents/guardians of symptomatic children are more likely to report the presence of dampness and mould, or if subjects living in damp houses are more likely to report respiratory symptoms for themselves and/or their children. This type of bias might be of particular interest in studies carried out in areas where home dampness is considered to

be a widespread problem (e.g. Strachan and Elton 1986; Martin *et al.* 1987; Waegemaekers *et al.* 1989). Three studies tried to investigate the influence of this type of bias by measuring pulmonary function as a more objective measure than reported respiratory symptoms (Strachan 1988; Brunekreef *et al.* 1989; Brunekreef 1992a). Comparison of the pulmonary function of children with reported respiratory symptoms living in homes with mould growth and in homes without reported mould growth showed no evidence of over-reporting of respiratory symptoms by parents living in damp and mouldy homes (Brunekreef *et al.* 1989; Brunekreef 1992a). In contrast, Strachan (1988) reported some evidence of reporting bias by comparing the pulmonary function of wheezy children in homes with and without reported mould growth. With the same pulmonary function, wheeze was more frequently reported by parents of children in homes with reported mould growth than those in homes without reported mould growth.

Two studies (Andrea *et al.* 1988; Platt *et al.* 1989) compared reported dampness by the residents and observed dampness by the investigators. In both studies, a good agreement was found between reported and observed dampness, suggesting no reporting bias with respect to home dampness. However, disagreement was reported for approximately 30% of the homes (Platt *et al.* 1989), or only part of the homes were inspected by the investigators (Andrae *et al.* 1988).

In three studies (Waegemaekers *et al.* 1989; Platt *et al.* 1989; Strachan *et al.* 1990) airborne fungal propagules were measured in an attempt to validate the resident-reported exposure variables. Waegemaekers *et al.* (1989) and Platt *et al.* (1989) reported positive associations between reported home dampness and the total numbers of CFU/m$^3$. Furthermore, they reported positive associations between the prevalence of (some of the) reported respiratory symptoms and the numbers of airborne fungal propagules. In contrast, Strachan and co-workers (1990) found no associations between reported dampness, the prevalence of wheeze, and airborne fungal counts. However, the value of the measurement of airborne fungal propagules as an estimator of the exposure to fungi as part of epidemiological studies is questionable (see Chapter 4). In only one study (Strachan and Sanders 1989) was the mean relative humidity (over one week) measured as an objective measure of home dampness. Weak associations were found between this and reported home dampness.

It is remarkable that in most studies only fungi and fungal products were put forward as possible causative agents for the associations between home dampness and respiratory morbidity. In the few studies in which measurements were carried out to assess the exposure, only airborne fungal propagules were measured. However, it is well known that not only fungi but also house dust mites encounter favourable conditions for their development in damp environments and that house dust mites produce inhalant allergens (see Chapter 3).

In conclusion, the results of recent epidemiological studies indicate an association between reported or observed home dampness and mould, and increased prevalences of reported respiratory symptoms in both children and adults. This association is independent of possible confounders like socio-economic status and other indoor environmental factors and is unaffected by outdoor environmental factors. No clear indications of reporting bias were found in most of the studies, where home dampness was not considered to be a widespread problem.

In order to validate the associations found, it is necessary to establish objective methods for measuring home dampness. Further research is also required concerning the mechanisms which connect home dampness with respiratory disease in children and adults. The assessment of the exposure to both fungi and house dust mites as possible causative agents should be part of epidemiological studies on the relationship between home dampness and respiratory morbidity.

Table 2.1 Summary of the main results of epidemiological studies on the relation between home dampness and respiratory morbidity in children

| reference | study design | exposure variables | outcome variables | odds ratios (95% CI) | remarks |
|---|---|---|---|---|---|
| Waegemaekers *et al.* (1989) Katwijk, the Netherlands | Cross-sectional (n=190) Questionnaire Inspection Airborne fungal propagules | reported dampness and mould | morning cough | 2.99 (1.28-6.97) | Conducted in an area with concern about home dampness. Positive association between prevalence of respiratory symptoms and numbers of CFU/$m^3$. |
| | | | day/night cough | 1.54 (0.77-3.10) | |
| | | | shortness of breath | 0.92 (0.32-2.61) | |
| | | | wheeze | 2.80 (1.18-6.64) | |
| | | | shortness + wheeze | 2.82 (1.08-7.38) | |
| | | | runny nose | 5.92 (3.03-12.57) | |
| | | | asthma | 2.80 (0.39-20.02) | |
| | | | bronchitis | 3.73 (1.63-8.51) | |
| | | | pneumonia | 4.46 (1.54-12.90) | |
| | | | allergy | 6.13 (2.12-17.80) | |
| Brunekreef (1992a) the Netherlands, south-east | Cross-sectional (n=1,051) Questionnaire Pulmonary function | reported damp stains | cough | 1.97 (0.88-4.41) | No association between reported home dampness and pulmonary function. Decline of pulmonary function for children with reported CNSRD independent of reported dampness. |
| | | | wheeze | 1.46 (0.72-2.96) | |
| | | | asthma | 1.70 (0.75-3.87) | |
| | | reported mould | cough | 3.06 (1.29-7.26) | |
| | | | wheeze | 1.37 (0.58-3.26) | |
| | | | asthma | 1.12 (0.91-3.38) | |
| Brunekreef (1992a) Helmond, the Netherlands | Cross-sectional (n=3,344) Questionnaire | reported damp stains | cough | 1.57 (1.06-2.32) | Indication of selection process by moving or taking remedial action by parents of symptomatic children. No relation between socio-economic status and mould reports. |
| | | | wheeze | 1.52 (1.23-2.13) | |
| | | | asthma | 1.30 (0.91-1.85) | |
| | | reported mould | cough | 2.05 (1.35-3.19) | |
| | | | wheeze | 1.90 (1.41-2.54) | |
| | | | asthma | 1.96 (1.50-2.60) | |
| Strachan (1988) Edinburgh, UK | Cross-sectional (n=873) Questionnaire Pulmonary function | reported mould | wheeze | 3.00 (1.72-5.25) | Indication of reporting bias. Prevalence of CNSRD and pulmonary function not related with mean RH (Strachan and Sanders 1990). No relation between wheeze and numbers of CFU/$m^3$ (Strachan *et al.* 1990). |
| | | | chest colds | 2.08 (1.22-3.51) | |
| | | | nasal discharge | 1.61 (0.89-2.90) | |
| | | | cough at night | 0.92 (not given) | |
| | | | cough during the day | 0.95 (not given) | |

Table 2.1 Continued

| reference | study design | exposure variables | outcome variables | odds ratios (95% CI) | remarks |
|---|---|---|---|---|---|
| Platt *et al.* (1989)<br>Glasgow, London, Edinburgh, UK | Cross-sectional<br>(n=1,169)<br>Questionnaire<br>Inspection<br>Airborne fungal propagules | observed dampness | wheezing | 1.15 (prevalence ratio) | Reported and observed dampness significantly associated. |
| | | | sore throat | 0.80 | |
| | | | fever | 1.58 | Reported dampness and mould correlated with numbers of CFU/$m^3$. |
| | | | persistent cough | 1.21 | |
| | | | runny nose | 1.03 | |
| | | observed mould | wheezing | 1.66 | Numbers of CFU/$m^3$ positively associated with the prevalence of wheeze, irritability and fever-high temperature. |
| | | | sore throat | 1.39 | |
| | | | fever | 2.15 | |
| | | | persistent cough | 1.38 | |
| | | | runny nose | 1.30 | |
| Andrae *et al.* (1988)<br>Norrköping, Sweden | Cross-sectional<br>(n=4,990)<br>Questionnaire | reported damage due to dampness | cough >2 weeks | 1.9 (1.3-2.9) | Figures are for children not exposed to ETS. |
| | | | exercise cough | 1.0 (not given) | |
| | | | allergic asthma | 1.3 (not given) | Association stronger for children with a family history of atopy. |
| | | | birch pollen asthma | 1.7 (not given) | |
| | | | allergic rhinitis | 1.1 (not given) | |
| Jaakkola *et al.* (1993)<br>Espoo, Finland | Cross-sectional<br>(n=2,568)<br>Questionnaire | reported mould | persistent cough | 2.85 (1.24-6.56) | Indication of dose-response relation between frequency of mould odour and respiratory symptoms. |
| | | | persistent phlegm | 4.71 (1.91-11.6) | |
| | | | persistent wheeze | 2.69 (0.80-9.02) | |
| | | | current asthma | 0.63 (0.08-4.76) | |
| | | reported damp | persistent cough | 2.29 (1.25-4.19) | Odds ratios similar for atopic and non-atopic children. |
| | | | persistent phlegm | 2.93 (1.47-5.82) | |
| | | | persistent wheeze | 1.96 (0.80-4.78) | |
| | | | current asthma | 0.24 (0.03-1.24) | |
| | | mould odour | persistent cough | 3.88 (1.88-8.01) | |
| | | | persistent phlegm | 6.87 (3.17-14.9) | |
| | | | persistent wheeze | 4.31 (1.61-11.6) | |
| | | | current asthma | 1.46 (0.34-6.29) | |
| Brunekreef *et al.* (1989)<br>Six studies, USA | Cross-sectional<br>(n=4,625)<br>Questionnaire<br>Pulmonary function | reported mould | wheeze | 1.79 (1.44-2.32) | Associations between reported respiratory symptoms and moulds were strongest for non-asthmatics and nonwheezers. |
| | | | cough | 2.12 (1.64-2.73) | |
| | | | bronchitis | 1.48 (1.17-1.87) | |
| | | | chest illness | 1.40 (1.11-1.78) | |

| | | | | | |
|---|---|---|---|---|---|
| | | | lower resp. illness | 1.57 (1.31-1.87) | No associations between reported dampness and mould and FVC and $FEV_1$. $FEF_{25-75}$ negatively associated with reported mould. $FEV_1$ and $FEF_{25-75}$ similarly reduced for wheezing children living in homes with and without reported mould. |
| | | | resp. illness before age 2 | 1.42 (1.12-1.80) | |
| | | | asthma | 1.27 (0.93-1.74) | |
| | | | hay fever | 1.57 (1.31-1.87) | |
| | | | nonchest illness | 1.40 (1.13-1.74) | |
| | | reported dampness | wheeze | 1.23 (1.10-1.39) | |
| | | | cough | 2.16 (1.64-2.84) | |
| | | | bronchitis | 1.32 (1.05-1.67) | |
| | | | chest illness | 1.52 (1.20-1.93) | |
| | | | lower resp. illness | 1.68 (1.41-2.01) | |
| | | | resp. illness before age 2 | 1.40 (1.11-1.78) | |
| | | | asthma | 1.42 (1.04-1.94) | |
| | | | hay fever | 1.26 (1.06-1.50) | |
| | | | nonchest illness | 1.39 (1.55-1.93) | |
| Dales *et al.* (1991) 30 communities, Canada | Cross-sectional (n=13,495) Questionnaire | reported dampness and mould | cough | 1.89 (1.63-2.20) | Figures are unadjusted odds ratios. The adjusted odds ratios were similar to the crude odds ratios. Lower odds ratios were found for children with physician-diagnosed mould allergy compared with controls. Lower odds ratios were found for children with physician-diagnosed dust allergy compared with controls. Stratification by 'accidents' or 'illnesses' (not of the chest) showed no differences. |
| | | | wheeze | 1.58 (1.42-1.76) | |
| | | | wheeze with dyspnea | 1.61 (1.40-1.86) | |
| | | | asthma | 1.45 (1.23-1.71) | |
| | | | bronchitis | 1.32 (1.18-1.48) | |
| | | | chest illness | 1.52 (1.37-1.70) | |
| | | | upper resp. symptoms | 1.46 (1.36-1.57) | |
| | | | eye irritation | 1.53 (1.33-1.76) | |
| | | | non resp. symptoms | 1.43 (1.33-1.55) | |
| | | moisture | cough | 1.91 (1.60-2.27) | |
| | | | wheeze | 1.74 (1.53-1.98) | |
| | | | wheeze with dyspnea | 1.68 (1.41-1.99) | |
| | | | asthma | 1.58 (1.29-1.94) | |
| | | | bronchitis | 1.49 (1.30-1.72) | |
| | | | chest illness | 1.47 (1.28-1.69) | |
| | | | upper resp. symptoms | 1.52 (1.38-1.68) | |
| | | | eye irritation | 1.60 (1.35-1.90) | |
| | | | non resp. symptoms | 1.45 (1.31-1.60) | |

Table 2.1 Continued

| reference | study design | exposure variables | outcome variables | odds ratios (95% CI) | remarks |
|---|---|---|---|---|---|
| Dales *et al.* (1991) *continued* | | no. of mould sites | cough | 2.26 (1.80-2.83) | |
| | | | wheeze | 1.73 (1.45-2.06) | |
| | | | wheeze with dyspnea | 2.00 (1.60-2.50) | |
| | | | asthma | 1.67 (1.27-2.19) | |
| | | | bronchitis | 1.46 (1.20-1.78) | |
| | | | chest illness | 1.81 (1.51-2.17) | |
| | | | upper resp. symptoms | 1.74 (1.52-2.00) | |
| | | | eye irritation | 1.74 (1.39-2.19) | |
| | | | non resp. symptoms | 1.72 (1.50-1.96) | |

Table 2.2 Summary of the main results of epidemiological studies on the relation between home dampness and respiratory morbidity in adults

| reference | study design | exposure variables | outcome variables | odds ratios (95% CI) | remarks |
|---|---|---|---|---|---|
| Waegemaekers *et al.* (1989) Katwijk, the Netherlands | Cross-sectional (n=328) Questionnaire Inspection | reported dampness and mould (men) | cough | 1.35 (not given) | Conducted in an area with concern about home dampness. |
| | | | phlegm | 18.1 (not given) | |
| | | | shortness of breath | 9.38 (not given) | |
| | | | wheeze | 4.06 (not given) | |
| | | | persistent wheeze | 8.73 (not given) | |
| | | | asthma | 1.15 (not given) | |
| | | | allergy | 3.45 (not given) | |
| | | reported dampness and mould (women) | cough | 3.48 (not given) | |
| | | | phlegm | 6.21 (not given) | |
| | | | shortness of breath | 2.25 (not given) | |
| | | | wheeze | 4.79 (not given) | |
| | | | persistent wheeze | 6.99 (not given) | |
| | | | asthma | 4.16 (not given) | |
| | | | allergy | 3.84 (not given) | |
| Brunekreef (1992b) Helmond, the Netherlands | Cross-sectional (n=3,344) Questionnaire | reported damp stains and/or mould (men) | cough | 2.56 (1.94-3.38) | Stratification by reported allergy for house dust or pollen showed no differences. |
| | | | phlegm | 2.26 (1.57-3.24) | |
| | | | wheeze | 1.63 (1.30-2.06) | |
| | | | asthma | 1.29 (0.92-1.81) | |
| | | | allergy | 1.24 (0.95-1.73) | |
| | | | lower resp. symptoms | 1.70 (1.38-2.09) | |
| | | reported damp stains and/or mould (women) | cough | 1.75 (1.30-2.36) | |
| | | | phlegm | 1.66 (1.16-2.38) | |
| | | | wheeze | 1.43 (1.15-1.77) | |
| | | | asthma | 1.25 (0.94-1.66) | |
| | | | allergy | 1.03 (0.79-1.35) | |
| | | | lower resp. symptoms | 1.55 (1.27-1.89) | |
| Platt *et al.* (1989) Glasgow, London, Edinburgh, UK | Cross-sectional (n=597) Questionnaire Inspection Airborne fungal propagules | observed dampness | wheezing | 1.18 (prevalence ratio) | Reported and observed dampness significantly associated. Reported dampness and mould correlated with numbers of CFU/$m^3$. |
| | | | bad nerves | 1.17 | |
| | | | aching joints | 1.09 | |
| | | | nausea-vomiting | 1.71 | |
| | | | blocked nose | 0.95 | |
| | | | breathlessness | 1.68 | |

Table 2.2 Continued

| reference | study design | exposure variables | outcome variables | odds ratios (95% CI) | remarks |
|---|---|---|---|---|---|
| Platt *et al.* (1989) *continued* | | observed mould | wheezing<br>bad nerves<br>aching joints<br>nausea-vomiting<br>blocked nose<br>breathlessness | 1.32<br>1.31<br>1.56<br>2.61<br>1.56<br>1.81 | Numbers of CFU/$m^3$ positively associated with the prevalence of high blood pressure, bad nerves, backache, palpitations and breathlessness. |
| Dales *et al.* (1991b) 30 communities, Canada | Cross-sectional (n=14,799) Questionnaire | reported dampness and mould | upper resp. symptoms<br>lower resp. symptoms<br>chronic respiratory disease<br>asthma<br>eye irritation<br>other symptoms | 1.50 (1.39-1.61)<br>1.62 (1.48-1.78)<br>1.45 (1.29-1.64)<br>1.56 (1.25-1.95)<br>1.63 (1.46-1.82)<br>1.46 (1.36-1.58) | A positive association was found between the number of mould sites and the prevalence of respiratory symptoms.<br>Stratification by physician-diagnosed allergies did not significantly influence the results.<br>Stratification by physician-diagnosed asthma did not significantly influence the results.<br>Stratification by 'accidents' or 'illnesses' (not of the chest) showed no differences. |

# Fungi and house dust mites: ecology and health effects

## 3.1 Introduction

In this Chapter a review is given of the ecology of fungi (3.2), the health effects of fungi on human beings (3.3), the ecology of house dust mites (3.4), and the health effects of house dust mites on human beings (3.5). Finally, conclusions are presented as to whether fungi and house dust mites, considering their ecology and health effects, can be regarded as causal factors in the relation between home dampness and respiratory symptoms.

## 3.2 Fungi: ecology

There are approximately 100,000 known species of fungi. Fungi are eukaryotic organisms that are basically formed of chains of cells - so called hyphae - with chitinous or cellulosic rigid cell walls. The polysaccharides make up about 80 to 90% of the dry weight of the fungal cell (Gravesen 1979). Reproduction of fungi occurs by units that may be simple fragments of the fungus body (mycelium) or highly specialized cells (spores) produced on elaborate fruiting structures. Many fungi produce sexual spores. Fungi are divided into four different groupings based on their method of sexual reproduction. Those of interest in indoor environments mainly belong to the class of Deuteromycetes or Fungi Imperfecti, which is an artificial grouping of asexual fungal stages. However, some fungi of interest in indoor environments belong to other classes (e.g. Mucorales, Ascomycetes, wood-rotting Basidiomycetes and some yeasts).

Asexual spores can be produced both within and outside the mycelium. In the first case, an enclosed cell develops, the sporangium, which contains many spores. However, when the spores are formed outside of the cell they are called conidia. The diameter of the spores or conidia produced by fungi ranges from 3 to 200 $\mu$m, but most of them have a diameter of approximately 5 to 10 $\mu$m. The spores are released into the air either by mechanical means or by specialized (active) discharge mechanisms (Burge 1985). Passive or mechanical dispersion occurs, in general, in relatively dry circumstances, where wind and other agitation is involved, or, during actual precipitation where rain splash occurs. Active or biological dispersal often depends on high moisture levels and consequently occurs during periods of high relative humidity. The spores are often liberated in massive concentrations and can remain airborne for a long time travelling over long distances (hundreds of kilometers). They are highly adapted for dispersal and survival with a wall which protects against desiccation. For example, spores of *Penicillium* and *Aspergillus* species sometimes remain viable for a period of more than 12 years in undisturbed conditions (Sussman 1968).

The majority of fungi are saprotrophic i.e. they utilize dead organic material for food. Providing temperature and moisture conditions are met, many species can utilize a wide range of organic materials, ranging from plant or animal remains to materials such as cellulose, paint, or stored products (Gravesen 1979). The water content of these materials is the most critical factor in the development of fungi. Scott (1957) introduced the concept of water activity ($a_w$) as the criterion of water availability in a material to microorganisms. The water activity is defined as the ratio of the vapour pressure above a substrate to the vapour pressure above pure water under the same temperature and pressure. The minimum water activity needed for the growth of fungi on building related substrates varies from 0.75 to more than 0.98 for different species (Grant *et al.* 1989). Grant and co-workers also observed successional colonisation of building related

substrates. They determined fungi as primary colonisers, growing at water activities below 0.80 (e.g. *Penicillium* spp. and *Aspergillus versicolor*), secondary colonisers, growing at water activities between 0.80 and 0.90 (e.g. *Cladosporium* spp.) and tertiary colonisers, growing at water activities higher than 0.90 (e.g. *Ulocladium* spp. and *Stachybotris atra*). Depending on the relative humidity at which fungi germinate, they are also divided into hydrophiles (RH>90%), mesohydrophiles (RH between 80 and 90%) and xerophiles (RH<80%).

The majority of the fungi in indoor environments grow at temperatures between 10 and 35°C, however, some fungi will grow at temperatures between 2 and 5°C (psychrophiles) and others at temperatures as high as 55 to 60°C (thermophiles).

Interior environments, unless scrupulously clean, offer a wide variety of substrates for growth. Damp, non-living organic material can be quickly colonized (Burge 1985; Grant *et al.* 1989). Condensation is the principal source of moisture on internal surfaces of dwellings and besides superficial condensation, interstitial condensation within porous building materials such as concrete, brick and plaster, may provide a moisture reservoir allowing fungal growth to continue in circumstances under which the surface would otherwise dry out. Interior dampness problems are usually related to construction faults, such as inadequate insulation or "cold-bridging", in combination with inadequate ventilation and/or the pattern of usage of these houses. Condensation and mould problems may also be encountered in "tight" houses built to preserve energy, particularly when there are no measures to prevent excessive generation of moisture.

### 3.3 Fungi: health effects

Although the causative role of fungi in individual cases of respiratory allergy and asthma has been known since the 18th century, their overall significance in respiratory health is still debated. Reported allergic reactions to single or clustered spores and hyphal elements of fungi in air include rhinitis, asthma and extrinsic allergic alveolitis (Tobin *et al.* 1987; Flannigan *et al.* 1991). Allergic reactions to fungi may be either immediate, developing within minutes of exposure (type I allergy, IgE-mediated), or delayed, occurring 4 to 8 h later (type III allergy, IgG-mediated). Rhinitis and asthmatic episodes fall into the type I category, whereas the much rarer instances of extrinsic allergic alveolitis (EAA; hypersensitivity pneumonitis) are type III reactions. EAA is most often associated with high levels of actonimycete or fungal spores ($10^6$-$10^{10}$ spores/$m^3$ air) released from moulded agricultural materials causing occupational diseases such as Farmer's Lung. However, EAA in both atopic and non-atopic individuals has also been associated with exposure to high concentrations of spores of allergenic fungi growing on damp surfaces within the home (Flannigan *et al.* 1991). For example, Jacobs and co-workers (1986) reported a case of EAA related to the growth of *Cladosporium* on the wooden ceiling of an unventilated hot-tub room in a dusty house. Bronchial provocation of the patient with a commercially available preparation of *C. cladosporioides* resulted some five hours later in identical symptoms to those experienced by the patient in the home. O'Brien *et al.* (1978) reported a case of asthma and EAA caused by the dry rot fungus *Serpula lacrymans*. In the serum of the patient both IgE and IgG antibodies to the fungus were detected, which were responsible for the type I and type III reactions respectively. *Penicillium chrysogenum* and *P. cyclopium* growing on a floor and its covering, wetted by a leaking central heating system, also caused allergic alveolitis (Fergusson *et al.* 1984).

The literature on the relationship between inhaled fungal spores and induction of respiratory allergy is still inadequate and controversial in many respects. Salvaggio and Aukrust (1981) concluded in their review on mould induced asthma that the evidence was primarily anecdotal. Among fungi which may be abundant in houses, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Mucor*, *Penicillium*, *Rhizopus* and *Ulocladium* have been revealed by skin prick tests to be allergenic. An extensive list of fungi reported as allergenic is included in a review on fungal allergens by Burge (1985). The isolation, purification and standardization of fungal allergens are major problems, because of the tendency of fungi to vary their antigen production, depending on the substrate on which they grow. This variation not only occurs between different species, but also between different strains of one species. This has lead to the postulation of Salvaggio and Aukrust (1981): "Regarding moulds, everything that can vary, will vary!". The inadequacy of commercial mould extracts used for diagnosis of mould allergy has also been demonstrated by Malling *et al.* (1985) among 68 children and 62 adults with suspected mould allergy. The patients were screened by skin prick tests using five fungal extracts (*Cladosporium herbarum*, *Alternaria tenuis*, *Penicillium notatum*, *Aspergillus fumigatus* and *Mucor racemosus*) from three different producers, and using a partially purified preparation of *C. herbarum*. Mould extracts of the same species from different manufacturers showed a wide range of positive skin prick tests (e.g. for *C. herbarum* this figure ranged from approximately 10% to 35%). Using the partially purified extract of *C. herbarum*, positive skin prick tests were found in approximately 60% of the patients. Many commercial fungal antigen preparations are made from fungal culture material with no spores and only vaguely resemble the original composition of the mycelium (Burge 1985). Therefore these products, although producing skin reactivity, might not contain the antigens relevant to inhalant allergic reactions. However, some progress has been made with regard to the isolation and purification of at least some allergens produced by fungi, especially by *Alternaria* and *Cladosporium* (Matthiesen *et al.* 1989; Matthiesen *et al.* 1994).

Allergy to fungi in the general population seems much less frequent than allergy to pollen, house dust mites and animal dander. De Vries (1973) reported that in a rural population in the Netherlands, positive skin prick test results to a mixed extract of common moulds were found in 4.8% of the 20-29 year old group, in 1.4% of the 30-39 year old group and in 6.1% in the 40-49 year old group. Barbee *et al.* (1976) reported that only 8% of a sample of more than 3,000 individuals from the general population in Arizona, USA, showed cutaneous hypersensitivity to a mixed extract of common moulds. Among a group of more than 700 children in New Zealand, Sears *et al.* (1989) found positive reactions to an *Alternaria* extract in only 6%, and to *Cladosporium* and *Penicillium* in 3% and 2% respectively. In the same group, nearly one-third had a positive reaction to a house dust mite extract. According to Miller (1990) 10-15% of the population is allergic to fungi.

Among patients with respiratory allergy, 2 to 30% are allergic to fungi (Gravesen 1979). In the Netherlands, Beaumont and co-workers (1985) found strong positive reactivity to fungi in only 3 to 4% of more than 800 out-patients referred to their allergological clinic because of recurrent obstructive airway complaints and suspected allergy. Hendrick *et al.* (1975) found that 13% of out-patients at an immunological clinic showed a positive reaction to *Aspergillus fumigatus*, and that 18% reacted to other moulds. In contrast, estimates of the prevalence of mould allergy among asthmatics in the USA have been as high as 80% (Salvaggio and Aukrust 1981). A study in Denmark revealed that the prevalence of fungal allergy in an atopic population was 8% in adults

and 23% in children (Osterballe 1981). Tarlo *et al.* (1988) prepared extracts of sixteen fungi sampled in the homes of 26 patients with allergic rhinitis or asthma, for skin prick testing. Fourteen patients (54%) showed one or more positive reactions to these extracts and of these, the most common were for *Cladosporium cladosporioides* (57%), *C. sphaerospernum* and *Alternaria tenuis* (both 50%), and *Fusarium* spp. (43%). Niemeijer and De Monchy (1992) reported an age dependency of sensitization to aero-allergens, including *Cladosporium herbarum*, in 499 Dutch asthmatic patients (aged 4-75 years) where sensitization was assessed by skin testing. For *Cladosporium* an age dependent relation was found with a high prevalence of sensitization at age 4 (42%), that declined rapidly to 10% or less in patients over 15 years of age.

Evidence of an association between asthma and indoor exposure to fungi was reported in a case-control study among 72 adults with asthma and 72 age and sex matched controls (Burr *et al.* 1988). Nineteen cases and nine controls reported mould patches in their homes. Furthermore, there was a statistically significant higher prevalence of positive radio allergosorbent test (RAST) responses to *Penicillium* among patients reporting mould in their homes. Holst and co-workers (1983) carried out a study lasting 9 months, on the relation between fungal propagules in the homes of 35 asthmatics and their symptoms of asthma and peak expiratory flow rate. In about one-third of the patients a positive association was found between symptoms of asthma or peak expiratory flow rate and the presence of specific fungi, especially *Aspergillus* spp.. However, none of these patients showed positive skin prick results conducted with commercially available fungal extracts. Beaumont *et al.* (1984) found no relation between the numbers of airborne propagules in indoor and outdoor air and sensitization to fungi in 28 asthmatic patients. In a study among eight asthmatic mould-sensitive patients, a significant difference was found between the mean peak flow on days with the highest numbers of CFU in outdoor air, compared with the mean peak flow on days with low numbers (Beaumont 1985). However, no relation was found between the mean peak flow and indoor mould counts. In the study by Tarlo *et al.* (1988) *Cladosporium cladosporioides* and *C. sphaerospernum* were isolated most frequently from indoor and outdoor samples, in concordance with the highest rates of positive skin reactions. However, *Alternaria tenuis* was found in only approximately 5% of the samples (sensitization rate 50%) and *Fusarium* spp. in less than 1% of the samples (sensitization rate 43%). Su *et al.* (1990) conducted a study on the relation between fungal propagules in the indoor air and reported respiratory symptoms in 150 children from the general population. Hay fever was associated with indoor concentrations of *Cladosporium*, *Epicoccum* and yeasts in winter. Furthermore, a positive association was found between the concentrations of *Aspergillus* during winter and reported wheezing and/or asthma. It should be stressed that viable and non-viable fungal particles are both important in relation to respiratory health effects. Only a few percent of the fungal particles are viable.

Some of the fungi that are found in indoor environments are also known to be opportunistic pathogens in man. For example, in exceptional cases the mould *Aspergillus fumigatus* may invade the lung tissue of debilitated individuals causing aspergillosis, and from there it becomes systemic, especially in the case of immunocompromised patients (Summerbell *et al.* 1992). Some well known fungal pathogens are associated with bird and bat droppings, e.g. the yeast *Cryptococcus neoformans* (Miller 1990).

Besides allergens, fungi may also produce mycotoxins. Mycotoxins are secondary metabolites with molecular weights generally greater than 200 but considerably smaller

than allergens. These secondary metabolites are produced when the mycelium is subjected to some kind of nutrient limitation during growth, the best-known is aflatoxin, an IARC class 1 human carcinogen (IARC 1993). Inhalation of aflatoxin containing spores has been shown to result in elevated liver cancer risk for those who handled material contaminated with the moulds *Aspergillus flavus* and *A. parasiticus* (Olsen *et al.* 1988). Spores of toxigenic fungi contain mycotoxins, for example, aflatoxins are present in high concentrations in spores of *Aspergillus flavus* and trichothecenes accumulate in the spores of *Strachybotris chartarum* (formerly *S. atra*) and *Trichoderma viride* (Tobin *et al.* 1987). Reviews of the mycotoxins produced by fungi common in homes, together with their toxic properties and acute toxicity assessed in laboratory animals, are presented by Tobin *et al.* (1987) and Hendry and Cole (1993).

It is well established that ingested mycotoxins can cause illness and death in humans and animals, however, toxicological data for many mycotoxins are limited, particularly for the dermal or inhalation exposure routes. Thus, the significance of mycotoxins in indoor environments is not yet clear (Miller 1990; Flannigan *et al.* 1991), but, it has been demonstrated in experimental animal studies that inhalation challenge with trichothecenes is 20 to 50 times more toxic than intravenous injection (Creasia *et al.* 1990). Furthermore, the trichothecenes produced by *Stachybotrys chartarum* and *Fusarium* spp., demonstrate acute toxicity to pulmonary alveolar macrophages in animals (Sorenson 1989). Trichothecenes and a number of other mycotoxins can be immuno-suppressive as well (Miller 1990). Therefore, although there is no definitive proof, it is nevertheless possible that inhalation of high concentrations of fungal spores may deleteriously affect macrophage functions such as phagocytosis of living and non-living particles in the lung, and therefore affect respiratory health. The effects on the immune system could also compromise health by reducing resistance to other microorganisms. Croft *et al.* (1986) reported that mycotoxins produced by *Stachybotrys chartarum*, which had colonised ducts, insulation and structural materials in a private residence, were the cause of chronic health problems in members of one household. These chronic health problems might have resulted from the effects of mycotoxins on the immune system. All health problems were reported to subside after removal of the contaminated material. Smith *et al.* (1992) investigated the cytotoxic properties of eighty-three fungal isolates collected and cultured from the air spora of damp houses in Scotland, UK. The biochemical assay system used revealed that 39 of the isolates possessed cytotoxic properties. Most of these strains belonged to *Penicillium* spp.. Thus, mycotoxins must certainly be regarded as potentially hazardous factors present in airborne particles in homes and non-industrial environments. However, considerably more research effort is needed before their actual impact on human health can be satisfactorily assessed.

Fungi also produce a variety of volatiles, including alcohols and ketones such as 1-octen-3-ol, 2-octen-1-ol and 1,10-dimethyl-trans-9-decalol (geosmin), which are often evident as "mouldy smells". The volatiles produced by moulds are complex mixtures of alcohols, esters, aldehydes, various hydrocarbons and aromatics, and a large number have been identified, the principal being ethanol. There is considerable variation in the production of volatiles, even between closely related genera or species.

Low toxicity to experimental animals has been demonstrated for 1-octen-3-ol and a range of other volatiles reported to be produced by moulds (Sorensen 1989). Symptoms like headaches, eye, nose and throat irritation or fatigue have been associated with volatile compounds produced by fungi (Samson 1985; Tobin *et al.* 1987; Flannigan *et al.* 1991). However, at present there is insufficient information for an assessment of the

health significance of fungal volatiles.

The cell wall of fungi contains ß-1,3-glucan, which can be extracted from the walls of fungal hyphae and spores. It acts as an inflammatory agent and has been implicated in increased reporting of mucous membrane irritation, dry cough and itching skin by occupants of buildings about which indoor air quality complaints had been made (Rylander *et al.* 1992). In experimental animal studies it has been shown that inhalation of ß-1,3-glucan results in a decrease in the numbers of pulmonary macrophages (Rylander and Goto 1991).

### 3.4 House dust mites: ecology

The term house dust mites applies to mites of the family Pyroglyphidae. At present 47 species in 17 genera are listed in this family. House dust mites are arachnids, not insects, and are related distantly to ticks and spiders. They belong to the order *Acari*. Ten species of the family Pyroglyphidae have been reported to occur in house dust more often than just occasionally, however, the following four species dominate: *Dermatophagoides pteronyssinus*, *D. farinae*, *D. microceras* and *Euroglyphus maynei*. Other mites may also occur in houses, these include several species regarded as storage mites (e.g. *Glyphagus destructor*, *Acarus siro* and *Tyrophagus putrescentiae*), as well as *Blomia tropicalis*, and species of the families Tarsonemidae and Cheyletidea (Platts-Mills *et al.* 1992). All these mites are small (0.1-0.6 mm) and whitish, and hence cannot be seen by the naked eye.

The natural food source of house dust mites appears to be skin scales, primarily from man, and/or fungi growing on skin scales. Several thousand mites can live for months on 250 mg of human dander. As one person produces 500 to 1000 mg of dander daily, nutrition seems always abundantly available for mites (Korsgaard and Iversen 1991), however, many other food sources may be used (Platts-Mills and De Weck 1989). Laboratory studies have shown that house dust mites require particular conditions of temperature and humidity to grow, e.g. for *Dermatophagoides pteronyssinus* the optimum conditions are 25°C and a relative humidity of 70 to 80%. Humidity seems to be the most critical factor in the survival and development of a house dust mite population (Mosbech 1985).

House dust mites have been found in most parts of the world and in almost every home as they live in dust that accumulates in carpets, bedding, fabrics, furniture, clothing and soft toys. House dust mite infestation has traditionally been assessed by counting isolated mite bodies under the light microscope. The earliest studies on mite counts reported seasonal and geographic variation in the numbers in house dust attributable to differences in humidity. For example, in temperate climates, the lowest numbers are found during winter and the highest numbers in summer, whereas in tropical countries, high numbers of mites can generally be found throughout the year. At high altitudes, the numbers of mites are lower. Environmental conditions also influence the species distribution. *D. pteronyssinus* usually dominates in continuously humid conditions, whereas *D. farinae* tends to predominate in areas where there is a prolonged period (more than three months) of dry weather. In most cases, it is unusual for other mite species to account for more than 10% of the mite population (Platts-Mills and Chapman 1987).

House dust mites are poikilothermic, which means that they cannot regulate internal body temperature or metabolic rates. Therefore both egg production and population growth decline at low temperatures, while at the same time mortality rates and the duration of the life-cycle increase (Colloff 1987). House dust mites develop at temperatures between 10 and 32°C, but they can survive temperatures as low as -15°C and as high as 50°C (Van Bronswijk 1981). The stages in the life-cycle are the egg, a six-legged larva, two eight-legged nymphal stages, and the adult males and females. At optimum conditions, egg to adult development of *D. pteronyssinus* takes 3 to 4 weeks. The adults live for about 6 weeks, during which time the females each produce 40-80 eggs (Arlian 1989). Their bodies contain 70-75% water by weight, most of which is water vapour actively extracted from unsaturated air. At relative humidities above 65-70%, mites can extract adequate amounts of water from unsaturated air to compensate for the loses by all avenues. Active mites do not survive at relative humidities below 50% longer than 6 to 11 days, however, they can survive extended dry periods by forming a desiccation-resistant protonymphal stage, that can survive for months at relative humidities below the critical humidity for active stages (Arlian 1992). As is the case for temperature, mites feed and multiply more rapidly at higher than at lower relative humidities (Arlian 1992).

### 3.5 House dust mites: health effects

House dust allergy was recognised as early as the 1920's when dust extracts were found to give clear positive reactions in skin tests on asthmatics. About 30 years ago, the role of mites of the family Pyroglyphidae as the most important source of house dust allergens, was established by researchers of the University of Leiden, the Netherlands (Voorhorst *et al.* 1964; Voorhorst *et al.* 1967). Therefore it was concluded that the importance of house dust mites in public health lay in their allergenic properties, which can be responsible for asthma and rhinitis. Based on the results of a number of studies on the relation between exposure to house dust mite allergens and asthma, Sporik *et al.* (1992) concluded that in many areas of the world the primary cause of asthma, especially in children and young adults, is inhalation of house dust mite allergens. Furthermore, there is increasing recognition that exposure to mite allergens can play an important role in the symptoms of atopic dermatitis (Platts-Mills *et al.* 1992). Allergy to mite allergens is fairly common in the atopic population. The reported prevalences among atopic asthmatics varies from 45-85% (Platts-Mills and De Weck 1989; Colloff *et al.* 1992), and among patients with perennial rhinitis, positive skin prick test responses have been found in 20-40% (Colloff *et al.* 1992). In the general population, the prevalences of house dust mite sensitization range from 5-30% (Platts-Mills and De Weck 1989). Niemeyer and de Monchy (1992) reported an age-dependency of sensitization to *D. pteronyssinus* in 499 Dutch asthmatic patients (aged 4-75 years), where sensitization was assessed by skin prick testing. High prevalences were found for patients between 4 and 25 years of age (40-70%), but after the age of 25 the sensitization rate declined to approximately 20%.

In the last decade, considerable progress has been made in the identification, purification and characterization of the allergens produced by *Dermatophagoides pteronyssinus*, *D. farinae* and *D. microceras*, using immunochemical techniques. Their major allergens (referred to as *Der* p I, *Der* p II, *Der* p III, *Der* f I, *Der* f II, *Der* f III, and *Der* m I, according to the unified nomenclature system of the International Union of Immunological Societies (IUIS 1986)) are well characterized and purified (Lind 1985; Platts-Mills and Chapman 1987; Platts-Mills *et al.* 1992). The production of monoclonal

antibodies (mAb) to mite allergens significantly contributed to the purification and quantification of the allergens. There are extensive structural and immunochemical data on the Group I and Group II allergens. So far, there are no indications that the two groups of allergens share structural similarities or show immunological cross reactivity (Schou and Lind 1991). More than 80% of mite allergic patients have specific IgE antibodies to these proteins, however, the relative importance of the Group III allergens needs more clarification (Platts-Mills *et al.* 1992). Recently, it was proposed that mite amylase, another component in mite extracts that binds IgE antibodies, was sufficiently well characterized to regard this protein and its analogues as Group IV mite allergens (Platts-Mills *et al.* 1992).

The Group I allergens have been shown to be faecal allergens, and very high concentrations of *Der* p I are present in mite faeces (Tovey *et al.* 1981a). One mite produces 6 to 40 faecal pellets a day, depending on the circumstances (Tovey *et al.* 1981b) and faecal pellets are about 25 $\mu$m in size (range 10-40 $\mu$m), but can break into smaller particles that can be inhaled (Platts-Mills and Chapman 1987). However, Tovey *et al.* (1981b) reported that only approximately 7% of the total amount of airborne *Der p* I was found on particles ranging from 2 to 15 $\mu$m in size. The majority (>80%) was found on particles with diameters of 6 to more than 20 $\mu$m. They used a cascade impactor consisting of 4 discs to collect particles with diameters of >20- 6 $\mu$m, 15-2 $\mu$m, 5-1 $\mu$m and 2.5-0.3 $\mu$m respectively and sampling was performed for 40 min. in eight bedrooms during domestic activity (e.g. bed making, vacuum cleaning of the floor). Several other studies have also shown that the bulk of airborne *Der p* I is associated with particles 10-40 $\mu$m in diameter (Platts-Mills *et al.* 1992) which means that most of the mite allergens will stay airborne for only short periods of time. Indeed, dust mite allergens are generally not detected in the air in undisturbed rooms (Tovey *et al.* 1981b; Platts-Mills *et al.* 1986a), but, during dust disturbing activities, like bed making or vacuuming, house dust mite allergens may become airborne. *Der f* I and *Der m* I have also been demonstrated to be faecal allergens (Lind 1986a).

The immune response to *Der p* I has been extensively studied in patients with asthma, rhinitis and atopic dermatitis. Most individuals with positive skin test to *D. pteronyssinus* extracts produce specific IgG, IgA, and IgE antibodies to *Der p* I, whereas most skin test negative subjects have no detectable amounts of specific IgE in their serum. Furthermore, there is a good quantitative correlation between the levels of IgG and IgE, and IgE is never found without IgG (Platts-Mills and Chapman 1987). In a prospective study among 92 children born to at least one atopic parent, Rowntree *et al.* (1985) found that specific IgG and IgE antibodies against *Der p* I developed from the second year of life onward. At five years of age, prevalences of specific IgG and IgE antibodies were 40% and 20-25% respectively. Interestingly, the prevalence of both IgG and IgE antibodies was positively associated with the development of respiratory symptoms and/or eczema.

A workshop held in 1987 under the auspices of the International Association of Allergology and Immunology, the UCB Institute of Allergy, the American Academy of Allergy and Immunology, and the World Health Organization, concluded on the epidemiological evidence that 2 $\mu$g *Der p* I/g dust (equivalent to 100 mites/g) should be regarded as representing a risk for the development of IgE antibody and asthma. A level of 10 $\mu$g *Der p* I/g dust (equivalent to 500 mites/g) should be regarded as a risk factor for acute attacks of asthma and a level at which most allergic patients will experience symptoms (Platts-Mills and De Weck 1989). These proposed threshold values were supported by a second international workshop on dust mite allergens and asthma held in

1990 (Platts-Mills *et al.* 1992). The proposed threshold limits assume that these values are of universal applicability. However, it was recognised that these guidelines will probably only be applicable to a part of the population, as the sensitivity of individuals varies enormously and the most sensitive individuals might well react to very low levels of allergen. Therefore, Tovey (1992) stated that at present safe levels of exposure to mite allergens are not known.

A number of studies investigated the relation between the exposure to house dust mite allergens and respiratory symptoms among children and adults. Lau *et al.* (1989) reported a dose-response relationship between the concentrations of *Der p* I and *Der f* I in house dust and the risk for sensitization of children to these allergens. Clearly elevated concentrations were found for atopic children sensitized to house dust mites compared with atopic children not-sensitized to house dust mites. Price *et al.* (1990) also reported a positive association between the level of house dust mite allergen in house dust and sensitization among asthmatic children. This association was not found at a cut-off point of 2 $\mu$g *Der p* I/g dust, but at cut-off points of 1 $\mu$g/g and 0.5 $\mu$g/g dust. Wickman *et al.* (1991) measured the levels of house dust mite allergen in the homes of 53 mite-sensitized children, 54 non-mite-sensitized atopic children and 53 non-allergic controls. Mite allergen levels were more often higher in the homes of children sensitized than in the homes of both groups of children not sensitized to house dust mite. The largest difference was found between the group of sensitized children and the group of non-sensitized atopic children. In a prospective study, Sporik *et al.* (1990a) reported a weak association between the degree of sensitization at age 11, and the *Der p* I concentrations in house dust at age one. None of the children in homes with concentrations less than 2 $\mu$g *Der p* I/g dust at the age of one year was sensitized at the age of 11. Studies performed by Wood *et al.* (1989) and Peat *et al.* (1987) indicated that asthma in mite allergic individuals was strongly associated with *Der p* I concentrations in house dust of more than 2 $\mu$g/g dust. However, at present it is not clear whether short periods of high exposure have more impact on human health than long term low level exposure. The results of these studies all add evidence for a causal relationship between exposure to house dust mite allergens and the development of sensitization and asthma. However, most of the information available has come from retrospective observation.

### 3.6 Concluding remarks

The present knowledge of the ecology of fungi and house dust mites shows that the development of both groups of organisms depends largely on environmental humidity. Humidity is the most critical factor in their development and it has been indicated that fungi as well as house dust mites are more abundant in damp environments.

Fungi and house dust mites produce a number of inhalant allergens, which might provoke symptoms like asthma and rhinitis (IgE-mediated allergies). The health effects of house dust mites are limited to allergic diseases, whereas fungi might also cause a number of other health effects, including infections, while the role of inhalation of mycotoxins and fungal volatiles have not yet been elucidated.

Considering their ecology and their possible health effects, fungi and house dust mites can both be regarded as causal factors in the relation between home dampness and respiratory symptoms in adults and children, as described in Chapter 2.

# Assessment of the exposure to fungi and house dust mites

This Chapter includes the following papers:

*Enumeration and identification of airborne viable mould propagules in houses; a field comparison of selected techniques*
A.P. Verhoeff, J.H. van Wijnen, J.S.M. Boleij, B. Brunekreef, E.S. van Reenen-Hoekstra, R.A. Samson
Allergy 45 (1990) 275-284

*Fungal propagules in house dust, I. Comparison of analytical methods and their value as estimators of potential exposure*
A.P. Verhoeff, E.S. van Reenen-Hoekstra, R.A. Samson, B. Brunekreef, J.H. van Wijnen
Allergy (in press)

## 4.1 Introduction

As reviewed in Chapter 2, in most studies on the relation between home dampness and respiratory symptoms, *reported* signs of dampness were used as exposure variables and *reported* respiratory symptoms as outcome variables. In Chapter 3 it has been suggested that fungi and house dust mites encounter favourable conditions for their development in damp environments. Evidence has also been presented in Chapter 3 for a causal relation between exposure to fungi and house dust mites, and respiratory symptoms. Therefore fungi and house dust mites can be regarded as causal factors in the relation between home dampness and respiratory symptoms. To further elucidate this relationship, it is essential to have valid measurement procedures to assess the exposure to fungi and house dust mites.

In epidemiology the term *exposure* denotes "any of a subject's attributes or any agent with which he or she may come in contact that may be relevant to his or her health" (Armstrong *et al.* 1992). The measurement of the exposure is an essential part of environmental epidemiology. For each exposure variable, the objective of taking measurements is to obtain those that maximize validity, or minimize error. Validity refers to the capacity of an exposure variable to measure the true exposure in a population of interest (Armstrong *et al.* 1992). Measurement error is one of the major sources of bias in epidemiological studies. The exposure measurement error for an individual is defined as the difference between the measured exposure and the true exposure. Two types of measurement error are distinguished: 1) systematic error or bias, that occurs for all measured subjects, and 2) the subject error, which varies from subject to subject. Two measures of measurement error are used to describe the validity of the measurement. The first is the difference between the observed population mean and the true population mean ("accuracy"). The second is the precision of the measurement, an estimate of the variation in the measurement error in the population (Armstrong *et al.* 1992).

This Chapter deals with the validity (accuracy and precision) of the available methods that are used in epidemiological studies to measure the exposure to fungi and house dust mites. Firstly, for house dust mites, a brief literature review is presented (4.2). For fungi - apart from a brief literature review on air sampling (4.3) - the results are presented of studies of the reliability of air sampling (4.4) and dust sampling (4.5) to assess the presence of these organisms in homes. The main hypotheses tested in these studies are: 1) that there are no differences in the results obtained with different techniques for the enumeration and identification of viable fungal particles in the indoor air of houses, 2) that there are no differences in the results obtained with different analytical methods for the enumeration and identification of fungal propagules in settled house dust, and 3) that measurements of fungi in single samples of indoor air or settled house dust do not provide a reliable estimation of the potential exposure.

## 4.2 Assessment of the exposure to house dust mites

Two different approaches are available to measure the presence of house dust mites and their allergens as indicators of environmental exposure: i.e. the sampling of air and settled dust. A brief literature review is presented on the validity of these measures as estimators of exposure.

### *4.2.1 Air sampling of house dust mites*

Several techniques have been described for volumetric sampling of airborne mite allergens, using cascade impactors or high and low volume samplers in combination with membrane filters (e.g. Swanson *et al.* 1985; Price *et al.* 1990; Sakaguchi *et al.* 1989). These techniques have the advantage that they sample airborne allergens and might therefore be more representative of the true exposure than assays of settled dust.

Mites themselves are not seen in air samples. Furthermore, in undisturbed rooms amounts of airborne mite allergens are small and therefore difficult to detect, even after prolonged sampling. Most of the mite allergens are bound to faecal pellets, which only become airborne as a result of disturbance, and very little allergen is associated with particles that will remain airborne for more than a few minutes (Pollart *et al.* 1988; Sakaguchi *et al.* 1989). Therefore, practical disadvantages of airborne sampling for mite allergens are that long sampling periods (2-24 hours) are required, whereas exposure to short periods of high mite allergen concentrations may clinically be (more) important, and that they require very sensitive assays (Platts-Mills and De Weck 1989).

