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# INDOOR ENVIRONMENT AND RECURRENT WHEEZING IN YOUNG CHILDREN

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# Indoor Environment and Recurrent Wheezing in Young Children

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*“For seeking and learning are in fact nothing but recollection”*

Plato (Meno)



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## ABSTRACT

Recurrent wheezing is one of the most common causes of morbidity and hospitalisation among infants and young children in many westernised countries. Respiratory infections and exposure to tobacco smoke have been identified as important risk factors. The indoor environment is also clearly of importance since we spend most of our time indoors. The aim of this thesis was to study the influence of various ventilation systems on indoor air quality, and to elucidate the impact of outdoor and indoor environment, primarily with focus on indoor air, on the development of recurrent wheezing in children up to the age of two years. The thesis is based on two main studies:

The first study assesses the impact of various ventilation systems on the indoor quality of single-family homes, located within a small residential area outside Stockholm. All houses were originally designed for natural ventilation. Twenty-two of the 59 investigated houses had been refitted with mechanical supply and exhaust ventilation systems. In another eight houses the original natural ventilation had been adjusted in order to improve the air change rate.

In the second study, we followed a birth cohort (BAMSE), comprising 4,089 children, born in predefined areas of Stockholm, during the two first years of the children's lives. Both urban and suburban districts were represented, including different types of buildings, dwellings with and without gas stoves for cooking, different socio-economic groups, and areas with various types of traffic exposure. Information on exposures was obtained from parental questionnaires. In addition, children with recurrent wheezing, and two age-matched controls per case, were identified and enrolled in a nested case-control study. Their homes were investigated and ventilation rate, humidity, temperature and NO<sub>2</sub> measured.

In BAMSE, an increased risk of recurrent wheezing was shown for children living in apartment buildings constructed after 1940 and single-family homes with crawl space/concrete slab foundation, compared with those living in buildings erected before 1940, OR 2.5 (1.3-4.8) and OR 2.5 (1.1-5.4). This was not primarily explained by differences in type of ventilation system, measured ventilation rate, occurrence of house dust mite allergen in the home, or other known risk factors for childhood wheezing.

Air change rate (ACH) was inversely related to indoor humidity, and increased humidity above median level 5.8 g/kg was associated with infant recurrent wheezing, OR 1.7 (1.0-2.9). In single-family homes, both studies show that mechanical ventilation increases the possibility of reaching an ACH of  $\geq 0.5$ , which in cold temperate regions protects buildings from increased indoor humidity, including levels that promote mite survival. Furthermore, occurrence of windowpane condensation on the interior side of double-glazed windows in wintertime indicated indoor humidity above 5.8 g/kg. Windowpane condensation, reported consistently over several years in the same home, was also associated with an increased risk of infant recurrent wheezing, OR 2.2 (1.1-4.5). There was also a higher proportion of recurrent wheezing in children exposed to signs of dampness, prospectively reported by parents, OR 1.4 (0.9-2.2) or observed at home inspections 1.6 (1.0-2.5). Moreover, recently painted surfaces in the child's bedroom were associated with an increased OR for recurrent wheezing, 1.7 (1.3-2.6).

It was further suggested that exposure to air pollution including NO<sub>2</sub>, particularly in combination with exposure to environmental tobacco smoke (ETS), increases the risk of recurrent wheezing in children: the OR was 3.1 (1.3-7.3) among children exposed to the highest quartile of indoor (NO<sub>2</sub>) and ETS.

It may be concluded that various building-related exposures such as certain types of building constructions, signs of dampness and newly painted interior surfaces, were associated with recurrent wheezing in children up to the age of two. In addition NO<sub>2</sub>, especially in combination with ETS seems to increase the risk of infant recurrent wheezing.

**Key words:** *Child, asthma, allergy, wheezing, air pollution, NO<sub>2</sub>, indoor air quality, building construction, ventilation system, indoor humidity, house dust mite, damp buildings, moisture, moulds*

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## SAMMANFATTNING PÅ SVENSKA

Återkommande episoder av nedre luftvägssymtom i form av pipande och väsande andning tillhör en av de vanligaste åkommorna hos små barn, och i många av västvärldens länder svarar sådana symtom för en betydande andel av de sjukhusinläggningar som görs inom denna åldersgrupp. I en del länder i Europa har man sedan början av 1900-talet kunnat se en gradvis ökning av olika allergisjukdomar och i Sverige beräknas att förekomsten av astma, allergisk snuva och atopiskt eksem har mer än fördubblats de senaste 30 åren. En liten andel av denna ökning kan troligtvis förklaras av bättre diagnostik, men det är sannolikt så att även vår förändrade livsstil har medfört att vi blivit mer känsliga. Vissa riskfaktorer för astma och allergiutveckling hos barn är väldokumenterade, t.ex. exponering för miljötabaksrök (ETS) och vissa infektionssjukdomar, men ytterligare kunskap erfordras om bl.a. sambanden mellan yttre miljö liksom faktorer i inomhusmiljö och tidiga astmasymtom hos barn.

Syftet med denna avhandling är att studera faktorer i miljön som kan ha betydelse för utvecklingen av tidiga och återkommande astmasymtom, och är framför allt inriktad på faktorer som byggnadskonstruktion och ventilationssystem samt luftomsättning, luftfuktighet och olika föroreningar i innemiljön, men innehåller även en studie av uteluftens inverkan på tidiga astmasymtom hos barn.

Avhandlingens fem delarbeten bygger helt på två olika grundstudier. I båda studierna har vi använt enkäter och besiktningsskyltar för att få information om hur olika byggnader var konstruerade, besiktningsskyltar avseende förekomst av fukt och mögelskador m.m., samt information om brukarvanor, som kan ha betydelse för inomhusmiljön (rökning, vädring mm). I studie 2, BAMSE-studien (*delarbete III-V*) har vi även tillgång till information som gäller eventuell astma eller annan allergisjukdom hos föräldrarna till de barn som ingår i studien. Vi har även information om astmasymtom hos barnen vid ett och två års ålder. I båda studierna har vi utfört mätningar av luftomsättning, temperatur och luftfuktigheten, samt analyserat förekomsten av kvalsterallergen i madrassdamm. Vidare har vi beräknat fuktillskottet inomhus, dvs. skillnaden i ånghalt ( $\text{g/m}^3$ ) utomhus respektive inomhus. I studie 1 har vi dessutom utfört mätningar av flyktiga kemiska ämnen i rumsluften (VOC) och i BAMSE ingår mätningar av kvävedioxid ( $\text{NO}_2$ ) utomhus och inomhus.

### **Studie 1 (delarbete I och II)**

Den första studien är utförd i 59 likvärdigt konstruerade enplans radhus, byggda under åren 1968-70, inom ett bostadsområde norr om Stockholm. I denna studie undersökte vi vilken inverkan olika ventilationssystem hade på luftomsättning, temperatur, luftfuktighet och halten av kemiska ämnen i inomhusluften samt för sannolikheten av höga nivåer av kvalsterallergen i madrassdamm (*delarbete I*). I epidemiologiska studier används ibland kondens på insidan av 2-glas fönsterrutor vintertid som indikator för ett undermåligt inomhusklimat. Vi beräknade därför också värdet av att använda kondens, och högt fuktillskott inomhus, som indikatorer för låg luftomsättning, hög luftfuktighet ( $\geq 7 \text{ g/kg}$ ), förhöjda halter av föroreningar inomhus, samt för kvalsterallergen i madrassdamm (*delarbete II*).

Resultaten från studie 1 (*delarbete I och II*) tyder på att mekanisk ventilation ökar möjligheten att uppnå en luftomsättning på 0,5 oms/h eller mer i moderna enfamiljsfastigheter och att detta minskar risken för luftfuktighetsnivåer inomhus vintertid som skapar en grogrund för kvalsterväxt, samt bidrar dessutom till att sänka föroreningshalten i rumsluften. Vi fann vidare att frånvaro av kondens och ett fuktillskott  $< 3 \text{ g/m}^3$  var tillförlitliga markörer (90-100%) för en luftfuktighet  $< 7 \text{ g/m}^3$  vilket förhindrar kvalsterväxt, men att det även om det förekommer kondens kan vara nödvändiga med ytterligare undersökningar för att fastställa om detta verkligen betyder att det också finns kvalster i bostaden. I några hus där garaget, som var sammanbyggt med huset, användes för bilparkering fann vi dessutom kemiska ämnen från bensinångor i bostadsenheten.

### **Studie 2, BAMSE- studien (delarbeten III-V)**

Denna studie utgår från BAMSE- projektet (Barn, Allergi och Miljö i Stockholm, ett Epidemiologiskt projekt), en longitudinell prospektiv studie som omfattar drygt 4000 barn, födda inom delar av

Storstockholm, under perioden februari 1994 till november 1996. Inom BAMSE-projektet finns också en s.k. fall- och kontrollstudie, en delstudie som består av 540 barn; 181 barn med astmasymtom ("fall") samt 359 barn utan astmasymtom ("kontroller"). Resultaten av studie 2 (*delarbete III-V*) avser framför allt denna delstudie. Alla de 540 barn som ingår i fall-kontroll studien bodde kvar i sin "första bostad" vid tidpunkten för rekryteringen till denna substudie. För att kunna bedöma skillnader i sjukdomsförekomst mellan exponerade och oexponerade fall och kontroller har vi utfört s.k. multipel regressionsanalys, där alla riskbedömningar är justerade för effekter av andra riskfaktorer för astma/allergisjukdom hos barn; kön, ärftlighet för astma/allergisjukdom, mammans rökning under graviditeten, amning samt desutom för bostadens byggnadsår.

I BAMSE-studien framkom det ingen generell skillnad mellan förekomsten av återkommande astmasymtom hos barn i flerbostadshus jämfört med barn i villor. Däremot var det vanligare med sådana symtom hos barn som bodde i flerbostadshus byggda efter 1939 och hos barn boende i villor byggda på kryppgrund eller platta på mark, än hos barn som bodde i flerbostadshus byggda före 1940. Det förelåg inga tydliga samband mellan återkommande astmasymtom och någon särskild typ av ventilationssystem eller med luftomsättningen i bostaden. Däremot förelåg en negativ korrelation mellan luftomsättning och luftfuktighetsnivåer inomhus (*delarbete IV*).

Vi fann att förhöjd luftfuktighet inomhus [ $\geq 5.8$  g/kg (medianen)] var relaterat till en högre risk för återkommande astmasymtom hos barn under 2 år. I hem där föräldrarna vid upprepade tillfällen konsekvent rapporterat att det förekom kondens på insidan av tvåglas fönsterrutor vintertid var risken för återkommande astmasymtom hos barnen dubblad, jämfört med barn som bodde i hem utan tecken på kondens under denna period - från födelsen till tiden för bostadsbesiktningen. Fukt- och mögelskador i hemmet ökade också risken för återkommande astmasymtom. Om barnets bostad vid besiktningstillfället hade både tecken på fukt/mögelskador och hög luftfuktighet ( $\geq 5.8$  g/kg) var risken för astmasymtom fördubblad jämfört barn vars hem inte hade tecken på fukt/mögelskador och där luftfuktigheten var låg ( $< 5.8$  g/kg). Ju fler tecken på fukt och mögelskador som noterades vid besiktningen, eller om exponeringen varit långvarig desto starkare tycktes samband med tidiga astmasymtom vara. Vidare sågs också en ökad risk för återkommande astmasymtom hos barn vars sovrum målats om under moderns graviditet eller under barnets första levnadsår. Vi fann också en samverkans effekt mellan olika miljöexponering, inklusive miljötabaksrök med en ökad risk för astmasymtom för barn som exponerats för flera riskfaktorer samtidigt (*delarbete V*).

Av de 540 barnen bodde 129 barn i stadsmiljö, 274 i förorter med i huvudsak flerbostadshus samt 137 i villaområden. Vi fann en trend till ökad risk för återkommande astmasymtom hos barn exponerade för förhöjda halter av luftföroreningar innehållande kvävedioxid ( $\text{NO}_2$ ), i förhållande till barn exponerade inom den lägsta kvartilen. Vidare framkom en samverkande effekt mellan de högsta halterna av  $\text{NO}_2$  (inom 4:e kvartilen) och miljötabaksrök (föräldrars rökning), med tre gånger högre risk för astmasymtom hos barn exponerade såväl för  $\text{NO}_2$  halter inom den 4:e kvartilen som föräldrars rökning, jämför med barn exponerade för lägre  $\text{NO}_2$  nivåer och utan rökande föräldrar. Endast 46 av 540 bostäder använde gasspis vid matlagning och någon statistiskt säkerställd riskökning för astmasymtom framkom inte i relation till användning av gasspis (*delarbete III*).

### **Sammanfattning**

Sammanfattningsvis tyder resultaten från samtliga fem delarbeten på att byggnadsrelaterade faktorer kan ha en stor betydelse för risken att små barn skall drabbas av tidiga återkommande astmasymtom, samt att en förbättrad inomhusmiljö därigenom kan ha en preventiv betydelse i detta sammanhang.

Resultaten tyder vidare på att exponering för  $\text{NO}_2$ , framför allt i kombination med exponering för miljötabaksrök, ökar risken för återkommande astmasymtom hos små barn redan vid nivåer som underskrider rekommenderade Europeiska riktnivåer för högsta årsmedelxponering ( $40 \mu\text{g}/\text{m}^3$ ).



## LIST OF PUBLICATIONS

This thesis is based on the following papers referred to in the text by their Roman numerals:

- I. Emenius G, Egmar AC, Wickman M.  
Mechanical ventilation protects one-storey single-dwelling houses against increased air humidity, domestic mite allergens and indoor pollutants in a cold climatic region. *Clin Exp Allergy* 1998;28:1389-96.
- II. Emenius G, Korsgaard J, Wickman M.  
Window-pane condensation and high indoor vapour contribution – markers of an unhealthy indoor climate? *Clin Exp Allergy* 2000;30:418-25.
- III. Emenius G, Pershagen G, Berglind N, Kwon HJ, Lewné M, Nordvall L, Wickman M.  
NO<sub>2</sub>, as a marker of air pollution, and recurrent wheezing in children - a nested case-control study within the BAMSE birth cohort. *Occup Environ Med*, in press.
- IV. Emenius G, Svartengren M, Korsgaard J, Nordvall L, Pershagen G, Wickman M.  
Building characteristics, indoor air quality and recurrent wheezing in very young children (BAMSE). *Indoor Air*, in press.
- V. Emenius G, Svartengren M, Korsgaard J, Nordvall L, Pershagen G, Wickman M.  
Indoor Exposures and Recurrent Wheezing in Infants – a Longitudinal Study in the BAMSE Cohort. Submitted.

I would like to thank; Blackwell Publishing for allowing me to reprint papers I and II, BMJ Publishing Group for the permission to reprint paper III and Indoor Air for permission to reprint paper IV.

## LIST OF ABBREVIATIONS

ACH	Air Changes per Hour
AIH/AOH	Absolute Indoor Humidity/ Absolute Outdoor Humidity
CI	Confidence Interval; encloses the true value with a specified probability. If “1” is not enclosed, the OR is “statistically significant”
ETS	Environmental Tobacco Smoke
HDM	House Dust Mites
l/m, pers	Litre per minute and person
MVOC	Microbial Volatile Organic Compounds
NO <sub>2</sub>	Nitrogen dioxide
NS (ns)	Not significant
OR	Odds Ratio; the odds of having disease in groups being compared; OR <sub>adj</sub> indicates the OR adjusted for potential confounders
p-value	A continuous measure of the compatibility between a hypothesis and data
RH	Relative Humidity
SD	Standard Deviation, an estimate of uncertainty of the average measurement
TVOC	Total Volatile Organic Compounds
VOC	Volatile Organic Compound
WPC	Window Pane Condensation
µg/g	Microgram per gram
µg/m <sup>3</sup>	Microgram per cubic metre
ng/g	Nanogram /gram

## INTRODUCTION

... “If the mildew has spread on the walls, he is to order that the contaminated stones be torn out and thrown into an unclean place outside the town. He must have all the inside walls of the house scraped and the material that is scraped off dumped into an unclean place outside the town. Then they are to take other stones to replace these and take new clay and plaster the house.

*If the mildew reappears in the house after the stones have been torn out and the house scraped and plastered, the priest is to go and examine it and, if the mildew has spread in the house, it is a destructive mildew; the house is unclean. It must be torn down - its stones, timbers and all the plaster - and taken out of the town to an unclean place”...*

*Leviticus 14:33-57 (3<sup>rd</sup> Book of Moses)<sup>1</sup>*

For centuries, certain indoor exposures has been suspected to cause negative consequences for human health and suggestions on how to improve the indoor environment have been given. Since the late 19th century the growing scientific understanding of this issue in Sweden is well documented. The risk of transmission of infectious diseases such as tuberculosis etc. through poor housing conditions was obvious, but also other topics were on the agenda.

“Helsövärdshörsningen i Stockholm” (The Stockholm Health Association) was founded in 1881, and indoor air quality was a topic given the highest priority. The chairman of the association, *Elias Heyman*, who was also the first professor of Hygiene at Karolinska Institutet, published two reports on the subject, entitled “The indoor air of homes” and “Contribution to the knowledge on the quality of air in schools”.<sup>2,3</sup> In 1913, *Germund Wirgin*, residential inspector, later professor in Uppsala, by use of “the growing perfection of the statistic science” found strengthened evidence for a causal association between damp buildings and health effects such as headache and “pain in the eyes” in adults, as well as bronchitis and pneumonia in children. These interpretations were made in view of overcrowded living, malnutrition and unhealthy working conditions. To prevent dampness in buildings, the main measure recommended was to shelter the building materials from water before and during construction of the building”.<sup>4,5</sup> In 1935, a *National official report* on physical and mental health also took up to the impact of building and housing conditions on health.<sup>6</sup> Effects of damp buildings were discussed and the report stressed the importance of building improvements, with focus on infants’ health because of their general susceptibility for diseases and because they mainly spent their time indoors. As another example, a *conference on “The ventilation of dwellings”* was held in Stockholm in 1940, and a conclusion of that conference was that an interdisciplinary approach to the problem was necessary to solve the existing problems of indoor air quality.<sup>7</sup> With the general improvement in the standard of living in Sweden, including the housing conditions, the debate on indoor air quality partly fell silent for some decades.

Personally, I was well aware of the report “Dirty-Sweden” by *Ludvig Nordström*\*;<sup>8</sup> but was not prepared for the new dimension of complaints about the home environment that I met at end of 1970s, while working as an environmental health officer. An increasing number of individuals reported at that time health effects that could be associated with living in new built and well repaired homes; homes with a building standard far better than that described by “Lubbe” Nordström. Many years later, this development brought me into this field of research.

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\*Ludvig Nordström was a writer and journalist who in 1938 published a book, “Dirty-Sweden”, based on a series of reports in Swedish national public radio, documenting the very low standards of living and housing in the Swedish countryside.

Today, in countries with an improved building and housing standard, complaints about the indoor environment and related health status reported by the residents may be difficult to verify - both the suspected deficiencies in the building and the disease outcome in the person. Commonly used terms include “*sick building*”, with the adherent “*sick building syndrome*” (*SBS*), to describe situations in which building occupants experience negative effects on health and comfort, effects that appear to be linked to the building. Symptoms suggested to be associated with *SBS* have been fatigue, headache, dizziness, irritation of eye, nose, or throat, dry cough and nausea and sensitivity to odours, but also increased susceptibility to infections has been reported. Generally, symptoms cannot be clinically defined and decline or stop when the person leaves the building.<sup>9;10</sup> *SBS* should be distinguished from “*building related illness*” (*BRI*), a term that is used when symptoms of diagnosable illness are identified and can be attributed directly to airborne building contaminants such as the bacteria in central air-conditioning systems that may cause legionnaire’s disease. That is, *BRI* gives acute symptoms that can be clinically defined, have clearly identifiable causes, and the symptoms may remain even after the person leaves the building. With an increasing prevalence of allergic diseases in many westernised countries, it has also been hypothesised that unfavourable indoor conditions may promote development of *asthma and allergic hypersensitivity* and/or result in more severe symptoms in individuals with allergic disease.

## OUTCOME AND SUGGESTED RISK FACTORS

### **Hypersensitivity**

According to a new suggested nomenclature for allergic disease, “*hypersensitivity* causes objectively reproducible symptoms or signs, initiated by exposure to a defined stimulus at a dose tolerated by normal subjects”.<sup>11</sup> Hypersensitivity reactions may be either *non-allergic*, initiated by a process where no immunologic mechanism can be proven, or they may be *allergic* and initiated by immunologic mechanisms, in most cases allergen-specific IgE-mediated. Diseases that are commonly associated with allergen-specific allergy include asthma, rhinitis, conjunctivitis, skin disorders and food allergy.

### **Wheezing and childhood asthma**

*Asthma* has been defined as a “*chronic inflammatory disorder of the airways*”.<sup>12</sup> The most important symptoms of asthma are *wheeze, chestiness and symptoms of breathlessness*. Asthma is often caused and elicited by a non-allergic reaction. In very young children the term wheeze is used more often than asthma, since there is no clear definition of asthma for this age.<sup>13-16</sup> Wheezing is a relatively common symptom in infancy and most of the children who have wheezing during infancy will not develop persistent asthma. However, recurrent episodes of wheezing during this period of life remain an important predictor of for asthma later in life, and of sensitisation to allergens. There are also indications of impaired lung function among children with early episodes of recurrent wheezing.<sup>17;18</sup>

A number of epidemiological studies indicate an increased prevalence of asthma and atopy related diseases in westernised countries during recent decades, and wheezing has become one of the most common causes of morbidity and hospitalisation among infants and young children.<sup>19-23</sup> In Sweden, a tenfold increase in the number of school children receiving inhaled steroids has been reported during the period from 1985 to 1995.<sup>24</sup> The reason for the increase is not fully understood. Throughout the world, however, there are large variations between the countries, with the prevalence of wheezing ranging from 6% to 32% in younger children.<sup>22</sup> Thus, it has been suggested that the increased prevalence of asthma symptoms and allergic diseases must be explained by environmental factors.<sup>22;25;26</sup>

### ***Heredity and gender***

*Hereditary* factors play an important role in the development of asthma and sensitisation to allergens. It has been suggested that approximately 10-15% of the children without allergic heredity develop some kind of allergic disease, and the child's risk of having such disease is related to whether one or both parents have atopy.<sup>27</sup> In families where both parents have an identical type of allergic disease, the incidence of allergic diseases in children may be above 70%.<sup>27</sup> A three times greater odds of having a child with asthma has further been shown for families with one asthmatic parent, and six times greater in families with two asthmatic parents, compared with families where only one parent had inhalant allergy without asthma.<sup>28</sup>

*Gender* is likewise a well-known risk factor for infant wheezing, with a higher prevalence among young boys.<sup>20,29</sup> With the onset of puberty, however, the incidence of wheeze and asthma increases in girls and in adulthood women have been reported to have higher prevalence of asthma than men.<sup>30,31</sup> The reason for this shift in prevalence during adolescence is not understood.