There are only limited data available in the literature on the validity of air sampling as measure of exposure to house dust mite allergens. Tovey *et al.* (1981b) measured the airborne concentrations of *Der p* I in the bedrooms of 11 allergic patients, using a vacuum pump and a fibre glass filter. Allergen was detected in the air only when the room was actively disturbed. In undisturbed rooms, the amounts of *Der p* I collected were all below the limit of detection (0.3 ng). With standardized active disturbance, the amounts of *Der p* I collected varied from less than 0.3 ng to 30 ng. These findings indicate that the results of air sampling largely depend on the activities in a room during sampling. Similar results were obtained by Sakaguchi *et al.* (1989). Price *et al.* (1990) measured the airborne concentration of *Der p* I in the homes (bedroom and living room) of 68 allergic asthmatic children by low volume sampling (2 l/min) while normal domestic activities were in progress. The airborne *Der p* I concentration ranged from zero to 63 ng/m$^3$ and in 22 homes (32%) none was detected. In eleven homes, air sampling was repeated after one year. A high correlation ($r=0.82$, $p<0.001$) was found between the concentrations measured at both sampling periods, which lead to the conclusion that the results of air sampling of mite allergen are highly reproducible, although the absolute concentrations were considerably lower during the second sampling period. Swanson *et al.* (1985) performed air sampling for mite allergens in one home with an interval of one month between. The mite allergen concentrations (expressed in pg protein/m$^3$) measured in the bedroom were 1,440 and 2,120 pg protein/m$^3$ respectively and in the study 670 and 2,770 pg protein/m$^3$. Based on these results, the authors concluded that the concentrations of mite allergens were relatively consistent with time. In other studies (Swanson *et al.* 1989; Sakaguchi *et al.* 1992) no information is given on the reproducibility of the results of air sampling.

An alternative method for sampling airborne mite allergens was used by Tovey *et al.* (1992). They used large Petri dishes to collect settling dust in rooms over a period of 14 days. They collected up to 14 ng *Der p* I/day, representing a "fall out" of over 400 ng/m²/day. No information was presented on the reproducibility of this method either.

At present no reliable information is available that will support adoption of a standardized method for air sampling of house dust mite allergens. According to an International Workshop held in 1987 (Platts-Mills and De Weck 1989) airborne sampling has not been shown to be better than dust sampling to measure the level of mite infestation (note: *not* exposure). This was confirmed by a second International Workshop held in 1990 (Platts-Mills *et al.* 1992). It was also stated that there has been little or no data showing a relationship between airborne measurements and sensitization to house dust mites or symptoms. In contrast, a relationship has been found between the concentrations of mite allergens in settled dust and sensitization or symptoms (see section 3.5). Therefore, air sampling of house dust was not recommended. However, Price *et al.* (1990) reported a strong association between sensitivity against house dust mite and the presence or absence of airborne *Der p* I. The actual concentrations of airborne allergen regarded as positive were, however, not defined.

Further research is needed to assess the validity of sampling for airborne mite allergens as a measure of exposure and more data are also needed to compare air sampling with dust sampling. So, at present, air sampling should be combined with dust sampling (Sporik *et al.* 1990b; Platts-Mills *et al.* 1992).

### *4.2.2 Dust sampling of house dust mites*

Dust sampling for measurement of the level of mite infestation is generally accepted and recommended as the best validated "index of exposure" to house dust mite allergens. This approach assumes that either the quantity of allergens released into the air is a function of that present in settled dust, or, that the measurement of allergen in settled dust is a valid estimate of exposure to airborne allergens. Standardized procedures for dust sampling to measure house dust mites and their allergens have been proposed (Platts-Mills and De Weck 1989; Platt-Mills *et al.* 1992; Commission of the European Communities 1993). Sampling sites should be consistent and preferably include the upper mattress surface and the floors in the living room and bedroom. Sampling can be conducted with vacuum cleaners equipped with a special attachment to collect dust on a paper filter. Sampling time should be standardized and 2 min/m² is commonly used. Samples can also be obtained from upholstered furniture, soft toys and clothing. Alternative techniques for collecting dust samples include shaking blankets in a plastic bag and scraping flat surfaces higher than floor level with a piece of firm card. However, these techniques are less effective than collection by vacuum cleaner and not standardized. Before analysis, the dust samples may be sieved to obtain a sample of fine dust that can be weighed accurately, although dust samples may still vary in density after sieving.

There are three methods of estimating the concentrations of house dust mites or their allergens (Platts-Mills and De Weck 1989; Platt-Mills *et al.* 1992; Commission of the European Communities 1993). The prevalence of mites can be determined by counting under a microscope after separation from the dust by flotation or suspension. Although this method permits the identification of mite species, the disadvantages of this method include 1) the need for training and the development of skill in determining different

mite species, 2) the failure to quantify faecal pellets and therefore to reflect the true extent of the amount of mite allergen in the dust, and 3) the unsuitability for large-scale (epidemiological) studies, due to the time-consuming nature of the work.

In most studies, immunochemical assays are used to measure the concentrations of house dust mite allergens. Total mite allergen content can be assessed by inhibition radioallergosorbent tests (RAST inhibition). This method provides a good estimate of the relative potency of different allergen extracts, but cannot be used for absolute quantitation of mite allergens levels. An advantage of the method is that it measures "relevant" antigenic determinants that have elicited a response in allergic subjects, since human IgE is used. However, RAST inhibition results are difficult to reproduce over an extended period of time, because the results vary with the composition of the extract used on the solid phase and with the composition of the serum pool used for detecting bound allergen.

Individual mite allergens can be measured with enzyme-linked immunosorbent assays (ELISA) or inhibition radioimmuno assays (RIA). Sandwich radio- or enzyme immunoassays employ either rabbit polyclonal or mouse monoclonal antibody for capture, and affinity-purified antibody or a second monoclonal antibody for detection. These assays are more sensitive than RAST inhibition. Those using monoclonal antibodies in particular have also the great potential advantage of long-term reproducibility. Immunochemical assays are highly specific and the results obtained with these assays can be expressed in absolute units of a defined protein by unit weight of dust or by unit area sampled. Furthermore, they are suitable for large-scale surveys because they can be automated. The detection limits for these assays vary from approximately 10 ng/g dust for *Der f* I and *Der f* II, to 20 ng/g dust for *Der p* I and *Der p* II. Lind (1986b) reported on the basis of five duplicate analyses (including extraction) a coefficient of variation for the ELISA for *Der p* I of approximately 27%, for extracts of raw dust. For fine dust, the coefficient of variation ranged from 15% to 51%. For the ELISA assay for *Der p* I, an interassay variation for repeated assays of 18%, and an intra-assay variation of 6% have been reported (Munir *et al.* 1993).

Van Leeuwen and Aalberse (1991) investigated the variation with time of the concentrations of *Der p* I, *Der p* II and *Der f* I in house dust within one season. Sampling was performed once a week in fourteen bedrooms for a period of 4 to 6 consecutive weeks. The geometric mean concentrations of *Der p* I over the entire sampling period for the different bedrooms varied from 113 ng/g dust (GSD 1.25) to 22,925 ng/g dust (GSD 1.39). The variation with time in one bedroom was much smaller than the variation between bedrooms. Comparable results were obtained for *Der p* II and *Der f* I.

Overall, there is a good correlation between mite numbers found in house dust and the levels of Group I allergens as determined with immunochemical assays (Platts-Mills and De Weck 1989; Platts-Mills *et al.* 1992).

The third possibility is the measurement of guanine, which is a nitrogenous excretory product of arachnids, found in house dust. Since mites are predominant among arachnids in house dust, determination of guanine content in the dust is an indirect method for assessing faecal pellets. Analysis of guanine is based on a colour reaction between guanine and an azo-compound. The amounts of guanine can be measured quantitatively on a w/w basis using a spectrophotometer, or semi-quantitatively using a commercially available test-kit. This method is simple and economical, but does not identify the source, i.e. mite species. In addition, the test-kit only provides semi-quantitative results, and occasionally false-negative and false positive results are obtained. It has been found that very low and very high guanine levels correspond well with low and high concentra-

tions of *Der p* I and *Der f* I respectively (Lau *et al.* 1990). These authors concluded that the guanine test was less sensitive than the ELISA and that its reliability was unsatisfactory.

### 4.3 Assessment of the exposure to fungi

Three different approaches are available for measuring the presence of fungi, i.e. the sampling of air, dust and surfaces. The first two are used as indicators of potential environmental exposure, whereas surface sampling can only be used to obtain qualitative information about the fungi present and therefore cannot be considered.

Preceding the results of the studies of the reliability of air sampling (4.4) and dust sampling (4.5) for the assessment of the presence of fungi in homes, some additional information on air sampling of fungi is presented, because section 4.4 does not cover all relevant information.

Several techniques have been developed for volumetric sampling of fungi in indoor environments. Table 4.1 presents a summary of the techniques most commonly used for the sampling of fungi in aerobiological studies. Some of the techniques give total counts of all airborne particles, viable and non-viable, whereas others only give counts of viable fungal propagules, i.e. propagules or Colony Forming Units (CFU). A few methods are described that not only provide total counts, but also viable counts. It is accepted that no samplers collect all particles with equal efficiency. In general, the sampling efficacy of a bioaerosol sampler is both a physical and a biological problem. For air sampling of fungal propagules the following physical sampling principles may be distinguished: 1) impaction onto a solid or semi-solid surface (e.g. a culture medium or an adhesive), 2) centrifugal impaction, 3) filtration and 4) liquid impingement. All these sampling principles are based on the inertial behaviour of particles. With filtration, other mechanisms such as interception, diffusion and electrostatic attraction also contribute to the deposition of particles onto the filter. Liquid impingement also uses diffusion with the bubbles enhancing particle collection (Nevalainen *et al.* 1992).

Impaction is the most widely used sampling technique for fungal propagules. This process depends on the particles' inertial properties, such as size, density and velocity, and on the impactor's physical parameters, such as inlet-nozzle dimensions and airflow paths. Because of differences in characteristics, samplers differ in cut-off size ($d_{50}$), i.e. the particle size above which 50% or more of the particles are collected. As most impactors have very sharp cut-off characteristics, almost all particles larger than the $d_{50}$ are collected. So, $d_{50}$ is generally assumed to be the size above which all particles larger than that size are collected (Nevalainen *et al.* 1992). In Table 4.1 reported cut-off sizes are included for those samplers for which this information is available in the literature.

Table 4.1 Overview of sampling techniques for airborne fungal propagules

| method with examples | sampling rate and time | remarks |
|---|---|---|
| *Non-viable, non-volumetric* | | |
| * gravity slide | - | semi-quantitative, over-representation of larger particles |
| *Non-viable, volumetric* | | |
| * Burkard trap | 10 l/min; 7 days | cut-off unknown |
| * rotating arm impactors | 47 l/min; 15-60 sec | cut-off unknown |
| * filter methods | 1-4 l/min, hours | viable counts possible by plating washings from the filter |
| *Viable, non-volumetric* | | |
| * settlement plates | - | semi-quantitative, over-representation of larger particles |
| *Viable, volumetric* | | |
| * Andersen 6-stage impactor | 28.3 l/min; 1-30 min | cut-off 0.65 $\mu$m (a) |
| * Andersen 2-stage impactor | 28.3 l/min; 1-30 min | cut-off 0.65 $\mu$m (a) |
| * Andersen 1-stage impactor (N6-Andersen) | 28.3 l/min; 1-30 min | cut-off 0.65 $\mu$m (a) |
| * Surface Air System impactor (SAS) | 180 l/min; 20 sec-6 min | cut-off 1.9 $\mu$m (b) |
| * Reuter Centrifugal impactor (RCS) | ca. 40 l/min; 20 sec-8 min | cut-off 3.8 $\mu$m (c) |
| * Reuter Centrifugal Plus impactor (RCS Plus) | ca. 50 l/min; 30 sec-8 min | cut-off unknown |
| * slit samplers | 10-30 l/min | cut-off 0.7 $\mu$m (d) |
| * liquid impingers | 12.5 l/min | cut-off 0.3 $\mu$m (d) |

(a) Andersen 1958, (b) Lach 1985, (c) Macher and First 1983, (d) Nevalainen *et al.* 1992

As indicated in Chapter 3 it is important to realize that both viable and non-viable fungal particles are important in relation to health effects. The viable fungal particles may only comprise a few percent of the total number of fungal particles. Thus, in order to optimize the information available from air sampling for fungal particles, both types of particle should be sampled. However, even using the best method available, a large number of airborne spores will not grow in culture and cannot be visually identified using currently available methods.

The techniques available for sampling the total number of fungal particles do not give a good assessment of the composition of the air spora because only fungi with distinctive spores can be identified using a microscope. It is often difficult to identify the fungal spores to species or even genus. However, filter methods may be used not only to give total counts, e.g. by light or scanning electron microscopy, or direct epifluorescense microscopy, but also to obtain counts of viable fungal particles by plating washings from

the filter. Furthermore, filter samples may be analyzed for mycotoxins, endotoxins and for specific allergens (Eduard *et al.* 1990). Palmgren *et al.* (1986) used a filter method (Nuclepore filters) for sampling micro-organisms in highly contaminated working environments. They found a strong correlation between the total counts, determined by acridine orange staining and epifluorescence microscopy, and counts of viable fungal particles obtained by plating washings from the filters, in environments where the airborne flora was dominated by fungal spores. Filter sampling has been mainly used in highly contaminated occupational environments (e.g. Heikkilä *et al.* 1988; Karlsson and Malmberg 1989; Eduard *et al.* 1990).

Most published data on the presence of fungal particles in non-industrial indoor environments have been obtained using the Andersen 6-stage impactor (Andersen 1958). This impactor collects viable fungal particles, allowing identification of the air spora sampled. The use of settle plates or open petri dishes for sampling of viable fungal particles is generally considered the least reliable, because they give no quantitative information and qualitatively misrepresent the fungal flora (Burge *et al.* 1987). It should be stressed that no single collection medium will enable the entire range of viable fungi in the air to be isolated. Media which are generally accepted for aerobiological studies include Malt Extract Agar (MEA), V8 juice agar and Dichloran 18% glycerol agar (DG18) (Commission of the European Communities 1993).

For personal air sampling of fungal propagules and other airborne microorganisms, filter methods are used, as well as liquid impingers and personal cascade impactors (e.g. Macher and First 1984; Karlsson and Malmberg 1989; Eduard *et al.* 1990) Personal sampling of airborne fungi has so far, only been conducted in industrial environments, and no studies have yet been published on personal air sampling of fungi in homes.

## 4.4 Enumeration and identification of airborne viable mould propagules in houses; a field comparison of selected techniques [1]

### *4.4.1. Abstract*

A number of techniques for the enumeration and identification of viable mould propagules in the indoor air of houses were evaluated in order to document to what extent different results are obtained when different methods are used. A comparison was made between the results obtained with five commercially available air sampling devices (Slit-to agar sampler, N6-Andersen sampler, Surface Air System sampler, Reuter Centrifugal Air sampler, Gelatine Filter sampler) and a non-volumetric sampler (the Open Petri Dish), in combination with four culture media (malt extract agar, dichloran glycerol-18 agar, oxytetracycline glucose yeast extract agar and dichloran rose bengal chloramphenicol agar). The coefficients of variation were high (generally > 20%) for all combinations. Statistical analysis showed that the Slit sampler and the N6-Andersen sampler in combination with DG18 and MEA gave the best precision and the highest yield in terms of colony forming units per square cubic meter of air ($CFU/m^3$) and number of species isolated.

### *4.4.2. Introduction*

Damp houses are common in the Netherlands: about 15% of the homes may be affected to some degree. Several epidemiological studies have suggested a relation between living in damp houses and an elevated prevalence of respiratory symptoms for the residents (Andersen and Korsgaard 1986; Martin *et al.* 1987; Strachan 1988; Waegemaekers *et al.* 1989). Case histories indicate that moulds, which encounter favourable conditions in damp houses, can induce adverse health effects (Beaumont 1985; Samson 1985; Hunter *et al.* 1988;). For an inhalation-allergic population, prevalences of fungal allergy ranging from 2-30% are reported (Gravesen 1979). To get more clarity on the possible health effects, it is necessary to be able to measure the concentrations of moulds in indoor environments and to assess the relationship between home dampness and the presence of moulds. The presence of viable moulds in indoor environments has been studied by a number of authors. However, in most studies different (air) sampling devices and media were used for the enumeration and identification of viable mould propagules (e.g. Gravesen 1972; Beaumont 1985; Fradkin *et al.* 1987; Hunter *et al.* 1988; Waegemaekers *et al.* 1989). Until now, there is no standardized method for the sampling of airborne moulds, although a proposal was recently published by the American Conference of Industrial Hygienists (ACGIH) (Burge *et al.* 1987). However, the ACGIH recommends several air sampling devices for the sampling of viable mould propagules.

In this study, carried out before the ACGIH recommendation was published, several available methods for the enumeration and identification of viable mould propagules in the indoor air of houses were compared. A number of parallel measurements were

[1] Verhoeff, AP, JH van Wijnen, JSM Boleij, B Brunekreef, ES van Reenen-Hoekstra & RA Samson. Allergy 45 (1990) 275-284

This study was supported by a grant from the Ministry of Housing, Physical Planning and Environment and by a grant from the Ministry of Welfare, Culture and Public Health

performed under normal living conditions in order to document to what extent different results are obtained when different methods are used.

### *4.4.3. Material and methods*

**Sampling devices.** The following air sampling devices were used:

1. *Slit-to-agar sampler* (Slit)
   The Slit sampler used was a Slit-to-agar sampler (Apparatenfabriek van Doorn, De Bilt, the Netherlands). The sampler draws air at 30 l/min by a vacuum pump (Charles Austin F65), through a slit orifice and the mould propagules are impacted onto a rotating medium. The sampling time per rotation is limited to 2, 6 or 8 min. The impaction efficiency of the Slit-to-agar sampler seems to be high (Beaumont 1985). According to Gregory (1973) it is best used indoors for occasional or routine sampling of short periods.
2. *N6-Andersen sampler* (N6)
   The standard six-stage Andersen cascade sampler is widely used in aerobiological studies (Beaumont 1985). This sampler collects air at 28.3 l/min through six stages with decreasing diameter holes in each succeeding plate (Andersen 1958). In this study the N6 modification of the Andersen sampler (Jones *et al.* 1985) was used. The results obtained with the Andersen six-stage sampler and the N6-Andersen sampler are highly comparable (Jones *et al.* 1985). The N6-Andersen sampler, however, is more efficient regarding time and cost of sampling and is recommended by the National Institute of Occupational Safety and Health (Morey 1985). The N6-Andersen sampler draws air by a vacuum pump (Charles Austin, F65), through a sieve plate containing 400 holes over a medium. The air volume sampled was recorded by a gasmeter (G1.6 Meterfabriek, Dordrecht, the Netherlands).
3. *Surface air system sampler* (SAS)
   The SAS sampler (Pool Bioanalyse Italiana, Milan, Italy) collects air at 180 l/min through a single-stage sampling head with 220 holes (diameter 2 mm) (Ligugnana 1980). The mould propagules are impacted on an agar containing 50 mm special Petri dish (contact plates). The SAS sampler is portable, operates on a 12 V rechargeable battery and can be set to sample from 20 s to 6 min.
4. *Reuter centrifugal air sampler* (RCS)
   The RCS (Biotest - Frankfurt, FRG) is a battery operated portable air sampler which collects mould propagules by drawing air through a fan and impacting the propagules on a medium coated strip, inserted into a drum surrounding the fan. The rated flow is 40 l/min.
5. *Gelatine filter sampler* (GF)
   The GF has been shown to be suitable for the enumeration of airborne fungi in highly contaminated environments (Blomquist *et al.* 1984a). Using gelatine filters, the possibility of overloading culture plates can be avoided, because after sampling the filters are dissolved and then diluted stepwise prior to culturing. Gelatine filters (Satoerius) with a pore size of 3.0 $\mu$m and a diameter of 50 mm were placed in plastic filter cassettes (Schleiger and Schull filterholder). The filterholder was connected by rubber tubing to a Charles Austin F 65 pump. The sampling flow was set to 15 l/min and the volume recorded using a gasmeter (G1.6 Meterfabriek, Dordrecht, the Netherlands).

6. *Open petri dish* (OPD)
   For illustrative purposes, the OPD was also included in this study. This method relies on the gravitational deposition of mould propagules. This method has been and is still widely used, especially in clinical studies (Beaumont 1985). An advantage of this method is the possibility of a long period of sampling (up to several hours). Further, sampling with the OPD may be performed by untrained persons after simple instructions. However, the quantitative estimation of propagules in a given amount of air is not possible. In addition, the influence of wind and air turbulence is great, especially around the edge of the plate and larger particles might be over-represented (Gregory 1973).

**Collection media.** The following media were used for sampling viable airborne fungal propagules in combination with the sampling devices mentioned above:

1. *Malt extract agar* (MEA)
   MEA, as described by Galloway and Burgess (1952), is widely used as collection medium in aerobiological studies (Blomquist *et al.* 1984a; Blomquist *et al.* 1984b; Morey 1985). It was recently recommended by the ACGIH (Burge *et al.* 1987). It is a general purpose medium and most fungi can be isolated. However, if a high concentration of moulds is expected, the colonies of fast growing fungi easily overgrow the slow growing moulds.
2. *Dichloran 18% glycerol agar* (DG18)
   DG18, developed by Hocking and Pitt (1980), is supposed to be a selective medium with a low water activity ($a_w$=0.995), intended for isolation of xerophilic fungi. Dichloran restricts the growth of fast growing genera like *Rhizopus* and *Mucor*, facilitating the counting of colonies and the isolation of all species present.
3. *Oxytetracycline glucose yeast extract agar* (OGYA)
   OGYA, as developed by Mossel *et al.* (1970), is a general purpose medium for detecting moulds in food and is widely used in the Netherlands.
4. *Dichloran Rose Bengal chloramphenicol agar* (DRBC)
   DRBC, as developed by King *et al.* (1979) and modified by Hocking (1981) in replacing chlortetracycline with chloramphenicol, is recommended by NIOSH (Morey 1985). It is a general purpose medium for detecting moulds in food. However, Rose Bengal-containing media have also proved to be good for aerobiological studies (Morring *et al.* 1983). The growth of the mould colonies is restricted by Rose Bengal. This allows the isolation of the various species without overgrowing of *Mucor* and similar moulds. Further, some xerophilic moulds can also be isolated.

**Incubation, isolation and identification.** The Slit, the N6, the SAS and the RCS were used in direct combination with all of the selected media. The OPD was not used in combination with DRBC. After sampling, the plates were directly inoculated at 25°C for 4 days. Then the number of colony forming units (CFU) was counted.

The gelatine filters were first dissolved in 5 ml peptone solution (8.5 g peptone + 1 g NaCl/1 l distilled deionized water). After shaking for 15 min, the solution was diluted stepwise and 0.2 ml of each sample was applied in duplicate on the four different media. After 4 days of incubation at 25°C the colonies were counted. The number of CFU/m$^3$ was calculated as the ratio of the number of CFU counted and the collected air volume. With the N6 and the SAS, a so-called positive hole conversion table is necessary to derive a more accurate number of CFU/m$^3$.

After colony counting, fungal spores and/or mycelium were transferred onto appropriate media for identification up to species level, as described by Samson and Van Reenen-Hoekstra (1988).

**Sampling procedures.** The study was conducted in 11 different houses during the winter of 1987 (November 1986-March 1987). The samples were taken under usual living conditions in living room or bedroom, when visible mould growth was present. Sampling was performed at a central point in the room approximately 1.5 m above the floor. During air sampling, no other activities were carried out on the sampling locations. The sampling periods of all methods used depend on the expected number of CFU/m³. Especially when the sampling period is too long and/or the concentration of the mould propagules is high, the number of CFU will be too high to be counted properly. The following sampling periods were chosen: Slit sampler 2 min, N6-Andersen sampler 2 min, SAS 20 s, RCS 1 min and 30 s, GF 30 min and OPD 60 min. With these sampling periods similar amounts of air were sampled by the first four sampling devices.

Sequential duplicate samples were taken with the first four sampling devices. Parallel duplicate samples were taken with the GF and the OPD samplers, and also with the SAS and N6 samplers. No parallel samples were taken with the Slit and RCS samplers, because only one of these samplers were available for the study. The air sampling devices were run parallel for each medium. The samplers were cleaned between each run with 70% ethanol.

In nine houses sufficient data were obtained to make a complete comparison in terms of CFU/m³, whereas in 10 houses sufficient data were obtained for a comparison in terms of number of species isolated.

**Data analysis: comparison of the results.** The following quantities were used to compare the results obtained with the different measurement techniques:

a) yield in terms of CFU/m³, in order to compare the volumetric results;
b) yield in terms of number of species isolated;
c) reproducibility of parallel and sequential duplicate samples;
d) correlations between the results obtained with the different techniques.

### *4.4.4 Results*

During the fieldwork at the first five locations, it appeared that about 20% of the samples taken on OGYA were overgrown by fast growing mould genera such as *Rhizopus* and *Mucor*. Further, counting the number of CFU on OGYA was difficult, because the colonies spread widely. Therefore, OGYA was excluded from the further sampling program and the analysis of the results.

**Yield in terms of CFU/m³.** On nine locations sufficient data were obtained to make a complete comparison between the yields in terms of CFU/m³. The results obtained with the OPD were compared separately.

The average yields (geometric mean, GM) on these locations varied from 71 CFU/m³ (geometric standard deviation, GSD 3.54) to 1,266 CFU/m³ (GSD 2.25). The numbers of CFU/m³ obtained varied widely from 0 to 17,750 CFU/m³. The values were right skewed, but became normally distributed after logn-transformation. Therefore, the statistical analysis (apart from the analysis of reproducibility) was performed with the

logn-transformation. Before logn-transformation, any zero counts were given the value one. Further, the results obtained with the GF could only be compared with the results obtained with the SAS.

The geometric mean numbers of CFU/m$^3$, obtained with the Slit, N6, SAS and RCS in combination with MEA, DG18 and DRBC, are presented in Table 4.2. High yields were more consistently obtained with the Slit and the N6, in combination with DG18 and MEA. However, the RCS in combination with MEA gave the highest yield of all.

Table 4.2 Geometric mean number of CFU/m$^3$ obtained with the different combinations of air sampling device and culture medium

| medium | sampler | | | | GM per medium |
|---|---|---|---|---|---|
| | Slit | SAS | N6 | RCS | |
| MEA | 545 | 164 | 508 | 721 | 424 |
| DG18 | 713 | 478 | 518 | 327 | 493 |
| DRBC | 455 | 147 | 361 | 193 | 260 |
| GM/sampler | 561 | 226 | 455 | 358 | |

GM: geometric mean

An analysis of variance was performed for the Slit, N6, SAS, and RCS in combination with MEA, DG18 and DRBC, in order to assess the effects caused by sampling location, type of sampling device and type of medium. This analysis was based on 108 means of sequential duplicates. They refer to nine different locations with complete data sets (9 locations x 4 samplers x 3 media).

The results are presented in Table 4.3 and indicate that the location, type of air sampling device and the type of medium all significantly contributed to the variance of the results. Furthermore, there was a significant interaction between air sampling device and medium, which was caused by the relatively much higher yield of the RCS in combination with MEA than in combination with DG18 and DRBC.

Table 4.3 Analysis of variance of the yield in terms of CFU/m³

| source | DF | SS | MS | F | p |
|---|---|---|---|---|---|
| model | 59 | 140.09 | 2.37 | 6.49 | <0.001 |
| residual | 48 | 17.55 | 0.37 | | |
| location | 8 | 94.69 | | 32.37 | <0.001 |
| sampler | 3 | 12.51 | | 11.41 | <0.001 |
| medium | 2 | 7.91 | | 10.82 | <0.001 |
| medium x sampler | 6 | 9.39 | | 4.28 | 0.002 |
| location x sampler | 24 | 7.70 | | 0.88 | 0.627 |
| location x medium | 16 | 7.89 | | 1.35 | 0.209 |

DF: degrees of freedom; SS: sum of squares; MS: mean square; F: F-value

The results obtained with the GF were compared with the results obtained with the SAS, because a sufficient number of parallel duplicate samples were taken on the same locations with these samplers ($n$=8). The average yields in terms of CFU/m³ were compared by medium (t-test). No significant differences were found between the yields obtained with the GF and the SAS.

With the OPD in combination with MEA, the geometric mean number of CFU was 18 (GSD 1.42). Also in combination with DG18, the geometric mean number of CFU was 18 (GSD 1.50). For illustrative purposes a comparison was made between the yield obtained with the OPD and the yields obtained with the volumetric air sampling devices by calculating Pearson correlation coefficients. Rather high correlations were found between the yields obtained with the OPD and the Slit in combination with MEA and DG18 (0.92 and 0.79 respectively, $n$=11) and between the yields obtained with the OPD and the N6 in combination with MEA and DG18 (0.95 and 0.83 respectively, $n$=11).

**Yield in terms of number of species isolated.** The yield in terms of number of species isolated varied between the 10 different locations from 14 to 39. The number of isolated mould genera varied from 6 to 18. In total, 76 mould species belonging to 28 genera were found. Species belonging to the genera *Cladosporium, Eurotium* and *Penicillium* were found in all the investigated houses. Table 4.4 presents the most frequently identified mould species. The numbers of isolated mould species were normally distributed.

Table 4.4 Mould species identified in the indoor air of more than seven houses

| species | no. of houses |
|---|---|
| *Cladosporium herbarum* | 10 |
| *Eurotium herbariorum* | 10 |
| *Penicillium chrysogenum* | 10 |
| *Penicillium glabrum* | 10 |
| *Penicillium verrucosum* | 10 |
| *Aspergillus versicolor* | 9 |
| *Cladosporium cladosporioides* | 9 |
| *Cladosporium* spp. | 9 |
| *Penicillium brevicompactum* | 9 |
| *Cladosporium sphaerospernum* | 8 |
| *Penicillium olsonii* | 8 |
| *Wallemia sebi* | 8 |

The mean numbers of species isolated, obtained with the Slit, N6, SAS and RCS in combination with MEA, DG18 and DRBC are presented in Table 4.5. The highest number of species isolated was obtained with the Slit, followed by the N6, in combination with DG18. The yields obtained with MEA and DRBC were similar.

Table 4.5 Mean number of species isolated, obtained with the different combinations of air sampling device and culture medium

| medium | sampler | | | | mean/ medium |
|---|---|---|---|---|---|
| | Slit | SAS | N6 | RCS | |
| MEA | 4.5 | 3.8 | 5.2 | 4.3 | 4.5 |
| DG18 | 6.8 | 5.8 | 6.7 | 4.2 | 5.9 |
| DRBC | 4.8 | 4.7 | 4.2 | 4.3 | 4.5 |
| mean/sampler | 5.4 | 4.8 | 5.3 | 4.3 | |

To assess the effects caused by sampling location, type of air sampling device, and type of medium, an analysis of variance was performed, based on 72 observations referring to six different locations with complete qualitative results (6 locations x 4 samplers x 3 media). Table 4.6 presents the results.

Table 4.6 Analysis of variance of the yield in terms of number of species isolated

| source | DF | SS | MS | F | p |
|---|---|---|---|---|---|
| model | 41 | 279.58 | 6.82 | 4.85 | <0.001 |
| residual | 30 | 42.19 | 0.37 | | |
| location | 5 | 133.11 | | 18.93 | <0.001 |
| sampler | 3 | 14.78 | | 3.50 | 0.027 |
| medium | 2 | 31.19 | | 11.09 | <0.001 |
| medium x sampler | 6 | 19.14 | | 2.27 | 0.064 |
| location x sampler | 15 | 63.89 | | 3.03 | 0.005 |
| location x medium | 10 | 17.47 | | 1.24 | 0.306 |

Abbreviations: see Table 4.3

This analysis of variance indicates that the location, type of air sampling device and the type of medium all significantly contributed to the variance of the results. Furthermore, there was a significant interaction between air sampling device and location, which was caused by the relatively much lower yield of the RCS on location 1 and the relatively much higher yield of the RCS on location 5. When the results obtained with the RCS were excluded from this analysis, the influence of the type of air sampling device as well as the interaction between air sampler and location became insignificant.

The results obtained with the GF could not be compared with the results of the remaining air sampling devices, because the number of GF-samples analyzed qualitatively was too small.

The yield in terms of number of species isolated obtained with the OPD in combination with MEA and DG18 could be compared (Mann-Whitney test) with the yield obtained with the volumetric air sampling devices, except the GF. In combination with MEA, no significant differences were found. With DG18, the number of species isolated on the OPD was lower than the yield obtained with the Slit and the N6 ($p < 0.05$).

**Reproducibility.** The reproducibility in terms of CFU/m$^3$ was assessed by calculating the mean coefficients of variation (CV) of parallel and sequential duplicate samples. The results are presented in Table 4.7. The mean CV values of parallel duplicate samples varied from 7.6% (N6/MEA) to 43.6% (GF/DRBC), whereas the CV value of the sequential duplicate samples varied from 11.1% (SAS/DG18) to 38.7% (RCS/DRBC).

Table 4.7 Coefficients of variation (CFU/m³, %) of sequential and parallel duplicates

| sampler | sequential ($n$=10) | | | parallel[a] | | |
|---|---|---|---|---|---|---|
| | MEA | DG18 | DRBC | MEA | DG18 | DRBC |
| Slit | 12.2 | 17.6 | 17.8 | - | - | - |
| SAS | 23.0 | 11.1 | 19.8 | 26.6 | 19.3 | 30.6 |
| N6 | 14.0 | 17.0 | 16.1 | 7.6 | 23.5 | 8.3 |
| RCS | 21.4 | 18.3 | 38.7 | - | - | - |
| GF | - | - | - | 27.6 | 40.3 | 43.6 |
| OPD | - | - | - | 16.5 | 16.7 | - |

[a] number of parallel duplicates: SAS: 22; N6: 6; GF: 13; OPD: 14

The reproducibility of the results in terms of number of species isolated from sequential duplicate samples was calculated as the fraction of the number of identical species isolated in both samples and the total number of species isolated from both samples (agreement rate). With the Slit the mean percentages were respectively 22% (MEA, $n$=3), 63% (DG18, $n$=4) and 34% (DRBC, $n$=4). With the SAS the mean percentages were 10% ($n$=3), 36% ($n$=6) and 21% ($n$=6) respectively and with the N6 33% ($n$=3), 38% ($n$=4) and 40% ($n$=3). The agreement rate could not be calculated for the RCS, GF and OPD, nor for the parallel duplicate samples, because the number of samples was too small.

**Correlations.** Pearson correlation coefficients were calculated between the yields in terms of CFU/m³ obtained with the different air sampling devices by medium. The correlations varied from 0.30 (N6/RCS in combination with DRBC) to 0.97 (Slit/SAS in combination with DG18). In general, the correlations were weaker between the yields obtained with the different air sampling devices in combination with DRBC. Correlations were also calculated between the yields obtained with the different media by air sampling device. These correlations varied from 0.19 (DG18/DRBC in combination with the RCS) to 0.93 (DG18/MEA in combination with the Slit). In general, the correlations of the yields obtained with the different culture media were (much) better in combination with the Slit and the N6 than those in combination with the SAS and the RCS. Regarding the GF, only a sufficient number ($n$=8) of parallel samples was obtained in combination with the SAS. The correlations between the yields were 0.57 (MEA), 0.69 (DG18) and 0.67 (DRBC).

### 4.4.5 Discussion

Because the "accuracy" - i.e., agreement between the measured results and the "true" number of CFU/m³ and mould species - cannot be determined, the different measurement techniques could not be compared by this criterion. However, the validity of the measurements was partly assessed with the yields in terms of CFU/m³ and number of species isolated, and the precision of the results.

The results of this study indicate that high *yields in terms of CFU/m³* were more consistently obtained with the Slit and the N6 samplers, in combination with DG18 and MEA. However, the highest yield of all was obtained with the combination RCS/MEA. A comparison with data reported in the literature is hardly possible, because in most studies different types of air samplers and media were used. Lach (1985) reported that the collection efficiency of the SAS was 70-85% of the collection efficiency of the Slit sampler. The RCS has a single opening for air intake and discharge, making it difficult to quantify the flow rates.

Macher and First (1983) indicated that the total sampling rate of the RCS was somewhat lower than the calculated total sampling rate of the manufacturer (210 ± 27 l/min versus 280 l/min). Further, they reported that the effective sampling rate depended on the particle size. The manufacturer advises to assume that the effective sampling rate is 40 l/min, irrespective of the particle size. Macher and First concluded that it would be more convenient to calculate the number of CFU/m³ obtained with the RCS using the total sampling rate. This would imply that the yield obtained with the RCS will be about five times lower than as reported in this study. In this study, the flow rate of the RCS was not determined. The GF was shown to be suitable for the enumeration of airborne mould propagules in highly contaminated environments (Blomquist *et al.* 1984a). With low concentrations the yield obtained with this device may be unreliable due to the heterogeneous distribution of spores in the solution. Burge *et al.* (1977) stated that the yields on media containing Rose Bengal were lower than the yield on MEA. Apart from a lower yield, Rose Bengal has the disadvantage that is decomposes in light and might then become toxic to some mould species, which restricts the applicability in field studies.

The highest *yields in terms of number of species isolated* were obtained with the Slit and N6 samplers in combination with DG18. The yields on MEA and DRBC were comparable. Species belonging to the genera *Aspergillus, Cladosporium, Eurotium* and *Penicillium* were found in most of the investigated houses, which is - apart from *Eurotium* - in good agreement with the results presented in the literature (Beaumont 1985; Fradkin *et al.* 1987). Apart from the presence of *Wallemia sebi* on DG18 only, no striking differences were found in the occurrence of the isolated species on specific samplers, media or combinations. Regarding the isolation of often cited pathogenic mould species, e.g. species belonging to the genera *Alternaria, Aspergillus, Cladosporium* and *Penicillium* (Gravesen 1979; Beaumont 1985), no systematic differences were found between samplers and media.

The *reproducibility* of the parallel duplicate samples was only assessed for the SAS, N6, GF and OPD. The best reproducibility was obtained with the N6, in combination with MEA and DRBC. However, in combination with DG18, the best reproducibility was obtained with the OPD.

The reproducibility of the sequential duplicate samples was better with the Slit and the N6 with CV values between 10% and 20%, than with the remaining devices. With those devices, CV values above 20% and even 30% were found. For the culture media,

the lowest CV values were found for DG18, followed by MEA and DRBC. However, the value of the lowest CV found (11.1%) indicates that even samples taken within 2 min after each other at the same location show only a moderate reproducibility. This is in agreement with Hunter *et al.* (1988), who indicated that the numbers of CFU/m³ in the indoor air varied widely within dwellings over short periods.

The reproducibility in terms of number of species isolated in sequential samples was moderate. In general, the agreement rates were less than 50%. With the SAS and the Slit, the reproducibility was better in combination with DG18 than in combination with the other media. With the N6, no clear differences between media were found. It is concluded that the presence of moulds in indoor environments in terms of CFU/m³ and in terms of number of species, cannot be determined reliably with single samples.

The calculated *correlation coefficients* between the yields obtained with the different combinations of air sampling device and medium, demonstrated that, in general, the relations between the different air samplers were stronger with DG18 and MEA as culture medium than with DRBC. The relations between the media were stronger in combination with the Slit and the N6 than with the SAS and the RCS. The results of the OPD correlated strongly with the results obtained with the Slit and the N6.

The results of this study indicate that from the selected volumetric sampling methods, the SAS gave consistently lower results than the others. The RCS gave high results on MEA, but not on DG18 and DRBC. High yields were more consistently obtained with the Slit and the N6. Of the selected culture media, OGYA was not suitable and DRBC gave consistently lower values than MEA and DG18. The yield on DG18 was somewhat higher than on MEA, and colony counting was easier, because the colonies did not grow as fast on DG18 as on MEA. Further, xerophilic mould species as well were isolated on DG18.

From the available non-volumetric methods only the OPD was included in this study. Strong correlations were found between the OPD and the Slit and N6 (the most suitable volumetric samplers) in combination with DG18 and MEA (the most suitable culture media). However, with the OPD no information is obtained of the concentrations of viable mould propagules. On the other hand, the OPD has the advantage of (much) longer sampling periods, which provides a more time-integrated picture of the presence of viable mould propagules. This aspect may be important when the indicators of exposure to mould propagules are needed as part of studies in which the possible health effects of moulds are investigated. However, it should be noted that an interpretation of the health significance of the results of studies like this is impeded by several difficulties: not only viable mould propagules are potentially able to induce adverse health effects, but also non-viable moulds or mould products; different mould species do not have the same potency to induce health effects; and the immunological sensitivity differs widely between individuals. Furthermore, the type of exposure that may be relevant is not known. Short periods of increased concentrations of moulds in the air may induce allergic reactions, but also long-term mean exposures may cause adverse health effects.

The ACGIH recently published guidelines for the assessment of bio-aerosols in the indoor environment (Burge *et al.* 1987). According to the ACGIH, the highest yield in terms of CFU/m³ will be obtained using Slit-to-agar samplers and impingers. In most cases, however, this degree of accuracy is not considered essential by the ACGIH and more portable samplers may be used. Therefore, the ACGIH recommends several air sampling devices for viable microbiological aerosols (slit-to-agar samplers, sieve plate impactors, filter cassettes, glass impingers and centrifugal samplers). Malt Extract Agar is recommended by the ACGIH as collection medium. Methods relying on gravitational

deposition of particles are not recommended by the ACGIH, because they do not provide representative quantitative and qualitative results.

To be able to make proper comparisons between different studies, standardization is needed. In view of the results of this study, the Slit and the N6-Andersen air sampling devices can be recommended. With regard to the use of a medium, the results of this study are somewhat contrary to the recommendation of the ACGIH. This may be because DG18 has not been widely used in aerobiological studies and was therefore probably not evaluated by the ACGIH. However, given the favourable results obtained with DG18, further research to investigate the utility of this culture medium in comparison with MEA is recommended.

## 4.5 Fungal propagules in house dust; comparison of analytical methods and their value as estimators of potential exposure [1]

### *4.5.1. Abstract*

The presence of viable mould propagules in house dust was investigated by 10 different analytical methods, in order to determine to what extent different results are obtained when different analytical methods are used. Moreover, the value of this measurement as an estimator of the potential exposure to fungi in epidemiological studies was assessed. Floor and mattress dust was sampled in 60 homes in the Netherlands during autumn 1990. For investigation of the variability in time sampling was repeated in 20 homes after six weeks.

Each analytic method is characterized by a unique combination of culture medium, suspension medium, and dilution step. The highest mean number of Colony Forming Units (CFU)/g dust was obtained by suspension of at least 100 mg dust in a peptone or sucrose solution in a ratio of 1:50 (w/w), followed by 10-fold dilution and plating onto DG18 agar (geometric mean (GM) approximately 60,000 CFU/g dust). The lowest mean number of CFU/g dust was obtained by direct plating of 30 mg dust on V8 agar (GM approximately 5,300 CFU/g dust). The mean coefficient of variation of duplicate analyses varied from 11%, for suspension in sucrose and plating on DG18 agar, to 27%, for suspension and dilution in sucrose in combination with V8 agar. The highest mean number of species isolated was obtained by direct plating of 30 mg dust on DG18 agar (mean number of species: 17). Suspension and dilution on DG18 or V8 agars yielded an average of approximately six species. In duplicate analyses, the mean percentage of agreement for the species isolated varied from approximately 35%, for suspension and dilution, to 60%, for direct plating. The reproducibility of the number of CFU/g dust in time was better for mattress dust than for floor dust; however, also for mattress dust, the predictive value of a single measurement was rather low. The variability in time in species isolated was substantial, both for mattress dust and floor dust. We concluded that results of measurements of viable mold propagules in house dust, both quantitatively and qualitatively, depend greatly on the analytic methods used. Furthermore, a single measurement of fungal propagules in settled house dust does not provide a reliable measure of potential exposure to fungi in indoor environments.

### *4.5.2 Introduction*

Fungi and house-dust mites are thought to be a major cause of respiratory symptoms in damp houses. The ability to assess human exposure to fungi and house-dust mites might clarify the relation between living in damp houses and possible health effects, as reported in a number of recent epidemiologic studies (Martin *et al.* 1987; Strachan 1988; Brunekreef *et al.* 1989; Platt *et al.* 1989; Strachan and Sanders 1989; Waegemaekers *et al.* 1989; Dales *et al.* 1991a, 1991b; Brunekreef 1992). The major allergens produced by

[1] Verhoeff, AP, ES van Reenen-Hoekstra, RA Samson, B Brunekreef & JH van Wijnen. Allergy (in press).

This study was supported by a grant from the Ministry of Welfare, Culture and Public Health.

house dust mites are well characterized and standardized (Platt-Mills and De Weck 1989; Platt-Mills *et al.* 1992). There is therefore the possibility of assessing human exposure to these allergens indirectly by measuring the allergen content in settled house dust. Isolation, purification, and standardization of the major fungal allergens are still beset by major problems (Burge 1985; Lowenstein *et al.* 1987). Furthermore, the available methods for the measurement of fungal propagules (viable fungal particles) in indoor air do not provide accurate data for an estimation of the exposure to fungi in indoor environments. This is due to the large variability in time, even within very short time periods (Hunter *et al.* 1988; Verhoeff *et al.* 1990). Overall, the variation of airborne fungal propagules in time is larger within homes than between homes (Verhoeff *et al.* 1992). Therefore, air sampling of viable fungal particles does not provide a reliable assessment of the long-term exposure of occupants to fungi in indoor environments.

Like the measurement of house-dust mite allergens in settled house dust, the measurement of fungal propagules in settled house dust might also give a useful measure of potential exposure to fungi. Most reports on the presence of mold propagules in house dust are based on various analytic methods. In the studies of Gravesen (1978), Wood *et al.* (1988), and Wickman *et al.* (1992) 30 mg dust was plated directly onto a medium. In other studies (Van de Lustgraaf 1977; Ishii *et al.* 1979; Miller *et al.* 1988; Wassenaar 1988a; Yoshida *et al.* 1988; Schober 1988, 1991), the dust was suspended in a solution and diluted stepwise before being plated onto a medium. Furthermore, different culture media were used. The results of measurements of airborne fungal propagules depend highly on the air-sampling device and culture medium used (e.g. Burge *et al.* 1977; Henningson *et al.* 1981; Verhoeff *et al.* 1990). The question arises whether the results of different analytic methods for measuring the presence of fungal propagules in settled house dust also depend on the analytic method used. The present study was undertaken to document the extent to which results differ with the use of different analytic methods, to investigate the variability within and between homes of the presence of fungal propagules in house dust, and to assess the reliability of this method as a measure of potential exposure.

### *4.5.3. Material and methods*

**House-dust samples.** House-dust was sampled in 60 homes in the Netherlands. The homes were selected from a group of approximately 250 homes, included in a case-control study on home dampness and respiratory symptoms of children.

Dust was obtained from the bedroom floor and the mattress of the child with a vacuum cleaner (Philips Topomatic T518) equipped with a special attachment (the ALK allergen mouthpiece, ALK, Horsholm, Denmark) to collect dust on a cellulose filter (Schleicher & Schuell, 589[1] black ribbon 70 mm). The power of the vacuum cleaner was set at 1,000 W. From each bedroom floor, an area of 2 $m^2$ was sampled for 4 min. The entire upper mattress surface with an area of approximately 2 $m^2$, was vacuum cleaned for 2 min after the bedding had been removed. Between the samplings, the mouthpiece was thoroughly cleaned with 70% ethanol.

After sampling, the filters were placed in plastic Petri dishes and stored with silicagel in the dark at room temperature (for no more than 2 weeks) until analysis. Sampling was performed in October-November 1990. For investigation of the variability in time, sampling was repeated after a period of 6 weeks in the first 20 houses.

**Analysis of the samples for fungal propagules.** The various methods used to investigate the presence of fungal propagules in house dust are described below. Each method is a unique combination of culture medium, suspension fluid, and dilution step.

- *Direct plating*
  A 30-mg representative aliquot of unsieved dust was plated directly on either DG18 agar (Hocking and Pitt 1980) or V8 agar (Gravesen 1978), amended with chloramphenicol (100 mg/l) to prevent bacterial growth, with a sterile glass spreader, needle, and forceps. The water activity ($a_w$) of DG18 agar was 0.955 and of V8 agar 0.988.
- *Suspension in sucrose*
  At least 100 mg of representative dust was suspended in a 1:50 (w/w) sucrose solution (50% sucrose + 0.02% Tween 80 in distilled water, sterilised for 30 min at 110°C), shaken at 150 rpm for 10 min and plated (0.1 ml) onto DG18 and V8 agars. This primary solution was diluted by a factor of 10 and, after shaking, 0.1 ml of this dilution was plated onto both agars.
- *Suspension in peptone*
  At least 100 mg of representative dust was suspended in a 1:50 (w/w) peptone solution (0.1% peptone (Difco) + 0.85% NaCl + 0.02% Tween 80 in distilled water). After shaking as above 0.1 ml aliquots of the primary suspension and of a 10-fold dilution were plated onto DG18 and V8 agars.

As far as possible, all analyses were done in duplicate, for which each dust sample had to contain at least 320 mg dust. When less dust was gathered, only direct plating was done, and, if possible, suspension in sucrose. All methods described were applied to the samples taken in the first 20 homes. The samples obtained in the remaining 40 homes were analyzed only by direct plating on DG18 and V8 agars and by suspension in sucrose in combination with DG18 agar.

The plates were incubated in the dark for 5 days at 25°C. The number of Colony Forming Units (CFU) was counted by the naked eye with a colony counter (Gallenkamp, Loughborough, UK) and expressed as CFU/g dust, followed by transfer of inoculum from the colonies onto appropriate media for identification to species level according to standard mycological procedures.

**Statistical analyses.** Statistical analysis of the data was done with SPSS-PC version 4.01. Pearson correlation coefficients were calculated to assess the association between the results in CFU/g dust obtained with the various analytic methods. Paired t-tests were done to test differences between means in CFU/g dust and in number of species isolated. The reproducibility in time of the number of CFU/g dust was assessed by calculating the reliability coefficients from the two-way random effects ANOVA model, according to Armstrong *et al.* (1992).

### 4.5.4 Results

**Results in CFU/g dust.** The numbers of CFU/g dust obtained with the various analytic methods varied widely. The distributions were right-skewed and became normally distributed after ln-transformation. Table 4.8 presents the number of CFU/g dust (geometric mean, geometric standard deviation and range) obtained with the different analytic methods. Approximately 10% of the duplicate samples obtained by direct plating onto V8 and DG18 agars could not be counted because of the high density of the colonies on the agar plates. Furthermore, some dust samples did not contain sufficient dust for all the various analyses.