### ***Environmental factors***

In addition to heredity and gender, various environmental exposures are associated with asthma and recurrent wheezing in childhood and may either promote or reduce the risk of disease. In infancy wheezing is highly associated with viral respiratory infections, in particular with respiratory syncytial virus (RSV). Exposure to *maternal smoking during pregnancy* or postnatal exposure to *environmental tobacco smoke (ETS)* has been identified as a risk factor for infant bronchitis, recurrent wheezing and early onset of asthma, in a number of surveys.<sup>32-36</sup> In turn, breast-feeding seems to have a preventive effect on early development of allergic disease.<sup>37</sup> Studies focusing on the impact of early *infectious diseases* are inconsistent and both promoting effects (RSV) and a preventive effect have been suggested.<sup>38-41</sup> Thus, and in line with the hypothesis that early exposure to some infections decrease the risk for early wheeze, young children who have *older siblings*, or who started early at *day care centre* may have a lower prevalence of asthma and wheeze.<sup>42-45</sup> Further, the composition of the *intestinal microflora* seems to have an impact on the development of allergic diseases.<sup>46</sup> Closely related to this is the discussion about early treatment with various *antibiotics* discussed as a possible risk factor, but the data are inconsistent.<sup>47-50</sup> The effect of early exposure to *furred pets* on allergic diseases is inconsistent and intensely discussed.<sup>51,52</sup> Selection bias has been suggested as a plausible mechanism to explain these inconsistencies in results, since families with atopic diseases are more likely to remove pets from the home.<sup>53</sup> In recent years, data from cross-sectional studies have suggested a protective effect related to certain *lifestyle factors*, with a reduced risk for children living on a *farm*<sup>54,55</sup> or in families with an *anthroposophic* lifestyle.<sup>56</sup> In some of these studies, the role of indoor exposure to *endotoxins* has been brought into focus but the issue seems to be complex; exposure to certain species of endotoxins may offer some protection,<sup>57-59</sup> whereas exposure to others, not associated with animals, may result in adverse health effects.<sup>60-62</sup> Thus, the *environment, both outdoors and indoors* is likely to be of importance for children's health early in life in terms of development of respiratory symptoms and IgE-mediated allergy.

## **OUTDOOR ENVIRONMENT**

Exposure to ambient air pollution and its potential effect on the development of asthma has been a topic of growing interest for many years. Recent studies show traffic to be a major source of air pollutants in urban areas. In general, certain air pollutants are measured and those must be interpreted as indicators of air pollution and are not necessary signify to the causal agent. In this context, nitrogen dioxide (NO<sub>2</sub>), a by-product of high temperature combustion in air, constitutes a commonly used indicator. Other potentially health-related pollutants include particles, ozone (O<sub>3</sub>), volatile and semi-volatile organic com-

pounds such as benzene, formaldehyde and polycyclic aromatic hydrocarbons. Due to greater emissions from motor vehicles and emissions from central heating plants and local burning of oil and wood during cold periods, outdoor air pollutants including NO<sub>2</sub> are at their highest level during the wintertime in countries with a temperate climate. Levels of ozone and other photochemical oxidants that are favoured by sunlight may, however, increase particularly during spring and in summer. In contrast to nitrogen dioxide, measured levels of ozone are higher in areas some distance from the city centres than in the centre, as ozone reacts with nitrogen oxide (NO) to form NO<sub>2</sub>.

The current European limit value for yearly average nitrogen dioxide exposure, applicable also for Sweden, is 40 µg/m<sup>3</sup>.<sup>63</sup> As motor vehicles provide the main source of outdoor pollutants, levels of nitrogen dioxide vary with the time of day, peaking mornings and late afternoons following the rush-hour traffic. Within the Stockholm area, 74% of the yearly population-weighted average daytime NO<sub>2</sub> concentrations emanates from local road traffic, and at night traffic contributes approximately 62%.<sup>64</sup> The calculated average daily roof-level exposure concentration varies between 4 and 45 µg/m<sup>3</sup> in the county, with a corresponding night exposure of 4-37 µg/m<sup>3</sup>. Not surprisingly, the highest concentration occurs in the central part of the city and the lowest in the outskirts of the county. Further, exposures differs between street canyon and roof level, where measurements are generally performed. The actual difference in a certain location depends on the traffic composition, street width, building height and the direction of the street in relation to wind exposure as well as on the ozone concentration at street level. In busy streets, the calculated annual mean concentration at street level may be approximately a factor 1.6 to 2.0 higher than roof level exposure.<sup>64</sup>

In epidemiological surveys focusing on children's health, the evidence for an association between NO<sub>2</sub> exposure and respiratory disorders does not provide a clear picture. Many papers demonstrate an association between NO<sub>2</sub> exposure and respiratory symptoms,<sup>65-67</sup> and some find the exposure particularly important for the development of respiratory disorders in girls.<sup>68;69</sup> A recently presented paper from a prospective European birth cohort study, on children at two years of age, indicated a positive association between both wheezing and physician-diagnosed asthma and air pollutants.<sup>70</sup> A prospective study on Californian school children and young adolescents found important losses in various lung functions, primarily in girls, in association with air pollution levels.<sup>71</sup> Some papers also suggest that episodes with exposure to high levels of NO<sub>2</sub> are more strongly related to adverse health outcomes than prolonged exposure at lower exposure levels.<sup>72</sup> It has further been hypothesised, that NO<sub>2</sub> exposure may increase the risk of asthmatic exacerbation following respiratory infections, even at relatively low levels of exposure.<sup>73</sup>

In the literature, there are also papers on epidemiological studies that fail to demonstrate any association between respiratory disorders and exposure to air pollution including NO<sub>2</sub>,<sup>21;74-76</sup> and among them a prospective birth cohort study assessing the impact of NO<sub>2</sub> exposure on the occurrence of bronchial obstruction in children below 2 years.<sup>76</sup>

In controlled chamber tests on adults, the main effect of NO<sub>2</sub> has been an increase in airway resistance. Further, asthmatics and individuals with chronic pulmonary diseases seem to be more susceptible to the exposure than individuals free from such diseases. The same result has also been demonstrated for subjects voluntarily exposed to NO<sub>2</sub> in road tunnels. Such studies have also provided evidence of an enhanced effect of exposure to common airborne allergens after exposure to increased levels of NO<sub>2</sub>.<sup>77;78</sup>

Outdoor air also constitutes a main source of exposure to air-borne allergens deriving from the flora, such as pollen from various trees, grasses and weeds, and mould spores, etc. This exposure is without a doubt very important, especially for those already sensitised and suffering from symptoms after exposure to pollen and outdoor mould. However, the impact of such outdoor allergen exposures will not be

further discussed in this context, even though the allergens will also penetrate into the indoor environment.

## INDOOR ENVIRONMENT

A considerable alteration in building structure and building technology has taken place during recent decades. A hundred years ago, the different components needed to erect a building could almost be counted on one man's fingers, today many thousands are used. Energy conservation measures, requiring extensive insulation, have resulted in use of multi-layer wall constructions, which increase the risk of moisture and mould damages. The ground construction of single family houses, in earlier times usually either a "heated" suspended timber floor (*"torpargrund"*) or a cellar, has now generally been superseded by constructions as concrete slab and crawl space foundations. In countries with a temperate climate, outdoor air-ventilated (unheated) crawl spaces, as well as concrete floors with insulation laid above the concrete slab, will be highly prone to water damages as a result of vapour condensation in the ground construction.<sup>79</sup>

### ***Building age and various housing conditions***

In the literature, a few studies indicating that building age may be related to unspecific health effects are found. In a study among office workers, Sundell et al found a tendency towards an elevated prevalence of sick building symptoms in new buildings.<sup>80</sup> Further, when the buildings were allocated to categories according to year of construction or remodelling, a significant increased risk of SBS was found in relation to buildings of age-category 1977 to 1986, compared with buildings erected before 1977. Austin et al, report a relationship between building age and eczema in adults, but not with respiratory symptoms, without presenting any data on precisely when the buildings were erected.<sup>81</sup> In a Swedish study of 609 multi-family buildings in Stockholm, with 14,235 dwellings, Engvall et al demonstrate that subjects living in relatively new buildings reported more sick building symptoms, than subjects living in older houses, with the most elevated risk for buildings erected between 1976 and 1990.<sup>82</sup> The impact of building age on health has also been discussed by Krämer et al, who found an increased odds ratio for the association between NO<sub>2</sub> and some allergic diseases, such as wheezing and rhinitis, when building age was included in the analysis.<sup>83</sup> A study by Kilburne reported adverse effects, including wheezing and shortness of breath in adults, which were associated with the indoor air in manufactured homes and during renovation. However, pulmonary function as measured by spirometry was normal and not different from that in controls.<sup>84</sup>

### ***The role of ventilation***

Ventilation plays a fundamental role in maintaining good indoor air quality and thermal comfort in buildings, with a primary role of removing different pollutants that are emitted into the interior space, and supplying the building with clean air. Thus, the outdoor air quality is important for the indoor air quality. In a study with the aim to assess the transfer of outdoor air into an unoccupied, but furnished dwelling in an area with heavy traffic, a reduction of 30% was seen for NO<sub>2</sub> indoors compared with outdoor levels.<sup>85</sup> It has also been shown that reducing the influx of outdoor air during periods with high concentrations of outdoor air pollutants can significantly reduce the indoor concentrations of such pollutants in mechanically ventilated buildings.<sup>86</sup>

Inside the building, pollutants are generated partly by the human metabolism and by human activities such as preparation of food, washing clothes, general cleaning, smoking habits etc, and partly by emissions of materials used in construction and furnishing.

In Sweden, the commonly installed ventilation systems in dwellings fall into the following categories:

- ◇ *Natural (draft) ventilation systems*: function through the combined effects of wind and differences in outdoor and indoor temperature, without support of mechanical fans (though a kitchen fan may be installed); supply air through slot air valves and leakage through weather-strips or other openings in the external building envelope.
- ◇ *Mechanical exhaust ventilation*: uses an extract fan for the exhaust air which leads to reduced internal pressure; supply air enters through slot air valves, leakage through weather-strips, separate ventilation windows or other openings in the external building envelope.
- ◇ *Balanced mechanical supply and exhaust ventilation*, with or without heat exchangers: fans are used both for the supply and exhaust of air. When equal flow rates are fitted there will be no pressure differences generated - flow rates are *balanced*. With an imperfect adjustment of the ventilation air flows, however, either an indoor air pressurisation or depressurisation may occur. In dwellings, the most common solution is mixing ventilation systems aimed at uniformly mixing clean air into the room space, thus diluting the indoor pollutants.

The originally installed ventilation system in Swedish homes is fairly closely related to the year when the building was erected or refurbished. In general, older apartment buildings are exclusively built with natural ventilation systems and from about 1960 and some decades onwards the natural ventilation was either combined with a kitchen fan or with a mechanical exhaust ventilation system in the building. From the middle of the 1970s apartment buildings and single-family homes, are more often equipped with a balanced mechanical supply and exhaust ventilation system. However, the original installation in older buildings may have been altered. According to the Swedish building code, the ventilation in mechanically ventilated homes should be at least 0.35 l/sek,m<sup>2</sup>, corresponding to 0.5 air changes per hour (ACH). Since 1991 the regulations also prescribe that ventilation systems shall be regularly controlled (exception for one- and two-family houses); buildings equipped with a balanced supply and exhaust ventilation system every 3<sup>rd</sup> year, exhaust ventilation every 6<sup>th</sup> year, naturally draft ventilated every 9<sup>th</sup> year.<sup>87</sup>

The impact of certain ventilation systems in dwellings on respiratory symptoms is difficult to evaluate since most studies assess the association of measured/indicated ventilation. Available papers on risk assessments of ventilation systems of homes, do not show any specific system as being associated with asthmatic symptoms<sup>88;89</sup> even though active mechanical ventilation of the homes has been suggested to slightly reduce the prevalence of allergic diseases.<sup>90</sup> Within the European Community Respiratory Health Survey (ECRHS), Zock et al present data on housing characteristics and asthma in adults in 38 study centres, demonstrating that the presence of ducted air heating and air conditioning was positively associated with asthma with an increased prevalence of current asthma, but also with wheezing and symptoms of breathlessness.<sup>91</sup>

In a number of studies, insufficient ventilation of homes has been suggested to be associated with adverse health effects, primarily symptoms of airway disease, in both adults and children. In many surveys, windowpane condensation has been used as a sign of poor ventilation that would be responsible for a humid indoor environment, promoting infestation of house dust mites (HDM) and mould growth as well as increasing levels of indoor pollutants by trapping air indoors.<sup>92-96</sup>

In recent years, a refinement of the measuring technique has made it possible to perform prolonged objective measurements of the ventilation rate in homes. The results give support for an association between low air change rate, increased indoor humidity and an increased risk of house dust mite infestation.<sup>97-101</sup> Concerning the home environment, a recently published review on the impact of building ven-



tilation concludes that ventilation rates above 0.5 air changes per hour seemed to reduce infestation of house dust mites in Nordic counties.<sup>102</sup> However, the evidence for an association between the ventilation rates in dwellings and respiratory symptoms and allergic diseases has been less convincing. In a recent Norwegian prospective birth cohort study, ventilation rate was not associated to bronchial obstruction in young children.<sup>89</sup>

### **Indoor air humidity**

Air humidity may either be expressed as absolute humidity, in grams of water per cubic metre ( $\text{g/m}^3$ ) or grams per kilogram of dry air ( $\text{g/kg}$ ), or as relative humidity (RH) that specifies the amount of water in the air in relation to its maximum at a given temperature. Further, the difference between the absolute outdoor and indoor humidity may be calculated, to assess the indoor contribution of moisture. A high indoor vapour contribution may increase the risk of condensation within the building construction, with a subsequent risk of microbial growth.

According to recommendations from the Swedish National Board on Health and Welfare, the indoor vapour contribution should not exceed  $3 \text{ g/m}^3$ , or the indoor humidity exceed  $7 \text{ g/kg}$  dry air for a prolonged period during heating season. This correspond to approximately 45% RH at  $21^\circ\text{C}$ .<sup>103</sup>

The impact of low indoor humidity on respiratory diseases and other indoor-related disorders is unclear, and only a few studies are available. In experimental assessment of the impact of low indoor humidity on the nasal mucosal function at different levels of air humidity, no physiological effects could be demonstrated.<sup>104;105</sup> Research further indicates that other exposures, such as high indoor temperature, particles, chemical compounds or even *increased* indoor humidity may be experienced as “dry air”.<sup>106;107</sup>

In contrast, there is increasing evidence in the literature that building moisture and increased indoor air humidity in buildings increase the prevalence of asthmatic symptoms. Two recent reviews conclude that there is strong evidence for an association between “dampness” and human health, even if the mechanisms are still not known.<sup>108;109</sup> The demonstrated risks are partly associated with increased indoor air humidity and partly related to dampness and signs of mould in the building construction.<sup>88</sup>

In a number of studies, it has also been demonstrated that increased indoor humidity promotes mite growth,<sup>98;110-112</sup> and that moisture in the construction may result in mould- and microbiologic activities,<sup>113</sup> and also initiates and increases the emissions of volatile organic compounds (VOC).<sup>114;115</sup>

### **Moisture and mould problems in buildings**

Understanding moisture and mould damages in buildings require knowledge of building structures as well as moisture physics. Vapour may enter into the building structure in several ways and may primarily be “built into the construction” by negligence during the erection time; building materials have perhaps not been sheltered from water or insufficient time has been allowed for drying out the construction. Moisture damages may occur as a consequence of leaks in the roof or the plumbing or through capillary movement of water in the building structure. As described above, humidity may also increase as a consequence of moisture-generating activities of the inhabitants themselves in combination with poor ventilation. Excessive moisture in buildings may promote microbial deterioration of the building materials and increase the risk that the occupants will be exposed to microbes. Mycotoxins produced by fungi and several toxigenic fungi have been isolated from both air samples and building materials in buildings with moisture problems.<sup>60;116</sup> Bacterial cytotoxic activities have also been detected in samples from water-damaged ceiling material following incubation on blood agar.<sup>117</sup> The microbial activity may result in production of secondary metabolites from gram-negative bacteria and mould, as well as emission of certain volatile compounds related to mould-microbial volatile compounds, MVOC.<sup>118-120</sup> However, the presence of mould spores indoors seems to be a poor indicator of dampness in buildings.<sup>113</sup>

Norbäck et al found that building dampness and microbial growth were related to current asthma and signs of inflammation in adults, with dampness in the floor construction having a particularly strong influence, and that immediate type allergy to moulds could explain some of these findings.<sup>121</sup> Exposure to house-dust-associated (1→3)-β-D-glucan in homes is shown to increase peak flow variability in asthmatic children.<sup>122</sup> Some authors further suggest that sensitisation to outdoor mould is associated with more severe allergic diseases and asthma,<sup>93;123-125</sup> but studies on children exposed to indoor mould in school buildings<sup>126</sup> and adults with respiratory symptoms in a population based case-control study indicate a more complex pattern.<sup>127</sup> Thus, sensitisation to moulds seems more to be an indicator of severity of disease than a marker of exposure.

### **Indoor allergens**

House dust mites (HDM) of the species *Dermatophagoides* are common in countries with a warm and humid climate. Mites live under conditions where they do not have access to liquid water, and are dependent on a mechanism that actively extracts water directly from the air. This mechanism will stop functioning when the humidity of the air decreases below a critical level. In cold temperate regions, the low moisture content of the outdoor air in combination with a long heating season for homes normally creates a dry indoor climate during the winter-period. Thus, at high altitude and in cold areas of Europe, residential mite growth is rare. Consequently, mite infestation in homes within the Stockholm area is not very prevalent.<sup>92;128;129</sup> However, in homes with apparent increased indoor humidity, mites may still be present and proliferate.<sup>130</sup> In temperate countries, excessive growth of HDM has been estimated to occur in homes that have an absolute indoor humidity exceeding 7 g/kg ( $\approx 45\%$  RH at 21 °C) for a prolonged period in wintertime.<sup>110;131-133</sup> The mites' droppings, which to some extent may become airborne, constitute the most important source of inhalable house dust mite allergen. House dust mites/ mite allergens will also be distributed passively in clothing to new indoor environments.<sup>134</sup>

House dust mite exposure in homes has been associated with sensitisation, but also to an increase in frequency and severity of asthma, in a number of surveys.<sup>92;98;101;135</sup>

A threshold level of 2000 ng/g for the risk of sensitisation to mite allergen and 1000 ng/g for attacks of asthma has been proposed,<sup>136;137</sup> but sensitisation at lower levels has been demonstrated.<sup>138</sup> Today, the suggested threshold level has lost its currency.

High levels of pet allergens have been found in homes both with and without furred pets, but also in settled dust from other indoor environments where pets have never been present.<sup>139-142</sup> The impact of such exposure will, however, not be further discussed in this thesis as it has little relation to building characteristics.

### **Indoor nitrogen dioxide (NO<sub>2</sub>) sources**

The main sources for indoor-generated NO<sub>2</sub> are tobacco smoke, gas stoves for cooking and gas appliances for heating. In homes with unvented cooking or heating appliances indoor concentrations of nitrogen dioxide may exceed outdoor levels.<sup>143</sup> In the region of Stockholm, however, gas appliances for heating are rare, whereas gas stoves for cooking are primarily used in some older buildings within the urban area. In several studies the indoor, or personal, NO<sub>2</sub> exposure levels have been assessed when evaluating the effect of ambient air pollution on human health.<sup>68;76</sup>

Several authors have demonstrated an increased risk of respiratory symptoms in children in homes where gas stoves are used.<sup>66;69;144-146</sup> Many of those studies indicate an increased risk primarily in girls.<sup>69;147</sup> Some studies also find a stronger association for indoor NO<sub>2</sub> exposure and respiratory health than for outdoor exposure.<sup>68</sup> Tunnicliff et al further suggest that domestic nitrogen dioxide exposure seems to potentiate the specific airway response of patients with mild asthma to inhaled house dust mite

allergen.<sup>148</sup> This was further strengthened by Ponsonby et al who found that current use of home gas was associated with HDM sensitisation and in addition to a stronger reduction of the FEV(1):FVC ratio among HDM-sensitive children.<sup>149</sup> On the other hand, others have failed to demonstrate any association between health and indoor NO<sub>2</sub> exposure and the use of gas stove for cooking<sup>74</sup> or demonstrate a moderate association between health and outdoor NO<sub>2</sub> exposure but not to indoor exposure levels.<sup>150</sup>

### ***Environmental Tobacco Smoke (ETS)***

The effects of ETS on the development of wheezing and asthma have been studied for decades and a large number of surveys on infant wheezing and early childhood asthma have given consistent evidence of an increased risk of such symptoms in children exposed to ETS.<sup>32;36;95;144;151-153</sup> Likely, exposure to ETS also has the potential to enhance the effect of exposure to other pollutants and airborne allergens and some surveys demonstrate an interaction between exposure to ETS, signs of a humid indoor environment and exposure to furred pet allergens on childhood asthma.<sup>94;154</sup> Further, there are indications of a joint effect between exposure to ETS and allergic heredity in relation to atopy.<sup>36;155</sup>

### ***Volatile Organic Compounds (VOC)***

There is growing concern about the impact of chemicals emitted into the indoor air. Volatile Organic Compounds (VOC) may easily vaporise at room temperature and concentrations may be higher in new buildings than in old.<sup>156-160</sup> In addition to the building structure, indoor smoking, furnishings - including floor coverings - textiles and household products constitute important sources for indoor-generated volatile organic compounds.<sup>157;161-163</sup> Based on studies conducted in non industrial work-places and experimental laboratory studies, it has been suggested that that ozone and nitrogen dioxide may react with certain VOCs to form formaldehyde and other oxygen-containing reactive compounds indoors.<sup>164-167</sup> Formaldehyde, in turn, is a well-known irritant that may be emitted from different sources within the building including tobacco smoke, various building components, furniture and paint.<sup>160;168</sup>

A recent review of the literature concerning health effects of VOC exposure in non-industrial indoor environments, concluded that indoor air pollution including VOC most likely is a cause of health effects and discomfort in indoor environments in non-industrial buildings, but that the scientific literature on the use of the total level of volatile organic compounds (TVOC) as a risk index for health is inconclusive.<sup>169</sup>

Experimental chamber studies, with controlled and blinded exposure, show that subjective unspecific symptoms may be related to VOC exposure, albeit at higher exposure levels than normally occur in home environments.<sup>170</sup> The literature also describes, that VOCs may influence the airways by induction of an inflammatory response, and a relation between VOCs in dwellings and airway diseases in both adults and children has been reported.<sup>168;171;172</sup> Recently, it has been suggested that maternal exposure to VOC may have an influence on the immune status of the new-born child.<sup>173</sup> Other surveys provide evidence that floor coverings containing PVC and plastic wall materials may be related to asthma symptoms in children, and linked to the use of the plasticiser di(2-ethyl hexyl)phthalate (DEHP) in such materials.<sup>80;174-176</sup> Diez et al further demonstrate an association between newly painted indoor surfaces and pulmonary infections and wheezing in the one-year-old child.<sup>172</sup>

It has also been indicated that exposure to formaldehyde in homes might invoke an inflammatory response in the airways of healthy children and increase the risk of childhood asthma.<sup>177-179</sup> Norbäck et al have further suggested that asthma symptoms in adults may be related to increased humidity in concrete floor constructions and emissions of 2-ethyl-1-hexanol, an indicator of dampness-related alkaline degradation of the plasticiser (DEHP).<sup>180</sup>

## EPIDEMIOLOGICAL STUDY DESIGN

*Epidemiology* (epi=among, demos=people, logos=doctrine) has been defined as “the study of the distribution and determinants of disease frequency in man” and is primarily concerned with the relationships between disease agents and health outcomes.<sup>181</sup> Epidemiology is based on two fundamental assumptions; first that disease does not occur randomly, and second that it may be possible to identify the causal factor for the disease, and thus that the disease may be preventable. The measure of disease frequency is therefore important and is usually expressed as *prevalence*, the proportion of a population having a disease at a specific time point, or *incidence* that quantifies the number of new events or cases that develop in a population at risk during a specified time interval.