Table 4.8 Number of CFU/g dust (geometric mean (GM), geometric standard deviation (GSD), range) obtained with 10 different analytic methods, and corresponding coefficients of variation (mean, standard deviation)

| Analytic method | Number of CFU/g dust | | | | Coefficient of variation (%) | | |
|---|---|---|---|---|---|---|---|
| | n | GM | GSD | range | n | mean | sd |
| V8 direct | 140 | 5,310 | 1.96 | 730-41,440 | 116 | 12.5 | 11.8 |
| DG18 direct | 146 | 7,470 | 2.14 | 980-56,050 | 121 | 14.2 | 12.3 |
| V8 peptone | 52 | 28,830 | 2.09 | 6,000-130,480 | 51 | 15.3 | 14.0 |
| V8 sucrose | 58 | 28,770 | 2.22 | 5,740-248,950 | 57 | 16.4 | 14.9 |
| DG18 peptone | 52 | 36,900 | 2.25 | 6,750-140,510 | 52 | 12.0 | 11.2 |
| DG18 sucrose | 109 | 36,980 | 2.71 | 2,120-360,770 | 107 | 11.4 | 10.0 |
| V8 peptone($10^{-1}$) | 52 | 46,680 | 2.82 | 2,500-407,580 | 51 | 26.2 | 21.8 |
| V8 sucrose ($10^{-1}$) | 59 | 47,810 | 3.16 | 6,370-475,920 | 59 | 27.2 | 23.2 |
| DG18 peptone ($10^{-1}$) | 52 | 60,170 | 2.76 | 10,000-632,230 | 51 | 24.3 | 20.3 |
| DG18 sucrose ($10^{-1}$) | 109 | 59,460 | 3.43 | 2,130-1,102,400 | 109 | 24.9 | 22.8 |

Statistically significant Pearson correlation coefficients were found between the numbers of CFU/g dust obtained with the various analytic methods. The correlation coefficients ranged from 0.39 ($p<0.01$) between direct plating onto DG18 and suspension in peptone in combination with V8 agar, to 0.86 ($p<0.001$) between suspension in peptone in combination with DG18 and suspension and dilution in peptone in combination with DG18 agar.

The highest numbers of CFU/g dust (Table 4.8) were obtained by suspension in sucrose or peptone, followed by a 10-fold dilution, in combination with DG18 agar. The lowest number of CFU/g dust were obtained by direct plating onto V8 agar. The yields obtained by direct plating onto both media were significantly lower (paired t-test $p<0.001$) than those obtained after suspension and/or dilution in sucrose or peptone. The yields after primary suspension were significantly lower ($p<0.001$) than those after

suspension and dilution. Comparisons were also made between the numbers of CFU/g dust obtained with different agars and different suspension fluids. The yields in CFU/g dust on DG18 agar were significantly higher than the corresponding yields on V8 agar, for both direct plating and plating of suspensions. The composition of the suspension fluid did not significantly influence the number of CFU/g dust.

The coefficients of variation of the duplicate analyses for the 10 analytic methods were calculated with the non-transformed data (Table 4.8). The mean coefficients of variation varied from 11.4% (primary suspension in sucrose in combination with DG18) to 27.2% (suspension and dilution in sucrose in combination with V8). Overall, the variation coefficients were higher after suspension and dilution than those after direct plating and primary suspension.

**Species isolated.** Because of the high density of the colonies on the agar plates or overgrowth by the fast-growing genera *Mucor* and *Rhizopus*, the colonies from approximately 30% of plates obtained by direct plating could not be isolated. With the other methods, this occurred in 0-10% of the samples. Overgrowth by *Mucor* and *Rhizopus* occurred mainly on V8 agar.

In total, 108 different species belonging to 54 different mold genera were isolated. The most frequently isolated species from mattress dust were *Alternaria alternata*, *Aspergillus versicolor*, *Aureobasidium pullulans*, *Botrytis cinerea*, *Cladosporium cladosporioides*, *C. herbarum*, *Epicoccum nigrum*, *Fusarium* spp., *Mucor* spp., *Penicillium brevicompactum*, *P. chrysogenum*, *P. glabrum*, *Phoma* spp., *Scopulariopsis brevicaulis*, *Ulocladium chartarum*, and some yeasts. From floor dust, the same mold species, except for *S. brevicaulis*, were isolated most frequently, as well as *C. sphaerospermum*, *Eurotium herbariorum*, *Rhizopus* spp. and *Wallemia sebi*. The following yeasts were predominant: *Rhodotorula glutinis*, *R. minuta*, *R. mucilaginosa*, *Cryptococcus albidus*, and *C. laurentii*.

The highest mean number of species isolated was found after direct plating onto DG18 agar (Table 4.9). The numbers of species obtained after suspension and dilution were significantly lower ($p < 0.001$, paired t-test) than those obtained by suspension only. The numbers of species obtained after suspension were significantly lower than those obtained by direct plating. Comparisons were also made between the numbers of species isolated using different agars and suspension fluids. The numbers of species isolated with DG18 agar were significantly higher than those obtained with V8 agar. Furthermore, suspension in sucrose yielded significantly more species than suspension in peptone. Xerophilic fungi, i.e. *Eurotium* spp. and *W. sebi*, were isolated more frequently on DG18 agar, whereas *Fusarium* spp. and *Scopulariopsis* spp. were isolated more frequently on V8 agar.

Table 4.9 Number of species isolated (mean, standard deviation) for the different analytic methods and the reproducibility in terms of species isolated from duplicate analyses, on basis of samples from which all colonies were isolated and identified by species

| Method | n | Number of different species | | Agreement rate (%) | |
|---|---|---|---|---|---|
| | | mean | sd | mean | sd |
| V8 direct | 74 | 14.9 | 3.6 | 59.7 | 13.9 |
| DG18 direct | 88 | 17.3 | 4.2 | 61.0 | 11.9 |
| V8 peptone | 49 | 11.4 | 3.1 | 50.7 | 17.6 |
| V8 sucrose | 51 | 13.0 | 4.7 | 57.4 | 18.2 |
| DG18 peptone | 51 | 13.4 | 4.1 | 53.8 | 16.4 |
| DG18 sucrose | 106 | 13.2 | 4.4 | 54.5 | 17.4 |
| V8 peptone($10^{-1}$) | 50 | 4.8 | 2.5 | 39.4 | 38.9 |
| V8 sucrose ($10^{-1}$) | 58 | 6.0 | 3.4 | 35.4 | 35.9 |
| DG18 peptone ($10^{-1}$) | 50 | 5.7 | 3.3 | 36.6 | 22.8 |
| DG18 sucrose ($10^{-1}$) | 107 | 7.0 | 3.8 | 35.5 | 20.2 |

The reproducibility of the duplicate analyses in terms of species isolated was calculated as the agreement rate: the ratio of the number of identical species isolated from both plates and the total number of species isolated from both plates (Table 4.9).

The mean agreement rate varied from approximately 37%, for suspension and dilution, to approximately 60%, for direct plating.

**Variability in time.** For information about the variability in time of the presence of fungal propagules in house dust, sampling was repeated in 20 homes after 6 weeks. For mattress dust, there were no statistically significant differences (paired t-test) between the average numbers of CFU/g dust found in both periods by most analytic methods, except for direct plating onto V8 agar and suspension in sucrose in combination with both agars. For floor dust, the mean numbers of CFU/g dust of the first sampling period were significantly higher than those of the second sampling period, except for suspension in peptone in combination with V8 agar and suspension in sucrose in combination with both agars.

The reproducibility of the number of CFU/g dust in time was assessed by calculating the reliability coefficient from the two-way random effects ANOVA model (Armstrong *et al.* 1992). For the number of CFU/g in mattress dust, the reliability coefficients ranged from 0.06 to 0.74 (Table 4.10). For floor dust, the reliability coefficients ranged from 0.01 to 0.32. Several reliability coefficients were calculated as negative and are therefore not presented.

Table 4.10 Estimated reliability coefficients for number of CFU/g dust, for mattress dust and floor dust separately

| Method | Mattress | Floor |
|---|---|---|
| V8 direct | 0.52 | 0.32 |
| DG18 direct | 0.19 | 0.21 |
| V8 peptone | 0.06 | 0.20 |
| V8 sucrose | 0.74 | * |
| DG18 peptone | * | * |
| DG18 sucrose | 0.51 | * |
| V8 peptone ($10^{-1}$) | * | 0.01 |
| V8 sucrose ($10^{-1}$) | 0.58 | * |
| DG18 peptone ($10^{-1}$) | * | 0.05 |
| DG18 sucrose ($10^{-1}$) | 0.42 | * |

*: Negative reliability coefficient, due to larger within-home mean square of variance than between-home mean square of variance

The agreement rate over time was calculated between the species isolated from samples taken during the first and second sampling period. Only samples in which all colonies were identified at species level were included. The results of duplicate analyses per sampling period were paired randomly. The mean agreement rates for mattress dust and floor dust are presented in Table 4.11. For mattress dust, the highest mean agreement rates (approximately 43%) between the species isolated in both sampling periods were found for direct plating. However, the number of samples was limited. For floor dust, the highest mean agreement rates (approximately 40%) were obtained for suspension, irrespective of the composition of the suspension fluid or culture medium.

Table 4.11 Agreement rate over time (%) between species isolated from samples taken in 20 homes, within a period of 6 weeks, on basis of plates from which all colonies were identified

| Method | Agreement rate (%) | | | | | |
|---|---|---|---|---|---|---|
| | Mattress | | | Floor | | |
| | n | mean | sd | n | mean | sd |
| V8 direct | 6 | 42.4 | 11.4 | 2 | 29.2 | 20.9 |
| DG18 direct | 10 | 44.0 | 8.2 | 4 | 40.5 | 6.6 |
| V8 peptone | 24 | 34.9 | 14.9 | 21 | 39.9 | 10.1 |
| V8 sucrose | 22 | 35.2 | 12.1 | 19 | 43.0 | 10.7 |
| DG18 peptone | 24 | 34.9 | 13.0 | 24 | 38.7 | 9.4 |
| DG18 sucrose | 24 | 33.4 | 11.6 | 26 | 41.8 | 10.1 |
| V8 peptone ($10^{-1}$) | 24 | 17.0 | 16.3 | 23 | 23.1 | 18.8 |
| V8 sucrose ($10^{-1}$) | 24 | 18.6 | 14.8 | 29 | 27.5 | 15.9 |
| DG18 peptone ($10^{-1}$) | 24 | 14.8 | 20.2 | 24 | 22.3 | 15.4 |
| DG18 sucrose ($10^{-1}$) | 24 | 21.7 | 13.1 | 29 | 27.5 | 10.1 |

### *4.5.5. Discussion*

The comparison between the yields of CFU/g dust indicated that the highest numbers were obtained by suspension followed by 10-fold dilution, and the lowest by direct plating. This was most probably due to breaking up of aggregates of spores during suspension and dilution, which also occurs with the dilution plate count method for the enumeration of fungi in soil (Jensen 1962). Furthermore, the factors used to calculate the numbers of CFU/g dust vary widely for the various analytic methods, e.g. from 33.3 for direct plating, to 5,000, for suspension and dilution in peptone. In studies using suspension and dilution to assess the presence of fungal propagules in house dust, (very) high numbers of CFU/g dust up to $7x10^6$ have been reported (Schober 1988). In studies where the dust was analyzed by direct plating of 30 mg dust (Wood *et al.* 1988; Wickman *et al.* 1992) the median values were around $1\text{-}2x10^3$.

The composition of the suspension solution (peptone or sucrose) did not influence the yield of CFU. The agar did have an influence; the yield on DG18 was significantly higher than on V8 agar. However, the results obtained with the various analytic methods were highly correlated. Van de Lustgraaf (1977) compared the yields for the isolation of xerophilic fungi in mattress dust. He used three different agars: malt extract agar with 8% NaCl, 40% sucrose, or 64% sucrose ($a_w$ respectively 0.94, 0.94 and 0.82). Dust samples were suspended in a 1:50 (w/w) sucrose solution (50% sucrose + 0.02% Tween 80) and aliquots of 0.1 ml were plated onto the agar plates. Overall, the lowest counts were obtained on malt extract agar with 64% sucrose (mean approximately 7,000 CFU/g dust). The highest counts were found on malt extract agar with 40% sucrose (mean approximately 20,000 CFU/g dust). Miller *et al.* (1988) reported higher counts on rose

bengal 2% malt extract agar (mean $8x10^5$ CFU/g dust) than malt extract agar with 20% sucrose (mean $5x10^5$ CFU/g dust). Dust was suspended in sterile water and diluted by a factor of $10^6$. A comparison between the yields in CFU/m³ with different air-sampling devices and agars, including DG18, also showed higher numbers on DG18 (Verhoeff *et al.* 1990). To our knowledge no other comparative studies on the yields using DG18 and V8 agar have been published.

For direct plating, identification of the colonies was impossible for approximately 30% of the plates, especially on V8 agar, because of the high density of colonies on the plates or overgrowth by fast-growing genera. When information on fungal species in house dust is required, direct plating might not be appropriate. Nevertheless, when it was possible to isolate the colonies, the highest mean number of species was obtained by direct plating. The number of species isolated on DG18 was significantly higher than on V8 agar. This confirms the value of DG18 reported in an earlier comparative study of species in indoor air, in which higher yields were also obtained when DG18 was used as isolation medium (Verhoeff *et al.* 1990). Xerophilic fungi, i.e. *Eurotium* spp. and *Wallemia sebi*, were isolated more frequently on DG18 agar. In the study of Van de Lustgraaf (1977), xerophilic fungi were isolated most frequently on malt extract agar with 64% sucrose. However, with this medium, other common species in house dust, such as *Cladosporium* spp. and *Aureobasidium* spp., were not found at all. Besides the medium, the composition of the suspension solution also had a significant influence on the yield of species isolated. Suspension in sucrose yielded higher numbers of species than suspension in peptone.

We conclude that the results of the measurements of fungal propagules in house dust, both quantitatively and qualitatively, depend greatly on the analytic method used.

The reproducibility in terms of CFU/g dust of duplicate analyses was acceptable for direct plating and the primary suspension, with mean coefficients of variation of approximately 11-16%. For the species isolated, the reproducibility of duplicate analyses was only moderate: the highest mean agreement rate found was approximately 60% (direct plating).

Overall, the reproducibility of the number of CFU/g dust in time was better for mattress dust than floor dust; however, also for mattress dust, the predictive value of a single measurement was rather low. This difference might be explained by varying cleaning methods. Mattress dust is probably less frequently disturbed by cleaning than floor dust. The variability with time in the species isolated is substantial, for both mattress dust and floor dust. Thus, a single sample of house dust does not provide reliable information on the presence of fungal propagules (quantitatively as well as qualitatively) over a longer period. Therefore, the potential exposure can be assessed only to a limited extent. Brunekreef *et al.* (1987), pointed out that the error in the exposure assessment in epidemiological studies on the relationship between environmental pollution and health effects, reduces the power of a study to detect a significant association and might introduce a bias in estimates of the size of an effect. Our results do not confirm the suggestion made by Miller *et al.* (1988) that a single sample of house dust reflects the presence of fungal propagules (quantitatively) over a long period. They found that the number of CFU/g dust in dust sampled in a new vacuum bag differed by only 10% from that found in a sample taken from the vacuum bag present in the vacuum cleaner at the moment of sampling. Miller *et al.* (1988) did not provide information on the presence of fungal species.

We conclude that a single sample of settled house dust for the assessment of fungal propagules does not provide a reliable measure of the potential exposure to fungi in indoor environments. Earlier we concluded that measurements of airborne fungal propagules in non-industrial indoor environments show the same lack of reliability for the estimation of the exposure to fungi (Verhoeff *et al.* 1992). Therefore, other indicators of exposure to fungi in indoor environments should be considered. For example, sampling of cell wall components of fungi, such as 1,3-ß-glucan and lipopoly-saccharides, might give an overall estimate of airborne fungal matter. Glucan can be assayed by a modification of the procedure used for bacterial endotoxin (Rylander *et al.* 1992). Further evaluation of its value as measure of exposure to fungi is necessary. However, a disadvantage of using glucans as an indicator of exposure to fungi is that no information on the fungal species present can be obtained. Fungi also produce volatile compounds, frequently evident as "moldy smells". The principal volatile produced by various fungi is 1-octen-3-ol (Flannigan *et al.* 1991). Miller *et al.* (1988) suggested that 3-methyl-1-butanol, 2-hexanone and 2-heptanone are possibly more representative of fungal activity. However, the measurement of these volatiles as a measure of exposure to fungi has to be further evaluated, because these compounds may have other indoor sources as well.

# The presence of fungi and house dust mites in houses in relation to building characteristics

This Chapter includes the following papers:

*Presence of viable mould propagules in indoor air in relation to house damp and outdoor air*
A.P. Verhoeff, J.H. van Wijnen, B. Brunekreef, P. Fischer, E.S. van Reenen-Hoekstra, R.A. Samson
Allergy 47 (1992) 83-91

*Fungal propagules in house dust, II; relation with residential characteristics, and respiratory symptoms*
A.P. Verhoeff, J.H. van Wijnen, E.S. van Reenen-Hoekstra, R.A. Samson, R.T. van Strien, B. Brunekreef
Allergy (in press)

*Mite antigen in house dust: relationship with different housing characteristics in the Netherlands*
R.T. van Strien, A.P. Verhoeff, B. Brunekreef, J.H. van Wijnen
Clin. Exp. Allergy (provisionally accepted)

## 5.1 Introduction

As previously observed in Chapter 3, both fungi and house dust mites encounter favourable conditions for their development in damp environments and from this, it can be inferred that the presence of fungi and house dust mites may be related to home dampness. This Chapter presents information on the relations between home dampness and other home characteristics, and 1) the presence of fungal propagules in indoor air, 2) fungal propagules in settled house dust, and 3) house dust mite allergen (*Der p* I) in settled dust. Knowledge about these relationships is of interest on the one hand with regard to the possibility of predicting the presence of fungi and mites in homes from their characteristics, and on the other hand for the evaluation of control measures aimed at reducing the presence of fungi and mites in homes.

The results of three different studies will be presented here. The first concerns the relation between the presence of airborne fungal propagules and home dampness. In this investigation attention was also given to the relation between fungal propagules in indoor air and in outdoor air, the variability with time of the presence of fungal propagules, and a more detailed comparison between the results obtained with the N6-Andersen sampler and settlement plates. In the second study the relation between the presence of fungal propagules in house dust and residential characteristics, including indoor air humidity, was investigated. The presence of fungi in house dust in homes of children with and without reported respiratory symptoms was also studied. The third study concerns the relation between the presence of *Der p* I in house dust and home characteristics, including indoor air humidity.

The main hypotheses tested in this Chapter are: 1) that there is no relation between the presence of fungi in indoor air and home dampness, 2) that there is no relation between the presence of fungi in settled house dust and home dampness, and 3) that there is no relation between house dust mite allergen in settled house dust and home dampness.

## 5.2 Presence of viable mould propagules in indoor air in relation to house damp and outdoor air [1]

### *5.2.1 Abstract*

The presence of viable mould propagules in indoor air was investigated using the N6-Andersen sampler in combination with DG18-agar, in relation to house damp (characterized with a checklist) and in relation to the presence of moulds in outdoor air. The first part of the study was conducted in 46 houses in the autumn of 1987, the second part in 84 houses in May 1989. Further, in the second part, the results obtained with settlement plates (OPD) were compared with those obtained with the N6-Andersen sampler. The number of CFU/m$^3$ in the indoor and outdoor air varied widely. A large variety of mould genera and species was isolated. Species of *Cladosporium*, *Penicillium* and *Wallemia* predominated. The variability in time was high and the reproducibility of the measurements in terms of CFU/m$^3$ and of species isolated was only moderate. The low predictive value of these measurements limits their use in epidemiological studies of the relationship between exposure to moulds and respiratory symptoms. Overall, the geometric mean concentration was somewhat higher outdoors than indoors. However, the clear differences found between the number of CFU/m$^3$ belonging to different mould species in in- and outdoor air show that the presence of viable mould propagules in indoor air is not simply a reflection of the presence of moulds in outdoor air. The presence of moulds in indoor air was only weakly related to house damp as characterized by the checklist. High, statistically significant correlations were found between the CFU yield obtained with the OPD and the CFU/m$^3$ yield obtained with the N6-Andersen sampler. However, the number of species isolated with the OPD was significantly lower than that obtained with the N6-Andersen sampler.

### *5.2.2 Introduction*

Damp houses are common in the Netherlands; about 15% are estimated to be affected to some degree (Langeweg 1988). Several epidemiological studies suggested a relation between living in damp houses and an elevated prevalence of respiratory symptoms experienced by the residents (Varekamp and Leupen 1970; Martin *et al.* 1987; Strachan 1988; Waegemaekers *et al.* 1989; Brunekreef *et al.* 1989). In most of these studies, information about house damp and the prevalence of respiratory symptoms was obtained by questionnaires. Varekamp and Leupen (1970) used a checklist to scale the degree of damp.

There are indications that moulds and house dust mites, which both encounter favourable conditions in damp houses, are a major cause of illhealth to people living in damp houses (Andersen and Korsgaard 1986; Arundel *et al.* 1986). Prevalences of mould allergy ranging from 2-30% (Gravesen 1979) and of house dust mite allergy up to 85% (Murray *et al.* 1983) are reported in subjects with respiratory allergy.

[1] Verhoeff AP, JH van Wijnen, B Brunekreef, P Fischer, ES van Reenen-Hoekstra & RA Samson. Allergy 47 (1992) 83-91

This study was supported by a grant from the Ministry of Housing, Physical Planning and Environment and by a grant from the Ministry of Welfare, Culture and Public Health

The development of allergic diseases in a healthy individual is the result of an interaction between the genetic predisposition to react to antigens and exposure to environmental factors. In studies of twins, it has been found that in allergic diseases, environmental factors play an important role (Lubs 1971; Wütrich *et al.* 1981).

The ability to assess human exposure to moulds and house dust mites may clarify the relation between living in damp houses and possible effects on health. The major allergens produced by the house dust mite are well characterized and standardized, making it possible to assess human exposure to these allergens by means of environmental monitoring as well as by measuring specific IgE antibodies in serum to these allergens (Platts-Mills and Chapman 1987). The results of environmental monitoring of moulds depend highly on the method used (Verhoeff *et al.* 1990). Moreover, biological monitoring by measuring specific IgE antibodies to allergens produced by moulds is beset with difficulties (Burge 1985; Lowenstein *et al.* 1987). The isolation, purification and standardization of allergens produced by moulds are major problems.

In the present study, the presence of viable mould propagules in indoor air was investigated in relation to house damp. In the first part we investigated the variability in time of the presence of moulds in indoor air and in the second part, the relation between the presence of moulds in indoor and outdoor air.

### *5.2.3 Materials and methods*

#### Selection of houses

The first part of the study was conducted in 46 houses in the city of Amsterdam. The houses were obtained from the registry of the Housing Inspectorate of the Municipal Health Service of Amsterdam that also deals with complaints about damp. The selection of houses was based on the presence of visible mould growth, which is considered to be an indicator of house damp. About 80% of the houses had (some) visible mould growth. This part of the study (part I) was carried out in September-October 1987.

The second part (part II) was conducted in 84 houses in Wageningen during May 1989. They were randomly selected from approximately 220 homes in 4 neighbouring areas. According to the housing corporation damp houses were to be expected in these areas.

#### House damp

A checklist, developed by Varekamp and Leupen (1970), was used to scale several building characteristics related to: 1) the likelihood of damp problems (e.g. date of construction, building materials, the presence of a crawl space), 2) the possibilities of eliminating moisture (e.g. ventilation and heating facilities) and 3) visible signs of damp (damp spots, mould growth, wet crawl space, silver-fish or sow bugs, stale odour and wood rot). For each room in the house, a damp score (scale 0-55) was calculated. The average damp score for all the rooms represents the dampness of the house; the higher the score, the damper the house. As a working hypothesis, a room or house with a damp score (DS) of 20 or more is considered "damp". This cut-off point is based on the observation that the number of house dust mites was clearly increased in houses with an average DS>20 (Varekamp *et al.* 1966), and on the DS distribution after inspection of approximately 1,200 houses (Ter Kuile and Kraaiveld 1987).

**Airborne viable mould propagules**

In parts I and II airborne viable mould propagules were sampled using the N6-Andersen sampler (Jones *et al.* 1985), in combination with DG18 agar, developed by Hocking and Pitt (1980). In a previous study, this combination gave the highest yield and the best reproducibility in terms of number of Colony Forming Units (CFU) per $m^3$ and number of mould species isolated compared with several other combinations of samplers and culture media (Verhoeff *et al.* 1990). The sampling period was 2 min. The air volume sampled was recorded by a gasmeter (G1.6 Meterfabriek, Dordrecht, the Netherlands). Sequential duplicate samples were taken.

The samples were incubated at 25°C for 4 days. CFU were then counted and corrected according to the positive hole conversion table. In part II, sampling was also performed with the open petri dish (OPD), a method relying on the gravitational deposition of mould propagules. The OPD was used in combination with DG18 agar. This method was included because in our previous study (Verhoeff *et al.* 1990) strong correlations were found between the number of CFU/$m^3$ obtained with the N6-Andersen and the number of CFU obtained with the OPD. The OPD has the advantage of longer sampling periods, which may increase measurement reproducibility. Parallel duplicate samples were taken. The sampling period was 60 min.

In part I sampling was conducted at a central point in the living room, at a sampling height of 1.5 m, under normal living conditions. From the samples taken in 25 randomly selected houses, the colonies were transferred onto the appropriate media for identification up to *genus* level. To assess the variability in time, the measurements were repeated in the same houses after 5 weeks.

In part II sampling with the N6-Andersen was performed in the living room, the master-bedroom and outside, near the house. With the OPD, sampling was performed in the living room and master-bedroom. The residents were asked to close windows and doors at least 1 h before the measurements were taken. From the samples taken in the living-rooms of 18 randomly selected houses and the corresponding outdoor air samples, the colonies were identified up to *species* level. Isolation and identification of the fungi were performed according to standard mycological procedures.

Table 5.1 summarizes the sampling protocol for both parts of the study.

Table 5.1 Sampling protocol of viable mould propagules

| | method | location | n | remarks |
|---|---|---|---|---|
| part I | N6 | living room | 46 | sequential duplicate samples; sampling repeated after 5 weeks; identification up to genus level of samples taken in 25 randomly selected houses |
| part II | N6 | living room<br>master-bedroom<br>outdoors | 84<br>84<br>84 | sequential duplicate samples; identification up to species level of samples taken in 18 randomly selected houses (living room) and corresponding outdoor samples |
| | OPD | living room<br>master-bedroom | 84<br>84 | parallel duplicate samples; identification up to species level for samples taken in 18 randomly selected houses (living room) |

**Statistical analysis**

Statistical analysis of the data was performed with the Statistical Package for Social Sciences - PC (Norusis 1986) and -mainframe (Hull and Nie 1981). Pearson correlation coefficients were calculated to assess the association between different variables. T-tests were performed for testing differences between means.

To obtain information on the variability in time of the concentrations of viable mould propagules, an analysis of variance was performed as described by Brunekreef *et al.* (1987), which separates the within-home or "error" variance from the between-homes or "true" variance. The ratio of these components of variance is called the variance ratio or labda.

### *5.2.4 Results*

*House damp.* Table 5.2 presents the damp scores (mean and range) for 4 rooms separately and averaged over the entire house. The damp scores of the entire house and those of the separate rooms were all normally distributed. DS in part I were higher than those in part II. In part I, 13 houses of 45 were classified as "damp", whereas only one of the houses in part II was classified as "damp".

Table 5.2 Damp scores (mean and range) for four rooms separately and for the entire homes, and the number of "damp" rooms, c.q. houses for both parts of the study

| location | part I (n=45) damp score | | | part II (n=84) damp score | | |
|---|---|---|---|---|---|---|
| | mean | range | damp (n) | mean | range | damp (n) |
| living-room | 20.0 | 7.8-38.0 | 19 | 11.0 | 7.0-16.9 | 0 |
| master bedroom | 16.2 | 6.6-40.0 | 15 | 11.4 | 6.0-23.2 | 7 |
| kitchen | 19.8 | 7.8-36.0 | 18 | 10.7 | 6.5-19.2 | 0 |
| bathroom | 20.9 | 11.2-36.2 | 20 | 12.3 | 6.0-22.8 | 3 |
| entire home | 18.7 | 10.6-33.5 | 13 | 11.5 | 7.1-22.1 | 1 |

*Airborne viable mould propagules. Concentrations.* The number of CFU/m$^3$ (N6-Andersen) and CFU (OPD) in indoor and outdoor air varied widely. The distributions were right-skewed and became normally distributed after ln-transformation. Table 5.3 presents the number of CFU/m$^3$ and CFU (geometric mean, geometric SD and range) found in both parts of the study.

Table 5.3 Concentrations of viable mould propagules in CFU/m³ and CFU (geometric mean, geometric SD and range), found in both parts of the study

| method | location | period | n | GM | GSD | range |
|---|---|---|---|---|---|---|
| part I | | | | | | |
| N6 | living room | 1 | 40 | 645 | 2.6 | 62-7,332 |
| | | 2 | 34 | 640 | 2.0 | 159-1,703 |
| part II | | | | | | |
| N6 | living room | - | 84 | 807 | 3.1 | 96-43,045 |
| | bedroom | - | 84 | 822 | 3.2 | 69-23,860 |
| | outdoors | - | 84 | 882 | 2.5 | 124-6,248 |
| OPD | living room | - | 84 | 14 | 2.9 | 1-518 |
| | bedroom | - | 84 | 11 | 2.8 | 0-206 |

The average coefficients of variation of the sequential duplicate samples taken in part I were 39% for the first sampling period and 19% for the second period. The average coefficients of variation of the sequential duplicate samples taken with the N6-Andersen in part II were 21% for living room samples, 18% for bedroom samples, and 28% for the outdoor samples. The average coefficients of variation of the parallel duplicate samples taken with the OPD were 30% for living room samples and 27% for bedroom samples.

Table 5.3 shows that the geometric mean of CFU/m³ was somewhat higher outdoors than indoors (part II). However, the highest CFU/m³ were found in the indoor air of some homes. Pearson correlation coefficients were calculated between the numbers of CFU/m³ found in the living room, bedroom and outdoors. A high correlation coefficient was found ($r=0.73$; $p<0.001$) between the concentrations in living rooms and bedrooms. A correlation coefficient of only 0.13 was calculated between CFU/m³ in living rooms and outdoors. The correlation coefficient was similarly low (0.19) between concentrations in bedrooms and the outdoor air. The latter two correlation coefficients were not statistically significant. No statistically significant differences (paired t-test) were found between the geometric mean of CFU/m³ found in the living room, bedroom and outdoors. However, high statistically significant correlations were found between the results of the measurements obtained with the N6-Andersen and the OPD in the living room and master bedroom (Table 5.4). Fig. 5.1 presents a scattergram of CFU/m³ obtained with the N6-Andersen and the number of CFU obtained with the OPD in the living room.

Table 5.4 Pearson correlation coefficients between the number of CFU/$m^3$ (ln-transformed) obtained with the N6-Andersen and the number of CFU (ln-transformed) obtained with the OPD

| N6-Andersen | OPD | |
|---|---|---|
| | living room | master bedroom |
| living room | 0.78** | 0.65** |
| master bedroom | 0.70** | 0.83** |
| outdoors | 0.28* | 0.15 |

** $p<0.001$; * $p<0.01$

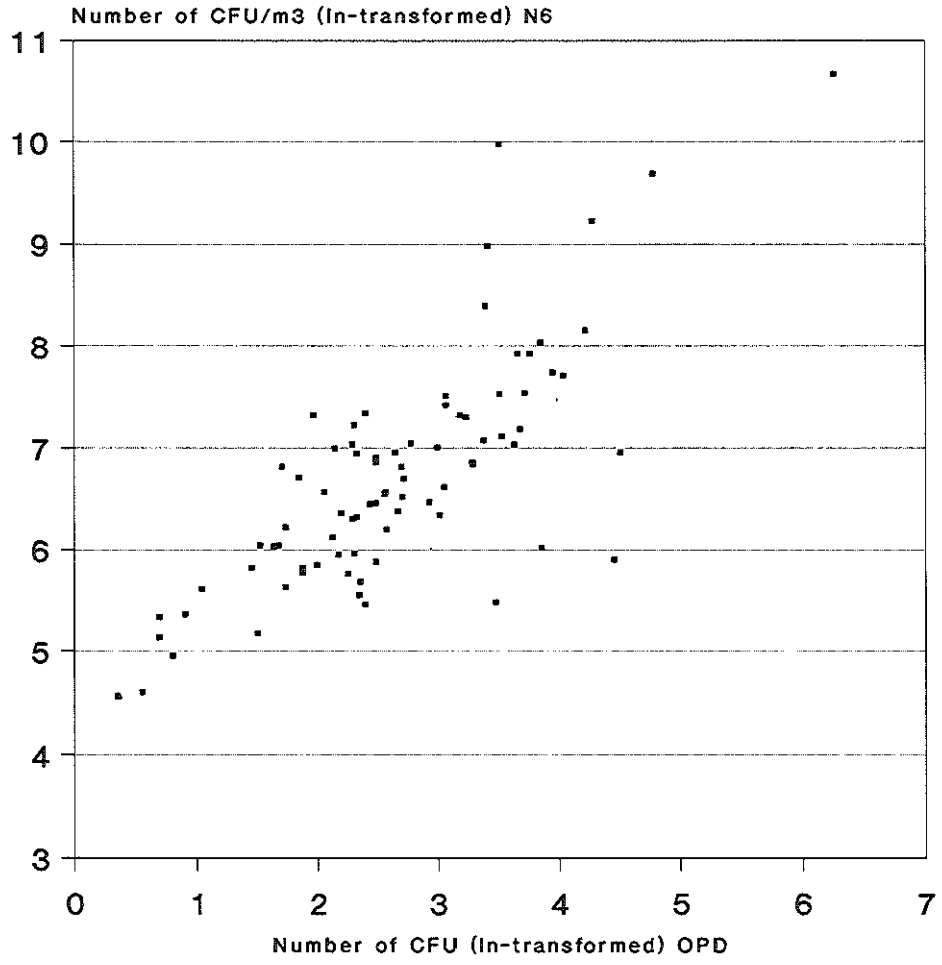


Figure 5.1 : Scatterplot of the number of CFU/m³ (ln-transformed) obtained with the N6-Andersen and the number of CFU (ln-transformed) obtained with the OPD in the living room

*Mould genera and species.* In part I the colonies were isolated and identified up to genus level from the samples taken during both periods in 25 randomly selected houses. However, for 22 of these samples, isolation and identification was impossible because the samples were overgrown by *Chrysonilia*, or because the medium was dehydrated. The qualitative results refer therefore to 78 samples taken in 25 houses.

In part II the fungi were isolated and identified up to species level, from the samples taken with the N6-Andersen sampler and the OPD in the living rooms of 18 randomly selected houses and corresponding outdoor samples.

In total, 19 different mould genera were found in part I whereas 66 different mould species belonging to 24 genera were found in part II.

Table 5.5 presents the most frequently isolated mould genera (part I) and species (part II) in indoor and outdoor air samples. *Alternaria, Aspergillus, Botrytis, Cladosporium, Penicillium* and *Wallemia* were found in almost all the investigated houses included in part I. In part II, *Cladosporium cladosporioides, C. herbarum* and *Penicillium brevi-compactum* were found in almost all investigated houses. *Cladosporium herbarum* was found in all outdoor samples.

The reproducibility of the duplicate samples of mould genera of species isolated was calculated as the agreement rate: the ratio of the number of identical mould genera or species isolated in both samples and the total number of mould genera or species isolated from both samples (Table 5.6).

A comparison (t-test) was made between the yield of species isolated using the N6-Andersen and the OPD. The yield obtained with the OPD (mean 5.6) was lower than that obtained with the N6-Andersen (mean 7.9). The mean agreement rate between the species isolated from the samples taken with the N6-Andersen and with the OPD was 26.0% (SD 10.2, $n=18$).

Table 5.5 Mould genera and species identified in 50% or more of the houses or corresponding outdoor samples

| genus/species | part I | part II | | |
|---|---|---|---|---|
| | | % of houses | | |
| | % of houses N6 | N6 | OPD | % outdoor samples N6 |
| *Alternaria* | 84 | 12 | 6 | 23 |
| *Aspergillus* | 96 | 78 | 61 | 61 |
| *A. penicillioides* | - | 56 | 33 | 28 |
| *A. versicolor* | - | 44 | 33 | 28 |
| *Aureobasidium* | 4 | 17 | 22 | 50 |
| *A. pullulans* | - | 17 | 22 | 50 |
| *Botrytis* | 80 | 39 | 50 | 78 |
| *B. cinerea* | - | 39 | 50 | 78 |
| *Cladosporium* | 100 | 100 | 100 | 100 |
| *C. cladosporioides* | - | 94 | 94 | 94 |
| *C. herbarum* | - | 89 | 78 | 100 |
| *Eurotium* | 64 | 56 | 44 | 72 |
| *E. herbariorum* | - | 56 | 44 | 72 |
| *Penicillium* | 100 | 100 | 83 | 94 |
| *P. aurantiogriseum* | - | 44 | 22 | 56 |
| *P. brevicompactum* | - | 89 | 50 | 67 |
| *P. olsonii* | - | 67 | 33 | 44 |
| *Wallemia* | 100 | 61 | 67 | 56 |
| *W. sebi* | - | 61 | 67 | 56 |

Table 5.6 Reproducibility in terms of mould genera (part I) or species (part II) isolated from duplicate samples (mean and SD of the agreement rate in %)

| | method | location | period | *n* | mean | SD |
|---|---|---|---|---|---|---|
| part I | N6 | living room | 1 | 22 | 74.6 | 15.2 |
| | | | 2 | 12 | 51.7 | 23.7 |
| part II | N6 | living room | | 18 | 42.3 | 20.1 |
| | | outdoors | | 18 | 32.2 | 10.3 |
| | OPD | living room | | 18 | 30.2 | 19.3 |

In part I the concentrations (in CFU/m³) were calculated for the most frequently isolated mould genera, *Aspergillus, Cladosporium, Penicillium* and *Wallemia*. From 13 houses reliable duplicate samples were obtained during both sampling periods.

Table 5.7 presents the concentrations of these genera for both sampling periods. In the first period the highest concentrations were found for *Penicillium*, followed by *Wallemia*, *Cladosporium* and *Aspergillus*. In the second period again the highest concentrations were found for *Penicillium*, whereas the lowest concentrations were found for *Wallemia*.

Table 5.7 Concentrations in CFU/m³ (GM, GSD and range) of *Aspergillus, Cladosporium, Penicillium* and *Wallemia* for both sampling periods in 13 houses (part I)

| genus | period 1 | | | period 2 | | |
|---|---|---|---|---|---|---|
| | GM | GSD | range | GM | GSD | range |
| *Aspergillus* | 32 | 4.7 | 0-215 | 17 | 3.9 | 0-112 |
| *Cladosporium* | 63 | 2.8 | 8-545 | 61 | 5.0 | 0-412 |
| *Penicillium* | 127 | 2.6 | 18-1043 | 167 | 2.7 | 17-561 |
| *Wallemia* | 72 | 3.8 | 18-982 | 2 | 4.8 | 0-224 |

Table 5.8 presents the concentrations of the most frequently isolated mould species found in living rooms and in the outdoor air in part II of the study. The highest concentrations were found for *Cladosporium*, indoors as well as outdoors.

Table 5.8 Comparison between concentrations in CFU/m³ (GM, GSD) of 17 different mould species in indoor and outdoor air (t-test; $n = 18$) (part II)

| species | indoors | | outdoors | | | |
|---|---|---|---|---|---|---|
| | GM | GSD | GM | GSD | T | p |
| Total number of CFU/m³ | 669 | 2.5 | 941 | 2.4 | -1.27 | 0.22 |
| *Aspergillus* (total) | 24 | 6.6 | 8 | 6.0 | 1.70 | 0.11 |
| *A. penicillioides* | 6 | 6.6 | 2 | 4.5 | 1.80 | 0.09 |
| *A. versicolor* | 4 | 5.4 | 2 | 2.3 | 1.69 | 0.11 |
| *Aureobasidium pullulans* | 1 | 2.2 | 3 | 4.4 | -2.19 | *0.04* |
| *Botrytis cinerea* | 2 | 2.7 | 6 | 4.0 | -2.54 | *0.02* |
| *Cladosporium* (total) | 245 | 2.8 | 501 | 3.0 | -3.08 | *0.01* |
| *C. cladosporioides* | 65 | 5.5 | 72 | 8.4 | -0.18 | 0.86 |
| *C. herbarum* | 59 | 7.4 | 224 | 3.8 | -3.49 | *<0.01* |
| *C. macrocarpum* | 2 | 2.3 | 1 | 1.6 | 1.86 | 0.08 |
| *C. sphaerospernum* | 3 | 5.3 | 3 | 3.9 | -0.03 | 0.98 |
| *Eurotium amstelodami* | 2 | 3.1 | 2 | 3.5 | -0.45 | 0.66 |
| *E. herbariorum* | 4 | 4.3 | 6 | 4.2 | -0.88 | 0.39 |
| *Penicillium* (total) | 154 | 3.6 | 65 | 4.4 | 2.04 | 0.06 |
| *P. aurantiogriseum* | 2 | 2.8 | 3 | 5.6 | -0.72 | 0.46 |
| *P. brevicompactum* | 15 | 7.6 | 6 | 4.8 | 1.85 | 0.08 |
| *P. glabrum* | 6 | 12.1 | 2 | 4.0 | 1.34 | 0.20 |
| *P. jensenii* | 2 | 4.5 | 2 | 2.4 | 0.87 | 0.40 |
| *P. olsonii* | 8 | 8.4 | 3 | 3.3 | 1.88 | 0.08 |
| *Ramularia deusta* | 1 | 2.0 | 1 | 2.1 | -0.35 | 0.73 |
| *Wallemia sebi* | 5 | 5.3 | 4 | 5.3 | 0.55 | 0.59 |

*Relation with house-damp.* Pearson correlation coefficients were calculated between total CFU/m³ (ln-transformed) and damp scores of living-room, master bedroom, kitchen, bathroom and the entire house. The results for both parts of the study are presented in Table 5.9.

In part I statistically significant correlations were found between bathroom DS and total numbers of CFU/m³, for both sampling periods. The DS of the entire home and total CFU/m³ correlated significantly only in the first sampling period. Pearson correlation coefficients were also calculated between numbers of CFU/m³ belonging to the most frequently isolated genera and DS. For *Aspergillus* and *Penicillium* the correlation coefficients were all positive (varying from 0.01 to 0.44), but not statistically significant. For *Cladosporium* negative correlation coefficients were found; in the first sampling period 3 of 5 correlation coefficients were statistically significant. For *Wallemia* the correlation coefficients were positive for the first sampling period, but negative for the second sampling period. However, these coefficients were not statistically significant.

Table 5.9 Pearson correlation coefficients between the total of CFU/m³ and CFU (ln-transformed) and the damp score of four different rooms and the entire homes for both parts of the study

| | damp score | | | | |
|---|---|---|---|---|---|
| | living room | master bedroom | kitchen | bathroom | entire home |
| part I (N6) | | | | | |
| period 1 ($n$=40) | 0.10 | 0.19 | 0.10 | 0.39** | 0.22* |
| period 2 ($n$=40) | -0.18 | -0.02 | -0.20 | 0.23** | 0.13 |
| part II (N6) | | | | | |
| living room ($n$=84) | 0.17 | 0.01 | 0.18 | -0.04 | 0.02 |
| master bedroom ($n$=84) | 0.26* | 0.18 | 0.20 | 0.08 | 0.20 |
| part II (OPD) | | | | | |
| living room ($n$=84) | 0.28** | 0.06 | 0.19 | -0.04 | 0.07 |
| master bedroom ($n$=84) | 0.30** | 0.20 | 0.22 | 0.06 | 0.20 |

* $p<0.05$; ** $p<0.01$

The geometric mean concentrations of mould propagules in "dry" and "damp" houses were compared per sampling period (t-test). These comparisons were made for total CFU/m³, as well as for concentrations of the 4 most frequently isolated genera. In both sampling periods, the total geometric mean of CFU/m³ and the geometric mean of CFU/m³ for the different mould genera were generally higher in "damp" houses than in "dry" houses. For *Cladosporium*, however, the geometric mean of CFU/m³ for the first sampling period was higher in "dry" houses than in "damp" houses. None of these differences was statistically significant. Also, the concentrations found in houses where mould growth was visible in at most one room ($n$=19) and those where mould growth was visible in 2 rooms or more ($n$=3) were compared. The geometric mean concentrations of viable mould propagules were higher in houses with visible mould growth in at least 2 rooms for both sampling periods. However, the differences were small and not statistically significant. The number of houses with no visible mould growth was too small to make a proper comparison.

In part II, significant correlations were found only between living-room damp scores and numbers of CFU in living rooms and bedrooms, as well as the numbers of CFU/m³ in bedrooms. No statistically significant correlations were found between CFU/m³ belonging to the most frequently isolated mould species and damp scores.

A comparison between the geometric mean concentrations of mould propagules in "dry" and "damp" houses was not possible, because only one of the investigated houses was classified as "damp". However, the numbers of CFU/m³ and CFU found in the living-rooms without visible mould growth ($n$=78) could be compared with those found in the living rooms with visible mould growth ($n$=6). CFU/m³ and CFU were significantly higher ($t$=2.75, $p$=0.01 and $t$=2.12, $p$=0.04, respectively) in living rooms with visible mould growth. However, the same comparisons between master bedrooms showed no statistically significant differences.

*Variability in time.* In part I sampling was repeated after 5 weeks, to obtain information about the variability in time of the presence of viable mould propagules in the indoor air. There was no statistically significant difference between the average total number of CFU/m³ found in both periods ($t=1.43$; $p=0.16$). Further, there were no statistically significant difference between the concentrations found in both periods for the most frequently isolated mould genera, except for *Wallemia* ($t=6.43$; $p<0.01$).

An analysis of variance was performed to estimate the within-house and between-house components of variance. The analysis of variance was conducted for the total number of CFU/m³, as well as for the concentrations of the most frequently isolated mould genera. The results are presented in Table 5.10.

The estimated variance ratios of "error" to "true" variance were generally high. This indicates that in this group of houses, the differences between houses regarding the presence of viable mould propagules were small compared with the variation in time within houses.

The mean agreement rate between the mould genera isolated in both sampling periods was 50.8% ($n=11$, SD 22.4%).

Table 5.10 Estimated within-house and between-house components of variance and variance ratio for the total concentrations of viable mould propagules and for *Aspergillus*, *Cladosporium*, *Penicillium* and *Wallemia* separately (part I)

| | estimated within-house variance | estimated between-house variance | variance ratio |
|---|---|---|---|
| Total CFU/m³ | 0.665 | 0.167 | 3.982 |
| *Aspergillus* | 2.133 | -0.027 | * |
| *Cladosporium* | 1.283 | 0.335 | 3.829 |
| *Penicillium* | 0.503 | 0.426 | 1.181 |
| *Wallemia* | 2.019 | 0.107 | 18.794 |

* variance ratio not calculated because of the estimated negative between-home variance

*Comparison of mould species in in- and outdoor air.* In total, 66 different mould species were isolated. Eighteen species were isolated only from indoor air samples, whereas 14 species were isolated only from outdoor samples. However, these species were not isolated more than twice out of 36 samples. The mean agreement rate between the species isolated from in- and outdoor samples taken with the N6-Andersen was 26.2% (SD 7.5, $n=18$). The mean agreement rate between species isolated from indoor samples taken with the OPD and the outdoor samples was 23.5% (SD 7.1, $n=18$).

A comparison (t-test) was made between the numbers of CFU/m³ found for the most frequently isolated species in the indoor and outdoor air.

Table 5.8 shows that the total number of CFU/m³ was somewhat higher in the outdoor air than in the indoor air of these 18 houses. However, the mean numbers of CFU/m³ belonging to *Aspergillus* (total), *A. penicillioides* and *A. versicolor* were higher in the indoor air, as also for most of the *Penicillium* species. For *Cladosporium* (total) as well as *C. herbarum*, the mean numbers of CFU/m³ were significantly higher in the

outdoor air, whereas no differences were found for the remaining species belonging to the genus *Cladosporium*. The mean number of CFU/m³ of *Aureobasidium pullulans* was significantly higher in outdoor air than in indoor air; no differences were found for *Ramularia deusta* and *Wallemia sebi*.

### *5.2.5 Discussion*

#### House damp

The number of "damp" houses found in part I was much higher than that found in part II. The selection of houses included in part I was based on the presence of visible mould growth, whereas those in part II were selected randomly. Further, the weather during part II was unseasonably dry and warm. This may have affected the results, because some of the checklist items apply to the actual presence of visible signs of dampness in the house.

In most epidemiological studies investigating the relation between house damp and respiratory health, information was obtained by questionnaires (Martin *et al.* 1987; Strachan 1988; Waegemaekers *et al.* 1989; Brunekreef *et al.* 1989). However, as indicated by Strachan (1988) and others, this method can incur potential responder bias. Yet also it is not clear whether the checklist used in this study, that scales several building characteristics in relation to house damp, is a better method for classifying houses as "damp" or "dry". Although a responder bias is avoided, the question remains whether the characteristics that favour the production of inhalatory allergens, such as moulds, are distinguished and weighted properly with this checklist. It is worthwhile pursuing this issue further, because a simple and validated checklist that enables a discrimination between "damp" and "dry" houses would be very useful in epidemiological studies.

#### Viable mould propagules

The geometric mean of CFU/m³ found in indoor air was higher than that reported by Holmberg (1987) and Waegemaekers *et al.* (1989), but comparable with that reported by others (Binnie 1987; Fradkin *et al.* 1987; Hunter *et al.* 1988). In part I 19 different mould genera were isolated. *Alternaria, Aspergillus, Botrytis, Cladosporium, Penicillium* and *Wallemia* were found in almost all investigated houses. In part II, 66 different mould species belonging to 24 different genera were isolated. *Cladosporium cladosporioides, C. herbarum, Eurotium herbariorum, Penicillium brevicompactum* and *Wallemia sebi* were isolated from most indoor and outdoor air samples. In part I the highest numbers of CFU/m³ were found for *Penicillium*, followed by *Cladosporium*. In part II the highest numbers of CFU/m³ were found for *Cladosporium cladosporioides*, followed by *C. herbarum* and some of the species belonging to *Penicillium*. These results agree generally with those reported by others (e.g. Gravesen 1972; Fradkin *et al.* 1987; Hunter *et al.* 1988), although in most studies the fungi were only identified up to genus level.

However, a proper comparison between the quantitative and qualitative results of different studies is difficult because in most studies different methods were used for the sampling of airborne viable mould propagules. In our previous study it was shown that the results in terms of CFU/m³ and of species isolated depend on the combination of sampler and culture medium used (Verhoeff *et al.* 1990). Also, the studies were performed in different seasons. It is well known that the presence of viable moulds

outdoors as well as indoors varies widely between seasons (Gravesen 1972; Hunter *et al.* 1988). This may also have contributed to the differences found between part I and part II of this study. Thirdly, the reproducibility of the measurements in terms of CFU/m³ and of moulds species isolated is low. The coefficients of variation of samples taken within minutes of each other were high, which is in agreement with results published previously (Smid *et al.* 1989; Waegemaekers *et al.* 1989; Verhoeff *et al.* 1990). The reproducibility of mould genera or species isolated was not very good either. Another possible source of variation might have been the considerable influence of air disturbances caused by indoor activities on the number of CFU/m³, as reported by Hunter *et al.* (1988).

The results of part I indicated that the within-house variation in the concentrations of mould propagules was much higher than the between-house variation. The ratios of "error" to "true" variance were generally high. Thus, the measurement of viable mould propagules in air with the techniques used in this study approximated the true exposure to only a limited extent (Brunekreef *et al.* 1987).

High, significant correlations were found between the CFU/m³ obtained with the N6-Andersen in the living and bedroom and the number of CFU obtained with the OPD. However, the mould species isolated with the OPD were significantly fewer than those isolated with the N6-Andersen. These findings are in agreement with our previous results (Verhoeff *et al.* 1990). Thus, with the OPD only a semi-quantitative but less complete picture about the presence of viable moulds in indoor air may be obtained.

The geometric mean concentrations of CFU/m³ found in the outdoor air during part II was somewhat higher than those found in living and bedrooms. However, the differences were not statistically significant. Further, no statistically significant correlations were found between the concentrations in indoor and outdoor air, but, a statistically significant correlation was found between the concentrations in living rooms and bedrooms.

A comparison, between the CFU/m³ concentrations of mould species in indoor and outdoor air showed clear differences. For *Aspergillus*- and *Penicillium*-species, the concentrations were higher indoors than outdoors. For most species belonging to *Cladosporium* and for *Aureobasidium pullulans* and *Botrytis cinerea*, the concentrations in outdoor air were higher than in indoor air. These results indicate that outdoor air may influence the presence of viable mould propagules in indoor environments, but that the presence of viable moulds in indoor air is not simply a reflection of the presence of moulds in outdoor air. Also, the high correlation found between the total numbers of CFU/m³ in the living room and bedroom indicates that indoor sources of moulds are present. These results are in agreement with those reported by Fradkin *et al.* (1987). This suggests that in aerobiological studies, attention should be given to the identification of Colony Forming Units up to species levels.

Despite the high within-house variability, it is notable that the total mean numbers of CFU/m³ and the mean number of CFU/m³ of the different genera were higher in "damp" houses than in "dry" houses during both sampling periods in part I of this study. However, these differences were not significant. Further, the correlations between house damp and indoor viable mould concentrations in both parts of the study were generally positive but low. Thus, the presence of viable mould propagules was only weakly related to house damp as characterized by the checklist. Nevertheless, Hunter *et al.* (1988) and Waegemaekers *et al.* (1989) also reported higher numbers of CFU/m³ in houses which were classified as "damp".

It is concluded that, due to the large variability, the measurement of viable mould propagules in indoor air with the techniques used in this study does not provide a method to distinguish the exposure to moulds in "damp" and "dry" homes. The question thus remains whether house characteristics favourable for moulds are indeed properly distinguished and weighted with the checklist used in this study.

## 5.3 Fungal propagules in house dust; relation with residential characteristics, and respiratory symptoms [1]

### *5.3.1 Abstract*

As part of a case-control study on the relation between home dampness and respiratory symptoms of children, house-dust samples were collected from bedroom floors and mattresses in 60 homes in the Netherlands. The house-dust samples were analyzed for the presence of fungal propagules by plating 30 mg of dust directly onto DG18 agar. A checklist and questionnaire were used to obtain information on the home characteristics and occupant behavior, that may have an effect on the presence of fungal propagules in house dust. The geometric mean (GM) number of CFU/g dust collected from the floors was 8,990. The number of CFU/g dust was significantly higher in dust from carpeted floors than in dust from smooth floors (GM respectively 12,880 CFU/g dust and 3,530 CFU/g dust). The GM number of CFU/g dust collected from mattresses was 6,760. Overall, the mean numbers of CFU/g dust collected from floors and mattresses were higher in bedrooms where damp spots and/or mold growth was observed. However, these differences were not statistically significant. The relation between home characteristics and the number of CFU/g dust of the most frequently isolated mold species (n=17), including *Alternaria alternata*, *Cladosporium cladosporioides*, *Penicillium brevicompactum* and *Scopulariopsis brevicaulis*, was also investigated. Only the type of flooring had a significant and consistent effect on the number of CFU/g floor dust of the different mold species. For *P. brevicompactum*, the number of CFU/g floor dust was significantly higher in bedrooms where damp spots were observed. The number of CFU/g mattress dust of *S. brevicaulis* was also significantly higher for those bedrooms where damp spots were observed. However, in view of the large number of statistical comparisons made, these two significant relationships might have been caused by chance alone. The total numbers of CFU/g mattress and floor dust were not related to the average relative indoor humidity measured over 6 weeks. Furthermore, there was no association between the presence of fungi in house dust and respiratory symptoms. We conclude that there was only a very weak relationship between the home characteristics and occupant behavior, as determined by checklist and questionnaire, and the presence of fungal propagules in floor dust and mattress dust. Only the type of flooring had a substantial and statistically significant effect on the presence of fungal propagules in floor dust. Therefore, the presence of fungal propagules in house dust cannot be predicted reliably by home characteristics.

[1] Verhoeff AP, JH van Wijnen, ES van Reenen-Hockstra, RA Samson, RT van Strien & B Brunekreef. Allergy (in press).

This study was supported by a grant from the Ministry of Welfare, Culture and Public Health.

### 5.3.2 *Introduction*

House dust is recognized as a major source of allergens in nonindustrial indoor environments. Over the last 25 years, it has been shown that pyroglyphid mites are a major source of allergens in house dust in many areas of the world (Platts-Mills and De Weck 1989; Platts-Mills *et al.* 1992). It is also clear that house dust is a complex mixture that may contain various other potent allergens such as animal danders (Lind *et al.* 1987; Wood *et al.* 1988) and moulds (Gravesen 1978; Van Bronswijk *et al.* 1986; Wood *et al.* 1988; Wickman *et al.* 1992).

A number of recent epidemiologic studies have indicated a relation between home dampness and respiratory symptoms (e.g. Brunekreef *et al.* 1989; Platt *et al.* 1989; Strachan and Sanders 1989; Waegemaekers *et al.* 1989; Dales *et al.* 1991b; Brunekreef 1992a). House-dust mites and fungi, which both encounter favourable conditions in damp houses, are thought to be a major cause of respiratory symptoms in damp houses. Several authors have suggested that molds are primarily harmful through production of allergens, toxins, and volatile compounds (Tobin *et al.* 1987; Flannigan *et al.* 1991). Evidence of an association between asthma and indoor exposure to fungi comes from a case-control study among 72 adults with asthma and 72 age- and sex-matched controls (Burr *et al.* 1988). Nineteen patients and nine controls reported mold spots in their homes. Furthermore, there was a significantly higher prevalence of positive radioallergosorbent test (RAST) responses to *Penicillium* among patients reporting mold in their homes.

The prevalence of house-dust mites and the potential exposure to house-dust mite allergens is generally assessed by sampling settled house dust (Platts-Mills and De Weck 1989; Platts-Mills *et al.* 1992). The presence of fungi in indoor environments is generally assessed by air sampling or surface sampling. However, air sampling of viable fungal particles does not provide reliable data for an estimation of the exposure to fungi in nonindustrial indoor environments (Verhoeff *et al.* 1992). Assessment of viable fungal particles (fungal propagules) in settled house dust might be a useful measure of potential exposure to fungi. Several studies report on the presence of fungal propagules in house dust (Van de Lustgraaf 1977; Gravesen 1978; Ishii *et al.* 1979; Wood *et al.* 1988; Yoshida *et al.* 1988; Wickman *et al.* 1992). However, little is known about the presence of fungal propagules in house dust in relation to home characteristics, e.g., signs of dampness, type of flooring, cleaning methods and indoor humidity. There is no information about the predictive value of these home characteristics regarding the presence of fungal propagules in house dust. Furthermore, the relation between fungal propagules in dust and respiratory symptoms in occupants is (largely) unknown. Therefore, as part of a case-control study on respiratory symptoms in children and home dampness, the presence of viable fungal particles in house dust was assessed, together with indoor relative humidity and other home characteristics that might influence the presence of fungal propagules in house dust.

### *5.3.3 Material and methods*

**Study population**

Samples of settled house dust were obtained in October and November 1990 from 60 homes in the Netherlands. The homes were selected from a group of 281 in the province of Noord-Holland that was included in a case-control study on home dampness and respiratory symptoms of children aged 6-12 years. Selection criteria were the presence of signs of dampness anywhere in the home observed by the investigators, and reported respiratory symptoms of the children. Four different groups of 15 homes each were selected: 1) homes of children without reported respiratory symptoms (controls) and without observed dampness; 2) homes of children without respiratory symptoms but with observed dampness anywhere inside; 3) homes of children with reported respiratory symptoms (chronic wheeze, chronic cough, attacks of shortness of breath with wheezing, or doctor-diagnosed asthma) denoted as cases, without observed dampness; and 4) homes of children with respiratory symptoms and with observed signs of dampness. These groups were chosen to increase the possibility of rejecting the hypotheses of the study: there is no difference in the presence of fungal propagules (quantitatively and qualitatively measured) in bedroom floor dust and mattress dust 1) in bedrooms with or without observed dampness, or 2) in bedrooms of children with reported respiratory symptoms and controls.

Finally, 31 cases and 29 controls were included in the study. Some characteristics of the study population are described in Table 5.11.

Table 5.11 Characteristics of the study population

| | Cases (n=31) | Controls (n=29) |
|---|---|---|
| Age (mean, years) | 8.74 | 8.38 |
| Sex (% boys) | 54.8 | 27.6 |
| Total IgE (mean, IU/ml) | 310.4 | 126.1 |
| Increased total IgE levels (%) (adjusted for age) | 63.1 | 55.2 |
| Mold allergy (RAST>0, %) * | 6.6 | 3.6 |
| House-dust mite allergy (RAST>0, %) | 36.7 | 10.7 |
| Difference of pulmonary function (%) (cases compared with controls) | | |
| - Forced vital capacity | -3.2 | |
| - Forced expiratory volume in 1 s | -3.9 | |
| - Peak expiratory flow | -4.4 | |
| - Maximum midexpiratory flow | -4.5 | |
| Visible signs of dampness in home (%) | 51.6 | 48.3 |
| Textile floor covering in bedroom (%) | 71.0 | 79.3 |

*: Mold mixture used in RAST panel included *Penicillium*, *Alternaria*, *Aspergillus*, and *Cladosporium*

**House-dust samples**

Dust from the bedroom floor and the mattress of each child was collected with a vacuum cleaner (Philips Topomatic T 518) equipped with a special attachment (the ALK allergen mouthpiece, ALK, Horsholm, Denmark) to retain dust on a cellulose filter (Schleicher & Schuell, 589[1] 70-mm black ribbon). The power of the vacuum cleaner was set at 1,000 W. In each bedroom, a floor area of 2 $m^2$ was sampled. Each square meter was sampled for 2 min. The entire upper mattress surface (an area of approximately 2 $m^2$) was vacuum-cleaned for 2 min, after the bedding had been removed. Between each sampling, the mouthpiece was thoroughly cleaned with 70% ethanol. After sampling, the filters were placed in plastic Petri dishes and stored with silicagel in the dark for a maximum of 2 weeks at room temperature until analysis.

**Fungal propagules in house dust**

For investigation of the fungal propagules in house dust, 30 mg representative dust (unsieved) was plated directly onto DG18 agar (Hocking and Pitt 1980), with a sterile glass spreader, needle, and forceps. The analyses were done in duplicate. At present, there is no standardized method for the analysis of house-dust samples for the presence of fungal propagules. In a comparative study on 10 different analytic methods, direct plating of 30 mg dust onto DG18 agar was shown to be one of the better methods (Verhoeff *et al.* 1994). The plates were incubated in darkness for 5 d at 25°C, and then

the number of Colony Forming Units (CFU) was counted with the naked eye with a colony counter (Gallenkamp, Loughborough, UK) and expressed as CFU/g dust. After counting, the colonies were transferred onto appropriate media for identification by species according to standard mycologic procedures.

**Home characteristics**

For characterization of those variables of the home environment and the occupant behavior, that might affect the presence of fungal propagules in house dust, a checklist and a questionnaire were used. The checklist, completed by the investigators, consisted of 59 items, including type of home, ventilation facilities, heating facilities, building materials, home insulation, home design, type of flooring, type of mattress, and visible signs of dampness (damp patches and/or fungal growth on outer and inner walls, ceilings, and floors). The questionnaire, completed by a principal occupant, consisted of 90 items, including the number of occupants, the presence of pets, cleaning methods, age of flooring and mattress, and visible signs of dampness over the past 2 years.

**Relative humidity**

Relative humidity and temperature were measured with thermohygrometers continuously for 6 weeks in the bedrooms of 11 children with respiratory symptoms and of eight children without respiratory symptoms. The thermohygrometers were calibrated at the beginning of the measuring period and then every 2 weeks.

**Statistical analysis**

Statistical analysis of the data was performed with SPSS-PC version 4.01. Pearson correlation coefficients were calculated to assess associations among continuous variables. For testing differences between means t-tests were performed. Bivariate and multivariate linear regression analyses were conducted to investigate the association between the numbers of CFU/g dust and home characteristics. For that purpose, all variables regarding home characteristics were dichotomized with regard to their possible effect on the presence of fungal propagules in house dust. A variable was given the value '0' if it was considered not to favor the development of fungi in house dust, and the value '1', if it was considered to favor development.

### *5.3.4 Results*

**Number of CFU/g dust**

The total number of CFU/g dust in both floor and mattress dust varied widely (Table 5.12). The values were right-skewed, but normally distributed after ln-transformation. For approximately 9% of the samples, the count could not be made because of the high density of colonies on the agar plates. This loss of samples was equal for floor dust and mattress dust, but it was somewhat higher for controls than for cases (14% and 5%).

Table 5.12 Frequencies (geometric mean (GM), geometric standard deviation (GSD)) of numbers of CFU/g dust from bedroom floors and mattresses for total population and cases and controls separately

| Location | Total population | | | Cases | | | Controls | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | GM | GSD | n | GM | GSD | n | GM | GSD |
| Bedroom floor | 54 | 8,990 | 2.30 | 30 | 8,300 | 2.6 | 24 | 9,940 | 2.0 |
| Smooth | 15 | 3,530 | 1.93 | 9 | 2,760 | 1.8 | 6 | 5,050 | 1.8 |
| Textile | 39 | 12,880 | 1.79 | 21 | 13,250 | 1.8 | 18 | 12,460 | 1.8 |
| Mattress | 55 | 6,760 | 2.11 | 29 | 6,810 | 2.0 | 26 | 6,690 | 2.3 |

The total number of CFU/g dust from textile floorings (Table 5.12) was significantly higher than that for smooth floorings (t-test, $p<0.001$). The number in mattress dust was significantly lower than that in dust from carpeted floors, but significantly higher than that in dust from smooth floors. However, the total number of CFU/g in mattress dust correlated significantly with the number in dust from smooth floors ($r=0.58$; $p<0.05$), but not with that in dust from textile floorings.

In total, 64 different fungal species belonging to 26 genera were isolated by the analytic method described. The following species were isolated from more than 50% of the floor and/or mattress dust samples: *Alternaria alternata*, *Aspergillus versicolor*, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *C. herbarum*, *Eurotium herbariorum*, *Mucor* spp, *Penicillium aurantiogriseum*, *P. brevicompactum*, *Ulocladium chartarum*, and yeasts. *Scopulariopsis* spp. were isolated mainly from mattress-dust samples, whereas the xerophilic mold *Wallemia sebi* was isolated from approximately 30% of both mattress and floor dust samples. The following yeasts were predominant: *Rhodoturula glutinis*, *R. minuta*, *R. mucilaginosa*, *Cryptococcus albidus*, and *C. laurentii*. Table 5.13 presents the geometric mean numbers for the most frequently isolated mold species and genera, and the yeasts, in floor and mattress dust. Table 5.13 shows that the colonies from approximately 40% of the samples could not be isolated and identified because of the high density of the colonies on the plates. This loss of samples was comparable for floor dust and mattress dust, as well as for cases and controls. The mean numbers of CFU/g of the various species in mattress dust were generally higher than those in the dust of smooth floors. This difference was significant ($p<0.05$) for *U. chartarum* only. The mean numbers in dust from textile floorings were generally higher than those in mattress dust. These differences were significant for *C. cladosporioides*, *Cladosporium* spp., and *Penicillium* spp.

Table 5.13 Frequencies (GM and GSD) of numbers of CFU/g dust from bedroom floors and mattresses for eight different mold species and yeasts

| Species | Floor dust | | | Mattress dust | | |
|---|---|---|---|---|---|---|
| | n | GM | GSD | n | GM | GSD |
| *Alternaria alternata* | 33 | 145 | 6.2 | 35 | 160 | 6.0 |
| *Alternaria* spp. | 36 | 168 | 6.2 | 37 | 244 | 2.5 |
| *Aspergillus versicolor* | 36 | 33 | 13.8 | 37 | 38 | 9.2 |
| *Aspergillus* spp. | 36 | 44 | 13.0 | 37 | 58 | 8.7 |
| *Aureobasidium pullulans* | 36 | 493 | 6.4 | 37 | 275 | 6.8 |
| *Cladosporium cladosporioides* | 31 | 453 | 3.9 | 34 | 181 | 8.5 |
| *Cladosporium* spp. | 36 | 1461 | 2.8 | 37 | 617 | 5.2 |
| *Eurotium herbariorum* | 36 | 20 | 14.3 | 37 | 37 | 9.1 |
| *Penicillium brevicompactum* | 32 | 215 | 6.8 | 37 | 134 | 6.0 |
| *Penicillium* spp. | 36 | 994 | 2.8 | 37 | 451 | 2.9 |
| *Ulocladium chartarum* | 35 | 33 | 14.4 | 36 | 79 | 8.1 |
| *Wallemia sebi* | 36 | 6 | 14.9 | 37 | 4 | 9.2 |
| Yeasts | 36 | 498 | 5.8 | 37 | 457 | 9.3 |

**Relation with home characteristics**

The relation between home characteristics and the total number of CFU/g dust was studied by performing bivariate linear regression analyses with 46 explanatory variables. As indicated above, all variables were dichotomized with regard to their possible effect on the presence of fungal propagules in house dust. For floor dust, only five variables showed a (marginally) significant ($p<0.10$) influence on the number of CFU/g dust. For mattress dust, four variables were found to have a (marginally) significant influence. Multivariate linear regression analyses were performed including only those explanatory variables that showed at least a marginally significant effect on the number of CFU/g dust according to the bivariate linear regression analyses. Table 5.14 presents the results of the multivariate linear regression analyses for floor dust and mattress dust. Table 5.14 indicates for each variable, whether it is considered to favor, or not to favor, the presence of fungal propagules in house-dust. Furthermore, the regression coefficient and the coefficient to the power of e ("factor") are presented. The latter indicates to what extent the number of CFU/g will increase if the variable in question is expected to favor development of molds. Also $R^2$ is presented, as it indicates the proportion of the variance of the number of CFU/g dust explained by the regression analysis.

For floor dust, 76% of the variance was explained by the type of flooring, the presence of pets, and whether or not the occupants were at home most of the time. For mattress dust, 57% of the variance was explained by the presence of furniture against the outer wall, fungal growth on the inner wall, and damp spots on the outer wall. However, it should be mentioned that the distribution of each of these explanatory variables was right-skewed. For example, fungal growth on the inner wall was present in

only one of the bedrooms. The results obtained with the regression analysis might therefore have been determined mainly by outliers.

Bivariate linear regression analyses were also performed on the number of CFU/g of each of the most frequently isolated mold species (n=17) and genera (n=13) in floor dust and mattress dust. As mentioned before, the colonies could not be isolated from about 40% of the samples. Therefore, the number of homes included in the regression analyses was reduced, as was the number of explanatory variables (n=29). In total, 1,740 bivariate linear regression analyses were made. For each species or genus, approximately three varying explanatory variables had a marginally significant influence ($p<0.10$) on the number of CFU/g floor or mattress dust. Only the type of flooring had a consistent and substantial influence on the numbers of CFU of the different species and genera in floor dust: the numbers were higher in dust from textile floorings than in dust from smooth floorings. All other explanatory variables had no consistent influence on the numbers of CFU/g for the various species or genera in dust. This finding, together with the large number of regression analyses made, suggests that the significant relations found might have been caused by chance alone. Therefore, no multivariate linear regression analyses were done for the number of CFU/g dust of the various mold species and genera.

Table 5.14 Relation between total number of CFU/g dust from floors and mattresses and home characteristics (multivariate regression analyses)

| Characteristic (not favoring/favoring fungal growth) | Coefficient | Standard error | Factor* | p |
|---|---|---|---|---|
| **Floor dust** ($R^2=0.76$) | | | | |
| occupants at home most of time (yes/no) | -0.40 | 0.19 | 0.67 | 0.04 |
| presence of pets (no/yes) | -0.47 | 0.17 | 0.63 | 0.01 |
| type of floor (smooth/textile covering) | 1.33 | 0.17 | 3.78 | <0.01 |
| **Mattress dust** ($R^2=0.57$) | | | | |
| furniture against outer wall (no/yes) | -0.74 | 0.27 | 0.48 | 0.01 |
| mold patches on inner wall (no/yes) | 1.47 | 0.64 | 4.35 | 0.03 |
| damp patches on outer wall (no/yes) | 0.70 | 0.22 | 2.04 | <0.01 |

* Factor: regression coefficient to the power of e

**Relation with reported and observed dampness**

A comparison was made (t-test) between the total CFU/g in floor and mattress dust, and the reported and observed dampness of the bedroom (Table 5.15). For floor dust, the geometric mean number of CFU/g was lower in bedrooms where the presence of fungal growth or damp spots was reported by the occupants. However, these differences were not statistically significant. In contrast, the geometric mean in mattress dust was higher in bedrooms with reported fungal growth or damp spots. Only for reported damp spots was the difference statistically significant. Overall, the mean numbers of CFU/g in floor and mattress dust were higher in bedrooms where fungal growth or damp spots were observed by the investigators, but the differences were not statistically significant.

The same comparisons made for the different fungal species and genera revealed that the counts for *Mucor* spp. and *U. chartarum* in floor dust were significantly lower in bedrooms with reported signs of dampness. The number of CFU/g dust belonging to *P. brevicompactum* in floor dust was significantly higher in bedrooms where dampness was observed by the investigators. The number of CFU/g dust of *S. brevicaulis* in mattress dust was also significantly higher in bedrooms where dampness was observed. However, in view of the large number of comparisons made (n=240), these significant differences might have been caused by chance alone.

Table 5.15 Relation between total numbers of CFU/g in floor dust and mattress dust, and reported or observed dampness in bedroom (t-test)

| | | No signs of dampness | | Signs of dampness | | p |
|---|---|---|---|---|---|---|
| | | n | GM | n | GM | |
| Reported dampness | **floor dust** | | | | | |
| | fungal growth | 50 | 9,090 | 3 | 8,130 | 0.83 |
| | damp spots | 45 | 9,170 | 7 | 7,120 | 0.47 |
| | **mattress dust** | | | | | |
| | fungal growth | 51 | 6,540 | 3 | 10,870 | 0.26 |
| | damp spots | 46 | 6,010 | 7 | 11,870 | 0.02 |
| Observed dampness | **floor dust** | | | | | |
| | fungal growth | 50 | 9,010 | 1 | 5,100 | - |
| | damp spots | 42 | 8,370 | 9 | 10,710 | 0.44 |
| | **mattress dust** | | | | | |
| | fungal growth | 51 | 6,400 | 2 | 8,400 | 0.61 |
| | damp spots | 42 | 6,370 | 11 | 8,210 | 0.32 |

**Fungi in dust of bedrooms of cases and controls**

A comparison was made between the total number of CFU/g in dust gathered in bedrooms of cases and controls (Table 5.11). The numbers of CFU/g in mattress dust and in dust from textile floorings were slightly higher for cases than for controls, but these differences were not statistically significant. For smooth floorings, the mean numbers were lower for cases than for controls.

The same comparisons were made with the numbers of CFU of the different fungal species and genera. For textile floorings, there were only minor differences between cases and controls. In dust from smooth floors, the counts of *Aureobasidium pullulans* were significantly higher for controls than for cases. The number of yeasts in mattress dust of controls was significantly higher than that for cases, whereas the counts of *W. sebi* were significantly higher in mattress dust of cases than for controls. However, in view of the number of comparisons made, these significant differences might have been caused by chance alone.

**Relation with relative humidity**

The average relative humidities over the entire measuring period in the different bedrooms ranged from 52.3% (sd 5.5) to 79.0% (sd 7.1). Pearson correlation coefficients were calculated between the average relative humidities and the numbers of CFU/g mattress and floor dust. This was done separately for textile and smooth floorings. The calculated correlation coefficients were all negative, but not statistically significant. Furthermore, there was no association between reported or observed dampness and the average relative humidity. However, the average relative humidity in the bedrooms of cases (68.4%, sd 7.8) was significantly higher (t-test, $p=0.025$) than the average relative humidity in the bedrooms of controls (60.7%, sd 4.9%). Comparable results were obtained with the average absolute humidity over the entire measuring period, because the relative and absolute humidity were strongly associated.

### *5.3.5 Discussion*

The numbers of CFU/g dust found in the present study were higher than those reported in two other studies in which the presence of fungal propagules in house dust was investigated by plating 30 mg dust directly onto an agar plate. Wood *et al.* (1988) sampled house dust in 106 houses in the Baltimore area, MD, USA. The numbers ranged from 133 to 25,341 CFU/g dust (median 2,398). However, Wood *et al.* (1988) used a mixed dust sample from the bedroom floor, mattress, and the floors of the television area and the basement. The dust was analyzed by direct plating of 30 mg dust onto V8-agar. A valid comparison could not be made because of the different sampling and analytic methods. Wickman *et al.* (1992) investigated the presence of fungal propagules in 175 homes in Sweden by taking dust samples from the floor of the living-room and plating 30 mg portions directly onto V8-agar. The mean count was 1,349 CFU/g dust (median 999). We have compared the results obtained with 10 different analytic methods (Verhoeff *et al.* 1994). This comparison indicated that the yield depends on the medium used. Higher numbers of CFU were obtained with DG18 than with V8-agar. In the study of Gravesen (1978) no estimation of the numbers of CFU was made.

The type of flooring had a significant influence on the counts, which were approximately four times higher in dust from textile floorings than from smooth floorings. Wood *et al.* (1988) and Gravesen (1978) did not provide information on the type of flooring. Wickman *et al.* (1992) reported only slightly higher numbers of CFU in dust from wall-to-wall carpets. The presence of fungal propagules in floor dust was not associated with reported or observed signs of dampness in the bedroom. Wickman *et al.* (1992) did not find a relation between the number of CFU/g of floor dust (living-room) and reported damp patches or mold growth in the home either. However, in their study, higher counts were associated with shower bathing (>0.5 h/d) and reported windowpane condensation in winter. Lower counts of CFU/g floor dust in the present study were associated with the presence of pets in the home and the absence of the occupants during most of the day.

In our study, higher numbers in mattress dust were associated with observed mold growth and damp patches in the bedroom, whereas lower numbers were associated with the presence of furniture placed directly against the outer wall. However, these findings lack meaning because the distributions of these explanatory variables were right-skewed.

The results obtained might therefore have been determined mainly by outliers. Wood *et al.* (1988) reported a positive association between reported mold growth in the home and the number of CFU/g dust. As mentioned above, they used a mixed dust sample, including mattress and floor dust from four different locations in the home. They also found an association between the number of propagules in house dust and the age of the home. The highest numbers were found in homes less than 10 years and more than 30 years old. In our study, no relation was found with the age of the home.

As far as comparable, the fungal flora found in the present study is similar to that reported by Gravesen (1978), Wood *et al.* (1988) and Wickman *et al.* (1992). However, the xerophilic fungi *Eurotium* spp. and *W. sebi* were not found by these investigators because these species do not develop on V8-agar. Van de Lustgraaf (1977) also frequently isolated xerophilic fungi from mattress dust, using three different media with relatively low water activities ranging from 0.82 to 0.94.

The type of flooring had a significant and consistent influence on the numbers of CFU in floor dust of the various species and genera. Overall, for each species or genus, each time approximately three varying home characteristics had a marginally statistically significant influence ($p<0.10$) on the number of CFU/g floor or mattress dust. In view of the large number of statistical comparisons made, we believe that these associations might have been caused by chance alone. Therefore, they are not reported here. Wickman *et al.* (1992) reported that some construction characteristics of buildings were associated with the occurrence of certain mold species and genera. For example, *A. alternata* was more prevalent in dust samples from homes with mineral-wool thermal insulation than in homes without this insulation. Although Wickman *et al.* (1992) reported four statistically significant associations, they did not report the total number of comparisons made and the distributions of the explanatory variables.

In our study, no clear differences were found between the presence of fungi in mattress and floor dust (textile and smooth floorings separately) gathered in the homes of cases and controls. The loss of samples caused by the high density of colonies on the agar plates was somewhat higher for controls than for cases. Therefore, the actual difference between the numbers of CFU/g in mattress dust and in dust from textile floorings for cases and controls might even be smaller. Yoshida *et al.* (1988) investigated the presence of fungi in house dust of patients with summer-type hypersensitivity pneumonitis and controls. The total numbers of fungi and yeasts were not significantly different between the homes of the two groups. However, some yeasts (*Trichosporon* spp.) were isolated significantly more frequently from dust obtained in the homes of patients. Ishii *et al.* (1979) investigated the presence of fungi in house dust of asthmatic children with positive and negative skin tests to molds and in house dust of non-asthmatic controls in Japan. The average number of fungal colonies did not differ significantly among the three groups. Also, the mycoflora in the dust of the three different groups was comparable. Only *W. sebi* was isolated more frequently from the dust in homes of asthmatic children than of controls. In our study, the counts of *W. sebi* were also significantly higher in mattress dust of cases than controls. *W. sebi* is known to have allergenic properties (Van Bronswijk *et al.* 1986). In the study of Wood *et al.* (1988) among 106 allergic patients, no association was found among total colony counts in house dust and skin test results, a history of asthma, or a history of exacerbation of symptoms on mold exposure. Wickman *et al.* (1992) reported significantly lower numbers of CFU in dust obtained in homes of atopic children than in that of control children. They suggested that the fact that sanitation measures are taken more often in the homes of asthmatic children explains this difference. Textile floorings were present in 45% of

the living rooms of the control group, as compared with 31% for the atopic group. However, as mentioned earlier, the type of flooring only marginally affected their counts. Furthermore, they found no differences between the two groups in reported cleaning habits.

No correlations were found between the average relative humidity in the bedroom over the entire measuring period and the total number of CFU in mattress and floor dust. However, statistically significant correlations were found between the average relative humidity in the bedroom and the concentrations of *Der p* I (the major allergen of the mite *Dermatophagoides pteronyssinus*) in mattress and floor dust (results will be presented elsewhere). Wickman *et al.* (1992) did not find an association between total CFU count and absolute indoor humidity. In our study the average relative humidity in the bedrooms over the entire measuring period of cases was significantly higher than that in the bedrooms of controls. Wickman *et al.* (1992) found a higher average absolute indoor humidity in the houses of house-dust-mite sensitized children than of other atopic children, but not of the control group.

We conclude that only a weak relation was found between the home characteristics as compiled by a checklist and questionnaire, and the number of fungal propagules in floor and mattress dust. Only the type of flooring had a significant and substantial effect on the number of fungal propagules in floor dust. Therefore, the presence of fungal propagules in house dust cannot be reliably predicted by the home characteristics as registered in the checklist and the questionnaire. Furthermore, the presence of fungal propagules in house dust was not related to the average relative indoor humidity measured over 6 weeks. No clear differences were found between the presence of fungal propagules in house dust of children with reported respiratory symptoms and of controls.

## 5.4 Mite antigen in house dust: relationship with different housing characteristics in the Netherlands [1]

### *5.4.1 Abstract*

As part of a case-control study on the relationship between home dampness and respiratory symptoms of children, the concentration of the major allergen of *Dermatophagoides pteronyssinus* (*Der p* I) in floor dust and mattress dust in 516 dwellings in the Netherlands was measured. A checklist, completed by the investigators, was used to obtain information on home and occupant characteristics, which may have an impact on the *Der p* I concentration in house dust. The geometric mean mite antigen concentrations were 2,370 ng *Der p* I/g floor dust for the living room, 2,201 ng *Der p* I/g floor dust for the bedroom and 5,075 ng *Der p* I/g mattress dust. In 86% of the houses the maximum concentration was higher than 2,000 ng *Der p* I/g dust, that is regarded as representing a risk for genetically predisposed individuals for the development of specific IgE to house dust mite allergen. In 55% of the houses the maximum concentration exceeded 10,000 ng *Der p* I/g dust, regarded as a risk factor for acute attacks of asthma for mite allergic patients. The *Der p* I concentrations in dust from carpeted floors were six to fourteen times higher than in dust from floors with a smooth floor covering. Higher *Der p* I concentrations in floor dust were also significantly associated with increasing age of the dwelling and of the floor covering, with an increasing number of occupants and with the absence of floor insulation. For mattress dust, the age of the mattress, the presence of an outer cavity wall and mechanical ventilation were important factors. Older mattresses had higher levels, and mattress dust from bedrooms with solid brick outer walls had higher levels than that from bedrooms with outer cavity walls. Mattresses in homes with continuous mechanical ventilation had almost twice lower levels than mattresses in homes with natural ventilation. There was a tendency towards higher *Der p* I concentrations in dust in homes with reported or observed signs of dampness. The *Der p* I concentrations in dust from carpeted bedroom floors and mattresses were positively associated with the average relative humidity in the bedroom over a period of 3 to 6 weeks in a subset of the homes where relative humidity was measured. Similar results were obtained using the concentrations of *Der p* I in ng/m$^2$ instead of ng/g dust. The results obtained in this study are of importance for planning and evaluating allergen avoidance measures advised to mite allergic patients.

### *5.4.2 Introduction*

In 1964 the house dust mite *Dermatophagoides pteronyssinus* was reported to be a major source of "house dust allergen" (Voorhorst *et al.* 1964). Since then, several studies have been conducted to establish which conditions favour the development of *Dermatophagoides pteronyssinus*. At first, house dust mites were counted under a microscope to estimate exposure (Voorhorst *et al.* 1964; Arlian *et al.* 1978; Van Bronswijk *et al.* 1971).

[1] Strien RT van, AP Verhoeff, B Brunekreef & JH van Wijnen.
Clin Exp Allergy (provisionally accepted)

This study was supported by a grant from the Dutch Asthma Fund.

Several investigators found a relationship between the number of mites in house dust and respiratory symptoms (Voorhorst *et al.* 1969; Korsgaard 1983a). Later, the major allergens of different house dust mites were isolated and immunochemical techniques were developed to determine the amount of allergen in dust samples (Platts-Mills *et al.* 1986; Lind 1986b).

The numbers of mites per gram house dust, and the antigen concentrations in the dust have been reported to vary widely. Absolute indoor humidity was found to have a relationship with the number of mites and with mite antigen concentrations (Wickman *et al.* 1991; Korsgaard 1983b). Relative indoor humidity was also found to be associated with mite numbers (Hart and Whitehead 1990). Tovey *et al.* (1981b) suggested that dampness of the house and age/type of furnishing and bedding were associated with mite antigen concentrations. Arlian and co-workers (1982) reported that floor dust from carpeted floors contained higher numbers of mites than floor dust from smooth floorings. They reported no significant correlation of mite numbers with the frequency and thoroughness of cleaning, the amount of dust, the age of furnishings and the age of the dwelling. They also reported that repeated vacuuming of mattresses or floors did not significantly reduce mite numbers. In other studies, a significant reduction of mite numbers and amounts of mite allergens in carpets, rugs and mattresses was found by vacuuming, although the long term effect was not clear (Hart and Whitehead 1990; Wassenaar 1988b; De Boer 1990). Wickman and co-workers (1991) reported a close association between type of dwelling and absolute indoor humidity. Absolute indoor humidity was found to be higher in single-story individual houses than in multistory individual houses. Apartments in flats were shown to have an even lower absolute indoor humidity. Mite antigen concentrations reflected this difference between these types of dwellings.

This paper reports a study on the levels of *Der p* I (a major allergen of *Dermatophagoides pteronyssinus*) in dust collected in 516 homes in the Netherlands and the relationship between these levels and a number of housing and occupant characteristics.

### *5.4.3 Materials and methods*

#### Population

The homes included in this study were inhabited by children who participated in a case-control study on the relationship between home dampness, respiratory symptoms and *Der p* I exposure. Results of the case-control study will be published elsewhere.

The parents of 7,632 Dutch schoolchildren in the age of 6-12 years old were invited to complete a screening questionnaire on respiratory symptoms based on existing, validated respiratory symptom questionnaires (Brunekreef *et al.* 1992). From these questionnaires, 80.1% was returned. The participants in this study were selected on the basis of answers to the questions in the screening questionnaire. Children reporting at least one positive answer to questions on wheeze in the past year (1), attacks of shortness of breath with wheeze in the past year (2), chronic cough (3) and doctor diagnosed asthma (4) were regarded as 'cases', and children without reported respiratory symptoms were regarded as 'controls'. Of the 'cases' randomly invited to participate in the full study, 78.1% agreed. For 'controls' this figure was 59.8%. The relative lower participation rate among 'controls' was partly due to the presence of two schools in the sample with mostly children of a religious conviction that precludes participation in invasive studies (the case-control study involved taking venous blood samples). Such reservations were much

less frequent among parents of symptomatic children. One half of the children in the study was selected from the eastern part of the Netherlands and the other half from the western part, i.e. close to the two participating research groups. The Netherlands has a temperate climate, with daily average outdoor temperatures in summer ranging from 15 to 25°C, and in winter from zero to 10°C. The homes of all selected children were visited by trained investigators (n=20, mostly students in the Wageningen University Environmental Sciences Programme) in October and November 1990. Complete information was obtained from 259 'cases' and 257 'controls'.

**_Der p_ I determination in house dust**

In each house three dust samples were taken by vacuuming (using a Philips Topomatic T518 at a power of 1,000 W) a single area of two $m^2$ of the bedroom floor and the living room floor for four minutes each, and by vacuuming the entire mattress surface for two minutes after bedding, sheets and mattress covers had been removed. When in a room a smooth floor cover and a rug on top of it were encountered, one $m^2$ of the rug and one $m^2$ of the smooth floor cover were sampled. The dust was collected on cellulose filters (Schleicher & Schuell, 589[1] black ribbon 70mm) using sampling nozzles manufactured by ALK Laboratories, Horsholm, Denmark. After sampling, the filters with dust were stored in closed Petri dishes at -20°C. Between two samples the sampling nozzle was thoroughly cleaned with ethanol. The dust was not sieved. Filters were conditioned at 20°C before weighing, and the total amount of dust collected was determined using a Mettler analytical balance. Of each dust sample, approximately 200 mg was removed using a spatula and forceps. The amount removed was weighed on the analytical balance before extraction by shaking in two ml ammoniumbicarbonate buffer for two hours. Analysis for *Der p* I was done using a monoclonal EIA, manufactured by ALK Laboratories, Horsholm, Denmark (Lind 1986b). The detection limit of the assay was 20 ng *Der p* I/g dust. Coefficients of variation (CV) were determined on the basis of 169 floor dust samples of wall-to-wall carpets for which each of the two square meters were sampled separately, 23 floor dust samples of wall-to-wall carpets that were extracted in duplicate and 130 floor and mattress dust extracts that were analysed in duplicate. For ln-transformed floor dust concentrations the mean coefficient of variation was 5.3%, which was mainly due to sampling (sampling 4.7%, dust extraction 1.7%, analysis 1.5%). The results were also expressed in ng *Der p* I/$m^2$, for which we assumed that the extracted dust was representative for the total dust sample.

**Checklist on building and occupant characteristics**

A checklist on building and occupant characteristics was developed for assessment of the likelihood that a home might become infested with dust mites. A trained investigator completed the checklist in each dwelling. From this checklist, approximately 30 housing and occupant characteristics were derived for further evaluation. Table 5.16 presents an overview of these characteristics.

A questionnaire, completed by the principal occupant, was used to obtain information about visible signs of dampness during the past two years.

Table 5.16 Overview of housing and occupant characteristics potentially related to the level of dust mite infestation

1. Type of home
2. Age of the home
3. Construction of foundation
4. Presence of materials to block water seepage through capillary action
5. Floor construction
6. Floor insulation
7. Construction of walls
8. Insulation of walls and/or windows
9. Roof construction
10. Roof insulation
11. Location of room (floor)
12. Heating system
13. Ventilation system
14. Location of home (east or west)

15. Number of occupants
16. Type of floor cover
17. Age of floor cover
18. Presence of upholstered furniture
19. Placement of furniture directly against outer walls
20. Presence of pets
21. Number of non-succulent plants in the room
22. Indoor cloth drying
23. Frequency of vacuum cleaning
24. Usual indoor winter temperature

25. Dampness of crawling space
26. Observed damp stains on floor
27. Observed mouldy stains on floor
28. Observed damp or mouldy stains on other indoor surfaces (walls, ceiling)
29. Other signs of dampness such as the presence of saw bugs
30. Signs of dampness as reported by the occupants

**Measurement of indoor relative humidity**

In the child's bedroom of 19 dwellings, relative humidity and temperature were measured with thermohygrometers for periods of 22 to 42 days. These recordings all took place in the same two months (October/November 1990). The thermohygrometers were placed in the bedroom, roughly one meter above floor level. Relative humidity and temperature were recorded every two hours. The average relative humidity for the complete measuring period was used in further analyses. The thermohygrometers were calibrated using a wet-bulb thermometer at the beginning of the measuring period, and then every 14 days.

**Data analysis**

The distributions of the *Der p* I concentrations were examined for the three different sampling locations. The distributions were right skewed and became approximately normally distributed after ln transformation.

Most of the housing and occupant characteristics were dichotomized with regard to their possible impact on mite antigen levels. A variable was given the value '0' if it was considered not to favour house dust mite infestation, and the value '1' if it was considered to favour this infestation. A few were grouped into three or four categories. The relationship between the categorized characteristics and the *Der p* I concentration was first examined using bivariate linear regression analysis. For floor dust samples, this was done separately for smooth floors, smooth floors with rugs and carpeted floors. A floor was called 'smooth' when no carpet of any kind was present. Then, multivariate linear regression analyses were performed with the housing and occupant characteristics which were found to have a significant relationship ($p < 0.05$) with the *Der p* I concentration in the bivariate analyses. These characteristics were analysed in a backward elimination procedure in which variables with a p-value of more than 0.10 were eliminated in a stepwise fashion (Kleinbaum *et al.* 1988). Because only 10 homes were situated in apartment buildings, these homes were excluded from the multivariate analyses. All housing characteristics with frequencies of less than ten in either category were omitted from all analyses. Statistical significance was assumed when $p < 0.05$. Statistical analysis was done using SAS software (version 6.06) on a VAX mainframe computer.

*5.4.4 Results*

Table 5.17 presents the *Der p* I concentrations for the three different sampling locations. The *Der p* I concentrations in dust from smooth floors with rugs were four to seven times higher than in dust from smooth floors ($p < 0.001$), whereas *Der p* I concentrations in dust from wall-to-wall carpets were six to fourteen times higher ($p < 0.001$). The type of floor cover in the bedroom was not related to the *Der p* I concentration in mattress dust, although the geometric mean concentration was somewhat higher in mattress dust in bedrooms with wall-to-wall carpets. High statistically significant correlations were found between the amounts of *Der p* I expressed as ng/g dust and those expressed as ng/m$^2$, ranging from 0.57 (bedroom floor, smooth) to 0.92 (living room floor, smooth with rug and wall-to-wall carpet).

Table 5.17 *Der p* I concentrations in ng *Der p* I/g house dust (geometric mean (GM) and geometric standard deviation (GSD)) for the three different sampling locations, stratified for type of floor cover, and comparison of the *Der p* I concentrations in dust from smooth floors with those in dust from smooth floors with rugs and wall-to-wall carpets (t-test)

| location | n | GM | GSD | t |
|---|---|---|---|---|
| living room | 512 | 2370 | 6.3 | |
| smooth | 112 | 371 | 2.8 | |
| smooth with rug | 170 | 2652 | 5.8 | 11.95** |
| wall-to-wall carpet | 230 | 5378 | 4.8 | 19.03** |
| bedroom | 514 | 2201 | 4.9 | |
| smooth | 144 | 640 | 2.7 | |
| smooth with rug | 31 | 2870 | 4.4 | 5.41** |
| wall-to-wall carpet | 339 | 3626 | 4.5 | 14.78** |
| mattress | 512 | 5075 | 4.0 | |
| smooth bedroom floor | 145 | 4511 | 3.8 | |
| smooth bedroom floor with rug | 30 | 4416 | 4.4 | -0.08 |
| carpeted bedroom floor | 337 | 5405 | 4.0 | 1.33 |

** $p<0.001$

Table 5.18 shows the Pearson correlation coefficients between the *Der p* I concentrations for the different sampling locations. Floor dust concentrations were stratified according to type of floor covering. Most correlation coefficients were high, especially between the *Der p* I concentrations in mattress and bedroom floor dust.

Table 5.18 Pearson correlation coefficients for ln-transformed *Der p* I concentrations in floor dust (bedroom and living room) and mattress dust (number of observations in parentheses)

| | bedroom (smooth) | bedroom (smooth with rug) | bedroom (carpeted) | mattress |
|---|---|---|---|---|
| bedroom (smooth) | | | | 0.53** (144) |
| bedroom (smooth with rug) | | | | 0.69** (30) |
| bedroom (carpeted) | | | | 0.64** (337) |
| living room (smooth) | 0.20 (51) | 0.19 (8) | 0.54** (52) | 0.29** (110) |
| living room (smooth with rug) | 0.18 (51) | 0.41 (16) | 0.44** (103) | 0.33** (170) |
| living room (carpeted) | 0.26* (42) | 0.32 (7) | 0.40** (181) | 0.31** (229) |

* $p<0.05$
** $p<0.01$

For all appropriate characteristics mentioned in Table 5.16, the bivariate association with the *Der p* I concentration was investigated for the three sampling locations separately. For floor dust concentrations, the analyses were stratified according to the type of floor covering. Because many of the housing and occupant characteristics were found to be related, a multiple regression analysis was performed in which initially all variables were entered which had at least a marginally significant ($p<0.10$) relation-ship with the *Der p* I concentration in the dust. Table 5.19 shows the results of the multivariate linear regression analyses for the *Der p* I concentrations in dust from living room floors (smooth floors, smooth floors with rugs and wall-to-wall carpeted floors separately) including only those variables which had a statistically significant ($p<0.05$) relationship with the *Der p* I concentration in the dust. Table 5.20 shows the results of a similar analysis for the *Der p* I concentration in bedroom floor dust, and Table 5.21 for mattress dust. In the multivariate linear regression analyses with the bedroom floor *Der p* I concentration as dependent variable, the *Der p* I concentration in the mattress dust was considered as an independent variable as well. R-squared is presented in the tables, as it indicates the proportion of the variance of the *Der p* I concentrations explained by the variables in the regression analysis. The reference categories of *Der p* I concentrations included in the tables denote the ln-transformed intercepts of the regressions.

Table 5.19 Multivariate association of different housing characteristics and the *Der p* I concentration in *living room* floor dust

| exposed vs reference category | ratio of *Der p* I concentration in exposed vs reference category |
|---|---|
| smooth floor | n=101 / $R^2$=0.06 |
| *Der p* I concentration for reference category | 282 ng/gram |
| upholstered furniture present vs not present | 1.60 (1.10-2.33)[a] |
| smooth floor with rug | n=157 / $R^2$=0.13 |
| *Der p* I concentration for reference category | 1108 ng/gram |
| indoor cloth drying vs no indoor cloth drying | 1.77 (1.05-2.99) |
| non insulated floor vs insulated floor | 3.00 (1.78-5.05) |
| wall-to-wall carpet | n=192 / $R^2$=0.17 |
| *Der p* I concentration for reference category | 686 ng/gram |
| insulated walls vs not insulated walls | 1.72 (1.02-2.90) |
| wooden floor vs concrete floor | 1.83 (1.25-2.68) |
| 4 occupants vs <4 occupants | 2.63 (1.32-5.21) |
| 5 occupants vs <4 occupants | 2.66 (1.30-5.46) |
| >5 occupants vs <4 occupants | 2.80 (1.33-5.90) |
| floor cover 4-7 years old vs <4 years old | 2.07 (1.30-3.28) |
| floor cover >7 years old vs <4 years old | 2.28 (1.43-3.66) |

[a] 95% confidence interval

In living rooms with a smooth floor covering, only the presence of upholstered furniture was significantly correlated with the *Der p* I concentration. In living rooms with rugs on smooth floors, indoor cloth drying and floor insulation were found to be the only significant predictors of the *Der p* I concentration. In living rooms with wall-to-wall carpets, the number of occupants, the age of the floor coverings, wall insulation and wooden floors were correlated with higher *Der p* I concentrations in the dust. The regression analyses explained only 6%, 13% and 17% respectively of the variance of the *Der p* I concentrations. The amount of *Der p* I/m² from smooth floors was significantly higher with increasing numbers of occupants and when pets were present. In living rooms with wall-to-wall carpets the levels of *Der p* I/m² were correlated with the age of the floor cover, floor construction and number of non-succulent plants. For smooth floors with rugs on top, the same variables were significantly associated with the amount of *Der p* I/m² as for the concentrations of *Der p* I/g dust.