The two main types of epidemiological studies are cohort studies and case-control studies. The word “*cohort*” (from the Latin word for one of the ten divisions of a Roman legion) is used to designate a group of people who share a common experience or condition and that are followed over a period of time to assess the occurrence of outcome; a birth cohort share a time of birth, etc. In the *cohort study* the population may be divided into two or more groups according to the extent of exposure to a potential risk factor. The *case-control study* starts from the outcome “the cases” (for example those classified as having “recurrent wheezing”) and compares various exposures of those subjects, with the exposure of a sample of individuals from the same population, but who are free from the outcome under study, “the controls”. A *nested case-control study* is conducted within a well-defined cohort of exposed and unexposed individuals. Case-control studies are often efficient in terms of cost and make it possible to perform assessment of certain exposures; i.e. based on information obtained by measurements that would not be possible to perform for the entire cohort. In the case-control study, the cases are the same as in the corresponding cohort whereas the controls represent - but are not comprised of - the entire cohort.

In case-control studies measures of association are often made by calculating the *ratio of the odds (OR)* of exposure among the cases, to that among the controls. The *confidence interval (CI)* represents the range within which the true magnitude of an effect lies, with a certain degree of assurance; the 95% CI is commonly used. If the null value (i.e.1) is not included in the CI, the association is defined as statistically significant, either representing decreased or increased odds.

In case-control studies, *selection bias* can occur whenever the inclusion of cases or controls into the study depends in some way on the exposure or another covariate related to the exposure. *Information bias* can occur whenever there are errors in the measurements on subjects. *Differential misclassification*, such as recall bias, may either increase or decrease the estimated odds ratio; *non-differential misclassification* occurs when inaccuracies exist in the categorisation of subjects by exposure or disease status, and will primarily result in a dilution of any effect. *Confounding* can be controlled for through restrictions, stratification into subgroups and through adjustment in the analyses by use of multivariate regression models.

## AIMS OF THE STUDY

The principal aim of this work was to study aspects of the indoor environment and their impact on human health, in terms of asthma and allergic diseases, particularly in young children, and all data are based on two main studies. The first study (*papers I and II*) investigates the association between indoor environment and house dust mite infestation in 59 similarly constructed single-family homes. The second study, the BAMSE study (*papers III-V*), investigates the association between indoor exposure and recurrent wheezing in very young children, based on a nested-case control study performed within the BAMSE birth cohort (acronym in Swedish for B=children, A=allergy, M=environment, in S=Stockholm, an E=epidemiologic study).

### ***The specific aims were:***

- ◇ to study the impact of different ventilation systems on indoor ventilation rate in single-family houses, and the influence of indoor ventilation on indoor humidity levels, house dust mite (HDM) infestation, and levels of volatile organic compound in the homes
- ◇ to evaluate the significance of windowpane condensation (WPC) and indoor vapour contribution as indicators of poor ventilation ( $<0.5$  ACH), high indoor humidity ( $\geq 7$  g/kg and  $\geq 45$  % RH) and high mite allergen concentration in mattress dust ( $\geq 2$   $\mu\text{g/g}$ )
- ◇ to assess the impact of building-related exposures, such as building construction, ventilation rate and indoor signs of dampness, etc., on recurrent wheezing in children up to the age of two years
- ◇ to study the association between  $\text{NO}_2$  exposure, including use of gas stove for cooking, and recurrent wheezing in children up to the age of two years.

## MATERIAL AND METHODS

### RECRUITMENT OF THE STUDY GROUPS

#### **Study 1 (papers I and II)**

Data were collected in a residential district located in the northern part of Stockholm County, Sweden. Fifty-nine one storey single-family houses were included. The residential area consisted of 250 houses with similar design, built in two stages during 1968 and 1970. Houses erected during the first stage were built with walls of lightweight concrete on crawl space foundations, the second group consisted of timber frame houses with brick-facing and concrete slab foundations. All houses were originally designed for natural ventilation. Twenty-two of the 59 investigated houses had been refitted and a mechanical supply and exhaust ventilation system installed after construction. In another eight houses the original natural ventilation had been adjusted, in order to improve the air change rate, table I.

**Table 1.** Ventilation and foundation characteristics of 59 single-family houses outside Stockholm, Sweden

<i>Ventilation</i> <i>Foundation</i>	<i>Mechanical supply and exhaust ventilation</i>	<i>Improved natural ventilation*</i>	<i>Natural ventilation</i>	<i>Total</i>
Crawl space	13	1	6	20
Concrete slab	9	7	23	39
Total	22	8	29	59

\* Installation of cooker hood/bathroom fan, new slot air valves and similar measures

Ventilation rate, indoor temperature, indoor air humidity and levels of VOC were measured simultaneously. Mattress dust was further analysed for content of house dust mite allergen.

The different methods used for evaluation of the indoor air will be further described below.

#### **Study 2, BAMSE (papers III-V)**

A case-control study was conducted within a longitudinal birth cohort study, BAMSE, with the main aim of investigating the impact of environmental exposures on the development of asthma and other allergy related diseases in children.<sup>37,182</sup>

The children in the cohort were born in parts of central and northern Stockholm, between February 1994 and November 1996, and identified in the Swedish Medical Birth Register. Recruitment to the study was carried out by the Child Health Care Centres (CHCC), attended by 99.8% of the eligible families within the catchment area, before the child was three months (mean age 2 months). Families planning to move within a year (n=699), those with insufficient knowledge of Swedish (n=331) and families whose infant suffered from a severe disabling disease (n=57) were not included. Another 169 children, who had an older sibling already enrolled in the study, were also excluded. In total 1,256 children were excluded according to these criteria. Amongst 7,221 infants born during the recruitment period 1,399 families never answered the questionnaire or declined participation and another 477 could never be reached due to incorrect address. Thus, the final study cohort represents 4,089 children (2,065 boys and

2,024 girls), which constitutes 75% of the 5,488 eligible children born in the study area during the recruitment period.

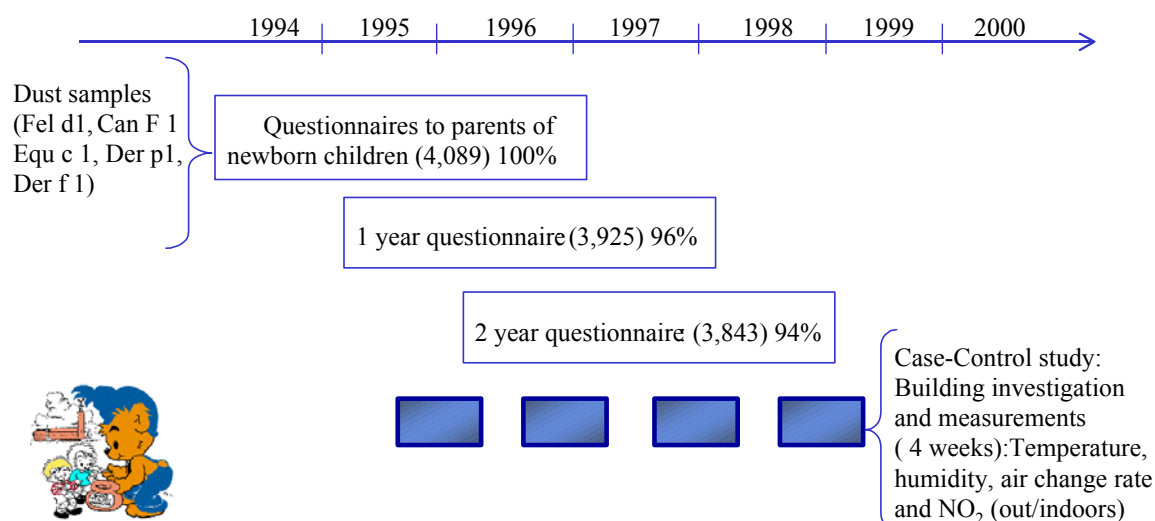
The parents of the children answered a first questionnaire, handed out by the CHCC nurse, focusing on allergic heredity, housing characteristics and various environmental exposures. In addition, dust was collected, by vacuuming the mother's bed, for further analyses of the content of house dust mite allergen. New parental questionnaires, focusing on symptoms of allergic diseases, were answered when the children were one and two years old, figure 1.

From the answers given in the symptom questionnaires, children with recurrent wheezing (for definition of outcome – see below, page 24) and two controls without recurrent wheezing were identified for inclusion in a nested case-control study. Cases and controls were age matched according to date of birth. Further, to be included in the nested case-control study, both cases and controls had to reside in the same dwelling as when they were born.

In total, 321 children with recurrent wheezing were identified from the cohort, but only 181 of these, 65 girls and 116 boys, had lived in the same home since birth. In the end, 540 children, both cases and controls, were recruited for the case-control study: 294 at the age of one year and 246 at the age of two. Three children, enrolled as controls at one year of age, fulfilled the criteria of recurrent wheezing at the age of two, and were included as cases at that age.

In the first winter season (October – March) following the child's recruitment to the case-control study, an environmental health officer performed a visual assessment of the child's home. Data on construction, ventilation system, water damage, etc, were collected using an inspection form, and indoor measurements were performed, figure 1.

**Figure 1.** *The BAMSE cohort study: flow chart*



### **Analyses of non-responders**

In 1996, an abridged version on two pages of the main questionnaire, with questions on environmental exposures, allergic heredity and recurrent wheeze, was sent to the families (n=1,418) who for different reasons did not participate in the BAMSE study. Nine hundred and fifty-four families (66%) answered this questionnaire and the answers were compared with those of the families included in the main study. Any or double heredity did not differ between the non-responders and excluded families compared to the study population of the 4,089 families, neither did exposure to pets. However, parental smoking was

significantly more prevalent among the non-responders and excluded families than among the participating families (maternal smoking: 18% compared to 9% in the BAMSE cohort; paternal smoking 23% vs 17% in the BAMSE-cohort). There was, however, no significant difference in parental smoking between non-responders and actively excluded families. Unfortunately, no questions on building related exposures were included in this substantially shortened questionnaire.

## **DEFINITION OF THE OUTCOME AND HEREDITY**

*Study I (paper I and II)* does not focus on outcome in terms of disease. Since the aim was to study the impact of different types of ventilation systems on indoor air quality, families with members suffering from asthma or any other allergy-associated disease were excluded from the study. This restriction was made to minimise the risk that measures had been undertaken to improve the indoor environment in other ways than through the change of ventilation system.