Table 5.20 Multivariate association of different housing characteristics and the *Der p* I concentration in *bedroom* floor dust

| | exposed vs reference category | ratio of *Der p* I concentration in exposed vs reference category |
|---|---|---|
| smooth floor | | n=130 / $R^2$=0.34 |
| | *Der p* I concentration for reference category | 336 ng/gram |
| | mattress *Der p* I concentration (per $\mu$g/g) | 1.39 (1.24-1.55)[a] |
| | house built 1920-1975 vs built after 1975 | 1.22 (0.88-1.68) |
| | house built before 1920 vs built after 1975 | 2.35 (1.46-3.77) |
| | bedroom situated on ground floor vs higher floor | 0.43 (0.22-0.86) |
| smooth floor with rug | | n=28 / $R^2$=0.48 |
| | *Der p* I concentration for reference category | 999 ng/gram |
| | mattress *Der p* I concentration (per $\mu$g/g) | 2.02 (1.52-2.68) |
| wall-to-wall carpet | | n=307 / $R^2$=0.42 |
| | *Der p* I concentration for reference category | 851 ng/gram |
| | mattress *Der p* I concentration (per $\mu$g/g) | 1.91 (1.74-2.10) |
| | floor cover 4-6 years old vs <4 years old | 1.75 (1.26-2.43) |
| | floor cover >6 years old vs <4 years old | 1.91 (1.43-2.56) |

[a] 95% confidence interval

In bedrooms with a smooth floor covering, the *Der p* I content of mattress dust, the age of the house and the floor level were significantly associated with the *Der p* I concentration. In older dwellings the *Der p* I concentrations were higher. In bedrooms with rugs on smooth floors, only the *Der p* I concentration in mattress dust was significantly related to the *Der p* I concentration in the floor dust. In carpeted bedrooms, the age of the floor covers and again the *Der p* I levels in mattress dust were positively associated with the *Der p* I concentrations in the floor dust. For smooth floors the same variables were significantly associated both with the amount of *Der p* I/m$^2$ and the concentrations per gram dust. The amount of *Der p* I/m$^2$ on smooth floors with rugs correlated with wall insulation (higher in case of insulated walls) and floor insulation (higher in the absence of floor insulation). For wall-to-wall carpets the amounts of *Der p* I/m$^2$ were significantly associated with the age of the floor covering, the *Der p* levels in mattress dust and the frequency of vacuuming (higher in case of a low frequency).

Table 5.21 Multivariate association of different housing characteristics and the *Der p* I concentration in *mattress* dust

| exposed vs reference category | ratio of *Der p* I concentration in exposed vs reference category |
|---|---|
| | n=390 / $R^2$=0.26 |
| *Der p* I concentration for reference category | 864 ng/gram |
| mattress 5-7 years old vs <5 years old | 1.43 (1.06-1.92)[a] |
| mattress >7 years old vs <5 years old | 1.58 (1.18-2.13) |
| vacuuming once a week vs more often | 1.54 (1.13-2.09) |
| no outer cavity wall vs outer cavity wall | 2.23 (1.49-3.33) |
| wooden floor vs concrete floor | 1.70 (1.32-2.18) |
| no mechanical vs mechanical ventilation | 1.84 (1.32-2.57) |
| residence in east vs west of the Netherlands | 2.04 (1.78-2.62) |

[a] 95% confidence interval

The *Der p* I concentrations in mattress dust were associated with age of the mattress, the construction of the outer wall (higher in the absence of an outer cavity wall), with residency in the east of the country, with floor construction, with vacuuming frequency, and ventilation system (lower in case of mechanical ventilation). The regression analysis explained 26% of the variance of the *Der p* I concentration. A possible impact of the type of mattress could not be assessed because all mattresses were made from polyether. Higher amounts of *Der p* I/m$^2$ were associated with increasing age of the mattress, the absence of an outer cavity wall, the presence of a wooden floor, the presence of roof insulation, a low indoor temperature during the winter season, and with residency in the east of the country.

A comparison was made (t-test) between the *Der p* I concentrations found in homes without observed or reported signs of dampness (i.e. damp spots and/or mould patches), and in homes with observed or reported signs of dampness (Table 5.22). There was a tendency towards higher *Der p* I concentrations in dust sampled on the different locations in homes with reported or observed dampness. The differences were more pronounced for smooth floors with rugs, wall-to-wall carpets and mattresses, and for reported dampness compared with observed dampness. In the multivariate regression analyses, however, the statistical significance of these differences was lost. Similar results were obtained comparing the amounts of *Der p* I/m$^2$ in homes with and without observed or reported signs of dampness.

Table 5.22 Comparison between the *Der p* I concentrations in homes without reported or observed dampness and those in homes with reported or observed dampness (t-test)

| | | no signs of dampness | | signs of dampness | | t |
|---|---|---|---|---|---|---|
| | | n | GM | n | GM | |
| reported dampness | living room | | | | | |
| | smooth | 53 | 348 | 57 | 390 | -0.58 |
| | smooth with rug | 80 | 1830 | 87 | 3774 | -2.71** |
| | wall-to-wall carpet | 113 | 5110 | 110 | 5682 | -0.51 |
| | bedroom | | | | | |
| | smooth | 64 | 616 | 77 | 649 | -0.28 |
| | smooth with rug | 10 | 1644 | 20 | 4073 | -2.04* |
| | wall-to-wall carpet | 173 | 3047 | 158 | 4399 | -2.21** |
| | mattress | 245 | 4438 | 255 | 5716 | -2.07** |
| observed dampness | living room | | | | | |
| | smooth | 45 | 379 | 64 | 366 | 0.18 |
| | smooth with rug | 77 | 2438 | 55 | 2771 | -0.47 |
| | wall-to-wall carpet | 93 | 5653 | 131 | 5394 | 0.22 |
| | bedroom | | | | | |
| | smooth | 59 | 654 | 83 | 637 | 0.16 |
| | smooth with rug | 11 | 1610 | 18 | 4073 | -1.93* |
| | wall-to-wall carpet | 146 | 3450 | 180 | 3886 | -0.71 |
| | mattress | 216 | 4699 | 280 | 5575 | -1.38 |

* $p<0.10$ ** $p<0.05$

The average relative humidity measured in 19 bedrooms over periods of 3 to 6 weeks in the late fall of 1990 ranged from about 50% to about 80%. Mean bedroom temperatures over the period of observation ranged from about 13°C to about 20°C, and absolute indoor humidity (as calculated from the measured relative humidity and temperature) ranged from over 6 g/kg to approximately 8.5 g/kg dry air, with one extreme at almost 11 g/kg. Relative and absolute indoor humidity were correlated ($r=0.39$ after removal of extreme in absolute humidity, $r=0.32$ without removal of outlier), but there was a strong negative correlation between indoor temperature and relative humidity, indicating that the warmer bedrooms had the lower relative humidities.

Figures 5.2 and 5.3 show the relationship between relative humidity in the bedroom (average of the entire measuring period) and the *Der p* I concentrations in bedrooms with carpeted floors and in mattresses respectively. Figures 5.2 and 5.3 also include the estimated Pearson correlation coefficients and the regression line obtained by linear regression analysis. The relative humidity in the bedroom was found to be positively associated with the bedroom floor *Der p* I concentration and with the mattress *Der p* I concentration. The mean absolute humidity was positively associated with the *Der p* I concentrations in floor dust ($r=0.22$, not significant), but not with those in mattress dust ($r=-0.01$). Similar correlation coefficients were obtained between the amounts of *Der p* I/m$^2$ and the relative and absolute indoor humidity. Correlations between bedroom temperature and *Der p* I were negative, reaching marginal statistical significance ($p<0.10$) for the correlation between temperature and *Der p* I in mattress dust.

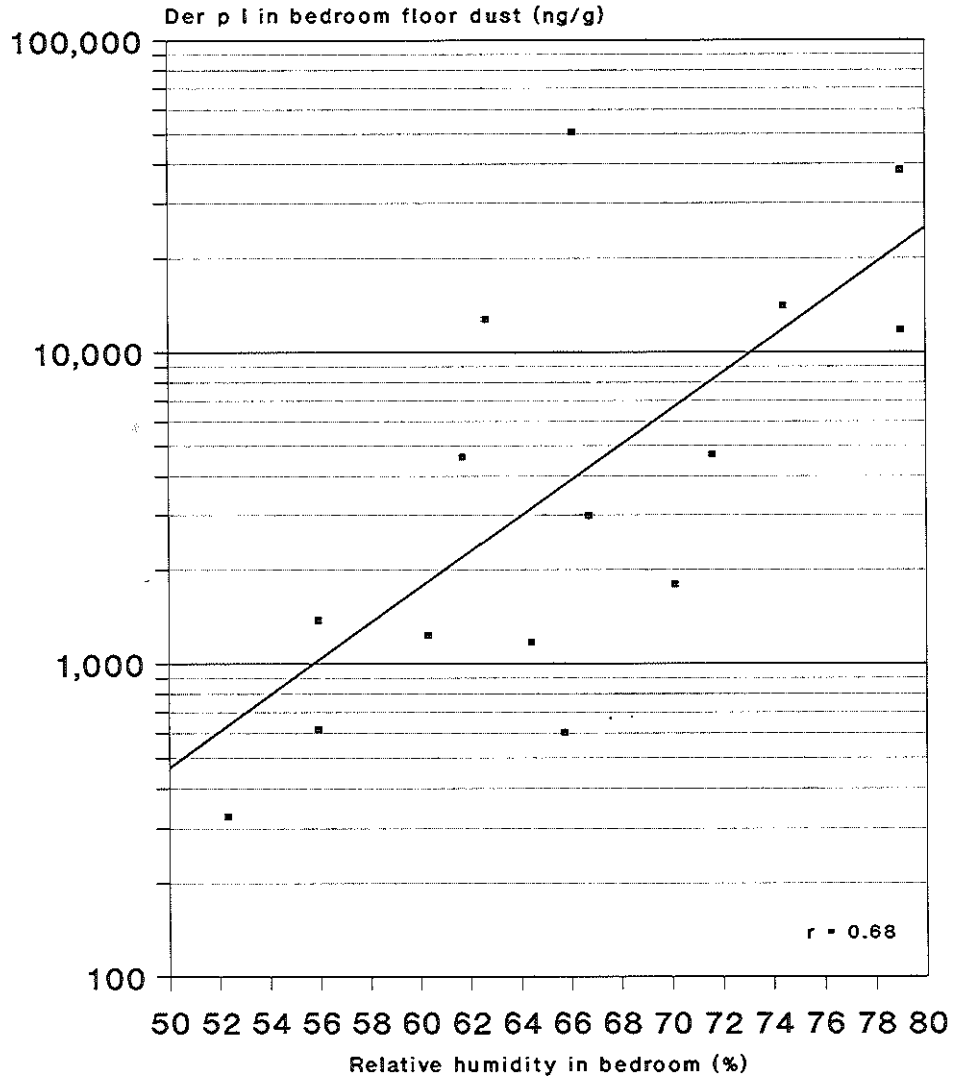


Figure 5.2 Correlation between mean relative humidity in the bedroom and *Der p* I concentration in the bedroom floor dust from *carpeted* floors ($n=15/p<0.01$)

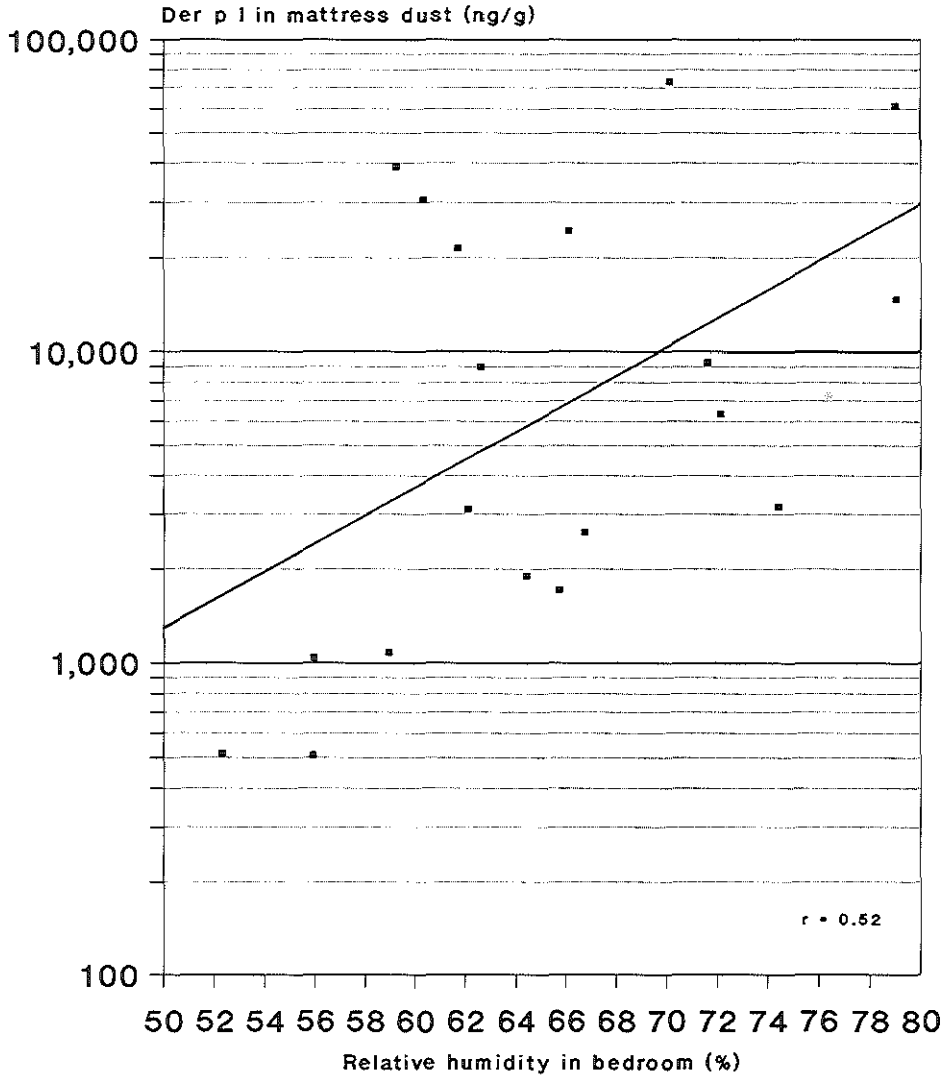


Figure 5.3 Correlation between mean relative humidity in the bedroom and *Der p* I concentration in mattress dust (n=19/p<0.05)

### *5.4.5 Discussion*

A large proportion of the dwellings in our study showed high *Der p* I concentrations in settled house dust. A WHO workshop has proposed provisional guidelines for sensitization (2,000 ng/g) and for an increased risk of asthma attacks for sensitized individuals (10,000 ng/g) (Platts-Mills and De Weck 1989). The maximum *Der p* I concentration in this study was below 2,000 ng/g dust in only 71 of the over 500 homes that were investigated. In 160 houses, the maximum concentration was between 2,000 and 10,000 ng/g dust. In 284 dwellings, the maximum *Der p* I concentration was higher than 10,000 ng/g of house dust. Lau *et al.* (1989) found median *Der p* I and *Der f* I concentrations in mattress dust in Berlin, Germany, of 600 ng/g (133 atopics) and 400 ng/g (55 non atopics). Price *et al.* (1990) reported carpet dust *Der p* I concentrations comparable to the results reported by Lau *et al.* (1989) for 46 atopic children with positive skinprick test against house dust mite and lower concentrations for 22 atopics with negative skin prick test against house dust mite in the UK. Wickman *et al.* (1991) found very low mattress dust *Der p* I concentrations in Stockholm, Sweden. In only 10% (54 children not sensitized for house dust mite) to 26% (53 house dust mite sensitized children) of the samples the concentrations were higher than the detection limit of 20 ng/g. Munir and co-workers (1993) found similar low *Der p* I concentrations in floor dust obtained in 39 homes in Sweden. The levels never exceeded 2,000 ng/g dust. Sporik *et al.* (1990a) reported in their study conducted in the UK higher *Der p* I concentrations than the *Der p* I concentrations found in the present study. The geometric mean *Der p* I concentrations were 2,400 ng/g in dust from living room floors, 4,300 ng/g in dust from the bedroom floor and 18,400 ng/g in mattress dust. The different concentrations reported in the literature reflect the different climatic conditions. The present study was conducted in the autumn, because in the Netherlands, the concentrations of house dust mite allergens are highest in this season (Van Leeuwen and Aalberse, 1991). The levels of house dust mite allergens in house dust in Dutch homes vary through the year by a factor of five (Van Leeuwen and Aalberse, 1991).

The correlations between the *Der p* I concentrations in living room floor dust, bedroom floor dust and mattress dust were high. This suggests that in general the amounts of *Der p* I on different locations within one home are strongly associated. The highest correlation coefficients were found between the *Der p* I concentrations in mattress dust and those in bedroom floor dust, for smooth floors as well as carpeted floors. Mattress dust *Der p* I levels were as high in bedrooms with smooth as in bedrooms with carpeted floors, suggesting allergen transport from mattress to floor rather than vice versa.

By far the largest influence on the *Der p* I levels in settled floor dust was found to be exerted by the type of floor cover. Dust from floors with a smooth cover has a six to fourteen times lower concentration of mite allergen than dust from carpeted floors. Dust from smooth floors with rugs contained four to seven times more *Der p* I than dust from smooth floors. Removal of carpets and rugs from living rooms and bedrooms seems to be the most efficient way to reduce exposure to dust mite allergen from floor dust under the conditions prevailing in the Netherlands. However, it is not yet clear to what extent the *Der p* I content in settled house dust represents airborne concentrations. The finding that carpeted floors contain more *Der p* I than floors with smooth floor coverings is in agreement with the results reported by Sporik *et al.* (1990). Arlian and co-workers (1982) found higher numbers of mites in dust from carpeted floors than in dust from smooth flooring. Our data suggest that some other housing characteristics were also associated

with the *Der p* I concentrations in settled house dust. Some of these factors point to possibilities for reduction of allergen exposure. Mattresses and upholstered furniture contributed to the allergen load on bedroom and living room floors respectively. Encasement of mattresses and replacement of upholstered furniture therefore will probably reduce allergen loads on floors. The age of the mattress and floor cover were both related to the allergen load, even after 7 or 8 years. More frequent replacement of mattresses and floor covers will therefore also reduce allergen exposure. Crowding, indoor cloth drying and mechanical ventilation were related to some of the allergen concentration variables. All of these point to possibilities to reduce allergen exposure by improving the balance between indoor moisture production and removal. In the literature, higher levels of mite antigen or mite numbers in house dust have been associated with single story individual houses (Wickman *et al.* 1991), with increasing age of furnishing and bedding (Tovey *et al.* 1981b) and increasing numbers of occupants (Van der Hoeven *et al.* 1992), and with energy conservation measures which reduce ventilation and thereby increase indoor humidity in winter (Harving *et al.* 1993). Energy conservation measures when associated with higher indoor temperatures, however, have also been associated with lower levels of mite numbers (Hart and Whitehead 1990). Lower levels of mite allergen have also been associated with the presence of a collective heating system, continuous heating of the home, and a relatively low water vapour production (Izard *et al.* 1993). Vacuuming has also been reported to reduce the numbers of mites and levels of mite allergens in carpet dust, mattresses and rugs, although the long term effect is not yet clear (Wassenaar 1988b; De Boer 1990; Hart and Whitehead 1990).

We found a tendency towards higher *Der p* I concentrations in dust in homes with reported or observed signs of dampness, especially for smooth floors with rugs, wall-to-wall carpets and mattresses. The differences were more pronounced for reported dampness. The observations made by the investigators can be characterized as 'spot-samples', whereas the occupants were asked about the occurrence of damp in the home for the previous two years. Munir *et al.* (1993) also found higher amounts of *Der p* I in carpet dust in homes with reported signs of dampness. They found no differences for smooth floors. Higher mite counts have also been associated with reported signs of dampness in the home (Hart and Whitehead 1990).

In our study, the *Der p* I content of dust from carpeted bedroom floors and dust from mattresses was positively associated with the average relative humidity over several weeks in the bedroom, but not with the absolute humidity. Hart and Whitehead (1990) reported a strong relation between the relative humidity in the bedroom and the number of *D. pteronyssinus* in mattress dust. They measured the relative humidity only once at the day of sampling. They found no relation between the relative humidity and the number of mites in carpets in the living room. In other studies a relationship was found or suggested between absolute indoor air humidity and the growth of house dust mite (Wickman *et al.* 1991; Hart and Whitehead 1990; Harving *et al.* 1993; Emenius *et al.* 1993). Reiser *et al.* (1990) did not find a correlation between *Der p* I concentrations and relative nor absolute humidity. They did, however, find a relationship between wall and *Der p* I concentrations. Our results show that relative humidity and *Der p* I concentration of house dust are positively correlated when relative humidity is measured over a somewhat longer period (3-6 weeks). Harving *et al.* (1993) reported a positive relationship between absolute indoor humidity and mite counts. Figure 2 presented in their paper makes clear, however, that this correlation was mainly due to the much lower mite counts observed in homes where the absolute air humidity was <6 g/kg dry air compa-

red to homes with higher absolute indoor humidities. The correlation between relative humidity and mite counts was not reported. We found no bedrooms with absolute indoor humidities below 6 g/kg dry air, and only four with absolute indoor humidity less than 7 g/kg. It seems that for the conditions prevailing in the Netherlands (and possibly in countries such as the UK (Hart and Whitehead 1990)), where winters are generally mild and damp, heating of bedrooms (which leads to a lower relative humidity in winter) may be effective in reducing mite allergen levels.

Colloff and co-workers (1992) recently suggested that the concentrations of *Der p* I expressed by square meter might give a better estimate of the potential than the amounts per gram dust. We found high statistically significant correlations between the amounts of *Der p* I per gram dust and per square meter. Therefore, the results of the analyses using the levels of *Der p* I/m$^2$ instead of those per gram dust, were similar. Given the high correlation, it probably does not make much difference whether one or the other is used as measure of exposure to *Der p* I.

In summary, the results of this study indicate that a small number of housing and occupant characteristics have a significant impact on the amounts of *Der p* I in house dust in homes in the Netherlands. For the *Der p* I content of floor dust, the major predictive variable is the type of flooring. In addition, the age of the floor cover appeared to be important. For bedroom floor dust, the allergen level in mattress dust was a major additional determinant. For mattress dust, a set of variables contributed to higher allergen concentrations. For future building practices, the positive influence of mechanical ventilation, seems noteworthy. The concentrations in dust from bedrooms floors and mattresses were also positively associated with the relative humidity measured over a longer period. The results were similar using the levels of *Der p* I in ng/g dust and ng/m$^2$.

# Home dampness and respiratory symptoms in children: a case-control study

This Chapter includes the following papers:

*Damp housing and childhood respiratory symptoms: the role of sensitization to dust mites and moulds*
A.P. Verhoeff, R.T. van Strien, J.H. van Wijnen, B. Brunekreef
Am. J. Epidemiol. (provisionally accepted)

*House dust mite allergen (Der p I) and respiratory symptoms in children; a case-control study*
A.P. Verhoeff, R.T. van Strien, J.H. van Wijnen, B. Brunekreef
Clin. Exp. Allergy (provisionally accepted)

## 6.1 Introduction

This Chapter presents the results of a case-control study on the relation between home dampness, reported respiratory symptoms in children, sensitization to house dust mites and moulds, and house dust mite allergen in house dust.

Section 6.2 deals with the role of sensitization to dust mites and moulds in the relation between home dampness and childhood respiratory symptoms. Concurrently, the possible role of reporting bias in the relation between these two will be addressed by comparing reported signs of dampness in the home by the occupants and observed signs of dampness by the investigators. The main hypothesis tested is that there is no relation between reported home dampness and reported respiratory symptoms in children, taking into account sensitization against fungal and house dust mite allergens.

Section 6.3 focusses on the relation between the presence of the major allergen of the mite *Dermatophagoides pteronyssinus* (*Der p* I) in settled house dust and respiratory symptoms in children and sensitization to house dust mites. The main hypothesis tested is that there is no relation between the levels of house dust mite allergen in settled house dust and reported respiratory symptoms in children, taking into account sensitization against house dust mite allergens.

## 6.2 Damp housing and childhood respiratory symptoms: the role of sensitization to dust mites and moulds [1]

### *6.2.1 Abstract*

In 1990, a case-control study was conducted in the Netherlands into the association between damp housing, childhood respiratory symptoms and sensitization to house dust mites and mould allergens. In this study 259 children with chronic respiratory symptoms and 257 control children were involved. Total serum IgE and specific IgE against house dust mite and a mixture of moulds were determined. A visual inspection for signs of home dampness was performed in all homes. A questionnaire was given to the parents of the children to elicit information about the presence of signs of dampness in the previous two years, and about a number of risk factors for childhood respiratory disease. In the case group, 94 children had elevated serum IgE levels to house dust mite and 24 children to the mixture of moulds. In the control group, house dust mite allergy was found in 31 children and mould allergy in two children. In a crude analysis, cases were slightly more likely to have been living in homes where damp or mould was reported or observed, than the controls. Restriction of the analysis to cases with elevated serum IgE levels against dust mites and/or moulds, and to controls without elevated serum IgE, increased the odds ratios, several now becoming significant at $p<0.05$. This supports a connection between sensitization to dust mites and/or moulds and damp housing and childhood respiratory symptoms. Only five children were sensitized to moulds without also being sensitized to dust mite allergen. It was therefore not possible to evaluate the role of sensitization to moulds in the absence of sensitization to dust mites. For the circumstances studied in the Netherlands, the results suggest that sensitization to dust mites and, possibly, mould allergen, plays an important role in the relationship between living in a damp home and childhood respiratory symptoms.

### *6.2.2 Introduction*

A number of recent studies have suggested that the occurrence of dampness and mould in modern homes is associated with an increased prevalence of respiratory symptoms in children (Martin *et al.* 1987; Andrae *et al.* 1988; Strachan 1988; Brunekreef *et al.* 1989; Platt *et al.* 1989; Strachan and Sanders 1989; Waegemaekers *et al.* 1989; Dijkstra *et al.* 1990; Strachan *et al.* 1990; Dales *et al.* 1991a, 1991b; Brunekreef *et al.* 1992a; Jaakkola *et al.* 1993). In most of these studies, information about house damp and respiratory symptoms was obtained by questionnaires and was not validated by objective methods, therefore the validity of this association has been questioned (Strachan 1988; Strachan *et al.* 1990).

The observation, either by residents or by investigators/inspectors, of damp and mouldy patches on interior surfaces in homes has been the main 'exposure variable' in most of these recent studies. Several authors have suggested that moulds are primarily implicated as

[1] Verhoeff, AP, RT van Strien, JH van Wijnen & B Brunekreef.
Am J Epidemiol (provisionally accepted)

This study was supported by a grant from the Dutch Asthma Fund.

harmful organisms, through the production of allergens, toxins and/or volatile components (Tobin *et al.* 1987; Flannigan *et al.* 1991). Evidence of an association between asthma and indoor exposure to fungi was reported in a case-control study of 72 adults with asthma and 72 age and gender matched controls (Burr *et al.* 1988). Of these, nineteen cases and nine controls reported mould spots in their homes. Furthermore, there was a significantly higher prevalence of positive radio allergosorbent test (RAST) responses to *Penicillium* among patients reporting mould in their homes. Several studies reported higher numbers of airborne fungal propagules in the indoor air of homes classified as 'damp' (Hunter *et al.* 1988; Waegemaekers *et al.* 1989; Verhoeff *et al.* 1992).

Dampness in homes is also associated with the level of dust mite infestation (Korsgaard 1983b; Wickman *et al.* 1991), and it is well known that dust mites produce strong allergens which play an important role in the development of asthma (Platts-Mills and De Weck 1989; Platts-Mills *et al.* 1992).

The present study was designed to investigate the role of sensitization to dust mites and moulds in the relation between home dampness and reported respiratory symptoms of children in a sample of the general population.

### *6.2.3 Materials and methods*

#### Study population

A case-control study was conducted in children aged 6 to 12 years. Cases and controls were selected from a random sample of 7,632 children from 18 schools in the province of Noord-Holland, and from 20 schools in the province of Gelderland. The children's parents completed a screening questionnaire regarding respiratory symptoms, based on existing, validated childhood respiratory symptom questionnaires (Brunekreef *et al.* 1992). The response to this questionnaire was 74.6% in Noord-Holland, and 85.5% in Gelderland. Cases were selected by the following reported symptoms and/or diagnosis: chronic wheeze in the past twelve months, cough on most days or nights for three consecutive months in the autumn/winter season, attacks of shortness of breath with wheezing in the past twelve months, and/or doctor-diagnosed asthma (ever in life). These respiratory symptoms were chosen on the basis of the results of questionnaire studies on the relation between home dampness and respiratory symptoms in children, which indicated that wheeze, asthma and especially persistent cough were associated with reported home dampness (e.g. Brunekreef *et al.* 1989; Brunekreef 1992; Dales *et al.* 1991a; Jaakkola *et al.* 1993; Waegemaekers *et al.* 1989). Controls were selected from the group of children without reported respiratory symptoms. Of all cases randomly requested to participate, 78% agreed, and of the controls, only 60% agreed. Two schools in Gelderland had a high proportion of children with an orthodox religious background and the response from the control children in these was low. Altogether, 259 cases and 257 controls participated in the study.

#### Sensitization and pulmonary function

Venous blood samples (20 ml) were taken from all children by a skilled nurse and after centrifugation, sera were stored at -20°C. Total IgE and specific IgE against dust mites (*Dermatophagoides pteronyssinus*) and against a mould mixture (*Penicillium, Alternaria, Aspergillus, Cladosporium*) were determined by RAST in the Central Laboratory of the Blood Transfusion Service in Amsterdam. Mould species belonging to the genera included

in the RAST panel make up most of the burden of the indoor airborne spore counts in Dutch homes (Verhoeff *et al.* 1992).

The pulmonary function of all children was measured using a Vicatest VCT-5 dry spirometer (Mijnhardt, Bunnik, the Netherlands) according to the protocol described by Houthuijs *et al.* (1989). The following pulmonary function parameters were assessed: forced vital capacity (FVC), forced expiratory volume in one second ($FEV_1$), peak expiratory flow (PEF) and maximum mid expiratory flow (MMEF).

**Home dampness**

A detailed questionnaire was given to the parents/guardians asking about a variety of home characteristics. These included the presence of visible damp patches and/or mould growth during the life of the child, and during the previous two years, in the living room, the bedroom of the child, the bathroom and elsewhere, and risk factors for childhood respiratory disease (the presence of an unvented water heater (a so called 'geyser'), smoking in the home, the presence of feathered or hairy pets and socio-economic status).

To verify the signs of dampness or mould, all homes were visited by trained investigators, who were blind regarding the case-control status of the children. The data were collected in the fall of 1990.

**Statistical analysis**

Statistical analysis was performed by means of the Statistical Analysis System (SAS, version 6.06). First the crude odds ratios of the association between respiratory symptoms and home dampness were calculated. Adjusted odds ratios were calculated using logistic regression. The results of the pulmonary function tests were analyzed using linear regression.

### *6.2.4 Results*

Table 6.1 presents information on some characteristics of the study group, including the distribution of sensitization (RAST > 0) and the percentage difference in pulmonary function of cases, asthmatics and children with chronic cough compared with controls. Of the cases, there were 76 children with doctor-diagnosed asthma, and 81 for whom chronic cough was reported without other symptoms of asthma. The case and control group were found not to be different regarding the proportion of smokers and pets in the home, however, the mean age of cases was marginally significantly lower ($t = -2.41$, $p < 0.10$) and there were more boys in the case group. For the asthmatics, the proportions of girls, and smokers and pets in the home were lower, whereas for children with chronic cough, the mean age was significantly lower ($t = -3.41$, $p < 0.05$) and the proportion of smokers in the home somewhat higher.

Evenly distributed among cases and controls, 23 subjects did not provide a usable blood sample. Of the controls 12,3% were sensitized to dust mites and one percent to moulds, but in cases, the proportions were higher. Of the doctor-diagnosed asthmatics, more than half were found to be sensitized against dust mites and 16% to moulds. Among the cases with chronic cough sensitization rates were lower. The proportions of cases, asthmatics, and children with chronic cough sensitized to house dust mites or moulds were significantly higher than those of controls (Chi-square test, $p < 0.05$). There was considerable overlap between case children sensitized to house dust mites and moulds. Of the 24 cases sensitized

to the mould mixture, only five were not sensitized to dust mites. Among cases, 176 (71%) had elevated total serum IgE levels and approximately half of those were sensitized to dust mites and/or moulds. Among controls, 117 (48%) had elevated total serum IgE levels with approximately 20% of them sensitized against dust mites and/or moulds. The amounts of total serum IgE were adjusted for age, according to the normal total IgE levels, as provided by the Central Laboratory of the Blood Transfusion Service (<7 years: 25 IU/ml; 7-10 years: 50 IU/ml; >10 years: 100 IU/ml). The proportions of cases and asthmatics with elevated total serum IgE were significantly higher than that of controls (Chi-square test, $p<0.05$), whereas this proportion of the children with chronic cough was only marginally significantly higher ($p<0.10$).

Table 6.1 Characteristics of the study population: selected Dutch children, 6-12 years old, 1990

| | Controls n=257 | Cases n=259 | Asthma n=76 | Chronic cough n=81 |
|---|---|---|---|---|
| Girls (%) | 58.8 | 47.9 | 38.0 | 53.5 |
| Age, mean (yr) | 8.7 | 8.3 | 8.5 | 7.9 |
| Smokers in the home (%) | 58.1 | 58.0 | 46.2 | 65.1 |
| Pets in the home (%) | 61.8 | 58.5 | 49.4 | 59.3 |
| Sensitized (RAST>0) to | | | | |
| - dust mites (%) | 12.3 | 37.8 | 54.7 | 27.2 |
| - moulds (%) | 0.8 | 9.8 | 16.0 | 7.4 |
| Total IgE (IU/ml) | | | | |
| geometric mean | 57 | 144 | 265 | 84 |
| range | 1-1809 | 0-8500 | 8-8500 | 0-2531 |
| Percentage decline in pulmonary function | | | | |
| FVC | | -0.6** | 0.4 | -1.8 |
| $FEV_1$ | | -2.7** | -5.1** | -1.8 |
| $FEV_1$/FVC | | -2.2** | -5.5** | 0.0 |
| PEF | | -3.2** | -5.7** | -2.2 |
| MMEF | | -8.4** | -18.3** | -2.1 |

** $p<0.05$

A valid pulmonary function test was obtained for 93% (473) of the children. The percentage difference of pulmonary function of cases, asthmatics and children with chronic cough, compared with the controls was calculated by linear regression, adjusting for age, height, weight and gender. For all cases and the children with doctor-diagnosed asthma there was a significant ($p<0.05$) difference of $FEV_1$, PEF, $FEV_1$/FVC and MMEF, compared with the control group (Table 6.1). For children with chronic cough, the parameter values of pulmonary function were lower compared to those of the controls, but these differences were not significant.

Table 6.2 shows the percentage of parents/guardians who had noted damp spots or mould in their home during the life of their child. These were reported somewhat more frequently and from more rooms in the house, by parents of cases than by parents of controls.

Approximately 50% of the parents of both cases and controls reported damp or mould in their home during the previous two years. Parents of cases had noted damp patches and mould in the living room during the previous two years more frequently. They also reported more frequently having removed mould during the last two years than the parents of controls (25% versus 14% respectively). On the whole, the investigators observed less damp and mould than was reported by the parents (Table 6.2), but they observed slightly more mould in the houses of cases than of controls.

Table 6.2 Percentage of homes where damp spots or mould were reported by the parents/guardians during the life of the child or observed by the investigators: selected Dutch children, 6-12 years old, 1990

| REPORTED: (during life) | controls (n=251) | cases (n=254) | asthma (n=78) | chronic cough (n=84) |
|---|---|---|---|---|
| no damp or mould | 75 (30%) | 62 (24%) | 18 (23%) | 17 (20%) |
| damp (house) | 166 (66%) | 181 (71%) | 58 (74%) | 64 (76%) |
| living room | 53 (21%) | 85 (33%) | 25 (32%) | 33 (39%) |
| bedroom | 43 (17%) | 55 (22%) | 12 (15%) | 28 (33%) |
| no. of damp rooms | 1.43 | 1.61 | 1.38 | 1.88 |
| mould (house) | 114 (45%) | 134 (53%) | 43 (55%) | 49 (58%) |
| living room | 24 (10%) | 43 (17%) | 17 (22%) | 10 (12%) |
| bedroom | 20 ( 8%) | 32 (13%) | 9 (12%) | 16 (19%) |
| no. of mouldy rooms | 1.22 | 1.51 | 1.49 | 1.53 |
| OBSERVED: | (n=247) | (n=252) | (n=76) | (n=84) |
| no damp or mould | 111 (45%) | 107 (42%) | 28 (37%) | 33 (39%) |
| damp (house) | 120 (49%) | 113 (45%) | 37 (49%) | 42 (50%) |
| living room | 40 (16%) | 38 (15%) | 12 (15%) | 15 (18%) |
| bedroom | 40 (16%) | 41 (16%) | 15 (20%) | 14 (17%) |
| mould (house) | 75 (30%) | 85 (34%) | 29 (38%) | 28 (33%) |
| living room | 19 (8%) | 20 (8%) | 10 (13%) | 2 (2%) |
| bedroom | 13 (5%) | 10 (4%) | 4 (5%) | 3 (4%) |

The agreement between observed and reported damp and mould in the home for the previous two years is shown in Table 6.3. For mould, the data were adjusted for mould removal by the parents. A reasonable agreement was found (kappa statistics ranging from 0.43 to 0.70) between reported and observed signs of dampness in the home. There was no indication of over-reporting of dampness and mould by the parents of cases relative to parents of controls. Comparisons made for the living room and bedroom separately gave the same results. Overall, the parents of cases were somewhat more in agreement with the investigators than the parents of controls, as indicated by higher kappa statistics.

Table 6.3 Agreement between observed damp and mould by the investigators and reported damp and mould for the last two years in the home, adjusted for mould removal: selected Dutch children, 6-12 years old, 1990

A. CASES

| damp patches (kappa = 0.56) | | | | mould (kappa = 0.70) | | | |
|---|---|---|---|---|---|---|---|
| reported | observed | | total | reported | observed | | total |
| | no | yes | | | no | yes | |
| no | 113 | 30 | 143 | no | 134 | 27 | 161 |
| yes | 24 | 81 | 105 | yes | 9 | 80 | 89 |
| total | 137 | 111 | 248 | total | 143 | 107 | 250 |

B. CONTROLS

| damp patches (kappa = 0.43) | | | | mould (kappa = 0.67) | | | |
|---|---|---|---|---|---|---|---|
| reported | observed | | total | reported | observed | | total |
| | no | yes | | | no | yes | |
| no | 97 | 41 | 138 | no | 144 | 26 | 170 |
| yes | 28 | 78 | 106 | yes | 11 | 65 | 76 |
| total | 125 | 119 | 244 | total | 155 | 91 | 246 |

Table 6.4 shows the crude odds ratios calculated from the frequencies in Table 6.2. Odds ratios for *reported* damp or mould during the life of the child were all greater than one. Odds ratios for the separate locations in the home were more often significant than for the whole house. This indicates that the relationship between reported damp, mould and respiratory symptoms is somewhat obscured by lumping all locations together. Odds ratios for cough were generally higher than for asthma. Using logistic regression analysis, the crude odds ratios were adjusted for passive smoking, the presence of an unvented geyser, gender, the presence of feathered or hairy pets and socio-economic status. The adjusted odds ratios were mostly smaller than the crude odds ratios, but still greater than one, except for reported damp in the bedroom for the asthma group (adjusted odds ratios 0.88).

The crude odds ratios for *observed* damp and/or mould (Table 6.4) were also generally higher than one, but not statistically significant. Adjusted odds ratios for observed dampness or mould were also mostly smaller than the corresponding crude odds ratios. Odds ratios were also calculated using reported signs of dampness over the previous two years as measure of exposure. The (adjusted) odds ratios were similar to those using observed dampness by the investigators as the exposure variable.

Table 6.4 Odds Ratios for reported (during the life of the child) or observed damp and/or mould in the home (95 percent confidence intervals in parentheses): selected Dutch children, 6-12 years old, 1990

| | cases | asthma | cough |
|---|---|---|---|
| reported damp (house) | 1.32 (0.89-1.96) | 1.46 (0.80-2.64) | 1.70 (0.94-3.09) * |
| living room | 1.94 (1.20-3.13)** | 1.97 (0.98-3.95) * | 2.75 (1.40-5.38)** |
| bedroom | 1.55 (0.92-2.61) | 1.16 (0.51-2.65) | 2.87 (1.43-5.78)** |
| reported mould (house) | 1.42 (0.94-2.16) | 1.57 (0.84-2.93) | 1.90 (1.02-3.52)** |
| living room | 2.17 (1.19-3.94)** | 2.95 (1.34-6.52)** | 1.84 (0.75-4.53) |
| bedroom | 1.94 (1.01-3.70)** | 1.88 (0.74-4.78) | 3.53 (1.55-8.03)** |
| observed damp (house) | 0.98 (0.68-1.41) | 1.22 (0.70-2.13) | 1.18 (0.70-1.99) |
| living room | 0.94 (0.59-1.51) | 0.94 (0.47-1.90) | 1.23 (0.63-2.40) |
| bedroom | 1.00 (0.61-1.64) | 1.33 (0.69-2.59) | 0.90 (0.43-1.92) |
| observed mould (house) | 1.18 (0.78-1.77) | 1.53 (0.85-2.78) | 1.26 (0.70-2.25) |
| living room | 1.10 (0.58-2.10) | 1.83 (0.81-4.13) | 0.34 (0.08-1.50) |
| bedroom | 0.68 (0.29-1.63) | 0.99 (0.31-3.14) | 0.50 (0.11-2.26) |

* $p<0.10$ ** $p<0.05$

The next step in the analysis was to investigate the relationship between damp and mould in the home and sensitization against dust mites and/or mould. We compared sensitized cases, asthmatics, children with chronic cough and controls, with non-sensitized cases, asthmatics, children with chronic cough and controls, respectively (Table 6.5).

Table 6.5 Odds Ratios for reported (during the life of the child) or observed damp and/or mould, comparing cases, asthmatics, children with chronic cough and controls with elevated serum IgE to house dust mites and/or mould with those without elevated serum IgE to dust mites and mould (95 percent confidence intervals in parentheses): selected Dutch children, 6-12 years old, 1990

| | cases | asthma | cough | controls |
|---|---|---|---|---|
| reported | | | | |
| damp | 1.68 (0.91-3.12) | 1.79 (0.61-5.28) | 8.08 (1.30-50.32)** | 1.98 (0.78-5.02) |
| mould | 1.53 (0.80-2.93) | 1.35 (0.44-4.17) | 8.79 (1.39-55.53)** | 2.02 (0.76-5.36) |
| observed | | | | |
| damp | 1.54 (0.88-2.71) | 3.33 (1.14-9.71)** | 3.20 (1.04-9.87)** | 1.46 (0.65-3.44) |
| mould | 1.87 (1.03-3.42)** | 3.57 (1.15-11.13)** | 3.67 (1.09-12.33)** | 1.88 (0.77-4.59) |

** $p<0.05$

All odds ratios were greater than one, and almost half of them were statistically significant. Although odds ratios for children with chronic cough were high, confidence intervals for these odds ratios were wide due to the small number of sensitized children with chronic cough living in homes without reported or observed signs of dampness. Similar results were obtained comparing atopic cases, children with chronic cough and controls, with non-atopic cases, children with chronic cough and controls respectively. Atopy was defined as having an elevated total serum IgE level. The odds ratios comparing atopic asthmatic children and non-atopic asthmatics were smaller than one, but confidence intervals were wide due to the low number (n=12) of non-atopic asthmatics. The results of these analyses indicate that home dampness is associated with sensitization to dust mites and moulds, and to atopy in general.

To investigate whether home dampness is associated with respiratory symptoms irrespective of sensitization to mites and/or moulds, cases *not* sensitized to dust mites and/or moulds were compared with controls who were also not sensitized to these allergens (Table 6.6, part I), and cases sensitized to dust mites and/or moulds were compared with controls also sensitized to these allergens (Table 6.6, part II).

Table 6.6 Odds Ratios for reported (during the life of the child) or observed damp and/or mould; (I) analysis restricted to cases and controls both *not* sensitized to house dust mites and moulds; (II) analysis restricted to cases and controls both sensitized to house dust mites and/or moulds (95 percent confidence intervals in parentheses): selected Dutch children, 6-12 years old, 1990

| | cases | asthma | cough |
|---|---|---|---|
| I | | | |
| reported damp | 1.18 (0.74-1.88) | 1.09 (0.47-2.54) | 1.34 (0.69-2.60) |
| reported mould | 1.35 (0.83-2.21) | 1.43 (0.60-3.40) | 1.46 (0.73-2.94) |
| observed damp | 0.83 (0.53-1.31) | 0.56 (0.24-1.33) | 0.93 (0.50-1.74) |
| observed mould | 0.96 (0.58-1.59) | 0.73 (0.28-1.90) | 0.94 (0.46-1.93) |
| II | | | |
| reported damp | 1.00 (0.36-2.82) | 0.99 (0.31-3.17) | 5.48 (0.72-41.67) |
| reported mould | 1.03 (0.35-3.03) | 0.96 (0.28-3.26) | 6.38 (0.83-49.25)* |
| observed damp | 0.86 (0.35-2.14) | 1.25 (0.43-3.60) | 2.00 (0.56-7.21) |
| observed mould | 0.96 (0.37-2.50) | 1.39 (0.46-4.22) | 1.83 (0.47-7.15) |

* $p<0.10$

The crude odds ratios were mostly close to unity, and only one was marginally statistically significant. The odds ratios for sensitized children with chronic cough were relatively high (Table 6.6, part II), but the confidence intervals for these odds ratios were wide due to the small number of children with chronic cough sensitized to house dust mite and/or moulds (n=24). Similar results were obtained comparing non-atopic cases with non-atopic controls, and atopic cases with atopic controls. As mentioned above, atopy was defined as having an elevated total serum IgE level.

Finally, cases sensitized to dust mites and/or mould were compared with controls who were not sensitized to these allergens, to investigate to what extent the relationship between damp and mould in the home and respiratory symptoms is associated with sensitization to house dust mites and/or mould (Table 6.7).

Table 6.7 Odds Ratios for reported (during the life of the child) or observed damp and/or mould; restricting the analysis to cases with elevated serum IgE to dust mites and/or moulds, and controls without elevated serum IgE to dust mites and mould (95 percent confidence interval in parentheses): selected Dutch children, 6-12 years old, 1990

| | cases | asthma | cough |
|---|---|---|---|
| reported damp | 1.98 (1.11-3.53)* | 1.95 (0.89-4.26)# | 10.82 (2.08-56.39)** |
| reported mould | 2.07 (1.13-3.80)* | 1.93 (0.85-4.41) | 12.84 (2.53-65.29)** |
| observed damp | 1.29 (0.76-2.17) | 1.86 (0.89-3.91) | 2.98 (1.09-8.14)* |
| observed mould | 1.80 (1.03-3.15)* | 2.61 (1.21-5.64)* | 3.45 (1.20-9.93)* |

# $p<0.10$ * $p<0.05$ ** $p<0.01$

Most odds ratios became statistically significant, indicating that home dampness is more strongly associated with sensitization in combination with respiratory symptoms than with respiratory symptoms as such. Similar results were obtained comparing atopic cases with non-atopic controls. Adjustment for the potential confounders mentioned above did not materially change the odds ratios. The odds ratios for reported damp and/or mould during the previous two years were mostly greater than one, but (marginally) significant for children with chronic cough only. Because only five children were sensitized to moulds without being also sensitized to dust mites, it was not possible to analyze these children separately.

The odds ratios comparing sensitized asthmatic children with non-sensitized controls were significantly ($p<0.05$) higher than those obtained comparing non-sensitized asthmatics with non-sensitized controls for observed dampness and mould. The odds ratios comparing sensitized children with chronic cough with non-sensitized controls were marginally significantly ($p<0.10$) higher than those obtained comparing non-sensitized children with chronic cough with non-sensitized controls for reported dampness, and observed dampness and mould. The difference was significant ($p<0.05$) for reported mould.

### *6.2.5 Discussion*

This study supports the association between reported respiratory symptoms and reported dampness and/or mould in the home, and is in agreement with the findings of other epidemiological studies (Martin *et al.* 1987; Andrae *et al.* 1988; Strachan 1988; Brunekreef *et al.* 1989; Platt *et al.* 1989; Strachan and Sanders 1989; Waegemaekers *et al.* 1989; Dijkstra *et al.* 1990; Strachan *et al.* 1990; Dales *et al.* 1991a, 1991b; Brunekreef 1992a; Jaakkola *et al.* 1993). Consistent with a causal relationship were the following observations. Firstly, the respondents were 'blinded' with regard to the hypothesis tested, as the screening questionnaire asked only about respiratory symptoms. Secondly, the observed relationship was

independent of possible confounding factors such as passive smoking, the presence of an unvented geyser and feathered or hairy pets, gender and socio-economic status. Thirdly, general over- or under-reporting is unlikely to have biased the results. A reasonable agreement existed between reported signs of dampness during the previous two years and observed signs of dampness, after adjusting for mould removal. This comparison showed no over-reporting of damp and mould by parents of cases relative to parents of controls. Further, reported respiratory symptoms were validated by measuring the pulmonary function of all children. Most pulmonary function parameter values of the cases were significantly lower than those of the controls, with the largest differences found for children with doctor-diagnosed asthma. The differences in pulmonary function in this study were similar to those found in a study of 3,000 children in the Netherlands (Flach and Brunekreef 1991).

Fungi and house dust mites have been put forward as possible causative agents in the relation under study, through the production of allergens or possibly other harmful substances (Tobin *et al.* 1987; Platts-Mills and De Weck 1989; Flannigan *et al.* 1991; Platts-Mills *et al.* 1992). Therefore, we determined total serum IgE levels and specific IgE to house dust mites and a mould mixture. The percentages of cases with elevated total IgE levels, and of cases sensitized to allergens from house dust mites and moulds were significantly higher than those for the controls. For house dust mite allergy, reported prevalences range from 45-85% in asthmatics and from 5-50% in controls (Platts-Mills and De Weck 1989). Reported prevalences of mould allergy in subjects with respiratory allergy range from 2-30% (Gravesen 1979). Our figures are comparable with these, although a proper comparison between the results of several studies is difficult because different analytical methods and selection criteria are often used. The results of both the pulmonary function tests and the IgE determinations in our study support the classification of cases and controls.

Without taking into account sensitization to house dust mites and moulds, the unadjusted odds ratios for reported damp during the entire life of the child ranged from 1.16 to 2.87, and for reported mould from 1.24 to 3.53. Strachan (1988) reported an unadjusted odds ratio of 3.70 (95% CI 2.22-6.15) for wheeze in the past year and reported mould anywhere in the house. His adjusted odds ratio was 3.00 (1.72-5.25). Dales *et al.* (1991a) presented adjusted odds ratios ranging from 1.32 (1.06-1.39) for bronchitis to 1.89 (1.58-2.26) for cough in relation to reported mould and dampness. In a study of 4,625 eight to twelve year old children from six cities in the United States, Brunekreef *et al.* (1989) reported odds ratios for mould ranging from 1.27 to 2.12, and for dampness from 1.23 to 2.16, after adjustment for potential confounders. In a study among 2,568 one to six year old children in Finland, adjusted odds ratios in relation to reported mould ranged from 0.63 (0.08-4.76) for current asthma, to 4.71 (1.91-11.6) for persistent phlegm (Jaakkola *et al.* 1993). Two populations of Dutch children aged 6-12 years, showed adjusted odds ratios of 1.30 to 1.97 for reported damp, and 1.12 to 3.06 for reported mould (Brunekreef 1992a). The published odds ratios are similar to those found in the present study. The finding that lower odds ratios were found for asthmatic children and higher odds ratios for children with chronic cough is consistent with the literature. This might be caused by frequent remedial action taken by parents of asthmatic children compared with the parents of children with chronic cough.

Comparing sensitized cases and controls with non-sensitized cases and controls gave higher odds ratios. Similar results were obtained comparing atopic cases and controls with non-atopic cases and controls. Atopy was defined as having an elevated total serum IgE level. These results suggest that home dampness leads to sensitization to dust mites and moulds, and to atopy in general. Restricting the analyses to cases sensitized to house dust mite and/or and moulds and controls not sensitized to these allergens gave considerably

higher odds ratios, especially for children with chronic cough without wheeze and/or asthma. Although odds ratios for children with chronic cough were high, confidence intervals for these odds ratios were wide due to the small number of sensitized children with chronic cough living in homes without reported or observed signs of dampness. There was no significant relationship between damp and mould in the home and respiratory symptoms when the analysis was restricted to cases and controls not sensitized to house dust mites or mould, or to cases and controls both sensitized to these allergens. This suggests that sensitization to dust mite allergens and/or mould allergens plays a causal role in the association between home dampness and respiratory symptoms. These results do not support a causal role of a nonallergic mechanism in the association under study, as suggested by Dales et al. (1991a). In our study we were able to investigate the role of sensitization on the basis of measurements of total and specific serum IgE, whereas the Canadian study used questionnaire based definitions for dust and mould allergy.

On the whole, sensitization to house dust mite allergen was found to be much more prevalent than sensitization to mould and this suggests that (under the circumstances prevailing in the Netherlands) it is a more important factor in the association between home dampness and respiratory symptoms of children. However, the RAST panel used to investigate sensitization to moulds only included a proportion of the mould species present in homes. Furthermore, the isolation, purification and standardization of fungal allergens are still major problems. This means that the sensitivity and specificity of a RAST analysis for mould allergy is limited. In our study, only 50% of atopic cases and 20% of atopic controls were sensitized to house dust mite and/or moulds and both of these groups were more likely to live in damp homes. Although increased total serum IgE levels are not only caused by sensitization to inhalation allergens, the association between atopy and home dampness suggests that sensitization to mould allergens might play some more important role in explaining the association between home dampness and respiratory symptoms in children.

The highest crude and adjusted odds ratios were obtained using reported dampness and mould during the entire life of the child as an exposure variable. Using observed damp and/or mould, or reported signs of dampness for the previous two years as exposure variables, most of the odds ratios were still greater than one, but none was statistically significant. We believe that the lower odds ratios are related to the 'spot-sample' character of the observations made by the investigators. As all epidemiological studies on the relationship between home dampness and respiratory symptoms, our study involved prevalent cases and suitable controls. Since the highest odds ratios were found using reported dampness and mould during the entire life of the child as an exposure variable, it is conceivable that the exposure to the suspected causal factors earlier in life is more important than the actual presence of damp stains and mould patches. There are indications that at a very early age there might exist a sensitive period during which exposure to allergens would enhance the risk of sensitization in genetically predisposed children (Suoniemi *et al.* 1981; Businco *et al.* 1988). This hampers the estimation of the association between the presence of signs of dampness and the development of respiratory symptoms. Prospective studies, or case control studies based on incident cases are needed to further clarify this association. In conclusion, the results of this study support the association between reported respiratory symptoms and reported dampness and mould in the home as found in other recent epidemiological studies. Further, sensitization to house dust mites and possibly moulds plays a causal role in this association.

## 6.3 House dust mite allergen (*Der p* I) and respiratory symptoms in children; a case control study [1]

### *6.3.1 Abstract*

The association between house dust mite allergen in house dust and childhood respiratory symptoms was investigated in a case-control study among 259 children with reported chronic respiratory symptoms and 257 control children without reported respiratory symptoms. The *Der p* I concentration in floor dust of the living room and bedroom and in mattress dust was determined using an enzyme immuno assay. Venous blood samples were taken from all children for serum IgE determination against house dust mite (*Dermatophagoides pteronyssinus*) by RAST. A questionnaire was administered to the parents of the children to elicit information about the home, about changes made to the home in the past in relation to respiratory symptoms, and about a number of risk factors for childhood respiratory disease. In 86% of the dwellings *Der p* I concentrations higher than 2,000 ng/g were found, and in 55% of the dwellings the concentrations exceeded 10,000 ng/g dust. In a crude analysis, cases were generally exposed to lower *Der p* I concentrations than controls. Restriction of the analysis to cases with elevated serum IgE levels against dust mites, and to controls without elevated serum IgE, taking the type of floor covering into account, showed only slightly higher *Der p* I concentrations in bedroom floor dust of cases. However, restriction of the analysis to cases sensitized to dust mites and cases not sensitized to house dust mites - adjusting for allergen avoidance measures taken in the past - revealed a positive association between the *Der p* I concentrations in bedroom floor dust and mattress dust and sensitization. This finding indicates that allergen avoidance measures modify current exposure to *Der p* I differentially in sensitized cases, non-sensitized cases, and in controls. A relation between exposure to dust mite allergen, sensitization and chronic respiratory symptoms only became apparent in this case-control study after adjustment for allergen avoidance measures, taken in the past.

### *6.3.2 Introduction*

About thirty years ago house dust mites were identified as the major cause of house dust allergy (Voorhorst *et al.* 1964). Since then the major allergens of house dust mites have been characterized and standardized, allowing the assessment of the potential exposure to these allergens and the presence of specific IgE in serum, using immunochemical techniques (Lind 1986b; Platts-Mills *et al.* 1986b). It is now clear that house dust mites produce strong allergens. Exposure of genetically pre-disposed subjects to these allergens can lead to sensitization and subsequently to asthma (Platts-Mills and De Weck 1989; Platts-Mills *et al.* 1992). A number of studies investigated the relation between the exposure to house dust mite allergens and respiratory symptoms. Lau *et al.* (1989) reported a dose-response relationship between the concentrations of *Der p* I and *Der f* I (the major allergens of

[1] Verhoeff, AP, RT van Strien, JH van Wijnen & B Brunekreef.
Clin Exp Allergy (provisionally accepted)

This study was supported by a grant from the Dutch Asthma Fund.

*Dermatophagoides pteronyssinus* and *D. farinae* respectively) in house dust and the risk of sensitization to these allergens. In this study atopic children sensitized against house dust mites were compared with atopic children not sensitized. Price *et al.* (1990) also reported a positive association between the level of house dust mite allergen in house dust and sensitization among asthmatic children. Wickman *et al.* (1991) measured the levels of house dust mite allergens in house dust of 53 mite-sensitized children, 54 non-mite-sensitized atopic children and 53 non-allergic controls. Increased mite allergen levels were more often found in homes of children sensitized to house dust mite than in the homes of children not sensitized to house dust mite. The largest difference was found between the group of house dust mite sensitized children and the group of non-sensitized atopic children. Based on the results of studies on the relationship between the levels of *Der p* I in house dust and sensitization, provisional hygienic threshold limits have been proposed (Platts-Mills and De Weck 1989). Concentrations of more than 2,000 ng *Der p* I/g dust are regarded as representing a risk for the development of specific IgE to house dust mite allergen in genetically predisposed individuals, and levels higher than 10,000 ng *Der p* I/g are supposed to increase the risk for acute attacks of asthma. Based on the results of studies on the relation between exposure to house dust mite allergens and asthma, Sporik *et al.* (1992) concluded that the primary cause of asthma in many areas of the world is exposure to house dust mite allergens, especially in children and young adults.

A case-control study was conducted to explore the relationship between a number of indoor risk factors and respiratory symptoms in children. This paper focusses on differences in mite allergen exposure between children with and without chronic respiratory symptoms.

### *6.3.3 Materials and methods*

#### Subjects

A case-control study on the relationship between chronic respiratory symptoms and a number of indoor risk factors was conducted among children aged 6 to 12 years. Cases and controls were selected from a random sample of 7,632 children from 18 schools in the province of Noord-Holland, and from 20 schools in the province of Gelderland. The parents of the children completed a screening questionnaire regarding respiratory symptoms, based on existing, validated childhood respiratory symptom questionnaires (Brunekreef *et al.* 1992). The response to the screening questionnaire was 74.6% in Noord-Holland, and 85.5% in Gelderland. Cases were selected from children reporting chronic wheeze in the past twelve months, cough on most days or nights for three consecutive months in the autumn/winter season, attacks of shortness of breath with wheezing in the past twelve months and/or doctor-diagnosed asthma (ever in life). Controls were selected from the group of children without reported respiratory symptoms. Of all cases randomly requested to participate, 78% agreed. For the controls, this percentage was 60%. The latter value was partly caused by a very low response among children of two schools in Gelderland with a high proportion of children with an orthodox religious background. Altogether, 259 cases and 257 controls participated in the study. As prevalent cases may have had symptoms for some time already, it is conceivable that changes in exposure to allergens have occurred, e.g., due to mattress renewals etc. Therefore we enquired about allergen reduction measures taken in the past in great detail.

**Specific IgE determination**

Venous blood samples (20 ml) were taken from all children by a skilled nurse. After centrifugation, sera were stored at -20°C. Specific IgE against house dust mite (*Dermatophagoides pteronyssinus*) was determined by RAST. The analyses were performed by the Central Laboratory of the Blood Transfusion Service in Amsterdam. Specific IgE determinations were done to permit more stringent case definitions and more detailed case-control comparisons (cf. analysis section).

**Pulmonary function**

Spirometry was performed according to the protocol of the ECCS (Quanjer 1983). A detailed description of our protocol has been provided by Houthuijs *et al.* (1989). A rolling-seal dry spirometer (Vicatest 5) coupled with automatic data acquisition software has been used. From a minimum of three valid expiratory manoeuvres the highest forced vital capacity (FVC), forced expiratory volume in one second ($FEV_1$), and peak flow (PEF) were selected. The highest maximal mid-expiratory flow (MMEF) was selected from a manoeuvre with a FVC within 5% or 100 ml of the highest FVC. Thus, selected pulmonary function values could be obtained from different curves. All pulmonary function data fulfilling the general acceptability criteria of the ECCS (such as no hesitant start, no early termination of the manoeuvre) were used in the analysis, including tests for which the highest FVC or $FVC_1$ was more than 5% or 100 ml larger than the second highest. This is in agreement with the recommendations of the ATS (American Thoracic Society 1987).

**Dust sampling and assay for *Der p* I**

Dust was sampled from the floors of the living room and the bedroom of the child, and the mattress of the child. Dust samples were obtained using a vacuum cleaner (Philips Topomatic T518), equipped with a special nozzle (ALK, Horsholm, Denmark) to collect dust on a cellulose filter (Schleicher & Schuell, 589 black ribbon 70 mm). The power of the vacuum cleaner was set at 1,000 W. From each living room and bedroom floor an area of 2 $m^2$ was sampled for 4 min. The entire upper mattress surface with an area of approximately 2 $m^2$, was vacuum cleaned for 2 min after the bedding had been removed. Between each sampling, the sampling nozzle was cleaned thoroughly with 70% ethanol. After sampling the filters were placed in Petri dishes and stored at -20°C until analysis.
A representative part (approximately 200 mg) of the fine dust (the dust was not sieved) from each sample was extracted by shaking for two hours in 2 ml ammoniumbicarbonate buffer. The amount of *Der p* I was measured with a monoclonal enzyme immunosorbent assay (EIA), manufactured by ALK, Horsholm, Denmark, according to Lind (1986b). The detection limit of the assay was 20 ng *Der p* I/g dust. The results were also expressed in ng *Der p* I/$m^2$. Sampling was performed in October-November 1990.

**Questionnaire**

A detailed questionnaire was administered to the parents/guardians, about a variety of home characteristics. These included allergen avoidance measures taken in the past, types of floor coverings, and a number of risk factors for childhood respiratory disease (among others, the presence of an unvented gas fired water heater (a so-called 'geyser'), smoking in the home, the presence of pets and socio-economic status).

**Statistical analyses**

Statistical analysis was performed by means of the Statistical Analysis System (SAS, version 6.06). The distributions of the *Der p* I concentrations were right skewed and became by approximation normally distributed after ln-transformation. To evaluate the relationship between case-control status and *Der p* I exposure, *Der p* I concentrations were grouped into three categories: <2,000 ng/g dust, 2,000 - 10,000 ng/g dust, and >10,000 ng/g dust. These levels correspond with the provisional hygienic thresholds discussed earlier. In the analysis, the lowest exposure level was used as a reference category, and odds ratios were estimated for intermediate and high exposure. The 'odds ratio' is a common measure of statistical association between exposure and disease in case-control studies (Rothman and Schlesselman 1982). Using three instead of just two categories of exposure, has the advantage that a crude exposure-response relationship can be detected if there is one.

The following comparisons were made: 1) Cases to controls, as defined by the screening questionnaire; 2) Asthmatic cases and controls; 3) Children with chronic cough, without other symptoms of asthma, to controls; 4) Sensitized cases to non-sensitized controls. This analysis was based on the assumption that *Der p* I exposure leads to sensitization among genetically pre-disposed subjects, and continued exposure leads subsequently to symptoms. This analysis was done for all three case definitions in 1-3; 5) Sensitized cases to non-sensitized *cases*. This analysis was based on the findings reported by Lau *et al.* (1989) and Wickman *et al.* (1991), referred to in the introduction; 6) Cases to controls (as defined in 1-4), removing all subjects from the analysis in whose homes allergen measures had been taken; 7) Sensitized cases to non-sensitized controls, with the same restriction; 8) Finally the analyses were repeated using *Der p* I/$m^2$ as exposure variable in stead of *Der p* I/g dust.

All comparisons were made with and without adjustment (by logistic regression cf. (Rothman and Schlesselman 1982)) for a number of potential confounders. As such, passive smoking, the presence of an unvented geyser, the presence of feathered or furred pets, age, gender, and socio-economic status were considered.

Some additional analyses were made to compare geometric mean *Der p* I levels between cases and controls. This was done to gain insight into the actual differences in exposure between the two groups. Some analyses were also made using other cut-off points than 2,000 and 10,000 ng/g dust. This was done to assess the sensitivity of the results to the level of the cut-off point.

The results of some of the major analyses are being reported in tabular form. The results of others are referred to in the text.

*6.3.4 Results*

Table 6.8 presents information on some characteristics of the study population. The case and control groups were found not to be different regarding mean age, the proportion of smokers and pets in the home, and the type of floor covering in the living room. However, there were more boys among the cases, and textile flooring in the bedroom was less common among cases. Among the cases, there were 76 children with doctor-diagnosed asthma, and 81 children for whom chronic cough was reported without other symptoms of asthma. For the asthmatics, the proportions of girls, smokers and pets in the home, and textile floorings in the living- and bedroom were lower compared with the entire group of cases. A higher proportion of cases was sensitized (RAST > 0) against house dust mite compared with the controls (37.9% vs 12.3%). The highest sensitization rate was found for

asthmatic children. Allergen avoidance measures taken in the home because of respiratory symptoms of one or more of the occupants, were reported only for cases, more frequently for asthmatic children than for children with chronic cough.

Table 6.8 Characteristics of the study population

| | Controls (n=257) | Cases (n=259) | Asthma (n=76) | Chronic cough (n=81) |
|---|---|---|---|---|
| Girls (%) | 58.8 | 47.9 | 38.0 | 53.5 |
| Age, mean (yr) | 8.6 | 8.3 | 8.5 | 8.0 |
| Smokers in the home (%) | 58.1 | 58.0 | 46.2 | 65.1 |
| Textile flooring in living room (%) | 78.6 | 78.0 | 73.4 | 76.7 |
| Textile flooring in bedroom (%) | 77.8 | 66.0 | 46.8 | 77.9 |
| Pets present in the home (%) | 61.8 | 58.5 | 49.4 | 59.3 |
| Never kept pets in the home (%) | 16.3 | 17.8 | 29.1 | 11.6 |
| Sensitized against dust mite (RAST>0, %) | 12.3 | 37.8 | 54.7 | 27.2 |
| Percentage decline of pulmonary function | | | | |
| FVC | | -0.6 | 0.4 | -1.8 |
| $FEV_1$ | | -2.7* | -5.1* | -1.8 |
| $FEV_1$/FVC | | -2.2* | -5.5* | 0.0 |
| PEF | | -3.2* | -5.7* | -2.2 |
| MMEF | | -8.4* | -18.3* | -2.1 |
| Reported allergen avoidance measures taken because of respiratory complaints (%): | | | | |
| Change of living room floor cover | 0.0 | 13.1 | 25.3 | 9.3 |
| Change of bedroom floor cover | 0.0 | 22.0 | 36.7 | 12.8 |
| Change of mattress | 0.0 | 7.3 | 15.2 | 6.9 |

* $p<0.05$

A valid pulmonary function test was obtained for 93% (473) of the children. The percentage difference of pulmonary function of cases, asthmatics and children with chronic cough, compared with the controls, was calculated by linear regression, adjusting for age, height, weight and gender. For all cases and the children with doctor-diagnosed asthma there was a significant ($p<0.05$) difference of $FEV_1$, PEF, $FEV_1$/FVC and MMEF, compared with the control group. For children with chronic cough, the same pulmonary function parameters were also lower compared to those of the controls, but these differences were not statistically significant (Table 6.8).

The *Der p* I concentrations per gram dust and per square meter varied widely (Table 6.9). Due to sampling errors, data were missing for the living rooms of two cases and two controls, for the bedrooms of one case and one control, and for the mattresses of one case and three controls. The highest geometric mean *Der p* I concentration was found for mattress dust. The geometric mean concentrations in dust from smooth floorings were

significantly lower than those found in dust from textile floorings (t-test, $p < 0.001$). The *Der p* I concentrations expressed in ng/g dust were divided into three categories as described above. In 86.3% of the homes, *Der p* I concentrations higher than 2,000 ng/g dust were found. In 55% of the homes the highest *Der p* I concentration found exceeded the proposed threshold limit value of 10,000 ng/g dust.

Table 6.9 Concentrations of *Der p* I (geometric mean (GM), geometric standard deviation (GSD), range) in house dust

| Location | n | ng/g dust | | | ng/m$^2$ | | |
|---|---|---|---|---|---|---|---|
| | | GM | GSD | range | GM | GSD | range |
| Living room | 512 | 2,370 | 6.3 | 85-150,519 | 593 | 10.8 | 5-391,993 |
| smooth floor covering | 112 | 371 | 2.8 | 99-31,084 | 27 | 4.0 | 5-28,141 |
| textile floor covering | 400 | 3,983 | 5.4 | 85-150,519 | 1,391 | 6.3 | 12-391,993 |
| Bedroom | 514 | 2,201 | 4.9 | 86-103,559 | 435 | 10.0 | 4-57,354 |
| smooth floor covering | 144 | 640 | 2.7 | 86-14,103 | 32 | 3.2 | 4-1,547 |
| textile floor covering | 370 | 3,558 | 4.5 | 98-103,559 | 1,202 | 5.8 | 11-57,354 |
| Mattress | 512 | 5,075 | 4.0 | 95-280,880 | 1,572 | 5.7 | 2-153,123 |
| in bedroom with smooth floor cover | 145 | 4,511 | 3.8 | 170-68,506 | 1,474 | 5.4 | 21-48,387 |
| in bedroom with textile floor cover | 367 | 5,317 | 4.0 | 95-280,880 | 1,630 | 5.7 | 2-153,123 |

Table 6.10 shows the crude odds ratios for the comparison of cases and controls. The lowest category of the *Der p* I concentrations (< 2,000 ng/g dust) was used as reference. The estimated odds ratios were mostly smaller than one. Only for bedrooms with textile floorings an odds ratio of 1.00 was found when the lowest and highest categories of *Der p* I were compared. For asthmatic children and children with chronic cough similar odds ratios were found. This suggests a negative association between the *Der p* I concentrations in house dust and reported respiratory symptoms. Using logistic regression analysis, the crude odds ratios were adjusted for passive smoking, the presence of an unvented geyser, the presence of feathered or furred pets, age, gender and socio-economic status. The adjusted odds ratios were similar to the crude odds ratios. Odds ratios could not be calculated for smooth floorings separately, because there were very few dust samples with *Der p* I levels higher than 2,000 ng/g.

Table 6.10 Crude odds ratios for elevated *Der p* I concentrations in house dust; comparison of cases and controls (95% confidence limits in parentheses)

| Location | 2,000 - 10,000 ng/g vs < 2,000 ng/g | > 10,000 ng/g vs <2,000 ng/g |
|---|---|---|
| Living room | 0.76 (0.50-1.16) | 0.72 (0.47-1.10) |
| Living room (textile flooring) | 0.63 (0.39-1.03) # | 0.59 (0.36-0.96) * |
| Bedroom | 0.75 (0.50-1.12) | 0.79 (0.47-1.10) |
| Bedroom (textile flooring) | 0.80 (0.49-1.31) | 1.00 (0.60-1.67) |
| Mattress | 0.62 (0.40-0.98) * | 0.69 (0.44-1.10) |

# $p<0.10$ * $p<0.05$

In a second analysis, a comparison was made between cases sensitized against house dust mites, and controls who were not sensitized against house dust mites. Again, most of the crude odds ratios were smaller than one. Only for textile floorings in the bedroom the calculated odds ratios were greater than one and marginally significant ($p<0.10$), when the lowest and highest categories of *Der p* I were compared (odds ratio 2.20). The comparison of sensitized asthmatics and non-sensitized controls gave similar odds ratios. For sensitized children with chronic cough most crude odds ratios were larger than one but not statistically significant. The adjusted odds ratios were comparable with the crude odds ratios.

The next step was a restriction of the analysis to cases who were sensitized against dust mites and cases who were not sensitized against dust mites (Table 6.11). Most of the crude odds ratios became greater than one after this restriction. The highest odds ratios were found comparing the lowest and highest categories of *Der p* I concentrations. However, the crude odds ratios were not statistically significant. Adjustment for confounding variables did not change the odds ratios substantially. When comparing sensitized asthmatics with non-sensitized asthmatics, and sensitized children with chronic cough with non-sensitized children with chronic cough, most odds ratios became also greater than one. For both asthmatics and children with chronic cough the odds ratios were higher than for the entire group of cases. For example, the following odds ratios were found comparing the lowest and highest categories of *Der p* I concentrations for sensitized asthmatics versus non-sensitized asthmatics: 1.46 (95% confidence interval 0.47-4.49) for the living room, 1.92 (95% confidence interval 0.43-8.54) for the bedroom, and 2.75 (95% confidence interval 0.75-10.10) for mattresses. However, due to the smaller numbers of children involved, the confidence intervals became wider. A comparison was also made between controls sensitized against dust mite and controls not sensitized against dust mites. The odds ratios were comparable with those found comparing sensitized cases with non-sensitized cases.

Table 6.11 Crude odds ratios for elevated *Der p* I concentrations in house dust; comparison of cases with elevated serum IgE against dust mites and cases without elevated serum IgE levels against dust mites, 1) irrespective of allergen avoidance measures taken in the homes (I), and 2) restricted to those living in homes where no allergen avoidance measures had been taken in the past (II) (95% confidence limits in parentheses)

| Location | 2,000 - 10,000 ng/g vs < 2,000 ng/g | > 10,000 ng/g vs <2,000 ng/g |
|---|---|---|
| I | | |
| Living room | 1.13 (0.60-2.12) | 1.34 (0.71-2.53) |
| Bedroom | 0.68 (0.36-1.26) | 1.13 (0.58-2.21) |
| Mattress | 1.04 (0.54-2.02) | 1.49 (0.78-2.85) |
| II | | |
| Living room | 1.29 (0.62-2.67) | 1.54 (0.74-3.19) |
| Bedroom | 1.19 (0.50-2.83) | 2.54 (1.11-5.83) * |
| Mattress | 1.19 (0.58-2.46) | 1.80 (0.90-3.62) # |

# $p<0.10$ * $p<0.05$

As shown in Table 6.8, parents of children with respiratory symptoms reported to have taken allergen avoidance measures in the past more often than parents of control children. This may have caused lower *Der p* I concentrations in dust of the homes of cases. The relation between exposure to house dust mite allergen and sensitization might therefore be obscured when current *Der p* I concentrations in house dust are compared between cases and controls. Therefore, the analysis was repeated by comparing cases sensitized against dust mites and cases not sensitized against house dust mites, both living in homes where no allergen avoidance measures had been taken in the past. This implied that the analysis was restricted to cases with textile floorings in living room and/or bedroom, and cases whose mattresses never had been changed because of respiratory symptoms. The results are presented in Table 6.11. All odds ratios increased after this restriction, and some now became statistically significant. The highest odds ratios were found by comparing the lowest and highest categories of *Der p* I content in house dust. The same picture was obtained comparing sensitized asthmatics with non-sensitized asthmatics, and sensitized children with chronic cough with non-sensitized children with chronic cough, all living in homes where no allergen avoidance measures had been taken. Due to the smaller numbers of children involved the confidence intervals became wider. The adjusted odds ratios were generally higher than the crude odds ratios, and some became statistically significant.

Comparisons were also made between the geometric mean concentrations of *Der p* I for the different groups of cases and controls. The results were essentially the same. For example, the geometric mean concentrations in dust of the living room with textile floorings were 3,207 and 4,949 ng/g dust for cases and controls respectively ($t=-2.61$, $p<0.01$). The geometric mean concentration for cases sensitized against house dust mites was 4,256 ng/g dust, compared with 2,816 ng/g dust for non-sensitized cases ($t=1.58$, $p=0.11$).

For the sensitized and non-sensitized cases, both living in homes where no allergen avoidance measures had been taken, adjusted odds ratios were also calculated using *Der p* I concentrations below 1,000 ng/g dust as reference and concentrations equal to or higher than 1,000 ng/g dust as exposed. The adjusted odds ratio for living rooms with textile floorings was 1.17 (95% confidence interval 0.54-2.54). For bedrooms with textile floorings the adjusted odds ratio was 1.62 (95% confidence interval 0.62-4.20), and for mattresses 3.09

(95% confidence interval 1.13-8.39).

Using the amount of *Der p* I/m$^2$ as exposure variable, comparisons were made between the geometric mean concentrations for the different groups of cases and controls. The results of these comparisons were not different from the results using the amount of *Der p* I/g dust as exposure variable (data not shown).

### *6.3.5. Discussion*

In this study, high concentrations of *Der p* I were found in the dust of textile floorings and mattresses. In 86% of the houses included in the study, *Der p* I concentrations exceeded the provisional threshold limit of 2,000 ng/g dust. This limit is regarded as representing a risk for the development of specific IgE to house dust mite allergen in genetically predisposed individuals (Platts-Mills and De Weck 1989). In 55% of the homes, concentrations of more than 10,000 ng *Der p* I/g dust were found, representing an increased risk for acute attacks of asthma (Platts-Mills and De Weck 1989). The concentrations found in dust from smooth floorings were 5 to 10 times lower than the concentrations found in dust from textile floorings. The *Der p* I concentrations (in ng/g dust) found in this study were comparable with those reported by Price *et al.* (1991) and Reiser *et al.* (1990). Lower concentrations were reported by Lau *et al.* (1989) and Wickman *et al.* (1991), whereas Sporik *et al.* (1990a) reported somewhat higher concentrations of *Der p* I/g dust. We did not sieve the dust prior to analysis. However, this seems not the explanation for the higher concentrations found in our study, as Reiser *et al.* (1990a) and Sporik *et al.* (1990) sieved the dust prior to analysis, whereas Wickman *et al.* (1991), Lau *et al.* (1989) and Price *et al.* (1990) analyzed unsieved dust.

The prevalences of house dust mite sensitization among cases and controls were comparable with those reported in the literature. Reported prevalences range from 45-85% among asthmatics and from 5-50% among controls (Platts-Mills and De Weck 1989). Pulmonary function was measured in all children. Most pulmonary parameter values of the cases were significantly lower than those of the controls. The largest differences were found for children with doctor-diagnosed asthma. These results are in agreement with an earlier Dutch study comparing children with chronic respiratory symptoms to controls (Flach and Brunekreef 1991).

In a crude analysis, without taking into account sensitization against house dust mite and allergen avoidance measures taken in the past, cases were generally exposed to lower *Der p* I concentrations than controls. Similar results were found for asthmatic children and children with chronic cough. Restriction of the analysis to cases with elevated serum IgE against dust mites, and to controls without elevated serum IgE levels, taking the type of floor covering into account, increased the odds ratios. However, only the estimated odds ratios for the *Der p* I content in dust of bedrooms with textile floorings became greater than one.

When the analysis was restricted to cases with elevated serum IgE against dust mites and cases without elevated serum IgE against dust mites, most odds ratios became greater than one. The highest odds ratios were found comparing the lowest and highest categories of *Der p* I content in house dust, which is indicative of an exposure-response relationship. Similar results were obtained comparing sensitized asthmatics and sensitized children with chronic cough with non-sensitized asthmatics and non-sensitized children with chronic cough respectively. However, the odds ratios were not statistically significant. Odds ratios increased again when the analysis was restricted to homes where no allergen avoidance measures had

been taken in the past. This indicates that parents of children with chronic respiratory symptoms take effective allergen reduction measures, and that they do so more often when their children are sensitized against house dust mites. These results are in agreement with those of other cross-sectional studies on the relation between the exposure to house dust mite allergens and sensitization among atopic children. Lau *et al.* (1989) reported similar concentrations of house dust mite allergens for atopic and non-atopic children. However, higher concentrations were found for atopic children sensitized to house dust mites than for atopic children not-sensitized to house dust mites. Price *et al.* (1990) found a positive association between the *Der p* I concentrations in house dust and skin sensitivity, comparing atopic children sensitized to dust mites and atopic children not-sensitized to dust mites. Wickman *et al.* (1991) also reported higher *Der p* I concentrations in homes of children sensitized to dust mites compared with the concentrations found in the homes of non-sensitized atopic children.

When we compared sensitized to non-sensitized cases, the estimated odds ratios at a cut-off point of 1,000 ng *Der p* I/g dust, were also all greater than one. These results suggest that the risk for sensitization might also be increased at *Der p* I concentrations below the proposed threshold limit of 2,000 ng/g dust, above which the risk for genetically predisposed individuals for the development of specific IgE to house dust mites is supposed to be increased (Platts-Mills and De Weck 1989). Our results are in agreement with those presented by Price *et al.* (1990). They found no association between skin sensitivity and the *Der p* I concentration in carpet dust at a cut-off point of 2,000 ng/g dust. However, with cut-off points of 1,000 ng *Der p* I/g dust and 500 ng/g dust, statistically significant differences between atopic children sensitized to house dust mites and atopic children not-sensitized to house dust mites were found. Lau *et al.* (1989) also reported a relationship between the concentrations of *Der p* I and *Der f* I in house dust below 2,000 ng/g dust and the risk for sensitization. Using concentrations of *Der p* I plus *Der f* I below 400 ng/g dust as reference and concentrations equal to or higher than 2,000 ng *Der p* I plus *Der f* I /g dust as exposed, they found an odds ratio of 7 (95% confidence interval 3-18).

Colloff and co-workers (1992) recently suggested that the concentrations of *Der p* I expressed by square meter might be a better estimation of the potential exposure. However, using the amount of *Der p* I per square meter as exposure variable, we found the same associations between exposure to house dust mite allergen and sensitization to house dust mite among children with respiratory symptoms.

Sporik *et al.* (1990a) used the highest allergen level in the home as exposure variable, irrespective of the location of sampling. In our study, in most houses (51%) the highest *Der p* I were found in mattress dust. Therefore, the results using the highest *Der p* I concentrations found in the home as exposure variable were similar with those using the levels in mattress dust as exposure variable.

Parents of children with chronic respiratory symptoms were found to regularly have taken allergen avoidance measures in the home. Therefore, lower *Der p* I concentrations might be measured in the houses of children with respiratory symptoms. A comparison of the present concentrations in house dust of cases and controls therefore obscures the relation between the exposure to house dust mite allergens and sensitization. This hampers the estimation of the association between exposure to house dust mite allergen, sensitization and the development of respiratory symptoms. Prospective studies, or case-control studies based on incident cases, are needed to further clarify the relationship between dust mite allergen exposure, sensitization and childhood respiratory symptoms.

# General discussion

## 7.1 Introduction

The studies presented in this thesis have contributed to the further clarification of the relationship between home dampness and respiratory symptoms in occupants, as outlined in Figure 1.1 in Chapter 1. Figure 7.1 summarizes the specific contributions of the studies reported here.

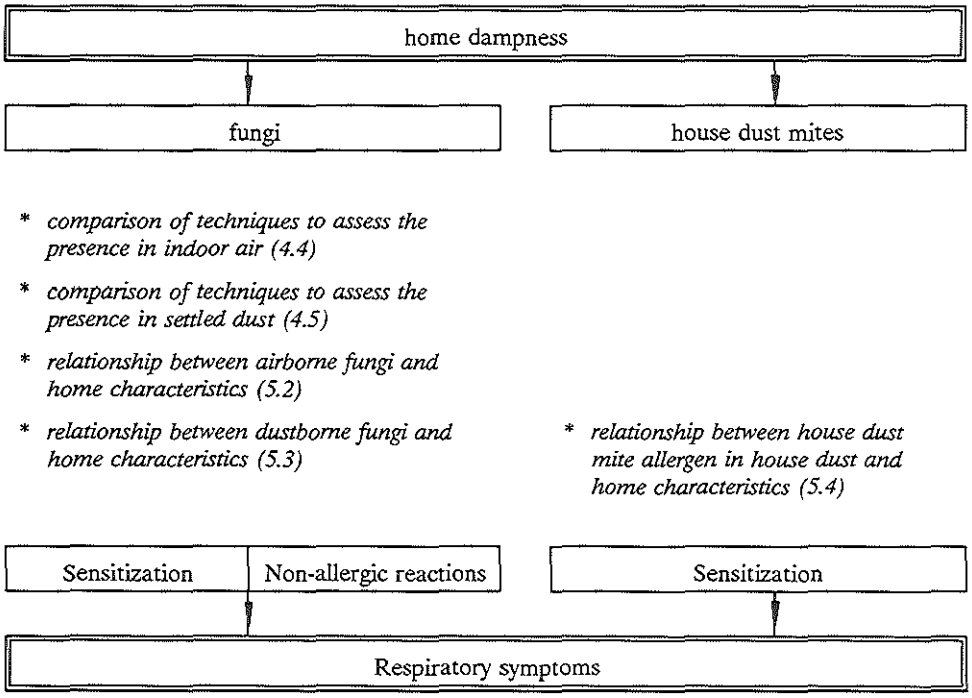


Figure 7.1 Overview of the contributions of the studies presented in this thesis to the further clarification of the relationship between home dampness and respiratory symptoms

The main null hypotheses tested in the studies mentioned in Figure 7.1 were:

1) There are no differences in the results obtained with different techniques for the enumeration and identification of viable fungal particles in the indoor air of houses;
2) There are no differences in the results obtained with different analytical methods for the enumeration and identification of viable fungal particles in settled house dust;
3) Measurement of fungi in single samples of indoor air or settled house dust do not provide a reliable estimation of the potential exposure;
4) There is no relation between the presence of fungi in indoor air and home dampness;
5) There is no relation between the presence of fungi in settled house dust and home dampness;
6) There is no relation between house dust mite allergen in settled house dust and home dampness;
7) There is no relation between reported home dampness and reported respiratory symptoms in children, taking into account sensitization against fungal and house dust mite allergens;
8) There is no relation between the levels of house dust mite allergen in settled house dust and reported respiratory symptoms in children, taking into account sensitization against house dust mite allergens.

In the remainder of this Chapter, we will discuss the results of our studies concerning these hypotheses in the larger framework of the overall relationship between home dampness and respiratory symptoms in children.

## 7.2 Epidemiology

The results of recent epidemiological studies conducted in the Netherlands and elsewhere, reviewed in Chapter 2, show a positive association between reported or observed home dampness and mould growth in modern homes, and the prevalence of reported respiratory symptoms in both children and adults. The epidemiological studies on home dampness and respiratory symptoms in occupants have several features in common. The design of all the studies was cross-sectional. Prospective studies on the relation between home dampness and the development of respiratory symptoms have not yet been published. In most studies questionnaires, completed by the occupants, were used to obtain information on the exposure variables (signs of home dampness) and the outcome variables (respiratory symptoms).

*Reported home dampness and reported respiratory symptoms*

In the larger studies conducted in the Netherlands among children, odds ratios for reported respiratory symptoms in relation to reported damp patches ranged from 1.30 (95% CI 0.91-1.85) for asthma, to 1.97 (95% CI 0.88-4.41) for chronic cough. For reported mould growth, the odds ratios ranged from 1.12 (95% CI 0.91-3.38) for asthma, to 3.06 (95% CI 1.29-7.26) for chronic cough (Brunekreef 1992a). In adults, odds ratios ranging from 1.03 (95% CI 0.79-1.35) for allergy, to 2.56 (95% CI 1.94-3.38) for cough in relation to reported damp stains and/or mould were found (Brunekreef 1992b). The odds ratios found in studies conducted in the UK, Scandinavia, US and Canada, are comparable with those found in the Netherlands (see Tables 2.1 and 2.2, Chapter 2). In

our case-control study reported in section 6.2 the odds ratios are similar to those presented in the literature. For reported dampness, comparing cases with controls, the odds ratio was 1.32 (95% CI 0.89-1.96), whereas reported mould had an odds ratio of 1.42 (95% CI 0.94-2.16). Asthmatic children achieved odds ratios of 1.46 (95% CI 0.80-2.64) and 1.57 (95% CI 0.84-2.93), respectively, while children with chronic cough had an odds ratio of 1.70 (95% CI 0.94-3.09) in relation to reported dampness, and of 1.90 (95% CI 1.02-3.52) in relation to reported mould growth.

In general, the associations are stronger using reported moulds as exposure variable than reported dampness. This might be explained by the fact that damp patches are not always associated with fungal growth, and are not automatically related to increased levels of fungi in indoor air. Furthermore, we found that the odds ratios for the separate locations in the home reached statistical significance more often than those for the whole house. This suggests that the relationship between reported dampness, mould growth and respiratory symptoms, is somewhat obscured by lumping all locations together (section 6.2).

*Observed home dampness and reported respiratory symptoms*

In two studies (Martin *et al.* 1987; Platt *et al.* 1989) observed signs of dampness by the investigators were used as exposure variables. The prevalence ratios for reported symptoms in relation to observed dampness ranged from 0.80 for sore throat, to 1.58 for fever. For observed mould growth, the prevalence ratios ranged from 1.30 for runny nose, to 2.15 for fever.

In our case-control study (section 6.2), observed signs of dampness by the investigators were also used as exposure variables. The odds ratios found using these were smaller than those using reported dampness as exposure variable. In addition, the odds ratios for the separate locations were comparable with those for the whole house when using observed signs of dampness. The observations made by the investigators can be regarded as 'spot-samples', whereas the signs of dampness reported by the residents included a period of two years or longer, during which more signs of dampness might have occurred.

*The role of information bias*

As in most studies reported signs of dampness were used as exposure variables, and reported respiratory symptoms as outcome variables, the associations found may have been (partly) caused by information bias. Two type of information bias may be important.

Firstly, that which is introduced by respondents with a tendency towards over-reporting and by respondents who generally under-report. In other words, those who report more respiratory symptoms also report more signs of dampness, and vice versa. This type of bias may be assessed by including questions on health symptoms, other than the outcome variables of interest. Dales *et al.* (1991a, 1991b) stated in their studies, that this type of information bias could not explain the observed associations between home dampness and respiratory symptoms.

The second type of information bias is introduced if symptomatic subjects or parents/guardians of symptomatic children are more likely to report the presence of dampness and mould, or if subjects living in damp homes are more likely to report respiratory symptoms for themselves and/or their children. Such misreporting can be of

major importance in studies conducted in areas where home dampness is a public concern. The possible role of this type of bias has been investigated by measuring the pulmonary function of respondents as a more objective measure (Strachan 1988; Brunekreef *et al.* 1989; Brunekreef 1992a). Strachan (1988) showed indications of reporting bias, when comparing the pulmonary function of wheezy children in homes with and without reported mould growth. He found that for any degree of bronchial lability, wheeze in children was more commonly reported by parents who also reported visible mould growth in the home. In the two other studies comparisons were made between the pulmonary function of children with reported respiratory symptoms living in homes with visible mould growth and in homes without reported mould growth. The reduction of pulmonary function was comparable for both groups of children. Therefore, these comparisons revealed no evidence of over-reporting of respiratory symptoms by parents living in damp and mouldy homes (Brunekreef *et al.* 1989; Brunekreef 1992a). In our case-control study reported in Chapter 6, lung function was measured as well. Most pulmonary function parameter values of children with reported respiratory symptoms were statistically significantly lower than those of the controls. This supported the classification of cases and controls. The lowest pulmonary function parameter values were found for children with doctor-diagnosed asthma, as expected from the literature (Flach and Brunekreef 1991).

In an attempt to validate the resident-reported exposure variables, Andrae *et al.* (1988) and Platt *et al.* (1989) compared reported signs of dampness by the residents with observed signs of dampness by the investigators. In both studies a reasonable agreement was found between reported and observed dampness. In our study, reported dampness and mould growth in the home some time during the last two years were compared with observed dampness and mould (section 6.2). For mould, the data were adjusted for mould removal by the parents. A reasonable agreement was found (kappa statistics ranging from 0.43 to 0.70) between reported and observed signs of dampness. The agreement was somewhat higher for reported mould than for reported dampness. Furthermore, the agreement between the parents of cases and investigators was somewhat higher than that between the parents of controls and the investigators, as indicated by higher kappa statistics (damp patches 0.56 versus 0.43, mould 0.70 versus 0.67). We found no indication of over-reporting of damp and mould by parents of children with reported respiratory symptoms relative to parents of controls.

*The role of potential confounders*

Bias by potential confounders might also have resulted in an artificial relationship between home dampness and respiratory symptoms. Respiratory symptoms and home dampness might be indirectly related through socio-economic status, or they might be indirectly related through other indoor environmental factors such as inadequate ventilation, type of heating and cooking fuels used, the presence of furred or feathered pets, or smoking in the home. In most studies, the crude associations between home dampness and respiratory symptoms did not change substantially after adjustment for these potential confounders. This was also the case in our case-control study (section 6.2). In another study conducted in the Netherlands, no clear relationship was found between educational level of the respondents and the reporting of mould in the home (Brunekreef 1992a). Overall then, the associations between reported signs of dampness and reported respiratory symptoms do not seem to be caused by confounding.

The outdoor environment might also affect the prevalence of reported respiratory symptoms and reported dampness indoors. The reviewed epidemiological studies have been carried out in a wide range of different climatic conditions, from areas with prolonged periods with cold and dry weather (Finland, Sweden, Canada), to areas with a more temperate climate (UK, the Netherlands). Even within one country (Canada, US), studies have been carried out in different regions, representing differences in temperature, humidity and ambient pollution. In all these studies, positive associations between home dampness and respiratory symptoms were found. So, these associations do not seem to be confounded by outdoor environmental conditions either.

*Conclusion*

It is therefore concluded that the associations between reported home dampness and reported respiratory symptoms in occupants found in recent epidemiological studies are, in general, not attributable to reporting bias or confounding by other indoor or outdoor factors possibly related with home dampness and/or respiratory health.

### 7.3 Suspected causal factors: fungi and house dust mites

If the association between home dampness and respiratory symptoms is real, the next important question is whether it is causal. In most epidemiological studies fungi and fungal products are proposed as the possible causative agents in this association, and little attention has been given to the possible role of house dust mites.

*Fungi*

The present knowledge of the ecology of fungi, reviewed in Chapter 3, shows that the development of fungi depends on environmental humidity. It is the most critical factor for their development, especially the water activity of the substrates on which fungi grow.

Fungi produce inhalant allergens, which may lead to sensitization in genetically predisposed individuals, and subsequently provoke asthma, rhinitis and extrinsic allergic alveolitis (Tobin *et al.* 1987; Flannigan *et al.* 1991). Most fungi have been shown to be allergenic by skin prick testing (e.g. Burge 1985). However, the isolation, purification and standardization of allergens produced by fungi are still major problems (Salvaggio and Aukrust 1981; Burge 1985; Malling *et al.* 1985; Commission of the European Communities 1993). This implies a low specificity and sensitivity of the immunological tests used (RAST) to investigate sensitization to moulds. In the Netherlands, the reported prevalence of sensitization against fungi is rather low, approximately 5% (De Vries 1973; Beaumont *et al.* 1985). In the case-control study presented in Chapter 6, 10% of cases and only 1% of the controls were sensitized against a mould mixture, including *Penicillium* spp., *Alternaria* spp., *Aspergillus* spp. and *Cladosporium* spp., assessed by RAST. Among the asthmatic children, 16% were sensitized against this mould mixture. Considering the low sensitivity and specificity of the RAST tests used to assess sensitization against fungi, fungal allergy may be more important than can be estimated at present using the commercially available RAST panels.

*House dust mites*

Besides fungi, house dust mites encounter favourable conditions in damp environments as humidity is the most critical factor in their development and survival. The criterion value for excessive mite growth in indoor environments is considered to be an absolute indoor humidity of approximately 7 g/kg dry air (Korsgaard 1983b; Platts-Mills *et al.* 1992). This value is within the normal range of absolute indoor humidity in Dutch homes. In a number of studies, absolute indoor humidity was found to be positively associated with the number of mites in house dust and with mite antigen concentrations (Korsgaard 1983b; Wickman et al 1991; Emenius *et al.* 1993; Harving *et al.* 1993). In the present study a strong positive relation was found between mite allergen levels in mattress dust and in dust from carpeted bedroom floors, and the average relative humidity in the bedroom measured over several weeks (section 5.4). The average absolute humidity over several weeks in the bedrooms involved ranged from 6.36 g/kg to 10.55 g/kg dry air. In most bedrooms this value was above the criterion value for excessive mite growth.

In the 1960's, mites of the family Pyroglyphidae were identified as the most important source of house dust allergens (Voorhorst *et al.* 1964, 1967). Since then, considerable progress has been made in the identification, purification and standardization of the major allergens produced by *Dermatophagoides* spp. (Lind 1985; Platts-Mills and Chapman 1987; Platts-Mills *et al.* 1992). Inhalation of house dust mite allergens can cause sensitization in genetically pre-disposed individuals, and subsequently allergic reactions such as asthma, rhinitis and atopic dermatitis. Allergy to mite allergens is very common in atopic populations. The reported prevalences among atopic asthmatics range from 45% to 85%, but in the general population, the prevalence of house dust mite sensitization ranges from 5% to 30% (Platts-Mills and De Weck 1989; Colloff *et al.* 1992). In the case-control study described in Chapter 6, 38% of the cases and 12% of the controls were sensitized against *D. pteronyssinus*, the highest prevalence being among asthmatics (54%).

*Conclusion*

The present knowledge on the ecology and the effects on human health of fungi and house dust mites gives sufficient evidence to consider both groups of organisms as potentially causal factors in the relation between reported or observed home dampness and reported respiratory symptoms in children and adults.

### 7.4 Assessment of the exposure to fungi and house dust mites

In epidemiological studies a positive relation between home dampness and respiratory symptoms is reported. In order to substantiate this relation, it is essential to be able to measure the suspected causal factors, i.e. fungi and house dust mites (house dust mite allergens). Furthermore, the existence of an exposure-effect relation should be demonstrated. The method(s) used to measure the exposure should preferably maximize validity and minimize error (Armstrong *et al.* 1992).

Inhalation of fungi and house dust mites allergens is the most interesting exposure route in relation to home dampness. Ideally, an air sampling device should be available for personal air sampling of fungi and house dust mites (or their allergens, or, in the

case of fungi, secondary metabolites), which takes air samples in the breathing zone, with high accuracy and precision.

*Assessment of the exposure to house dust mites*

Section 4.2 contains a brief review of the available methods for measuring the potential exposure to house dust mites and their allergens. In the studies reported in sections 5.4 and 6.3, it was decided to assess the potential exposure by measuring the amounts of *Der p* I in settled dust, in accordance with the recommendations of a WHO workshop (Platts-Mills and De Weck 1989) and of a report issued by the Commission of the European Communities (1993).

The reproducibility of the assay used in our study to determine the amount of *Der p* I in settled dust was acceptable. For floor dust, the mean coefficient of variation of duplicate samples was 5.3% (based on the ln-transformed concentrations). This coefficient was mainly determined by the variation due to sampling (4.7%), and to a minor extent by the variation due to extraction of the dust (1.7%) and the variation due to the final analysis (1.5%). For mattress dust no duplicate samples could be taken; the coefficients of variation due to extraction and analysis were both 1.5%. The variation on different locations within homes was much smaller than the variation between homes. Van Leeuwen and Aalberse (1991) reported that for the *Der p* I concentrations in settled floor dust, the variation in time within one bedroom was much smaller than the variation between bedrooms. This implies that the measurement of house dust mite allergens in settled house dust can be used as a reliable estimator of the potential exposure to these allergens in different homes.

Nevertheless, it is realized that (personal) air sampling for mite allergens may be a more accurate estimate of exposure than assays of settled dust. However, at present no reliable information is available that will support adoption of a standardized method for air sampling of house dust mite allergens. According to two international workshops (Platts-Mills and De Weck 1989; Platts-Mills *et al.* 1992) airborne dust sampling has not proved to be a better method than settled dust sampling for the assessment of the potential exposure to house dust mite allergens. It was also stated that there has been little or no data showing a relationship between airborne measurements and sensitization against house dust mites or symptoms, whereas relations have been found between the concentrations of mite allergens in settled dust and sensitization or symptoms. Further research is needed to compare the validity of sampling air and dust for an assessment of the (potential) exposure in epidemiological studies.

*Assessment of the exposure to fungi*

The most widely used method to measure the presence of fungi in dwellings as estimator of exposure is air sampling of viable fungal particles. Personal air sampling of fungi in homes has so far not been performed. As reviewed in section 4.3, several air sampling devices are available, all distinguished by their own sampling characteristics (e.g. principle of sampling, cut-off point).