In the *BAMSE study (paper III-V)* recurrent wheezing was defined as follows: three reported episodes of wheezing or more, after three months of age, combined with use of inhaled steroids or symptoms of suspected bronchial hyperreactivity (wheezing or cough during excitement or play) excluding those with symptoms only when the child had a common cold. Episodes of wheezing during the first three months were not included in the criteria of definition, due to risk of misclassification of the disease.

Heredity for allergic diseases was based on information regarding parental atopy: doctor-diagnosed asthma and asthma medication and/or rhinitis, in combination with reported allergy to furred pets or to pollen, in one or both parents.

### ***Ethical aspects***

*Study I*, does not includes data on individuals, and ethical permission for this study was not needed.

*The BAMSE study (papers III-V)*, was approved by the ethical committee of Karolinska Institutet, Stockholm, Sweden. Parents were supplied with written information concerning the study and assented to participate with their child by filling in the first questionnaire.

## **ASSESSMENTS OF THE EXPOSURE**

### ***Questionnaires and inspection forms:***

*Study I*: a short inspection form was used.

*The BAMSE study*: three parental questionnaires were answered when the child was approximately two months, one year and two years of age, respectively. Information on environmental exposures was mainly asked for in the first questionnaire, but questions on windowpane condensation were asked in all three questionnaires. In addition, the inspector filled in an extensive inspection form at the time of inspection of the dwellings of cases and controls.

### ***Measurements:***

*Study I*: Measurements were performed for all homes during two consecutive weeks in March.

*BAMSE*: Four-week measurements were performed, once for each child, in the first winter period (October to March) following recruitment into the case-control study, i.e. measurements were performed in four different consecutive winter periods; first measurements in the autumn of 1995 and the last in the spring of 1999.

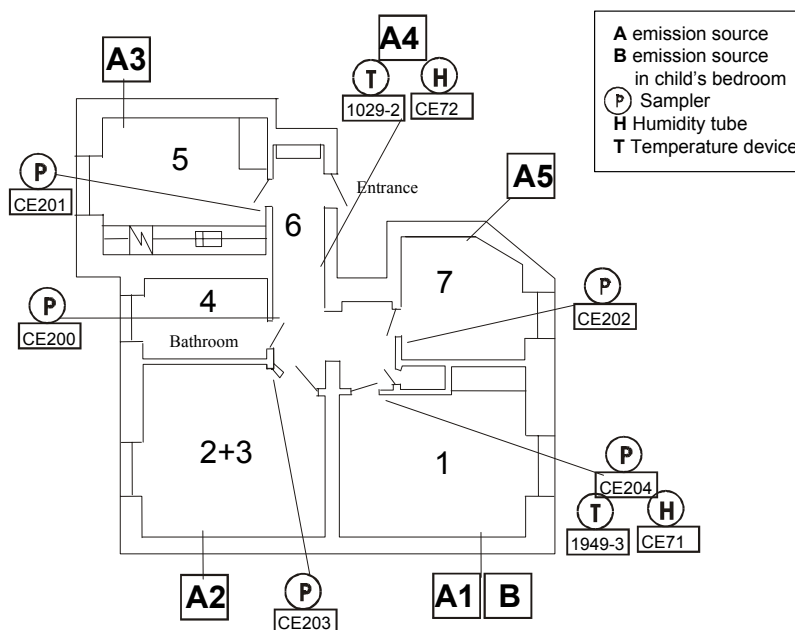


### Air Change Rate (I-V)

Indoor air change rate per hour (ACH) was measured using a passive tracer gas technique.

Tracer gas sources with a constant emission of perfluorocarbon tracer (PFT) were distributed in the dwellings, positioned at 1.8 m above floor level. In *Study 1*, two different sources emitting PFT were used, one for the bedroom and the other for the living room and the hall. In *BAMSE* the PTF sources (A) were distributed into each room of the home, adjusted to the volume of the room and in addition with a specific PTF source (B) placed in the child's bedroom, figure 2.

**Figure 2.** Placement of emission sources and measuring points



Passive air samplers (i.e. diffusion tubes with an active carbon adsorbent) were used and left open to collect tracer gas continuously over the periods of measurements.<sup>183;183</sup> After the sampling period, the samplers were capped and sent by mail to the laboratory for analysis, together with a protocol on sampling time, room volumes and positions of the equipment. At the laboratory the adsorbed compounds were extracted with a solvent and injected into a gas chromatograph for separation and quantitative analysis. The total average ventilation rate ( $\text{m}^3/\text{h}$ ) was computed from the calculated average tracer concentration, which was calculated from the average of the analysed amount of tracer compounds in the samplers using known air sampling rate. The air change rate was computed from the calculated ventilation rate, divided by the total volume of the dwelling. In *the first study*, the samplers were placed in the following positions: kitchen (close to cooker hood and close to the door to the laundry room), hall (close to the door to the bathroom and to a bedroom) and two samplers in one of the bedrooms. In *BAMSE*, samplers were placed close to the door in each room where tracer gas was distributed, figure 2.

### Temperature (I-V)

The temperature was registered in two rooms of each house (living room and one bedroom) using an electronic device (GTM20, Mitec, Säffle, Sweden). The temperature was converted to pulses and at regular intervals the data were stored in a computer register. Each device registered the average temperature during the 14-day (*Study 1*) or four-week (*BAMSE*) study period.<sup>184</sup>

*Humidity (I-V)*

The average relative indoor air humidity (RH) was measured continuously using diffusion sampling tubes, with lithium chloride hydrate as absorbent, placed close to the temperature device and measured during the same period as mentioned above.<sup>185</sup> Absolute and relative humidity levels were calculated. Data on outdoor temperature and outdoor air humidity during the study period were obtained from the Swedish Meteorological and Hydrological Institute. Furthermore, in papers *II and IV* the outdoor as well as indoor water vapour concentration expressed as grams of water per cubic meter of air ( $\text{g}/\text{m}^3$ ) was calculated from data on relative humidity and temperature during the period of investigation. The difference between outdoor and indoor vapour concentrations constitutes the indoor vapour contribution.

*Volatile Organic Compounds (paper I and II)*

Indoor Volatile Organic Compounds (VOC) were measured using diffusion samplers with Tenax TA as an adsorbent (Chemik Lab AB, Norrtälje, Sweden). After sampling, the contents of the tubes were analysed using thermal desorption (ATD-400 Perkin-Elmer) followed by gas chromatographic separation in high-efficiency capillary columns. Detection and quantitative analysis were performed using a mass selective detector (Iontrap ITD 800) and the yield of adsorbed substances was quantified by comparison with a standard. All values were expressed as toluene equivalents.<sup>186</sup> By comparing the levels analysed in the different groups of houses,  $200 \mu\text{g}/\text{m}^3$  was chosen as the lowest recommended standard.<sup>187</sup>

*Nitrogen dioxide; NO<sub>2</sub> (BAMSE, paper III-IV)*

NO<sub>2</sub> was measured indoors, in the main living room at about 1.7-1.8 m above the floor level, and outdoors outside the window of this particular room, using a passive method (Palmer tube).<sup>188</sup> To assess the precision, double measurements were performed at random in some of the 540 dwellings [132 (24.4%) of dwellings for indoor NO<sub>2</sub> and 121 (22.4%) for outdoor NO<sub>2</sub>]. The intra-class correlation coefficient for both indoor and outdoor NO<sub>2</sub> was 0.99.<sup>189</sup> Further, a comparison was made with NO<sub>2</sub> levels recorded by central urban monitors using the Chemiluminescent nitrogen oxide analyser, model AC 31 M (Environnement S:A, France) and DOAS (Differential Optical Absorption Spectroscopy), model AR500 (OPSIS AB, Sweden).<sup>190;191</sup>

*Mite allergens (paper I, II and IV)*

Two different techniques were used for the analyses of house dust mites (HDM).

*In Study I*, dust from bedroom mattresses from each home was collected in a standardised fashion by one of the authors using a nozzle attached to the hose of a vacuum cleaner (Volta Lite U1840, Volta, Sweden).<sup>192</sup> Separate filter cassettes were used for each of the mattresses. Dust was collected from the same bed as in a previous study.<sup>193</sup> After removal of bed linen and pillows each mattress was vacuumed for 2.5 minutes. The nozzle was washed and dried thoroughly between each sampling. Dust samples were analysed for group I allergen concentrations of the house dust mite species *Dermatophagoides pteronyssinus* (Der p 1), *Dermatophagoides farinae* (Der f 1) and *Dermatophagoides microceras* (Der m 1), by an enzyme-linked immunosorbent assay (ALK, Hørsholm, Danmark) and expressed as  $\mu\text{g}$  per gram of dust.<sup>194</sup> The detection limit was set to  $0.007 \mu\text{g}/\text{g}$  dust.

*In the BAMSE study (paper IV)*, dust was collected from the mother's mattress at the child's median age of two months. The family's own vacuum cleaner was used. A family member vacuumed the mattress, and the dust samples were sent by mail to the laboratory and kept in frozen equipment until the time of the analysis. Concentrations of house dust mite allergen (HDM) from *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides farinae* (Der f 1) were determined by a two-site ELISA using mono-

clonal antibodies and expressed as  $\mu\text{g/g}$  of fine dust.<sup>195</sup> The detection limit of the ELISA was 0.055  $\mu\text{g/g}$ .<sup>196</sup>

## STATISTICAL METHODS

*In Study 1 (paper I)*, statistical analyses were performed for houses with natural ventilation and mechanical ventilation only, due to the heterogeneity of the eight houses with improved natural ventilation. However, in the comparisons regarding mite allergen concentrations and ventilation rate or indoor air humidity, the eight houses with improved natural ventilation were also included. Median values and range were used in comparisons between different groups. Odds ratios according to Mantel-Haenszel and with 95% confidence intervals (maximum likelihood method) were used<sup>197</sup> as well as the Fischer exact test when appropriate. The Wilcoxon's two-sample test and Spearman's rank correlation test were used with two-tailed p-values.

In addition (*paper II*), sensitivity, specificity, predictive values and accuracy were calculated in order to assess to what extent "owner reported windowpane condensation" and "indoor water vapour contribution  $\geq 3 \text{ g/m}^3$ " can be used as markers of reduced ventilation ( $< 0.5 \text{ ACH}$ ), high indoor air humidity ( $> 7 \text{ g/kg}$ ) and high mite allergen concentrations in mattress dust ( $> 2 \mu\text{g/g}$  dust) (*paper II*).

*In the BAMSE study (paper III-V)*, statistical analyses were carried out using STATA software 7.0. A conditional logistic multivariate regression model was used to control for potential confounding factors. Cases and controls were matched on date of birth and the analyses were adjusted for gender and heredity of allergic diseases (defined as diagnosed asthma and asthma medication and/or rhinitis, together with allergy to furred pets or to pollen, in one or both parents), maternal smoking ( $\geq 1$  cigarette/day during pregnancy and at the time of answering the questionnaire when her child was two months old), duration of any breast-feeding ( $< 6$  months/  $\geq 6$  months) and building age in three groups (construction year up to 1939/1940-1975/1976 and onwards). Analyses associated with air change rate (ACH) were also adjusted for outdoor temperature, and analyses of indoor humidity adjusted for outdoor humidity levels. To assess a potential confounding effect of socio-economic status, we used a classification in accordance with the Nordic standard occupational classification and Swedish socio-economic classification (Statistics Sweden).<sup>198</sup> When we stratified for sub-groups, the matched data sets were dissolved since too many sets were otherwise lost, and unconditional logistic regression was used adjusted as above. Pearson's correlation coefficient was calculated for comparison of exposures, and a p-value below 0.05 was considered as significant.