In the literature, information on the accuracy and precision of these methods as a measure of exposure is limited. We conducted a comparative study to document to what extent different results are obtained if different methods for sampling of airborne fungal propagules in homes are used (section 4.4). We compared the results obtained with five commercially available air sampling devices and a non-volumetric sampler, in

combination with four different culture media. The hypothesis was tested that there are no differences in the results obtained with different techniques for the enumeration and identification of viable fungal particles in the indoor air of houses. Because the accuracy (the agreement between the measured results and the "true" number of CFU/m$^3$ and mould species) cannot be determined, the different measurement techniques could not be compared by this criterion. Therefore, the validity of the measurements could only be assessed by comparing the yields in terms of CFU/m$^3$ and number of species isolated, and by measuring the precision of the results. This comparative study indicated that the results - quantitatively as well as qualitatively - depend to a great extent on the sampling device and culture medium used. The null-hypothesis was rejected. High yields in terms of CFU/m$^3$ were more consistently obtained with a Slit-sampler and the N6-Andersen sampler, in combination with DG18 agar and Malt Extract Agar. The highest yields in terms of species isolated were obtained with a Slit-sampler and the N6-Andersen sampler, in combination with DG18 agar. This particular agar, although specially designed for the isolation of xerophilic fungi from food, proved to be a good general purpose medium for aerobiological studies.

The precision of the methods both in terms of CFU/m$^3$ and in terms of species isolated was only moderate. The coefficients of variation of parallel duplicate samples ranged from approximately 8% (N6-Andersen sampler in combination with MEA or DBRC), to 44% (Gelatine Filters in combination with DBRC). The variability of sequential duplicate samples, taken within minutes of each other was also high, with coefficients of variation ranging from approximately 11% (SAS sampler in combination with DG18), to 39% (RCS sampler in combination with DRBC). For most combinations, these coefficients of variation were higher than 20%. This was confirmed in a second study, described in section 5.2. The average coefficients of variation of sequential duplicate samples taken with the N6-Andersen sampler in combination with DG18 agar (indoors as well as outdoors) were again generally higher than 20%. This variation may be partly due to sampling errors, but is also caused by the real variation in the number of fungal propagules in indoor air within short time intervals. Hunter *et al.* (1988) indicated that the number of CFU/m$^3$ varied widely within dwellings over short periods.

The reproducibility of species isolated in sequential samples, expressed as the agreement ratio was only moderate (generally less than 50%). This was also confirmed in the second study (section 5.2), where the applicability of air sampling of fungal propagules in epidemiological studies was further investigated by assessing the variability in time. Sampling was performed with the N6-Andersen sampler in combination with DG18 agar with an interval of five weeks between. The results of this study indicated that for the total number of CFU/m$^3$ as well as the numbers belonging to the most frequently isolated mould genera, the variation between homes was much smaller than the variation within homes. The agreement ratio between the genera isolated in both sampling periods was approximately 50%.

Although the use of settlement plates (Open Petri Dishes, OPD) is generally not recommended (no quantitative information and over-representation of larger particles), in both our studies (sections 4.4 and 5.2) high correlations were found between the number of CFU/m$^3$ obtained with the N6-Andersen sampler and the numbers of CFU obtained with the OPD. The number of species obtained with the OPD was considerably lower than those obtained with the volumetric methods. The precision of the results obtained with the OPD was comparable with that of the volumetric air sampling devices. Thus settlement plates can only be used to obtain a semi-quantitative reflection about the presence of fungal propagules in indoor air.

Considering these results it is concluded that the precision of air sampling of viable fungal particles is limited. The presence of fungal propagules in indoor air cannot be determined reliably - quantitatively as well as qualitatively - with single samples. Therefore, the usefulness of air sampling of viable fungal particles as estimator of the potential exposure is only limited.

As with the measurement of house dust mite allergens in settled house dust, the measurement of fungal propagules in settled house dust was considered as possibly a more precise measure as estimator of the potential exposure to fungi (section 4.5). The results of measurements of airborne fungal propagules depend largely on the sampling device and culture medium used. Therefore, the first step was to document the extent to which results differ with the use of different analytical methods for the assessment of the presence of fungal propagules in house dust. Ten different analytical methods were used, to test the hypothesis that there are no differences in the results obtained with different analytical methods for the enumeration and identification of fungal propagules in settled house dust. Each method was a unique combination of culture medium, suspension fluid and dilution step. Again, the accuracy cannot be determined. Therefore, the validity of the measurements could only be assessed by comparison of the yields in terms of CFU/g and number of species isolated, and by measurement of the precision of the results. Once more, we found that the results - quantitatively as well as qualitatively - largely depended on the analytical method used. The null-hypothesis was rejected. The highest yield in CFU/g dust was obtained after suspension followed by a 10-fold dilution, and the lowest by direct plating. The composition of the suspension fluid did not influence the yield in CFU, but higher counts were consistently obtained on DG18 agar compared with V8 agar. The highest numbers of different species were obtained after direct plating, although identification was difficult or even impossible due to the high density of the colonies on the plates for 30% of the plates. The latter problem did not occur with suspension. Higher numbers of different species were obtained on DG18 agar compared with V8 agar, and with suspension in sucrose compared with suspension in peptone. It should be stressed that it is not possible to isolate the entire range of fungi in dust (or air) using a single culture medium.

The reproducibility in terms of CFU/g dust of duplicate analyses was acceptable for direct plating and the primary suspension, with mean coefficients of variation of approximately 11% to 16%. The reproducibility of the species isolated by duplicate analyses was only moderate (highest agreement ratio approximately 60%). These figures cannot be compared with those obtained for air sampling of fungal propagules, because the latter were not based on duplicate analyses, but on parallel and serial samples. The reproducibility in time (assessed over a period of 6 weeks) of the number of CFU/g dust - expressed as the reliability coefficient (Armstrong *et al.* 1992) - was somewhat better for mattress dust compared with bedroom floor dust. The estimated reliability coefficients for the results obtained with the different analytical methods ranged from 0.06 to 0.74 for mattress dust, and from 0.01 to 0.32 for floor dust. However, also for mattress dust the predictive value of a single measurement is rather low. Furthermore, the variation in time with regard to the species isolated was substantial for both mattress and floor dust (maximum mean agreement ratio approximately 40%).

Considering these results it is concluded that the precision of dust sampling for viable fungal particles is limited. The presence of fungal propagules in settled house dust cannot be determined reliably - quantitatively as well as qualitatively - with single samples. Therefore, the usefulness of dust sampling for viable fungal particles as an

estimator of the potential exposure is only limited.

As both air- and dust-sampling do not provide precise measures of the potential exposure to viable fungal particles in homes, other indicators of exposure to fungi in indoor environments to be used in epidemiological studies have to be considered. For example, sampling of cell wall components of fungi, such as ß-1,3-glucan (Rylander *et al.* 1992) and lipopolysaccharides (Notermans *et al.* 1988) might give an overall estimate of the amount of airborne fungal matter. However, using these types of indicator, no information on the fungal species is obtained. The measurement of the volatiles produced by fungi might also be used as indicator of exposure (Miller *et al.* 1988; Flannigan *et al.* 1991), but their validity as an indicator of exposure should be further evaluated because these volatiles may have other indoor sources.

*Conclusions*

It is concluded that for house dust mites, the measurement of house dust mite allergens in settled house dust can be used as a precise estimator of the potential exposure to these allergens.

For fungi, the precision of both air and dust sampling for viable fungal particles is limited. The value of air and dust sampling for viable fungal particles as an estimation of the potential exposure is therefore limited as well.

## 7.5 The presence of fungi and house dust mites in relation to home dampness and other residential characteristics

Fungi and house dust mites both encounter favourable conditions in damp environments. This suggests a relationship between the presence of these organisms and reported and/or observed signs of dampness, providing that the latter are indicators of increased humidity in the micro-environments where these organisms live. In the daily public health practice and in epidemiological studies, it would be useful to be able to predict the presence of fungi and house dust mites on the basis of home and occupant characteristics. This presupposes a relation between signs of dampness and other home-characteristics and the presence of fungi and house dust mites.

*Fungal propagules in indoor air*

In our study (section 5.2) the mean total numbers of CFU/m$^3$ and the mean numbers of CFU/m$^3$ of the different genera appeared to be only weakly associated with home dampness expressed as dampness score. However, the mean numbers were consistently higher in homes classified as damp compared with those in dry homes. Also, positive correlations were found between the fungal spore counts and home dampness expressed as a dampness score. However, these differences and most of the correlation coefficients were not statistically significant, and therefore our null-hypothesis (there is no relation between the presence of fungi in indoor air and home dampness) could not be rejected. Higher numbers of CFU/m$^3$ in homes classified as damp were also found by Hunter *et al.* (1988), Platt *et al.* (1989) and Waegemaekers *et al.* (1989). Classification of the homes was either based on reported dampness by the occupants or observed dampness by the investigators. In our study, the mean numbers of CFU/m$^3$ in homes classified as damp

were on average 1.6 to 2.1 times higher than the mean numbers of CFU/m³ in homes classified as dry. The ranges of the numbers of CFU/m³ in homes classified as damp and in those classified as dry showed considerable overlap. The predictive value of observed home dampness with respect to the levels of airborne fungal propagules is, therefore, limited.

It was found that the indoor air fungal flora was not simply a reflection of the outdoor fungal flora (section 5.2). Previously it had been suggested (Sneller and Roby 1979; Tyndall *et al.* 1987; Reponen *et al.* 1992) that the outdoor air is the major source of fungi for non-industrial indoor environments, unless the ground is frozen and covered with snow. Our results indicate that even in spring, specific indoor sources of fungi influence the counts to such an extent that indoor and outdoor counts for specific genera were clearly different. This is in agreement with the results of other studies conducted in the Netherlands by Beaumont *et al.* (1984). In our study, the total numbers of CFU/m³ in outdoor air were not correlated with the indoor counts, whereas the numbers of CFU/m³ found in the living room and bedroom were highly correlated. For the different fungal species, the differences between indoors and outdoors were even more pronounced. This is in agreement with the results presented by Fradkin *et al.* (1987), and it implies that in aerobiological studies, attention should be given to the identification of colonies up to species level.

*Fungal propagules in settled house dust*

We also found weak positive relations between reported or observed signs of home dampness and the total number of CFU/g of settled house dust, and the numbers of CFU/g of the different fungal species in floor and mattress dust (section 5.3). Most differences were not statistically significant, and therefore the null-hypothesis (there is no relation between the presence of fungi in settled house dust and home dampness) could not be rejected. Wood *et al.* (1988) and Wickman *et al.* (1992) also reported weak positive associations between the numbers of fungal propagules in settled house dust and reported signs of dampness. In our study the mean numbers of CFU/g floor and mattress dust were approximately 1.3 times higher in homes with observed signs of dampness than in those without. In homes with reported signs of dampness the mean number of CFU/g floor dust was comparable with that for homes without, whereas the mean number of CFU/g mattress dust was approximately 1.8 times higher in homes with reported signs of dampness than in those without. The ranges of the numbers of CFU/g dust in homes with observed or reported signs of dampness showed considerable overlap with those in homes without observed or reported dampness respectively. This implies that observed and reported dampness predict the presence of fungal propagules in settled house dust only to a limited extent. Further, the total numbers of CFU/g dust and the numbers of CFU/g of the different species were not associated with the relative or absolute indoor humidity measured over a period of several weeks in a subsample of the homes.

For floor dust, the main predictive factor of the numbers of CFU/g was the type of flooring. The numbers in dust from textile floorings were four times higher than those in dust from smooth floorings. For mattress dust, no single housing factor or occupant characteristic included in our study, was of major importance.

*House dust mite allergen (Der p I) in settled house dust*

The levels of concentration of *Der p* I in settled house dust were in general higher in homes with reported or observed signs of dampness, compared with those in homes without reported or observed signs of dampness (section 5.4). For reported signs of dampness in the home, most differences were statistically significant. These findings are in concordance with the results presented by Hart and Whitehead (1990) and Munir *et al.* (1993). The major differences were found for the *Der p* I content in dust from floors with wall-to-wall carpets or rugs, and in dust from mattresses. In homes with reported signs of dampness, the mean concentrations of *Der p* I in dust sampled at these locations were on average 1.1 to 2.5 times higher than those sampled in homes without reported signs of dampness. In homes with observed signs of dampness, the mean concentrations of *Der p* I in dust sampled at the different locations were on average 1.0 to 2.5 times higher than those in homes without observed signs of dampness. The ranges of the *Der p* I concentrations in homes with or without observed signs of dampness showed more overlap than the ranges of the *Der p* I concentrations in homes with or without reported signs of dampness. Thus, the differences in relation to observed dampness were less pronounced than those for reported dampness. This is possibly due to the 'spot-sample' character of observations of dampness made by investigators during a single home visit. The predictive value of observed home dampness with respect to the concentrations of *Der p* I in settled dust is therefore lower than the predictive value of reported signs of home dampness. The average relative indoor humidity measured over a period of several weeks in the bedroom during autumn, was strongly correlated with the concentrations of *Der p* I in mattress dust and in dust from wall-to-wall carpets. This suggests that the indoor humidity measured over a period of several weeks predicts the levels of *Der p* I in mattress dust and in dust from wall-to-wall carpets fairly well. However, the applicability of thermohygrometers in large scale epidemiological studies is limited. In Sweden, recently an inexpensive passive sampler for estimating monthly averages of relative humidity has been developed (Norberg and Stymne 1993). The accuracy of this method was reported to be high for relative humidities up to 65%. At present this method is not suitable under the circumstances in the Netherlands. Our data further suggests that for the conditions prevailing in the Netherlands, where winters are generally mild and damp, heating of the bedrooms may be effective in reducing mite allergen levels.

By far the strongest influence on the *Der p* I levels in settled floor dust was exerted by the type of floor cover. This finding is in agreement with the results reported by Sporik *et al.* (1990a). Dust from carpeted floors contained six to fourteen times more mite allergen than dust from smooth floors, whereas dust from smooth floors with rugs contained four to seven times more *Der p* I than dust from smooth floors. Thus, for the *Der p* I content in floor dust, the major predictive variable is the type of flooring. This strongly suggests that removal of carpets and rugs is the most efficient way to reduce the potential exposure to dust mite allergen. However, it is not yet clear to what extent the *Der p* I content in settled dust represents airborne concentrations. Price *et al.* (1990) reported that, with similar concentrations per gram settled dust, the airborne levels above all-wool carpets were higher than those above synthetic carpets or hard floors, which did not differ from each other. The development of static charges on synthetic carpets may prevent the release of particles containing mite allergen and therefore result in lower airborne concentrations with all-wool carpets or hard floors.

A small number of other home characteristics were associated with the *Der p* I levels in settled dust. Some of these factors point to possibilities for reduction of allergen exposure, e.g. with smooth floors, a further reduction of mite allergen concentrations can be achieved by removal of upholstered furniture. For wall-to-wall carpets, the age of the carpets was positively associated with the *Der p* I content and this association was also found for mattresses. More frequent replacement of mattresses and floor covers will therefore also reduce allergen exposure. Mattresses contributed to the allergen load on bedroom floors. Encasement of mattresses therefore will probably reduce allergen levels on floors. Mattress covers have been shown to reduce mite counts and the amounts of *Der p* I in mattress dust (Sarsfield *et al.* 1974; Walshaw and Evans 1986; Owen *et al.* 1990). In our study mattress covers were not encountered, and therefore their effect on the *Der p* I concentration in mattress dust could not be assessed.

We found an inverse relation between allergen avoidance measures taken in the past by the occupants and mite allergen levels. The avoidance measures included a change of floor-covering in the living room and/or bedroom, a change of mattress and/or bedding, and moving to another home. These measures were taken because at least one of the occupants had respiratory symptoms. In homes where four or more avoidance measures had been taken, the geometric mean concentrations on the different locations were approximately 1,500 ng/g dust. In homes where no allergen avoidance measures were taken the levels varied from approximately 2,500 ng/g dust from the floor of the bedroom, to 5,000 ng/g dust for mattresses. Allergen avoidance measures, including change of floor-covering and the use of mattress covers, have been associated with clinical improvement of asthma (Murray and Ferguson 1983; Walshaw and Evans 1986).

*Conclusions*

The numbers of fungal propagules, both airborne and in settled house dust, and the concentrations of *Der p* I in settled house dust, are only related to a small number of home- and occupant characteristics. The presence of fungal propagules in air and settled dust was weakly but consistently positively associated with reported and/or observed home dampness. Thus, reported and observed signs of dampness predict the presence of fungi only to a limited extent. The presence of *Der p* I in settled house dust was more strongly positively associated with reported and/or observed home dampness. For floor dust, the type of flooring has by far the largest effect on the presence of both groups of organisms, and is therefore the most important predictive characteristic.

## 7.6 The role of sensitization to fungal and house dust mite allergens in explaining the relation between home dampness and respiratory symptoms

The role of sensitization against house dust mite allergens and against allergens produced by a number of common fungi in the relation between home dampness and respiratory symptoms was investigated in the case-control study presented in section 6.2. The results showed that a restriction of the analysis to cases sensitized against these allergens and controls not sensitized against these allergens increased the odds ratios. The results are summarized in Table 7.1.

Table 7.1 Odds ratios for reported (during the life of the child) or observed dampness and mould; comparison of cases and controls, and comparison of sensitized cases and non-sensitized controls (95% CI in parentheses)

| | cases vs controls | sensitized cases vs non-sensitized controls |
|---|---|---|
| reported damp | 1.32 (0.89-1.96) | 1.98 (1.11-3.53)* |
| reported mould | 1.42 (0.94-2.16) | 2.07 (1.13-3.80)* |
| observed damp | 0.98 (0.68-1.41) | 1.29 (0.76-2.17) |
| observed mould | 1.18 (0.78-1.77) | 1.80 (1.03-3.15)* |

* $p<0.05$

A similar increase was found when comparing sensitized asthmatic children and sensitized children with chronic cough only, to non-sensitized controls. When the comparison was restricted to cases and controls neither of which were sensitized against house dust mites and/or fungi, or cases and controls both sensitized to these allergens, all odds ratios became close to unity. The null-hypothesis that there is no relation between home dampness and reported respiratory symptoms, taking into account sensitization against fungal and house dust mite allergens, can therefore be rejected. These findings support the theory that sensitization to allergens of house dust mites and fungi plays a causal role in the association between home dampness and respiratory symptoms. They do not support, however, a causal role of a nonallergic mechanism in the association under study, as suggested by Dales *et al.* (1991a).

Among cases, sensitization against house dust mite allergen (*Der p* I) was far more prevalent than sensitization against the mould mixture included in the RAST assay (38% and 10% respectively). Furthermore, of those children sensitized to moulds, only five were not also sensitized to house dust mites. This suggests that - under the circumstances prevailing in the Netherlands - sensitization to house dust mite allergens is a more important causal factor in the association between home dampness and respiratory symptoms in children, than sensitization to moulds. However, as described above, the specificity and sensitivity of the RAST assay for the assessment of sensitization to moulds using commercially available fungal allergens, is low. Possibly, this is illustrated by the results of our study, as only 50% of the atopic cases, defined as having an elevated total serum IgE level, was sensitized against house dust mites and/or moulds. For atopic controls, this figure was 20%. Both atopic cases and atopic controls were more likely to live in damp homes than non-atopic cases and controls respectively. For example, the odds ratio for reported dampness comparing atopic and non-atopic cases was 1.58 (95% CI 0.86-2.93). Comparing atopic and non-atopic controls gave an odds ratios of 1.61 (95% CI 0.92-2.61). Although increased total serum IgE levels are not only caused by sensitization against inhalant allergens, these findings suggest that sensitization to mould allergens might be a more important causal factor in the association between home dampness and respiratory symptoms of children than can be assessed at present by means of the commercially available RAST panels for moulds.

*Conclusion*

It is concluded that sensitization to inhalant allergens produced by house dust mites and fungi plays a causal role in the relation between home dampness and respiratory symptoms.

## 7.7 The relation between exposure to fungi and house dust mites, and health effects

Sensitization to allergens of house dust mites and/or moulds plays a causal role in the relation between home dampness and respiratory symptoms. This implies that a relation should exist between the exposure to these allergens, sensitization to these allergens and respiratory symptoms. In the case-control study presented in Chapter 6, the *Der p* I concentrations in settled house dust were measured at three locations (living room floor, bedroom floor, mattress) in all (516) houses included in the study, while in 60 homes the numbers of fungal propagules in settled house dust at two locations (bedroom floor, mattress) were investigated.

*Fungal propagules in house dust, sensitization and respiratory symptoms*

The total numbers of fungal propagules in mattress and floor dust did not differ substantially in dust gathered in the homes of cases or controls (section 5.3). In view of the large variability in time of the presence of fungal propagules in settled house dust (section 4.5) and the relative small number of cases (n=31) and controls (n=29) involved, the power of this sub-study to detect significant associations was low.

For *Aureobasidium pullulans* the counts in dust from smooth floors were significantly higher for controls than for cases, but the opposite was true for the number of yeasts in mattress dust. For *Wallemia sebi*, which is known to have allergenic properties (Van Bronswijk *et al.* 1986), counts found in mattress dust of cases were significantly higher than those of controls. But, in view of the number of comparisons made, these significant differences might have been caused by chance alone. Another factor that might have obscured a relation between the presence of fungi in house dust and respiratory symptoms in our study (a case-control study based on prevalent cases) might be the fact that parents of cases take significantly more allergen avoidance measures than those of controls. Wickman *et al.* (1992) reported significantly lower numbers of CFU in homes of atopic children than in those of control children, probably due to allergen avoidance measures taken in these homes. Their study included 175 children, therefore, the power of the study to detect significant associations was higher than that of our study.

The relation between fungal propagules in house dust and sensitization to fungal allergens could not be assessed, because only 3 children included in this sub-study were found to be sensitized against moulds.

*House dust mite allergen (Der p I) in house dust, sensitization, and respiratory symptoms*

The relation between house dust mite allergen (*Der p* I) in house dust, sensitization, and respiratory symptoms was investigated in our case-control study presented in Chapter 6. The design of the study was based on cross-sectional studies on the relation between home dampness and respiratory symptoms, reviewed in Chapter 2, where respiratory symptom prevalence was related to various measures of home dampness.

Therefore, we compared prevalent cases with suitable control children. As the former may already have had symptoms for some time, it is conceivable that changes in exposure to allergens might have occurred, due to allergen avoidance measures taken in the past. Therefore, we enquired, in great detail, into allergen avoidance measures taken in the past.

In a crude analysis, without stratification for sensitization against house dust mite allergens and allergen avoidance measures taken in the past, cases were generally exposed to lower *Der p* I concentrations than controls (odds ratios smaller than one). For these analyses the *Der p* I concentrations were divided into three categories according to the provisional guidelines proposed by a WHO workshop (Platts-Mills and De Weck 1989).

The analysis was then restricted to cases sensitized against house dust mites and to controls not sensitized against house dust mites, taking the type of floor covering into account. This analysis was based on the assumption that *Der p* I exposure leads to sensitization among genetically pre-disposed subjects, and continued exposure leads subsequently to symptoms. The odds ratios increased after this restriction. However, only the estimated odds ratio for the *Der p* I content in dust of bedrooms with textile floorings became greater than one. A further restriction was made by comparing cases with elevated specific serum IgE levels against house dust mites and cases without elevated specific serum IgE. This analysis was based on the findings described by Lau *et al.* (1989) and Wickman *et al.* (1991). Lau *et al.* reported clearly elevated concentrations of *Der p* I and *Der f* I in house dust for atopic children sensitized to house dust mites, compared with atopic children not sensitized to house dust mites, whereas no differences were found with the non-atopic control group. Similar results were found by Wickman *et al.* (1991). Price *et al.* (1990) also reported a positive association between the level of house dust mite allergen in house dust and sensitization among asthmatic children. After this restriction, most odds ratios became greater than one, although not statistically significant. The highest odds ratios were found comparing the lowest and highest categories of *Der p* I content in house dust, and this would indicate an exposure-response relationship. The odds ratios increased again, when the analysis was further restricted to cases sensitized to house dust mites and cases not sensitized against house dust mites, both living in homes where no allergen avoidance measures had been taken. These findings suggest a positive relation between exposure to house dust mite allergens and sensitization to house mite among children with respiratory symptoms. Based on these results, our null-hypothesis that there is no relation between the levels of house dust mite allergen in settled house dust and reported respiratory symptoms in children, taking into account sensitization against house dust mite allergens, could not be rejected.

The results of our study indicate that parents/guardians of children with chronic respiratory symptoms take effective allergen reduction measures. Among cases, 22% reported to have changed the floor covering of the bedroom because of respiratory symptoms of the child. In 66% of the bedrooms of cases textile floor coverings were present, compared with 78% of the bedrooms of controls. As the type of floor covering strongly determines the *Der p* I content in floor dust, this might explain the overall lower mean concentrations for cases. Replacement of wall-to-wall carpets by smooth floor coverings has been associated with clinical improvement of asthma (Murray and Ferguson 1983; Walshaw and Evans 1986).

Due to the intervention measures in the past, a cross-sectional comparison of the *Der p* I concentrations in dust of homes of children with and without prevalent respiratory symptoms can obscure the relation between exposure to house dust mite allergens and sensitization. This hampers an assessment of the associations between the exposure to house dust mite allergen, sensitization and the development of respiratory symptoms. Prospective studies, that follow children for several years directly after birth, or case-control studies based on incident cases, are needed to further clarify the relationships between dust mite allergen exposure, sensitization and childhood respiratory symptoms. Such a study should involve not only house dust mite allergens, but also the relations between the exposure to fungi and other indoor allergens (e.g. allergens from pets) and sensitization and childhood respiratory symptoms.

*Conclusions*

No relation was found between the presence of fungal propagules in house dust and respiratory symptoms in children. However, the power of the sub-study to detect significant associations was low, because of the small number of cases and controls involved, and the low validity of the measurement of fungal propagules in house dust as estimator of exposure.

A positive relation was found between the levels of house dust mite allergen in settled house dust and sensitization among children with respiratory symptoms. In these children's homes allergen avoidance measures are taken that effectively reduce the levels of house dust mite allergen in settled house dust.

## 7.8 General conclusions

1. The associations between reported or observed home dampness and reported respiratory symptoms in occupants found in recent epidemiological studies and in our case-control study presented in Chapter 6, conducted in areas where home dampness was not a public concern, are not attributable to reporting bias or confounding by other indoor or outdoor factors possibly related to home dampness and/or respiratory health.

2. The present knowledge of the ecology and the effects on human health of fungi and house dust mites gives sufficient evidence to consider both groups of organisms as potential causal factors in the relation between reported or observed home dampness and reported respiratory symptoms in children and adults.

3. In order to establish the above mentioned relation, it is essential to be able to measure the suspected causal factors, and to demonstrate an exposure-effect relation.
   For house dust mites, the measurement of their allergens in settled house dust can be used as a reliable estimator of the potential exposure to these allergens.
   For fungi, the validity of both air and dust sampling for viable fungal particles as estimators of the potential exposure is limited.

4. The levels of airborne fungal propagules and the numbers of fungal propagules in settled house dust, are weakly but consistently positively associated with reported and/or observed home dampness. The presence of *Der p* I in settled house dust is more strongly positively associated with reported and/or observed home dampness. The type of floor covering is a good predictor of the presence of fungi and house dust mite allergens in floor dust.

5. Sensitization to inhalant allergens produced by house dust mites and fungi plays a causal role in the relation between home dampness and respiratory symptoms.

6. A positive relation was found between the levels of house dust mite allergen in settled dust and sensitization among children with respiratory symptoms. In the homes of children with respiratory symptoms, effective allergen avoidance measures are taken that reduce the levels of house dust mite allergens in settled house dust. The intervention measures taken in the past hamper the assessment of the associations between the exposure to house dust mite allergens and the development of sensitization and respiratory symptoms in cross-sectional studies based on prevalent cases and suitable controls.

# References

American Thoracic Society. Standardization of spirometry - 1987 update. Am Rev Respir Dis 136 (1987) 1285-1296

Andersen, AA. A new sampler for collection, sizing and enumeration of viable airborne particles. J Bacteriol 76 (1958) 471-484

Andersen, I & J Korsgaard. Asthma and the indoor environment: assessment of the health implications of high indoor air humidity. Environ Int 12 (1986) 121-127

Andrae, S, O Axelson, B Björkstén, M Fredriksson & N-IM Kjellman. Symptoms of bronchial hyperreactivity and asthma in relation to environmental factors. Arch Dis Child 63 (1988) 473-478

Arlian, LG. Biology and ecology of house dust mites *Dermatophagoides* spp. and *Euroglyphus* spp. Immunol Allergy Clin N Am 9 (1989) 339-356

Arlian, LG. Water balance and humidity requirements of house dust mites. Exp Appl Acarol 16 (1992) 15-35

Arlian, LG, RL Brandt & R Bernstein. Occurrence of house dust mites, *Dermatophagoides* spp. (Acari: Pyroglyphidae), during the heating season. J Med Entomol 15 (1978) 35-42

Arlian, LG, IL Bernstein & JS Gallagher. The prevalence of house dust mites, *Dermatophagoides* spp, and associated environmental conditions in homes in Ohio. J Allergy Clin Immunol 69 (1982) 527-532

Armstrong, BK, E White & R Saracci. Principles of exposure measurement in epidemiology. Monographs in Epidemiology and Biostatistics, Vol 21, Oxford University Press, Oxford, New York, Tokio, 1992

Arundel, AV, EM Sterling, JH Biggin & TD Sterling. Indirect health effects of relative humidity in indoor environments. Environ Health Pers 65 (1986) 351-361

Barbee, RA, MD Lebowitz, HC Thompson & B Burrows. Immediate skin-test reactivity in a general population sample. Ann Int Med 84 (1976) 129-133

Beaumont, F. Aerobiological and clinical studies in mould allergy. Thesis, University of Groningen, 1985

Beaumont, F, HF Kauffman, HJ Sluiter & K De Vries. A volumetric-aerobiological study of seasonal fungus prevalence inside and outside dwellings of asthmatic patients living in Northeast Netherlands. Ann Allergy 53 (1984) 487-493

Beaumont, F, HF Kauffman, HJ Sluiter & K De Vries. Volumetric aerobiological survey of conidial fungi in the North-East Netherlands II. Comparison of aerobiological data and skin tests with mould extracts in an asthmatic population. Allergy 40 (1985) 181-186

Binnie, PWH. Airborne microbial flora in Florida homes. In: Indoor Air '87. Proc 4th Conf Indoor Air Quality and Climate, Berlin, 1987, Vol 1, pp. 660-664

Blomquist, G, U Ström & LH Stromquist. Sampling of high concentrations of airborne fungi. Scand J Work Environ Health 10 (1984a) 109-113

Blomquist, G, U Palmgren & G Ström. Improved techniques for sampling airborne fungal particles in highly contaminated environments. Scand J Work Environ Health 10 (1984b) 253-258

Boer, R de. The control of house dust mite allergens in rugs. J Allergy Clin Immunol 86 (1990) 808-814

Bronswijk, JEMH van. House dust biology for allergists, acarologists and mycologist. Zeist, 1981

Bronswijk, JEMH van, JMCP Schoonen, MAF Berlie & FS Lukoschus. On the abundance of *Dermatophagoides pteronyssinus* (Trouessart, 1897) (Pyroglyphidae: Acarina) in house dust. Res Popul Ecol 8 (1971) 67-79

Bronswijk, JEMH van, G Rijckaert & B van de Lustgraaf. Indoor fungi, distribution and allergenicity. Acta Bot Neerl 35 (1986) 329-345

Brunekreef, B. Associations between questionnaire reports of home dampness and childhood respiratory symptoms. Science Total Environ 127 (1992a) 79-89

Brunekreef, B. Damp housing and adult respiratory symptoms. Allergy 47 (1992b) 498-502

Brunekreef, B, D Noy & P Clausing. Variability of exposure measurements in environmental epidemiology. Am J Epidemiol 125 (1987) 892-898

Brunekreef, B, DW Dockerey, FE Speizer, JH Ware, JD Spengler & BG Ferris. Home dampness and respiratory morbidity in children. Am Rev Respir Dis 140 (1989) 1363-1367

Brunekreef, B, B Groot, B Rijcken, G Hoek, A Steenbekkers & A De Boer. Reproducibility of childhood respiratory symptom questions. Eur Respir J 5 (1992) 930-935

Burge, HA. Fungus allergens. Clin Rev Allergy 3 (1985) 319-329

Burge, HP, WR Solomon & JR Boise. Comparative merits of eight popular media in aerometric studies of fungi. J Allergy Clin Immunol 60 (1977) 199-203

Burge, HA, M Chatigny, J Feeley, K Kreiss, P Morey, J Otten & K Peterson. Bioaerosols: guidelines for assessment and sampling saprophytic bioaerosols in the indoor environment. Appl Ind Hyg 9 (1987) 10-16

Burr, ML, J Mullins, TG Merrett & NCH Stott. Indoor moulds and asthma. J Roy Soc Health 3 (1988) 99-101

Businco, L, A Cantani & F Farinella. Month of birth and grass pollen or mite sensitization in children with respiratory allergy: a significant relationship. Clin Allergy 18 (1988) 269-274

Colloff, MJ. Effects of temperature and relative humidity on development times and mortality of eggs from laboratory and wild populations of the European house-dust mite *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). Exp Appl Acarol 3 (1987) 279-289

Colloff, MJ, J Ayres, F Carswell, PH Howarth, TG Merrett, EB Mitchell, MJ Walshaw, JO Warner, JA Warner & AA Woodcock. The control of allergens of dust mites and domestic pets: a position paper. Clin Exp Allergy 22 (1992) suppl.2, 1-28

Commission of the European Communities. European Collaborative Action Indoor Air Quality & its Impact on Man (formerly COST Project 613), Report No. 12, Biological particles in indoor environments. ECSC-EEC-EAEC, Brussels-Luxembourg, 1993, 81 pp.

Creasia, DA, JD Thurman, RW Wannemacher & DL Bunner. Acute inhalation toxicity of T-2 mycotoxin in the rat and guinea pig. Fund Appl Toxicol 14 (1990) 54-59

Croft, WA, BB Jarvis & CS Yataware. Airborne outbreak of trichothecene toxicosis. Atmos Environ 20 (1986) 549-552

Dales, RE, H Zwanenburg, R Burnett & CA Franklin. Respiratory health effects of home dampness and molds among Canadian children. Am J Epidemiol 134 (1991a) 196-203

Dales, RE, R Burnett & H Zwanenburg. Adverse health effects among adults exposed to home dampness and molds. Am Rev Respir Dis 143 (1991b) 505-509

Dijkstra, L, D Houthuijs, B Brunekreef, I Akkerman & JSM Boleij. Respiratory health effects of the indoor environment in a population of Dutch children. Am Rev Respir Dis 142 (1990) 1172-1178

Eduard, W, J Lacey, K Karlsson, U Palmgren, G Ström & G Blomquist. Evaluation of methods for enumerating microorganisms in filter samples from highly contaminated occupational environments. Am Ind Hyg Assoc J 51 (1990) 427-436

Emenius, G, L Egmar, G Axelsson, G Pershagen & M Wickman. Protective effect of balanced mechanical ventilation on mite allergens levels. J Allergy Clin Immunol 91 (1993) 353 (abstract)

Fergusson, RJ, LJR Milne & GK Crompton. *Penicillium* allergic alveolitis: faulty installation of central heating. Thorax 39 (1984) 294-298

Flach, KC & B Brunekreef. Respiratory symptoms and pulmonary function of children between 6 and 12 years old. (in Dutch) T Soc Gezondheidsz 69 (1991) 159-164

Flannigan, B, EM McCabe & F McGarry. Allergenic and toxigenic micro-organisms in houses. J Appl Bacteriol 70 (1991) 61s-73s

Fradkin, A, RS Tobin, SM Tarlo, M Tucic-Porretta & D Malloch. Species identification of airborne molds and its significance for the detection of indoor pollution. JAPCA 37 (1987) 51-53

Galloway, LD & R Burgess. In: Applied mycology and bacteriology, 3rd ed., Leonard Hill, London, 1952, pp. 54-57

Grant, C, CA Hunter, B Flannigan & AF Bravery. The moisture requirements of moulds isolated from domestic dwellings. Int Biodeterior 25 (1989) 259-284

Gravesen, S. Identification and quantification of indoor airborne microfungi during 12 months from 44 Danish homes. Acta Allergol 27 (1972) 337-354

Gravesen, S. Identification and prevalence of culturable mesophilic microfungi in house dust from 100 Danish homes; comparison between airborne and dust-bound fungi. Allergy 33 (1978) 268-272

Gravesen, S. Fungi as a cause of allergic disease. Allergy 34 (1979) 135-154

Gregory, PH. The microbiology of the atmosphere. Leonard Hill Books, Aylesbury, 1973

Hart, BJ & L Whitehead. Ecology of house dust mites in Oxfordshire. Clin Exp Allergy 20 (1990) 203-209

Harving, H, J Korsgaard & R Dahl. House-dust mites and associated environmental conditions in Danish homes. Allergy 48 (1993) 106-109

Heikkilä, P, M Kotimaa, T Tuomi, T Salmi & K Louhelainen. Identification and counting of fungal spores by scanning electron microscope. Ann Occup Hyg 32 (1988) 241-248

Hendrick, DJ, RJ Davies, MF d'Souza & J Pepys. An analysis of skin prick test reactions in 656 asthmatic patients. Thorax 30 (1975) 2-8

Hendry, KM & EC Cole. A review of mycotoxins in indoor air. J Toxicol Environ Health 38 (1993) 183-198

Henningson, E, R Roffey & Å Bovallius. A comparative study of apparatus for sampling airborne micro-organisms. Grana 20 (1981) 155-159

Hocking AD. Improved media for enumeration of fungi from foods. CSIRO Food Res Qr 41 (1981) 7-11

Hocking, AD & JI Pitt. Dichloran glycerol medium for enumeration of xerophilic fungi from low moisture foods. Appl Environ Microbiol 39 (1980) 488-492

Hoeven, WAD van der, R de Boer & J Bruin. The colonisation of new houses by house dust mites (Acari: Pyroglyphidae). Exp Appl Acarol 16 (1992) 75-84

Holmberg, K. Indoor mould exposure and health effects. In: Indoor Air '87. Proc 4th Conf Indoor Air Quality and Climate, Berlin, 1987, Vol. 1, pp. 637-642

Holst, PE, ED Coleman, JE Sheridan, TV O'Donnell & PT Sutthoff. Asthma and fungi in the home. NZ Med J 96 (1983) 718-720

Houthuijs, D, B Remijn, B Brunekreef & R de Koning. Estimation of maximum flow-volume variables in children. Pediatr Pulmonol 6 (1989) 127-132

Hull, CH & NH Nie. SPSS update 7-9. McGraw Hill, New York, 1981

Hunter, CA, C Grant, B. Flannigan & AF Bravery. Mould in buildings: the air spora of domestic dwellings. Int Biodeterior 24 (1988) 81-101

IARC. Some naturally-occurring substances: some food items and constituents, heterocyclic amines and mycotoxins. IARC Monograph 56, IARC, Lyon, 1993

Ishii, A, M Takaoka, M Ichinoe, Y Kabasawa & T Ouchi. Mite fauna and fungal flora in house dust from homes of asthmatic children. Allergy 34 (1979) 379-387

IUIS. Allergen nomenclature. Bull World Health Organiz 64 (1986) 767-770

Izard, JL, A Dornelas de Andrade, J Birnhaum, MF Artillian, P Corget, D Vervloet & D Charpin. Housing and mite-allergen levels. J Allergy Clin Immunol 91 (1993) 328 (abstract)

Jaakkola, JJK, N Jaakkola & R Ruotsalainen. Home dampness and molds as determinants of respiratory symptoms and asthma in pre-school children. J Exposure Analysis Environ Epidemiol 3 suppl 1 (1993) 129-142

Jacobs, RL, RE Thorne, JR Holcomb, LA Schwietz & FO Jacobs. Hypersensitivity pneumonitis caused by *Cladosporium* in an enclosed hot-tub area. Ann Int Med 105 (1986) 204-206

Jensen, V. Studies on the microflora of Danish beech forest soils. I. The dilution plate count technique for the enumeration of bacteria and fungi in soil. Zentralblatt für Bacteriologie, Parasitkunde, Infektionskrankheiten und Hygiene, Abt. II 116 (1962) 13-32

Jones W, K Morring, P Morey & W Sorensen. Evaluation of the Andersen viable impactor for single stage sampling. Am Ind Hyg Assoc J 46 (1985) 294-298

Karlsson, K & P Malmberg. Characterization of exposure to molds and actinomycetes in agricultural dusts by scanning electron microscopy, fluorescence microscopy and the culture method. Scand J Work Environ Health 15 (1989) 353-359

King, AD, AD Hocking & JI Pitt. Dichloran rose bengal medium for enumeration of molds from foods. Appl Environ Microbiol 37 (1979) 959-964

Kleinbaum DG, LL Kupper, KE Muller. Applied regression analysis and other multivariable methods. Second Edition. PWS-KENT Publishing Company, Boston, 1988

Korsgaard, J. Mite asthma and residency: a case-control study on the impact of exposure to house dust mites in dwellings. Am Rev Respir Dis 128 (1983a) 231

Korsgaard, J. House-dust mites and absolute indoor humidity. Allergy 38 (1983b) 85-92

Korsgaard, J & M Iversen. Epidemiology of house dust mite allergy. Allergy 46, suppl. 11 (1991) 14-18

Kuile, WM ter & JH Kraaiveld. Gravimetrical and morphological characterization of allergen-containing dust particles in houses (in Dutch). MT-TNO, Delft, 1987

Lach, V. Performance of the surface air system samplers. J Hospital Infect 6 (1985) 102-107

Langeweg, F (ed.). Caring for tomorrow; a national survey of the environment 1985-2010 (in Dutch). Samson HD, Tjeenk Willink, Alphen aan den Rijn, 1988

Lau, S, G Falkenhorst, A Weber, I Werthmann, P Lind, P Buettner-Goetz & U Wahn. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. J Allergy Clin Immunol 84 (1989) 718-725

Lau, S, A Rusche, A Weber, I Werthmann, Buettner-Goetz & U Wahn. Nachweis von Hausstaubmilbenallergenen-ELISA und Guaninbestimmung im Vergleich. Allergologie 13 (1990) 12-15

Leeuwen, L van & R Aalberse. Antigens in house dust. (in Dutch) Dutch Asthma Fund, Leusden, 1991

Ligugnana, R. A simpler approach in the training of food factory staff for health education. In: Proc World Congress Foodborne Infections and Intoxications, Berlin, 1980, pp. 6-16

Lind, P. Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. J Allergy Clin Immunol 76 (1985) 753-761

Lind, P. Demonstration of close physiochemical similarity and partial immunochemical identity between major allergens, Dp 42, of house dust mite, *D. pteronyssinus* and corresponding antigens of *D. farinae* (Df 6) and *D. microceras* (Dm 6). Int Arch Allergy Appl Immunol 79 (1986a) 60-65

Lind, P. Enzyme-linked immunosorbent assay for determination of major excrement allergens of house dust mite species *D. pteronyssinus*, *D. farinae* and *D. microceras*. Allergy 41 (1986b) 442-451

Lind, P, PS Norman, M Newton, H Lowenstein & B Schwartz. The prevalence of indoor allergens in the Baltimore area: house-dust-mite and animal dander antigens measured by immunochemical techniques. J Allergy Clin Immunol 80 (1987) 541-547

Lowenstein, H, K Ipsen, P Lind & F Matthiesen. The physiochemical and biological characteristics of allergens. In Lessof MH, Lee TH, Kemeny DM (eds): Allergy, an international textbook. John Wiley & Sons, Chichester, 1987, pp. 87-104

Lubs, MLE. Allergy in 7000 twin pairs. Acta Allergol 26 (1971) 249-285

Lustgraaf, B van de. Xerophilic fungi in mattress dust. Mykosen 20 (1977) 101-106

Maas, PJ van der. Chronic non-specific respiratory symptoms in children in relation to ambient air pollution and other factors. Thesis, Erasmus University of Rotterdam, 1979

Macher, JM & MW First. Reuter centrifugal air sampler: measurement of effective air flow rate and collection efficiency. Appl Environ Microbiol 45 (1983) 1960-1962

Macher, JM & MW First. Personal air samplers for measuring occupational exposures to biological hazards. Am Ind Hyg Assoc J 45 (1984) 76-83

Malling, H-J, B Agrell, S Croner, S Dreborg, T Foucard, M Kjellman, A Koivikko, A Roth & B Weeke. Diagnosis and immunotherapy of mould allergy. I. Screening for mould allergy. Allergy 40 (1985) 108-114

Martin, CJ, SD Platt & SM Hunt. Housing conditions and ill health. Br Med J 294 (1987) 1125-1127

Matthiesen, F, L Larsen & H Lowenstein. Purification and characterization of two important mold allergens, *Alt a* I and *Cla h* I (abstract). XIVth Congress of the EAACI 1989. Allergologie 12 (1989) 21

Matthiesen, F, L Friberg, M Olsen & H Lowenstein. Purification and characterization of the major allergen of *Alternaria alternata*, *Alt a* I. J Allergy Clin Immunol (1994) in press

Miller, JD, AM Laflamme, Y Sobol, P Lafontaine & R Greenhalgh. Fungi and fungal products in some Canadian houses. Int Biodeterior 24 (1988) 103-120

Miller, JD. Fungi as contaminants in indoor air. In: Indoor Air '90. Proc 5th Int Conf on Indoor Air Quality and Climate, Ottawa, 1990, Vol. 5, pp. 51-64

Ministry of Housing, Physical Planning and Environment. Qualitative Housing Registration 1989-1991. Results of a nationwide sample (in Dutch). Ministry of Housing, Physical Planning and Environment, The Hague, 1993

Morey, PR. Airborne viable micro-organisms in office environments: sampling protocol and analytical procedures. NIOSH Draft, Cincinnatti, 1985

Morring, KL, WG Sorenson & MD Attfield. Sampling for airborne fungi: a statistical comparison of media. Am Ind Hyg Assoc J 44 (1983) 662-664

Mosbech, H. House dust mite allergy. Allergy 40 (1985) 81-91

Mosbech, H, A Jensen, JH Heining & C Schou. House dust mite allergens on different types of mattresses. Clin Exp Allergy 21 (1991) 351-355

Mossel, DAA, AMC Kleynen-Semmeling, HD Vincentie, H Berens & M Calsaras. Oxytetracycline glucose yeast extract agar for selective enumeration of moulds and yeast in food and clinical material. J Appl Bacteriol 33 (1970) 454-457

Munir, AKM, R Einarsson, N-IM Kjellman & B Björkstén. Mite (*Der p* I, *Der f* I) and cat (*Fel d* I) allergens in the homes of babies with a family history of allergy. Allergy 48 (1993) 158-163

Murray, AB & AC Ferguson. Dust free bedrooms in the treatment of asthmatic children with house dust or house dust mite allergy: a controlled trial. Pediatrics 71 (1983) 418-422

Murray, AB, AC Ferguson & BJ Morrison. Diagnosis of house dust mite allergy in asthmatic children: what constitutes a positive history? J Allergy Clin Immunol 71 (1983) 21-28

Nevalainen, A, J Pastuszka, F Liebhaber & K Willeke. Performance of bioaerosol samplers: collection characteristics and sampler design considerations. Atm Environ 26a (1992) 531-540

Niemeijer, NR & JGR De Monchy. Age-dependency of sensitization to aero-allergens in asthmatics. Allergy 47 (1992) 431-435

Norberg, P & H Stymne. Measurement of indoor relative humidity using a passive sampler for water vapour. Indoor Air 3 (1993) 398-404

Norusis, MJ. SPSS/PC + for the IBM PC/XT/AT. SPSS Inc., Chicago, 1986

Notermans, S, J Dufrenne, LM Wijnands & HWB Engel. Human serum antibodies to extracellular polysaccharides (EPS) of moulds. J Med Vet Mycol 26 (1988) 41-48

O'Brien, IM, J Bull, B Creamer, R Sepulveda, M Harries, PS Burge & J Pepys. Asthma and extrinsic allergic alveolitis due to *Merulius lacrymans*. Clin Allergy 8 (1978) 535-542

Olsen, JH, L Dragsted & H Autrup. Cancer risk and occupational exposure to aflatoxins in Denmark. Br J Cancer 58 (1988) 392-396

Osterballe, O, A Dirksen, B Weeke & ER Weeke. Cutaneous allergy in a Danish Multi Centre Study. Ugeskr Laeg 143 (1981) 3211-3218

Owen, S, M Morganstern, J Hepworth & A Woodcock. Control of house dust mite antigen in bedding. Lancet 335 (1990) 396-397

Palmgren, U, G Ström, G Blomquist & P Malmberg. Collection of airborne micro-organisms on Nucleporefilters, estimation and analysis - CAMNEA method. J Appl Bacteriol 61 (1986) 401-406

Peat, JK, WJ Britton, CM Salome & AJ Woolcock. Bronchial hyperresponsiveness in two populations of Australian schoolchildren. III. Effect of exposure to environmental allergens. Clin Allergy 17 (1987) 297-300

Platt, SD, CJ Martin, SM Hunt & CW Lewis. Damp housing, mould growth, and symptomatic health state. Br Med J 298 (1989) 1673-1678

Platts-Mills, TAE, PW Heymann, JL Longbottom & SR Wilkins. Airborne allergens associated with asthma: particle sizes carrying dust mite and rat allergens measured with a cascade impactor. J Allergy Clin Immunol 77 (1986a) 850-857

Platts-Mills, TAE, PW Heymann, MD Chapman, ML Hayden & SR Wilkins. Cross-reacting and species specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and *D. farinae*: development of a radioimmunoassay for antigen $P_1$ equivalent in house dust and dust mite extracts. J Allergy Clin Immunol 78 (1986b) 398-407

Platts-Mills, TAE & MD Chapman. Dust mites: immunology, allergic disease, and environmental control. J Allergy Clin Immunol 80 (1987) 755-777

Platts-Mills, TAE & AL De Weck. Dust mite allergens and asthma: a world wide problem. International workshop report. J Allergy Clin Immunol 83 (1989) 416-427

Platts-Mills, TAE, WR Thomas, RC Aalberse, D Vervloet & MD Chapman. Dust mite allergens and asthma: report of a second international workshop. J Allergy Clin Immunol 89 (1992) 1046-1060

Pollart, S, MD Chapman & TAE Platts-Mills. House dust mite and dust control. Clin Rev Allergy 6 (1988) 23-33

Price, JA, I Pollock, SA Little, JL Longbottom & JO Warner. Measurement of airborne mite antigen in homes of asthmatic children. Lancet 336 (1990) 895-897

Quanjer, PH. Standardized lung function testing. Bull Eur Physiopath Resp 19 (1983) 1-95

Reiser, J, D Ingram, EB Mitchell & JO Warner. House dust mite allergen levels and an anti-mite mattress spray (natamycin) in the treatment of childhood asthma. Clin Exp Allergy 20 (1990) 561-567