Due to technical failure, some  $\text{NO}_2$  measurements were lost [7.2 % (40/540) of the indoor  $\text{NO}_2$  measurements and 7.7 % (42/540) of the outdoor  $\text{NO}_2$  measurements] (*paper III*). In order to reduce the loss of efficiency we used a multiple imputation method, in which we first generated 5 copies of the original data set; each of those with missing values replaced by values randomly generated by the imputation model.<sup>199</sup> After performing identical conditional logistic regressions on each of 5 data sets, the results were combined to produce overall estimates and standard errors. Multiple imputation was performed using NORM.<sup>200</sup>

Differences in proportions between certain reported exposures in the home, provided by parents with and without allergic asthma, were determined using two-sample tests for proportions (*paper V*).

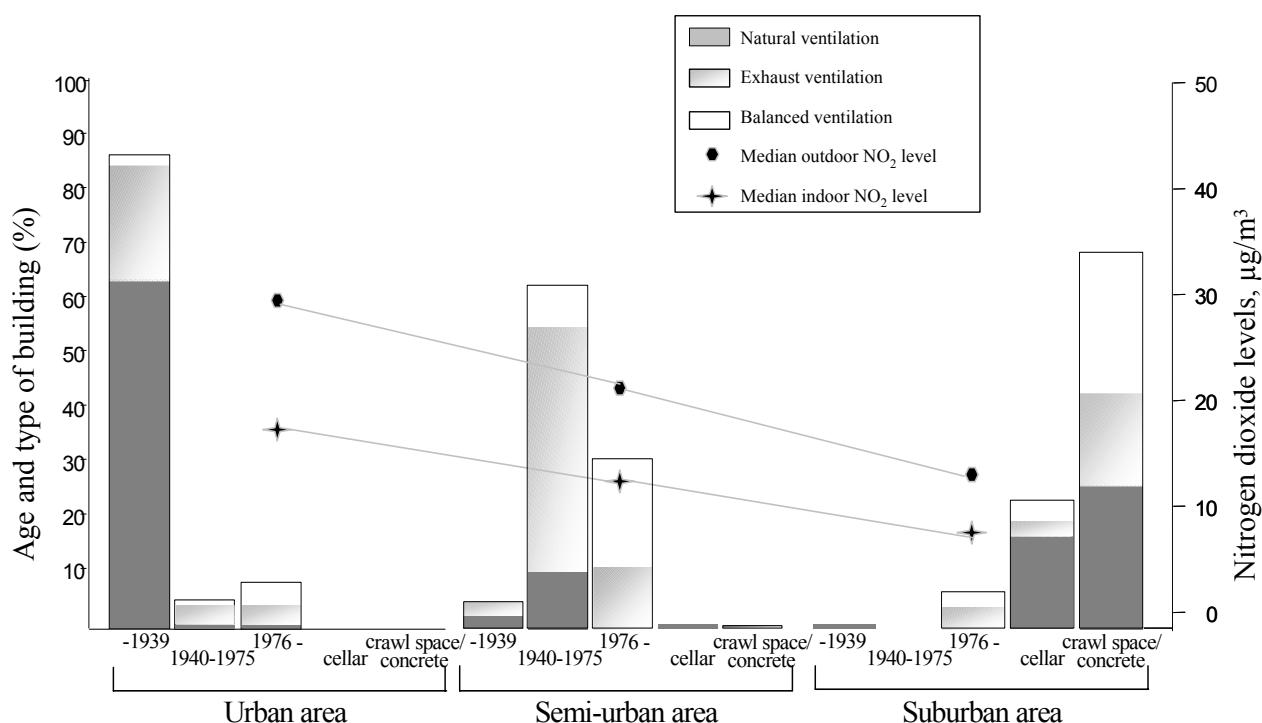
We also assessed the association between indicators of dampness and recurrent wheezing for the entire cohort, by use of unconditional logistic regression, adjusted as above (*paper V*).

## RESULTS

### BUILDING AGE AND VARIOUS HOUSING CONDITIONS

Most children (75%) in the BAMSE study (*paper III-V*) lived in apartment buildings and the rest in single-family homes (detached; semi-detached etc). The mean net residential floor area for apartments was 86.5 m<sup>2</sup> (SD 22.7) vs 126.0 m<sup>2</sup> (SD 28.6) for single-family homes. Both age and type of buildings were unevenly distributed within the study area. All children in the urban area lived in apartment buildings, of which 87% were built before 1940. In the area farthest away, approximately 25 kilometres from the urban centre, 57% of the children lived in single-family homes. The building's location within different geographical areas, also entails differences in NO<sub>2</sub> exposure, figure 3.

**Figure 3.** Distribution of buildings, including the frequency of installed ventilation system, and measured levels of NO<sub>2</sub> within the BAMSE study area



We found that building age was associated with infant recurrent wheezing, with an increased risk of wheezing in children living in buildings erected after 1940, and most pronounced for children living in homes built between 1976-1985, compared with those children living in houses built before 1940. This increased risk was further strengthened when nitrogen dioxide exposure (NO<sub>2</sub>) was included in the adjustment model, table 2.

There was no significant difference in recurrent wheezing, between the entire group of children living in single-family homes compared with those living in apartment buildings. Neither was home area (or room volume) associated with recurrent wheezing, either calculated as total area or available area per person. With buildings erected before 1940 used as reference category, however, primarily apartment buildings erected after 1939 and single-family homes with crawl space/concrete slab foundation appeared to be associated with an increased risk of recurrent wheezing, OR<sub>crude</sub> 1.7 (1.2-2.6) and OR<sub>crude</sub> 1.6 (0.9-2.8), respectively. Again, when adjustments were made for NO<sub>2</sub> exposure the odds ratios were further strengthened [OR<sub>adj</sub> 2.5 (1.3-4.8) and OR<sub>adj</sub> 2.5 (1.1-4.5) respectively]. It has to be

stressed that the year of construction is just a proxy variable and that the number of single-family homes with a cellar was low. In addition, due to loss of NO<sub>2</sub> data, risk assessments including NO<sub>2</sub> exposure are based on a reduced number of observations (498 vs 540).

**Table 2.** The influence of building age on recurrent wheezing in the children, up to two years, of the BAMSE case-control study

Exposure	Number (%) of cases N=181	Number (%) of controls N=359	Crude		Adjusted <sup>1</sup>		Adjusted <sup>2</sup>	
			OR	95% CI	OR	95% CI	OR	95% CI
Reference category: Buildings erected before 1940	35 (19.3)	102 (28.4)						
Buildings erected 1940-1960	34 (18.8)	65 (18.1)	1.5	0.9-2.7	1.8 <sup>1</sup>	1.0-3.2	2.5 <sup>2</sup>	1.3-4.8
Buildings erected 1961-1975	49 (27.1)	88 (24.5)	1.7	1.0-2.9	1.7 <sup>1</sup>	0.9-2.9	2.1 <sup>2</sup>	0.5-4.5
Buildings erected 1976-1985	37 (20.4)	51 (14.7)	2.2	1.2-4.0	2.1 <sup>1</sup>	1.1-3.9	2.7 <sup>2</sup>	1.1-5.4
Buildings erected 1985 -	26 (14.4)	53 (14.8)	1.4	0.8-2.7	1.6 <sup>1</sup>	0.8-3.2	2.2 <sup>2</sup>	1.1-8.0

1) Adjusted for gender, heredity, maternal smoking, breast feeding

2) Adjusted for gender, heredity, maternal smoking, breast feeding and NO<sub>2</sub> exposure ( based on 498 observations)

## Ventilation

In Study 1, homes with mechanical ventilation had higher air change rates than those still equipped with originally installed natural ventilation system (mean 0.59 ACH vs 0.27 ACH,  $p < 0.0001$ ) and mechanical ventilation correlated significantly with lower indoor humidity. Consequently, lower concentrations of volatile organic compound (TVOC) and lower levels of mite allergen in mattress dust were found in these houses compared with homes equipped with originally installed natural draft ventilation system (*paper I*), table 3.

**Table 3.** Median values and range for some measured indoor parameters in 59 single-family houses outside Stockholm

<i>Ventilation system</i>	Mechanical supply and exhaust ventilation N=22	Natural ventilation N=29	Improved natural ventilation* N=8	Significance for mechanical vent. vs natural vent**
<i>Measured indoor parameters</i>				
Air change rate (ACH)	0.59 (0.3-1.08)	0.27 (0.1-0.77)	0.25 (0.23-0.51)	$p < 0.0001$
Relative indoor humidity, %	31.3 (27.5-38.0)	40.3 (31.5-53.0)	37.3 (35.5-53.5)	$p < 0.0001$
Absolute indoor humidity, g/kg	4.9 (4.3-5.5)	5.9 (5.1-7.7)	5.8 (5.3-7.7)	$p < 0.0001$
Mite allergen in mattress dust, $\mu\text{g}/\text{m}^3$	0.02 (0.0-1.5)	0.07 (0.0-15.2)	0.1 (0.0-64.5)	$p = 0.04$
TVOC levels, $\mu\text{g}/\text{m}^3$	149 (57-483)	373 (144-4 819)	288 (192-3 208)	$p < 0.0001$

\* Installation of cooker hood/bathroom fan, new slot air valves and similar measures

\*\* Statistical analyse were performed for houses with natural ventilation and mechanical ventilation only, due to the heterogeneity of the eight houses with improved natural ventilation

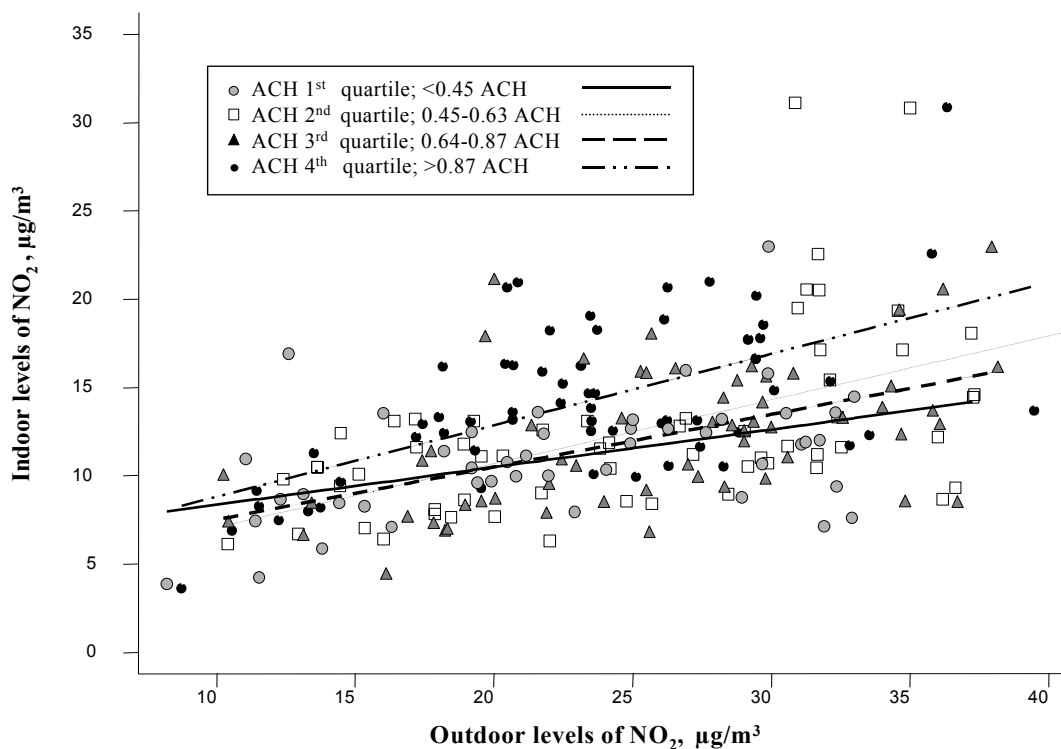
The influence of the type of ventilation system on air change rate (ACH) and indoor humidity also held true for the entire group of BAMSE homes (*paper IV*), but air change rates were generally higher in this study, 0.73 ACH for mechanically ventilated homes vs 0.57 ACH for homes with natural ventilation,  $p > 0.0001$ . For a sub-group of homes of the BAMSE study, with a ground construction similar to that in

the homes of Study 1 (crawl space/concrete slab), the difference was still apparent though not significant, 0.53 ACH vs 0.39 ACH,  $p=0.173$ . In the BAMSE study, no association was demonstrated between the type of ventilation system and levels of house dust mite allergen. The amount of TVOC was not measured in this study.