Reponen, T, A Nevalainen, M Jantunen, M Pellika & P Kalliokoski. Normal range criteria for indoor air bacteria and fungal spores in a subarctic climate. Indoor Air 2 (1992) 26-31

Ross, A, M Collins & C Sanders. Upper respiratory tract infection in children, domestic temperatures, and humidity. J Epidemiol Comm Health 44 (1990) 142-146

Rothman, KJ & JJ Schlesselman. Case-control studies. Oxford University Press, Oxford, 1982

Rowntree, S, JJ Cogswell, TAE Platts-Mills & EB Mitchell. Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. Arch Dis Child 60 (1985) 727-735

Rylander, R & H Goto. First glucan lung toxicity workshop. Report 4/91. Committee on Organic Dusts, ICOH, Sweden, 1991

Rylander, R, K Persson, H Goto, K Yuasa & S Tanaka. Airborne beta-1,3-glucan may be related to symptoms in sick buildings. Indoor Environ 1 (1992) 263-267

Sakaguchi, M, S Inouye, H Yasueda, T Irie, S Yoshizawa & T Shida. Measurement of allergens associated with dust mite allergy. II. Concentrations of airborne mite allergens (*Der* I and *Der* II) in the house. Int Arch Allergy Appl Immunol 90 (1989) 190-193

Sakaguchi, M, S Inouye, H Yasueda & T Shida. Concentration of airborne mite allergens (*Der* I and *Der* II) during sleep. Allergy 47 (1992) 55-57

Salvaggio, J & L Aukrust. Mold-induced asthma. J Allergy Clin Immunol 68 (1981) 327-346

Samson, RA. Occurrence of moulds in modern living and working environments. Eur J Epidemiol 1 (1985) 54-61

Samson, RA & ES van Reenen-Hoekstra. Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures, Baarn, 1988

Sarsfield, JK, G Gowland, R Toy & ALE Norman. Mite sensitive asthma of childhood; trial avoidance measures. Arch Dis Child 49 (1974) 761-721

Schober, G. Der Einfluss der Wasseraktivität des Nährmediums auf die Ausbeute von Schimmelpilzen in Staubproben. Mycoses 31 (1988) 255-258

Schober, G. Fungi in carpeting and furniture dust. Allergy 46 (1991) 639-643

Schou, C & P Lind. The antigenicity of house dust mites. Allergy 46, suppl 11 (1991) 10-13

Scott, WJ. Water relations of food spoilage microorganisms. Advances in Food Research 7 (1957) 83-127

Sears, MR, GP Herbison, MD Holdaway, CJ Hewitt, EM Flannery & PA Silva. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. Clin Exp Allergy 19 (1989) 419-424

Smid, T, E Schokkin, JSM Boleij & D Heederik. Enumeration of viable fungi in occupational environments: a comparison of samplers and media. Am Ind Hyg Assoc J 50 (1989) 235-239

Smith, JE, JG Anderson, CW Lewis & YM Murad. Cytotoxic fungal spores in the indoor atmosphere of damp domestic environment. FEMS Microbiol. Letters 100 (1992) 337-344

Sneller, MR & RR Roby. Incidence of fungal spores at the homes of allergic patients in an agricultural community. I. A 12-month study in and out of doors. Ann Allergy 43 (1979) 225-228

Sorenson, WG. Health impact of mycotoxins in the home and workplace: an overview. In: Biodeterioration Research 2, eds CE O'Rear and GC Llewellyn, pp. 201-15, New York, Plenum, 1989

Sporik, R, ST Holgate, TAE Platts-Mills & JJ Cogswell. Exposure to house dust mite allergen (*Der p* I) and development of asthma in childhood. A prospective study. N Engl J Med 323 (1990a) 502-507

Sporik, R, M Chapman & T Platts-Mills. Airborne mite antigen. Lancet 336 (1990b) 1507-1508

Sporik, R, MD Chapman & TAE Platts-Mills. House dust mite exposure as a cause of asthma. Clin Exp Allergy 22 (1992) 897-906

Storm van Leeuwen, W, Z Bien & H Varekamp. Neuere Erfahrungen über Diagnose und Therapie von Ueberempfindlichkeitskrankheiten (allergische Krankheiten) Zeitschrift für Immunitätsforschung und experimentelle Therapie 37 (1923) 77-105

Strachan, DP. Damp housing and childhood asthma: validation of reporting of symptoms. Br Med J 297 (1988) 1223-1226

Strachan, DP & RA Elton. Relationship between respiratory morbidity in children and the home environment. Family Practice 3 (1986) 137-142

Strachan, DP & CH Sanders. Damp housing and childhood asthma; respiratory effects of indoor air temperature and relative humidity. J Epidemiol Comm Health 43 (1989) 7-14

Strachan, DP, B Flannigan, EM McCabe & F McGarry. Quantification of airborne moulds in the homes of children with and without wheeze. Thorax 45 (1990) 382-387

Su, HJ, JD Spengler & HA Burge. Examination of microbiological concentrations and association with childhood respiratory health. In: Indoor Air '90. Proc. of the 5th Int. Conf. on Indoor Air Quality and Climate, Ottawa, 1990, Vol. 2, pp. 21-26

Summerbell, RC, F Staib, R Dales, N Nolard, J Kane, H Zwanenburg, R Burnett, S Krajden, D Fung & D Leong. Ecology of fungi in human dwellings. J Med Vet Mycol 30 (1992), suppl 1, 279-285

Suoniemi, I, F Björksten & T Haahtela. Dependance of immediate hypersensitivity in the adolescent period on factors encountered in infancy. Allergy 36 (1981) 263-268

Sussman, AS. Longevity and survivability of fungi. In: The Fungi vol II. GC Ainsworth and AS Sussman (eds), Academic Press, 1968

Swanson, MC, MK Agarwal & CE Reed. An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: studies with mite, roach, cat, mouse, and guinea pigs antigens. J Allergy Clin Immunol 76 (1985) 724-729

Swanson, MC, AR Campbell, MJ Klauck & CE Reed. Correlations between levels of mite and cat allergens in settled and airborne dust. J Allergy Clin Immunol 83 (1989) 776-783

Tammes, E, AR De Borst & BM Hutink. Inventarisation of dampness- and mould problems in dwellings (in Dutch), Bouwcentrum, Rotterdam, 1985

Tarlo, SM, A Fradkin & RS Tobin. Skin testing with extracts of fungal species derived from the homes of allergy clinic patients in Toronto, Canada. Clin Allergy 18 (1988) 45-52

Tobin, RS, E Baranowski, AP Gilman, T Kuiper-Goodman, JD Miller & M Giddings. Significance of fungi in indoor air: report of a working group. Can J Public Health 78 (1987) s1-s14

Tovey, ER. Allergen exposure and control. Exp Appl Acarol 16 (1992) 181-202

Tovey, ER, MD Chapman & TAE Platts-Mills. Mite faeces are a major source of house dust allergens. Nature 289 (1981a) 592-593

Tovey, ER, MD Chapman, CW Wells & TAE Platts-Mills. The distribution of dust mite allergen in the houses of patients with asthma. Am Rev Respir Dis 124 (1981b) 630-635

Tovey, ER, GB Marks, M Matthews, WF Green & A Woolcock. Changes in mite allergen *Der p* I in house dust following spraying with a tannic acid/acaricide solution. Clin Exp Allergy 22 (1992) 67-74

Tyndall, RL, CS Dudney, AR Hawthorne, R Jernigan, K Ironside & P Metler. Microflora of the typical home. In: Indoor Air '87. Proc 4th Int Conf Indoor Air Quality and Climate, Berlin, 1987, Vol 1., pp. 617-621

Varekamp, H. The exogenous causes of asthma bronchiale (in Dutch). Thesis, University of Leiden, Leiden, 1925

Varekamp, H & R Voorhorst. The influence of climate and housing on patients with asthma and rhinitis vasomotoria (in Dutch). Ned T Geneesk 105 (1961) 2022-2028

Varekamp, H, FTM Spieksma, MJ Leupen & AW Lijklema. House dust mites in their relation to dampness of houses and the allergen content of home dust (IV). Proc. 5th Interasthma Congress, Pressa Trajecta, Utrecht, 1966

Varekamp, H & MJ Leupen. Investigations on the relation between the dampness of homes and chronic airway complaints in minors (in Dutch). Ned Tijdschr Geneesk 114 (1970) 352-355

Verhoeff, AP, JH van Wijnen, JSM Boleij, B Brunekreef, ES van Reenen-Hoekstra & RA Samson. Enumeration and identification of airborne viable mould propagules in houses; a field comparison of selected techniques. Allergy 45 (1990) 275-284

Verhoeff, AP, JH van Wijnen, B Brunekreef, P Fischer, ES van Reenen-Hoekstra & RA Samson. The presence of viable mould propagules in indoor air in relation to house damp and outdoor air. Allergy 47 (1992) 83-91

Verhoeff, AP, ES van Reenen-Hoekstra, RA Samson, B Brunekreef & JH van Wijnen. Mold propagules in house dust, I; Comparison of analytic methods and their value as estimators of potential exposure. Allergy (in press)

Voorhorst, R, MIA Spieksma-Boezeman & FThM Spieksma. Is a mite (*Dermatophagoides* sp.) the producer of the house-dust allergen? Allerg Asthma 10 (1964) 329-334

Voorhorst, R, Fthm Spieksma, H Varekamp, MJ Leupen & AW Lyklema. The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house dust allergen. J Allergy 39 (1967) 325-339

Voorhorst, R, Fthm Spieksma & H Varekamp. House dust atopy and the house dust mite *Dermatophagoides pteronyssinus*. Stafleu Leiden, 1969

Vries, K De, TJ Smid, IE Robertus-Koster & NGM Orie. Prevalence of skintests and precipitins in a randomly selected group of a rural population. In: Hers, JFP, Winkler, Kc. (eds.), Airborne transmission and airborne infection. Oosthoek Publishing, Utrecht, 1973

Waegemaekers, M, N van Wageningen, B Brunekreef & JSM Boleij. Respiratory symptoms in damp homes: a pilot study. Allergy 44 (1989) 192-198

Walshaw, MJ & CC Evans. Allergen avoidance in house dust mite sensitive adult asthma. Quart J Med 58 (1986) 199-215

Wassenaar, DPJ. Staubzaugen zur allergologischen Sanierung eines synthetischen Teppichbodens. Allergologie 11 (1988a) 268-273

Wassenaar, DPJ. Effectiveness of vacuum cleaning and wet cleaning in reducing house-dust mites, fungi and mite allergen in cotton carpet: a case study. Exp Appl Acarol 4 (1988b) 53-62

Wickman, M, SL Nordvall, G Pershagen, J Sundell & B Schwartz. House dust mite sensitization in children and residential characteristics in a temperate region. J Allergy Clin Immunol 88 (1991) 89-95

Wickman, M, S Gravesen, SL Nordvall, G Pershagen & J Sundell. Indoor viable dust-bound microfungi in relation to residential characteristics, living habits, and symptoms in atopic and control children. J Allergy Clin Immunol 89 (1992) 752-759

Wood, RA, PA Eggleston, P Lind, L Ingemann, B Schwartz, S Gravesen, D Terry, B Wheeler & NF Adkinson. Antigenic analysis of household dust samples. Am Rev Respir Dis 137 (1988) 358-363

Wood, RA, PA Eggleston, KE Mudd & NF Adkinson. Indoor allergen levels as a risk factor for allergic sensitization. J Allergy Clin Immunol 83 (1989) 199

Wütrich, B, E Baumann, RA Fries & EW Schnyder. Total and specific IgE (RAST) in atopic twins. Clin Allergy 11 (1981) 47-54

Yoshida, K, M Ando, T Sakata & S Araki. Environmental mycological studies on the causative agent of summer-type hypersensitivity pneumonitis. J Allergy Clin Immunol 81 (1988) 475-483

Summary

This thesis details seven investigations into the relation between home dampness and respiratory symptoms in occupants.

Chapter 1 presents the background of the studies. Damp houses are common in the Netherlands and approximately 20% of the total housing stock is affected. As early as the 1920's attention had been drawn to the possible influence of home dampness on the prevalence of asthma. In recent years, epidemiological studies carried out in Western-Europe and North-America again showed increased respiratory complaints in children and adults, associated with the presence of dampness in modern homes. However, the causal relationship between home dampness and respiratory illnesses had not been fully elucidated. The hypothetical relationship between home dampness and respiratory symptoms of occupants is outlined in this Chapter, i.e. that home dampness promotes the development of fungi and house dust mites. Both of these produce inhalant allergens and susceptible individuals may develop allergic reactions to them. This would lead to an increased prevalence of respiratory symptoms among occupants of damp houses. Finally, the goals of the studies presented in this thesis are stated in this Chapter. The studies concentrate on 1) the measurement of the presence of, and the exposure to fungi in homes, 2) the relation between the presence of fungi and house dust mite allergens in homes and home dampness, as well as other home and occupant characteristics, and 3) the relation between home dampness, the presence of mite allergens and fungi, and respiratory symptoms in children.

Chapter 2 provides a review of recent epidemiological studies on the relation between home dampness and respiratory symptoms in children and adults, conducted in the Netherlands and elsewhere. These studies have several features in common. They were all designed to be cross-sectional, and in most studies questionnaires completed by the occupants, were used to obtain information on the exposure variables (signs of home dampness) and the outcome variables (respiratory symptoms). The role of information bias and bias by potential confounders in the associations found, is discussed. It is concluded that the associations between reported or observed home dampness and mould, and increased prevalences of reported respiratory symptoms found in recent epidemiological studies conducted in areas where home dampness is not a public concern, are not attributable to reporting bias or confounding by other indoor or outdoor factors possibly related with home dampness and/or respiratory health.

If the association between home dampness and respiratory symptoms is real, the next question is, whether it is causal. Fungi and house dust mites are the suspected causative agents in this association. Chapter 3 reviews the present knowledge of the ecology and health effects of fungi and house dust mites. Humidity is the most critical factor in the development and survival of fungi and house dust mites. They encounter favourable conditions in damp environments and both groups of organisms produce inhalant allergens. Inhalation of these can cause sensitization in genetically pre-disposed individuals, and subsequently allergic reactions, such as asthma and rhinitis.

Chapter 4 deals with the assessment of the exposure to fungi and house dust mites. To substantiate the relation between home dampness and respiratory symptoms, it is essential to be able to measure the suspected causal factors, and to demonstrate an exposure-effect relation. The method(s) used to measure the exposure should preferably maximize validity, and minimize error.

For house dust mites, a brief literature review is presented (section 4.2). Enzyme immuno assays have been developed, that are highly reproducible, to measure the amounts of the major house dust mite allergens in airborne or settled dust. The potential exposure to house dust mite allergens is usually assessed by measuring the amounts of these allergens in settled dust. In our study, the variation of the concentrations of *Der p* I (the major allergen of *Dermatophagoides pteronyssinus*) in house dust on different locations within homes, was much smaller than the variation between homes. Furthermore, for the *Der p* I concentrations in settled house dust, the variation in time within homes is much smaller than the variation between homes. Therefore, it is concluded that the measurement of house dust mite allergens in settled house dust can be used as a reliable estimator of the potential exposure to these allergens in different homes.

The most widely used method to measure the presence of fungi in dwellings as estimator of exposure, is air sampling of viable fungal particles. In the literature, information on the accuracy and precision of these methods as a measure of exposure was scarce. Therefore, a comparative study was conducted to document to what extent different results are obtained if different methods for airborne fungal propagules are used (section 4.4). This study showed that the results, quantitatively as well as qualitatively, depend to a large extent on the sampling device and collection medium used. Further, the precision of the methods both in terms of CFU/m$^3$ and in terms of species isolated was only moderate. Statistical analysis showed that the Slit sampler and the N6-Andersen in combination with DG18 and MEA, gave the best precision and the highest yields in terms of CFU/m$^3$ and number of species. The variability in time of the results of air sampling of fungal propagules was assessed by sampling twice with an interval of five weeks (section 5.2). The results showed that for the numbers of CFU/m$^3$, the variation between homes was much smaller than the variation within homes. The agreement ratio between the genera isolated in both sampling periods was approximately 50%. It is concluded that the precision of air sampling of viable fungal particles is limited. The usefulness of air sampling of fungal propagules as estimator of potential exposure is therefore limited as well.

The measurement of fungal propagules in settled house dust was considered as possibly a more precise estimation of the potential exposure (section 4.5). The first step was to document the extent to which results differ with the use of different analytical methods for the assessment of the presence of fungal propagules in house dust. The results obtained with ten different analytical methods were compared. Each method was a unique combination of culture medium, suspension fluid, and dilution step. Our study showed that the results, quantitatively as well as qualitatively, largely depended on the analytical method used. The highest numbers of CFU/g dust were obtained after suspension followed by a 10-fold dilution, and the lowest by direct plating. In contrast, the highest numbers of different species were obtained by direct plating, and the lowest numbers after suspension followed by a 10-fold dilution. The precision in terms of CFU/g dust of duplicate analyses was acceptable for most methods, with mean coefficients of variation of approximately 11% to 16%. The reproducibility of the species isolated by duplicate analyses was only moderate (highest agreement ratio approximately 60%) for all methods. It is concluded that the precision of dust sampling for viable fungal particles is limited. The reproducibility in time (assessed over a period of 6 weeks) of the number of CFU/g dust, was somewhat better for mattress dust than for bedroom floor dust. The variation in time with regard to the species isolated was substantial for both mattress and floor dust. The usefulness of dust sampling for viable

fungal propagules as estimator of the potential exposure is therefore limited as well.

Chapter 5 deals with the presence of fungi and house dust mites in relation to home dampness and other home and occupant characteristics. In the daily public health practice and in epidemiological studies, it would be useful to be able to predict the presence of fungi and house dust mites on the basis of home and occupant characteristics.

The total mean numbers of CFU/m$^3$ and the mean numbers of CFU/m$^3$ of the different genera were all weakly but positively associated with observed home dampness (section 5.2). However, these associations were not statistically significant. The mean numbers in homes with observed signs of dampness were on average 1.6 to 2.1 times higher than those in homes without observed signs of dampness. As the ranges of the numbers of CFU/m$^3$ in homes with and without observed signs of dampness showed considerable overlap, the predictive value of home dampness with respect to the levels of airborne fungal propagules is limited.

We also found weak positive relations between reported or observed signs of home dampness and the total number of CFU/g settled house dust, and the numbers of CFU/g of the various fungal species in floor and mattress dust (section 5.3). The mean numbers in floor and mattress dust were approximately 1.3 times higher in homes with observed signs of dampness than in those without observed signs of dampness. The numbers of CFU/g floor dust were comparable in homes with and without reported signs of dampness, whereas the mean number of CFU/g mattress dust was approximately 1.8 times higher in homes with reported signs of dampness. As the ranges of the numbers of CFU/g dust in homes with or without observed or reported signs of dampness showed considerable overlap, the predictive value of signs of home dampness with regard to the presence of fungal propagules in house dust is limited. No associations were found between the mean relative or absolute indoor humidity in the bedroom measured over a period of several weeks, and the total numbers of CFU/g dust or the counts of the various fungal species. For floor dust, the main predictive factor of the numbers of CFU/g dust was the type of floor covering. From textile floor coverings, the mean number of CFU/g dust was four times higher than in dust from smooth floorings.

The concentrations of *Der p* I in house dust were in general higher in homes with reported or observed signs of dampness, compared to those without either (section 5.4). The mean concentrations in homes with reported signs of dampness were on average 1.1 to 2.5 times higher than those in homes without reported signs of dampness. In homes with observed signs of dampness, the mean concentrations were on average 1.0 to 2.5 times higher than in homes without observed signs of dampness. The ranges of the *Der p* I concentrations in homes with or without observed signs of dampness showed more overlap than the ranges of the *Der p* I concentrations in homes with or without reported signs of dampness. Therefore, the predictive value of observed dampness with respect to the concentrations of *Der p* I in house dust is lower than the predictive value of reported signs of dampness. The average relative indoor humidity in the bedroom measured over a period of several weeks, was strongly correlated with the concentrations of *Der p* I in mattress dust and in dust from wall-to-wall carpets. For floor dust, the main predictive factor of the levels of *Der p* I in dust was the type of flooring. Dust from smooth floorings had mean concentrations of *Der p* I/g dust six to fourteen times lower than those from smooth floors with rugs or wall-to-wall carpets.

Chapter 6 presents the results of a case-control study on the role of sensitization to fungal and house dust mite allergens in the relationship between reported home dampness and reported respiratory symptoms in children. The odds ratios for these symptoms in relation to reported signs of dampness by the occupants found in this study were comparable with those found in other recent epidemiological studies (section 6.2). Using observed signs of dampness by the investigators as exposure variable, the odds ratios became smaller. The observations made by the investigators can be regarded as 'spot-samples', whereas the signs of dampness reported by the residents included a period of two years or more. A reasonable agreement was found between reported signs of dampness by the occupants and observed signs of dampness by the investigators. No indication was found of over-reporting of damp and mould by parents of children with reported respiratory symptoms relative to parents of controls.

Restriction of the analysis to cases sensitized against allergens of house dust mite and fungi, and controls not sensitized against these allergens, increased the odds ratios. A similar increase was found comparing sensitized asthmatic children and sensitized children with chronic cough only, to non-sensitized controls. These findings support the theory that sensitization to allergens of house dust mites and fungi plays a causal role in the association between home dampness and respiratory symptoms.

As part of this case-control study, the relation between the levels of *Der p* I in settled house dust, sensitization and respiratory symptoms was investigated (section 6.3). In a crude analysis, without stratification for sensitization against house dust mite allergens and allergen avoidance measures taken in the past, cases were generally exposed to lower *Der p* I concentrations than controls. Restriction of the analysis to cases with elevated serum IgE levels against dust mites, and to controls without elevated serum IgE against dust mites, taken the type of floor covering into account, showed only slightly higher *Der p* I concentrations in the bedroom floor dust of cases. However, restriction of the analysis to cases sensitized to dust mites and cases not sensitized to house dust mites - adjusting for allergen avoidance measures taken in the past - revealed a positive association between the *Der p* I concentrations in bedroom floor dust and mattress dust, and sensitization. These findings indicate that parents/guardians of children with chronic respiratory symptoms take effective allergen reduction measures.

In the general discussion (Chapter 7), a summary is given of the specific contributions of the studies reported in this thesis to the study of the relation between home dampness and respiratory symptoms in occupants. The conclusions about the main hypotheses tested in these studies are:

- The associations between reported or observed home dampness, and reported respiratory symptoms in occupants, found in recent epidemiological studies and in our case-control study, conducted in areas where home dampness was not a public concern, are not attributable to reporting bias or confounding by other indoor or outdoor factors possibly related with home dampness and/or respiratory symptoms.
- The present knowledge of the ecology and the effects on human health of fungi and house dust mites gives sufficient evidence to consider both groups of organisms as potential causal factors in the relation between reported or observed home dampness and reported respiratory symptoms in children and adults.
- For house dust mites, the measurement of their allergens in settled house dust can be used as a reliable estimator of the potential exposure to these allergens.
  For fungi, the validity of both air and dust sampling for viable fungal particles as estimators of the potential exposure is only limited.

- The levels of airborne fungal propagules and the numbers of fungal propagules in settled house dust are weakly but consistently positively associated with reported and/or observed signs of home dampness. The presence of *Der p* I in settled house dust is more strongly positively associated with reported and/or observed dampness. For floor dust, the type of flooring is a good predictor of the presence of fungi and house dust mite allergens.
- Sensitization to inhalant allergens produced by house dust mites and fungi plays a causal role in the relation between home dampness and respiratory symptoms.
- A positive relation was found between the levels of house dust mite allergen in settled house dust and sensitization among children with respiratory symptoms. Allergen avoidance measures taken in the past hamper the assessment of the associations between exposure to house dust mite allergens and the development of sensitization and respiratory symptoms in cross-sectional studies based on prevalent cases and suitable controls. Prospective studies, that follow children for several years directly after birth, or case-control studies based on incident cases, are needed to further clarify the relationships between exposure to indoor allergens, including fungi and house dust mites, sensitization and childhood respiratory symptoms.

# Samenvatting

Dit proefschrift beschrijft de resultaten van zeven onderzoeken in de relatie tussen vochtige woningen en luchtwegsymptomen bij bewoners.

In hoofdstuk 1 worden de achtergronden van de studies weergegeven. Vochtige woningen komen in Nederland algemeen voor. Circa 20% van het totale Nederlandse woningbestand heeft te kampen met vochtproblemen. Al in de twintiger jaren werd gewezen op het mogelijke belang van vocht in woningen op de prevalentie van astma. Recent epidemiologisch onderzoek uitgevoerd in West-Europa en Noord-Amerika, liet een toename zien van luchtwegklachten bij kinderen en volwassenen, gerelateerd aan de aanwezigheid van vochtproblemen in moderne woningen. Echter, de causale relatie tussen vocht in woningen en luchtwegaandoeningen is nog steeds niet volledig opgehelderd. De hypothetische causale relatie tussen vocht in woningen en luchtwegsymptomen bij de bewoners, wordt in dit hoofdstuk beschreven. De hypothese is dat een vochtige woning de ontwikkeling van schimmels en huisstofmijten bevorderd. Schimmels en huisstofmijten produceren beiden inhalatie-allergenen. Gevoelige individuen kunnen allergische reacties ontwikkelen tegen deze allergenen. Dit zou vervolgens leiden tot een verhoogde prevalentie van luchtwegsymptomen onder bewoners van vochtige woningen. De doelstellingen van de verschillende studies worden in dit hoofdstuk besproken. Het accent van de verschillende studies ligt bij: 1) het meten van de aanwezigheid van, en de blootstelling aan schimmels in woningen, 2) de relatie tussen de aanwezigheid van schimmels en huisstofmijtallergeen en vocht in woningen, en andere woning- en bewonerskenmerken, en 3) de relatie tussen vocht in woningen, de aanwezigheid van huisstofmijtallergeen en schimmels, en luchtwegsymptomen bij kinderen.

Hoofdstuk 2 geeft een overzicht van recent epidemiologisch onderzoek naar de relatie tussen vochtigheid van woningen en luchtwegsymptomen bij kinderen en volwassenen, uitgevoerd in Nederland en elders. Deze epidemiologische studies hebben een aantal gemeenschappelijke kenmerken. Het betreft allemaal dwars-doorsnede onderzoeken, en in de meeste van deze studies werden vragenlijsten gebruikt om informatie te verzamelen ten aanzien van de blootstelling (zichtbare vochtverschijnselen), en ten aanzien van de relevante effecten (luchtwegsymptomen). De mogelijke rol van vertekening van de resultaten door informatie bias en door mogelijk verstorende variabelen in de gevonden associaties, wordt besproken. Er wordt geconcludeerd dat de associaties tussen gerapporteerde of waargenomen vochtverschijnselen, en verhoogde prevalenties van gerapporteerde luchtwegsymptomen, zoals gevonden in studies uitgevoerd in gebieden waar vochtproblemen in woningen niet als een publieksprobleem werden ervaren, niet toe te schrijven zijn aan informatie bias of vertekening door andere factoren in het binnen- of buitenmilieu die mogelijk samenhangen met de vochtigheid van woningen of luchtwegsymptomen.

Indien de associatie tussen vochtige woningen en luchtwegsymptomen reëel is, dan is de volgende vraag of deze causaal is. Schimmels en huisstofmijten zijn de verdachte oorzakelijke factoren in deze associatie. Hoofdstuk 3 geeft een overzicht van de beschikbare kennis met betrekking tot de ecologie en gezondheidseffecten van schimmels en huisstofmijten. Vocht blijkt de meest kritische factor voor de ontwikkeling en overleving van schimmels en huisstofmijten. Beide groepen organismen produceren inhalatie allergenen. Inhalatie van deze allergenen kan leiden tot sensibilisatie bij genetisch gepredisponeerde individuen, en vervolgens tot allergische reacties als astma en rhinitis.

Hoofdstuk 4 betreft het bepalen van de blootstelling aan schimmels en huisstofmijten. Om de relatie tussen vocht in woningen en luchtwegsymptomen op te helderen, is het van essentieel belang om de verdachte oorzakelijke factoren te kunnen meten, en om dosis-effect relaties aan te tonen. De methoden die gebruikt worden moeten valide zijn, en slechts leiden tot een zo klein mogelijke meetfout.

Voor huisstofmijten wordt een kort overzicht gegeven van de beschikbare methoden (hoofdstuk 4.2) Er zijn enzyme-immuno-assays ontwikkeld waarmee de concentraties van de belangrijkste allergenen van huisstofmijten kunnen worden gemeten in zowel de lucht als in gedeponeerd stof. Deze assays zijn goed reproduceerbaar. De potentiële blootstelling aan huisstofmijtallergenen wordt in het algemeen bepaald door het meten van de concentraties van deze allergenen in gedeponeerd huisstof. In ons onderzoek was de variatie in de concentraties van *Der p* I (het belangrijkste allergeen van de mijt *Dermatophagoides pteronyssinus*) op verschillende lokaties in één woning veel kleiner dan de variatie tussen woningen. Bovendien is de variatie in tijd van de *Der p* I concentraties in één woning veel kleiner dan de variatie tussen woningen. Daarom wordt geconcludeerd dat het bepalen van de concentraties huisstofmijtallergeen in gedeponeerd huisstof gebruikt kan worden als een betrouwbare schatting van de potentiële blootstelling aan deze allergenen in verschillende woningen.

De potentiële blootstelling aan schimmels in woningen wordt algemeen bepaald door monstername van levensvatbare schimmeldelen in de binnenlucht. In de literatuur waren slechts weinig gegevens beschikbaar ten aanzien van de juistheid en de precisie van deze methoden als schatting van de potentiële blootstelling aan schimmels. Daarom hebben wij een vergelijkende studie uitgevoerd, om na te gaan in hoeverre de resultaten verkregen met verschillende methoden voor het bepalen van het vóórkomen van levensvatbare schimmeldelen in de binnenlucht met elkaar overeenkomen (hoofdstuk 4.4). Deze studie gaf aan dat de resultaten, zowel kwantitatief als kwalitatief, sterk afhangen van de gebruikte monstername apparatuur en voedingsbodems. De precisie van de verschillende methoden in termen van CFU/m$^3$ en in termen van geïsoleerde schimmelsoorten was slechts matig. Statistische analyse liet zien dat de beste precisie en de hoogste opbrengsten in termen van CFU/m$^3$ en schimmelsoorten, werden verkregen met de Slit en de N6-Andersen monstername-apparaten, in combinatie met DG18 en MEA als voedingsbodems. De variatie in de tijd in het vóórkomen van levensvatbare schimmeldelen in de binnenlucht werd bepaald over een periode van vijf weken (hoofdstuk 5.2). De resultaten gaven aan dat voor de aantallen CFU/m$^3$ de variatie tussen woningen veel kleiner was dan de variatie in de tijd binnen één woning. De mate van overeenstemming in geïsoleerde schimmelgeslachten op beide momenten van monstername was circa 50%. Op grond van deze resultaten wordt geconcludeerd dat de precisie van de monstername van levensvatbare schimmeldelen in de binnenlucht beperkt is. De waarde van het meten van levensvatbare schimmeldelen in de binnenlucht als schatting van de potentiële blootstelling is daarom ook beperkt.

In een tweede studie (hoofdstuk 4.5) werd nagegaan of het meten van levensvatbare schimmeldelen in gedeponeerd huisstof mogelijk een meer precieze schatting zou kunnen geven van de potentiële blootstelling. Als eerste werden de resultaten vergeleken verkregen met tien verschillende analysemethoden. Elke methode was een unieke combinatie van voedingsbodem, suspensie vloeistof, en verdunningsstap. Ons onderzoek gaf aan dat de resultaten, kwantitatief en kwalitatief, sterk afhankelijk waren van de toegepaste analysetechniek. De hoogste aantallen CFU/g stof werden verkregen na suspensie van het stof, gevolgd door een verdunning met een factor 10. De laagste aantallen CFU/g stof werden verkregen door het direct uitplaten van het stof. Echter, de

hoogste aantallen geïsoleerde schimmelsoorten werden juist verkregen bij het direct uitplaten, terwijl de laagste aantallen soorten werden verkregen bij suspensie gevolgd door verdunning. De precisie in termen van aantallen CFU/g stof van duplo-analyses was acceptabel voor de meeste analysemethoden, met gemiddelde variatie-coëfficiënten van circa 11% tot 16%. De mate van overeenstemming in geïsoleerde schimmelsoorten was slechts matig (de hoogste mate van overeenstemming was circa 60%) voor alle analysemethoden. Op grond van deze resultaten wordt geconcludeerd dat de precisie van de monstername van levensvatbare schimmeldelen in huisstof beperkt is. De reproduceerbaarheid in de tijd, bepaald over een periode van zes weken, in aantallen CFU/g stof was beter voor matrasstof dan voor vloerstof. De variatie in de tijd in termen van geïsoleerde schimmelsoorten was aanzienlijk voor zowel matras- als vloerstof. De waarde van het meten van levensvatbare schimmeldelen in huisstof als schatting van de potentiële blootstelling is daarom ook beperkt.

In hoofdstuk 5 worden de resultaten gepresenteerd van het onderzoek naar de relaties tussen het vóórkomen van schimmels en huisstofmijten in woningen, en vochtverschijnselen en andere woning- en bewonerskenmerken. Voor de dagelijkse praktijk van de behandeling van gezondheidsklachten in relatie tot vocht in de woning, en voor epidemiologisch onderzoek, is het waardevol om het vóórkomen van schimmels en huisstofmijten te kunnen voorspellen op grond van woning- en bewoners karakteristieken.

De totale gemiddelde aantallen CFU/m$^3$, en de gemiddelde aantallen CFU/m$^3$ voor de verschillende genera, waren allen zwak maar positief geassocieerd met waargenomen vochtverschijnselen (hoofdstuk 5.2). Deze associaties waren echter niet statistisch significant. De gemiddelde aantallen in woningen met waargenomen vochtverschijnselen waren 1.6 tot 2.1 maal hoger dan de aantallen in woningen zonder waargenomen vochtverschijnselen. De bereiken van de aantallen CFU/m$^3$ in woningen met en zonder waargenomen vochtverschijnselen, vertoonden een aanzienlijke overlap. Daarom is de voorspellende waarde van waargenomen vochtverschijnselen voor de aantallen CFU/m$^3$ slechts beperkt.

Wij vonden ook zwakke positieve verbanden tussen gerapporteerde of waargenomen vochtverschijnselen en de totaal aantallen CFU/g stof, en de aantallen CFU/g voor de verschillende schimmelsoorten in matras- en vloerstof (hoofdstuk 5.3). De gemiddelde aantallen in vloer- en matrasstof waren circa 1.3 maal hoger in woningen met waargenomen vochtverschijnselen dan in woningen zonder waargenomen vochtverschijnselen. De aantallen CFU/g vloerstof in woningen met of zonder gerapporteerde vochtverschijnselen waren vergelijkbaar, terwijl het gemiddelde aantal CFU/g matrasstof, 1.8 maal hoger was in woningen met gerapporteerde vochtverschijnselen vergeleken met het gemiddelde aantal in woningen zonder gerapporteerde vochtverschijnselen. De bereiken van de aantallen CFU/g stof in woningen met en zonder waargenomen of gerapporteerde vochtverschijnselen vertoonden een aanzienlijke overlap. Daarom is de voorspellende waarde van waargenomen en gerapporteerde vochtverschijnselen voor de aantallen CFU/g huisstof slechts beperkt. Er werden geen associaties gevonden tussen de gemiddelde relatieve of absolute luchtvochtigheid in de slaapkamer, gemeten over een periode van meerdere weken, en het totaal aantal CFU/g stof of de aantallen CFU/g stof voor de verschillende schimmelsoorten. Voor vloerstof is de belangrijkste voorspellende factor het type vloerbedekking; de gemiddelde aantallen CFU/g stof van textiele vloerbedekking waren 4 maal hoger dan de aantallen in het stof van gladde vloeren.

De concentraties *Der p* I in huisstof waren over het algemeen hoger in woningen met

gerapporteerde of waargenomen vochtverschijnselen, in vergelijking met de concentraties in woningen zonder deze vochtverschijnselen (hoofdstuk 5.4). De gemiddelde concentraties in woningen met gerapporteerde vochtverschijnselen waren 1.1 tot 2.5 maal hoger dan in woningen zonder gerapporteerde vochtverschijnselen. In woningen met waargenomen vochtverschijnselen waren de gemiddelde concentraties 1.0 tot 2.5 maal hoger dan in woningen zonder waargenomen vochtverschijnselen. De bereiken van de concentraties *Der p* I in woningen met en zonder waargenomen vochtverschijnselen vertoonden een grotere overlap dan de bereiken van de concentraties in woningen met of zonder gerapporteerde vochtverschijnselen. Daarom is de voorspellende waarde van waargenomen vochtverschijnselen voor de *Der p* I concentraties minder dan de voorspellende waarde van gerapporteerde vochtverschijnselen. De gemiddelde relatieve luchtvochtigheid in de slaapkamer gemeten over een periode van enkele weken was sterk positief gecorreleerd met de *Der p* I gehaltes in stof van matrassen en textiele vloerbedekking. Voor vloerstof was de belangrijkste voorspellende factor het type vloerbedekking; in het stof bemonsterd van gladde vloeren waren de gemiddelde concentraties zes tot veertien maal lager dan in het stof bemonsterd van gladde vloeren met een kleed of van textiele vloerbedekking.

Hoofdstuk 6 beschrijft de resultaten van een patiënt-controle onderzoek naar de rol van sensibilisatie tegen schimmel en huisstofmijtallergenen in de relatie tussen gerapporteerde vochtverschijnselen in woningen en gerapporteerde luchtwegsymptomen. De odds ratio's voor gerapporteerde luchtwegsymptomen in relatie met gerapporteerde vochtverschijnselen door de bewoners, waren vergelijkbaar met de odds ratio's gerapporteerd in andere recente epidemiologische studies (hoofdstuk 6.2). Indien waargenomen vochtverschijnselen door de onderzoekers als blootstellings variabelen werden gehanteerd, dan werden lagere odds ratio's gevonden. De waarnemingen van de onderzoekers kunnen gekarakteriseerd worden als 'moment opname', terwijl de vochtverschijnselen gerapporteerd door de bewoners een periode van twee jaar of langer besloegen. Wij vonden een redelijke overeenkomst tussen gerapporteerde vochtverschijnselen door de bewoners, en waargenomen vochtverschijnselen door de onderzoekers. Er werd geen aanwijzing gevonden voor een over-rapportage van vocht- en schimmelplekken door de ouders van kinderen met gerapporteerde luchtwegsymptomen ten opzichte van de ouders van controles.

Beperking van de analyse tot patiënten gesensibiliseerd tegen allergenen van de huisstofmijt en schimmels, en controles die niet gesensibiliseerd waren tegen deze allergenen, verhoogde de odds ratio's. Een vergelijkbare verhoging werd gevonden bij vergelijking van gesensibiliseerde astmatische kinderen en gesensibiliseerde kinderen met chronische hoest, met niet-gesensibiliseerde controles. Deze resultaten ondersteunen de theorie dat sensibilisatie tegen allergenen van huisstofmijten en schimmels een causale rol speelt in de associatie tussen vocht in woningen en luchtwegsymptomen.

Als onderdeel van dit patiënt-controle onderzoek onderzochten we de relatie tussen de concentraties *Der p* I in huisstof en luchtwegsymptomen (hoofdstuk 6.3). Een vergelijking tussen patiënten en controles, zonder stratificatie naar sensibilisatie tegen de huisstofmijt en saneringsmaatregelen genomen in het verleden, gaf aan dat patiënten aan lagere *Der p* I concentraties waren blootgesteld dan controles. Restrictie van de analyse tot patiënten met verhoogd serum IgE tegen huisstofmijt, en controles zonder verhoogd serum IgE tegen de huisstofmijt, rekening houdend met het type vloerbedekking, gaf aan dat alleen de *Der p* I concentraties in het vloerstof van de slaapkamer hoger waren voor

patiënten. Echter, restrictie van de analyse tot patiënten gesensibiliseerd tegen de huisstofmijt en patiënten die niet gesensibiliseerd waren tegen de huisstofmijt - gecorrigeerd voor saneringsmaatregelen genomen in het verleden - gaf een positieve associatie tussen de *Der p* I concentraties in stof van de vloer van de slaapkamer en de matras, en sensibilisatie. Deze resultaten geven aan dat ouders/verzorgers van kinderen met chronische luchtwegaandoeningen effectieve saneringsmaatregelen nemen om de *Der p* I concentraties te reduceren.

In de algemene discussie (hoofdstuk 7) wordt een samenvatting gegeven van de specifieke bijdragen van de onderzoeken gerapporteerd in dit proefschrift aan het onderzoek naar de relatie tussen vocht in woningen en luchtwegsymptomen bij bewoners. De conclusies met betrekking tot de belangrijkste onderzochte hypotheses luiden:

- De associaties tussen gerapporteerde of waargenomen vochtverschijnselen in woningen, en gerapporteerde luchtwegsymptomen bij bewoners, zoals gevonden in recente epidemiologische studies en in ons patiënt-controle onderzoek, uitgevoerd in gebieden waar vocht in woningen geen publieksprobleem was, zijn niet toe te schrijven aan informatie bias of vertekening door andere binnen- of buitenmilieu factoren die mogelijk gerelateerd zijn aan vocht in woningen en/of luchtwegsymptomen.
- De beschikbare kennis over de ecologie en gezondheidseffecten van schimmels en huisstofmijten levert voldoende bewijs om beide groepen van organismen als mogelijke causale factoren te beschouwen in de relatie tussen gerapporteerde of waargenomen vochtverschijnselen in woningen en gerapporteerde luchtwegsymptomen in kinderen en volwassenen.
- De bepaling van de concentraties huisstofmijtallergenen in gedeponeerd huisstof kan gebruikt worden als betrouwbare schatting van de potentiële blootstelling aan deze allergenen.
  Voor schimmels is de betrouwbaarheid van de monstername van lucht en gedeponeerd stof als schatting van de potentiële blootstelling aan levensvatbare schimmeldelen, beperkt.
- De aantallen levensvatbare schimmeldelen in de binnenlucht en in gedeponeerd huisstof zijn zwak maar consistent positief geassocieerd met gerapporteerde en/of waargenomen vochtverschijnselen. De concentraties *Der p* I in huisstof zijn sterker positief geassocieerd met gerapporteerde en/of waargenomen vochtverschijnselen.
  Voor vloerstof is het type vloerbedekking een redelijke voorspeller voor het vóórkomen van schimmels en huisstofmijtallergenen.
- Sensibilisatie tegen inhalatie-allergenen geproduceerd door huisstofmijten en schimmels speelt een causale rol in de relatie tussen vocht in woningen en luchtwegsymptomen.
- Er werd een positieve relatie gevonden tussen de hoeveelheid huisstofmijt allergeen in gedeponeerd huisstof en sensibilisatie onder kinderen met luchtwegsymptomen. Saneringsmaatregelen genomen in het verleden vormen een belemmering voor het vaststellen van de associaties tussen de blootstelling aan huisstofmijtallergenen, het optreden van sensibilisatie en de ontwikkeling van luchtwegsymptomen in dwarsdoorsnede onderzoek, gebaseerd op prevalente patiënten en geschikte controles. Prospectieve studies, waarin kinderen gedurende enkele jaren vanaf hun geboorte worden gevolgd, of patiënt-controle studies gebaseerd op nieuw-gediagnostiseerde patiënten, zijn nodig om de relaties tussen de blootstelling aan allergenen in het binnenmilieu (waaronder schimmels en huisstofmijten), sensibilisatie en luchtwegsymptomen bij kinderen, nader vast te stellen.

# List of abbreviations

| | |
|---|---|
| ACGIH | American Conference of Industrial Hygienists |
| $a_w$ | water activity, the ratio of the vapour pressure above a substrate to the vapour pressure above pure water under the same temperature and pressure |
| CFU | colony forming unit |
| CI | confidence interval |
| CNSRD | chronic non-specific respiratory diseases |
| CV | coefficient of variation (relative standard deviation) |
| $d_{50}$ | cut-off size, the particle size above which 50% or more of the particles are collected |
| DF | degrees of freedom |
| DG18 | dichloran 18% glycerol agar |
| DRBC | dichloran rose bengal chloramphenicol agar |
| DS | damp score |
| EAA | extrinsic allergic alveolitis |
| EIA | enzyme immuno assay |
| ELISA | enzyme-linked immunosorbent assay |
| $FEF_{25-75}$ | forced expiratory flow between 25 and 75% of the forced vital capacity (litre/sec) |
| $FEV_1$ | forced expiratory volume in one second, volume that can be exhaled in one second during a forced expiratory manoeuvre after a maximal inhalation (litre/sec) |

| | |
|---|---|
| FVC | forced vital capacity, maximal volume that can be exhaled during a forced expiratory manoeuvre after a maximal inhalation (litre) |
| GF | gelatine filter sampler |
| GM | geometric mean |
| GSD | geometric standard deviation |
| IU | international unit |
| IUIS | International Union of Immunological Societies |
| mAb | monoclonal antibodies |
| MEA | malt extract agar |
| MMEF | maximal mid-expiratory flow, mean expiratory flow between 25 and 75% of FVC during a forced expiratory manoeuvre after a maximal inhalation |
| MS | mean square |
| N6-Andersen | Andersen 1-stage impactor |
| NIOSH | National Institute of Occupational Safety and Health |
| OGYA | oxytetracycline glucose yeast extract agar |
| OPD | open petri dish |
| OR | odds ratio |
| PEF | peak flow, maximal expiratory flow during a forced expiratory manoeuvre after a maximal inhalation |
| PRIST | paper radioimmunosorbent test |
| RAST | radio-allergosorbent test |
| RCS | Reuter Centrifugal Sampler |
| RH | relative humidity |
| RIA | radioimmuno assay |
| SAS | Surface Air System impactor |
| SD | standard deviation |
| SS | sum of squares |
| V8 | V8 juice agar |

# Acknowledgements

Over the past six years, many people have contributed to the work presented in this thesis. There are some people who I would like to acknowledge particularly.

Regarding my daily work, I am much obliged to Joop van Wijnen (head of the Department of Environmental Medicine), with whom I am working close together now for almost nine years. He has trained me in Environmental Medicine and gave me, within the daily work of the Department, all opportunities to carry out the research presented in this thesis. I also thank him for guidance and the many encouraging discussions about the research.

All studies have been conducted in close cooperation with the Department of Epidemiology and Public Health of the University of Wageningen. Without this cooperation, which was extremely useful and pleasant, this thesis had not been written. I specially thank Bert Brunekreef for guidance and many discussions about the design and analyses of the studies. Rob van Strien and Paul Fischer (presently National Institute of Public Health) for the pleasant cooperation in conducting the case-control study, and the study on fungi in indoor and outdoor air, respectively. Jan Boleij for his advice and assistance at the start of the studies. The technical staff of the Department for their technical assistance, and Lonneke de Rijk, Rita Slob and Nicole Janssen for their contributions. Further, I thank all the students who participated in the fieldwork.

The skill of Rob Samson, Ellen Hoekstra and Marjolein van der Horst of the Centraalbureau voor Schimmelcultures was indispensable. Without their cooperation, the research on fungi would not have been possible. I also thank Rob Samson for his comments on part of the thesis.

I thank Paul van der Maas (University of Rotterdam) for the discussions about the research and his comments on the draft thesis.

I thank Brian Flannigan (Heriot-Watt University, Edinburgh, UK), Suzanne Gravesen (ALK Laboratories, Horsholm, Denmark), Aino Nevalainen (National Institute of Public Health, Kuopio, Finland), and Hans-Urs Wanner (Technical University, Zurich, Switzerland) for our fruitful and pleasant discussions within Working Group 5 "Biological Particles in Indoor Environments" of the European Collaborative Action Indoor Air Quality & its Impact on Man, that significantly contributed to my knowledge of this field.

The Central Laboratory of the Blood Transfusion Service in Amsterdam (Rob Aalberse and Stephan Stapel) kindly performed the serum IgE analyses.

The Dutch Ministry of Welfare, Culture and Public Health, the Ministry of Housing, Physical Planning and Environment, and the Dutch Asthma Fund, funded large parts of the studies.

I also would like to thank the workers of the Public Health Services (GGD Zaanstad, GGD Waterland, GGD Amstelland- de Meerlanden, GGD Gooi- en Vechtstreek, GGD West-Veluwe Valleigebied) of the towns where the studies were conducted, as well as all participants of the studies. Without their willingness to participate, there would have been no thesis. Froukje Versteeg-Hospes prevented with her experience that the children participating in the studies were traumatized by taking blood samples.

Lenneke Brachel improved the lay-out of the thesis, whereas Margaret Flannigan kindly translated my Dutch English into British English.

Finally, I thank all current and former colleagues of the Public Health Service Amsterdam, especially at the Department of Sanitary Housing Inspection and the Department of Public Health and Environment, for providing a very pleasant work environment during the last years.

# Curriculum vitae

Arnoud Paul Verhoeff werd geboren op 13 maart 1960 te 's-Gravenzande. De lagere school werd doorlopen in Wageningen. Van 1972 tot 1978 doorliep hij het Atheneum-B aan het Christelijk Lyceum te Zeist. Van 1978 tot 1985 studeerde hij biologie. Het kandidaats-examen werd afgelegd aan de Rijksuniversiteit Utrecht. Het doctoraal diploma (cum laude) werd behaald aan de Universiteit van Amsterdam. Het doctoraal pakket bestond uit het bijvak Gezondheidsleer en Luchtverontreiniging aan de Landbouwuniversiteit Wageningen, het bijvak Maatschappelijke Biologie aan de Rijksuniversiteit Utrecht, en het hoofdvak Gezondheidswetenschappen aan de Universiteit van Amsterdam.

Van augustus 1985 tot maart 1987 was hij als erkend gewetensbezwaarde militaire dienst tewerkgesteld bij de afdeling Hygiënisch Woningtoezicht van de GG en GD Amsterdam. In deze periode lag het accent op het ondersteunen van de behandeling van (gezondheids)klachten van Amsterdammers, die in verband worden gebracht met de kwaliteit van het binnenmilieu van woningen. Tijdens deze periode werd de basis gelegd voor het onderzoek beschreven in dit proefschrift.

Vanaf 1987 is hij als stafmedewerker verbonden aan de afdeling Medische Milieukunde van de GG en GD Amsterdam. Naast uiteenlopende werkzaamheden in het brede veld van de medische milieukunde, werd binnen deze afdeling het onderzoek verricht dat in dit proefschrift is beschreven. Het onderzoek werd uitgevoerd in nauwe samenwerking met de vakgroep Humane Epidemiologie en Gezondheidsleer van de Landbouwuniversiteit Wageningen, en het Centraalbureau voor Schimmelcultures in Baarn.