Air change rate ACH  $<0.5$  was primarily related to single-family houses with natural ventilation. In Study 1, only 5 of the houses with mechanical supply and exhaust ventilation fell below 0.5 ACH compared with 24 of the 29 houses with natural ventilation OR 0.06 (0.01-0.2) (*paper I*). In study 2, the BAMSE study, single-family homes built on crawl space or concrete slab foundation and equipped with a natural ventilation system, was the only category of buildings with a group mean below 0.5 ACH (*paper IV and appendix*). In Study 1, none of the 23 houses with an ACH  $\geq 0.5$  had an absolute indoor humidity (AIH) of 7 g/kg air or more, compared with 10 of the 36 houses with an ACH  $<0.5$  ( $p=0.01$ ). In none of the 23 houses with an ACH  $\geq 0.5$  were concentrations of mite allergen exceeding 2  $\mu\text{g/g}$  of dust found, compared with six (17%) of the 36 houses with an ACH below 0.5 ( $p=0.04$ ) (*paper I*).

In the BAMSE study, no significant relation was found between type of ventilation system and indoor humidity levels. In 69% of the homes air change rate exceeded 0.5 ACH. Among those houses with ACH  $<0.5$ , most still held humidity levels  $<7$  g/kg. Increased indoor humidity  $\geq 7$  g/kg, was, however, significantly more common in homes with low ventilation rate compared with homes exceeding 0.5 ACH, 31.0% vs 9.2%,  $p<0.0001$ , with similar proportion for the entire group of homes as for the subgroup of single-family houses with crawl space/concrete slab foundation. House dust mite (HDM) allergens were detected in 7% of the dust samples. Only nine homes held levels above 0.5  $\mu\text{g/g}$  dust, and four of these had levels above 1  $\mu\text{g/g}$  dust (2 cases and 2 controls) (*paper IV*). Thus, no meaningful assessment of the relationship between ACH/indoor humidity and mite allergen concentrations in mattress dust could be made.

Figure 4. Correlation between outdoor and indoor  $\text{NO}_2$  levels in homes, without internal sources of  $\text{NO}_2$ , with the regression lines for different quartiles of measured air change rate



For both studies (*paper I and III*), a negative correlation was demonstrated between air change rate and absolute indoor air humidity, even though somewhat weaker for the homes of BAMSE compared with the homes in Study 1;  $r=-0.64$   $p<0.0001$  for Study 1 (*paper I*),  $r=-0.42$ ,  $p<0.001$  for the entire group of

BAMSE homes (*paper IV*) and  $r=-0.38$ ,  $p=0.002$  for the subgroup of single-family houses with crawl space/concrete slab foundation.

In contrast, a weak positive correlation,  $r=0.35$ ,  $p<0.001$ , was found between ACH and indoor  $\text{NO}_2$  levels for homes without internal sources of  $\text{NO}_2$ , i.e. no use of gas stove for cooking or heating and no tobacco smoke (*BAMSE, paper III, IV and appendix*), figure 4.

In the BAMSE study (*paper IV*), neither any certain ventilation system, nor the air change rate, was found to be directly associated with recurrent wheezing in children up to the age of two years, table 4.

**Table 4.** The influence of installed ventilation system and measured levels of air change rate, indoor temperature and humidity on recurrent wheezing in children, up to two years of age, of the BAMSE case-control study

Exposure	Number (%) of cases N=181	Number (%) of controls N=359	Adjusted <sup>1-3</sup>	
			OR	95% CI
Exhaust ventilation (vs natural)	85 (47.0)	151 (42.1)	1.1 <sup>1</sup>	0.6-2.0
Balanced ventilation (vs natural)	43 (23.8)	78 (21.8)	0.8 <sup>1</sup>	0.4-1.7
Ventilation rate $\geq 0.5$ ACH	130 (71.8)	240 (67.0)	1.3 <sup>2</sup>	0.8-2.0
Absolute Indoor Humidity >median, 5.8 g/kg	102 (56.4)	167 (46.7)	1.7 <sup>3</sup>	1.0-2.9
Relative Humidity >45%	25 (13.8)	43 (12.0)	0.8 <sup>3</sup>	0.4-1.5
Indoor temperature >median, 21.7 °C	93 (51.4)	182 (50.7)	0.9 <sup>1</sup>	0.6-1.4
Window pane condensation 4 questionnaires	26 (14.4)	26 (7.2)	2.2 <sup>1</sup>	1.1-4.5

1) Adjusted for gender, heredity, maternal smoking, breast feeding and building age

2) In addition, adjusted for outdoor temperature

3) In addition, adjusted for absolute outdoor humidity

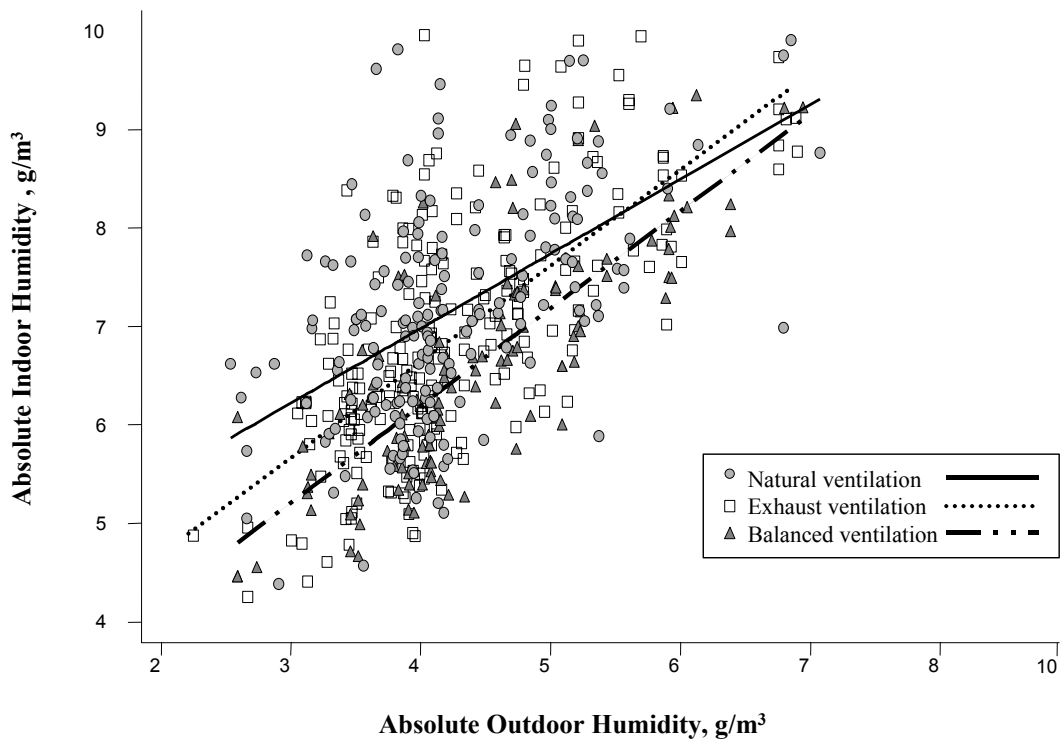
## SIGNS OF DAMPNES

### Indoor humidity

In the BAMSE study, measurements of absolute indoor humidity showed a mean for all measurements of 5.8 g/kg (6.9 g/m<sup>3</sup>) and there was a strong correlation between the two indoor measurements of humidity that were performed simultaneously in different parts of the home,  $r=0.95$ ,  $p<0.001$  (*paper I and appendix*). Even though ventilation rate was not directly associated with recurrent wheezing, the risk of such symptom was greater for children living in homes with indoor absolute humidity above the median, >5.8 g/kg, than for children exposed to lower levels of humidity, OR 1.7 (1.0-2.9) (*paper IV*), table 4. This increased risk was still present in homes with a ventilation rate above 0.5 ACH (data not shown). No association was seen between vapour contribution within the home and infant recurrent wheezing (data not shown).

The indoor humidity levels were strongly affected by outdoor humidity, and  $r=0.63$ ,  $p<0.001$ , Figure 5.

Figure 5. Correlation between outdoor and indoor humidity in homes of the children in the BAMSE case-control study, and the regression lines for different types of ventilation systems

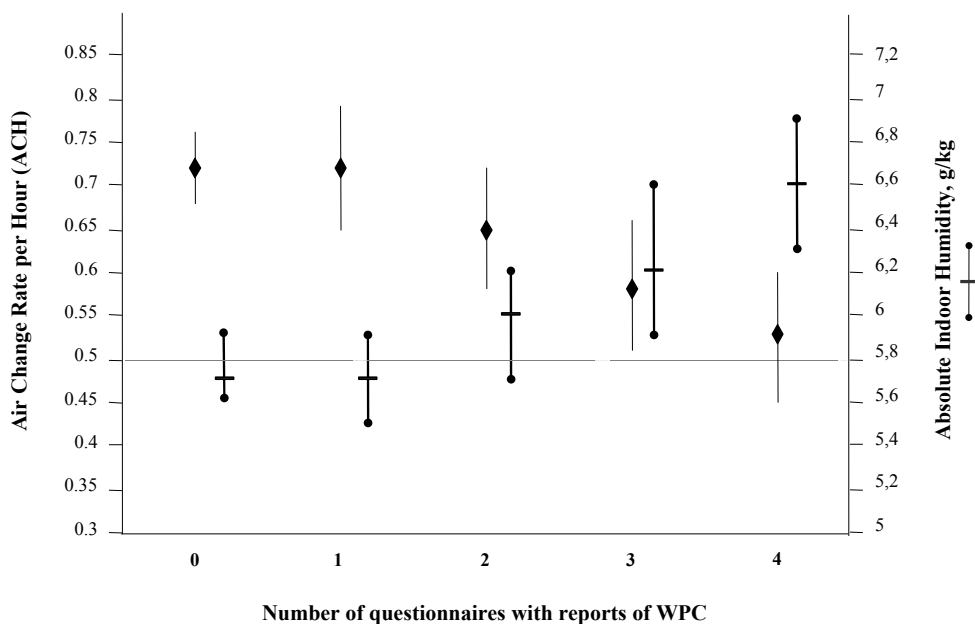


**Window pane condensation and indoor vapour contribution**

In *Study I*, ACH was significant lower and indoor humidity and levels of dust mite allergens higher in homes with reported window pane condensation and/or a measured vapour contribution  $\geq 3 \text{ g/m}^3$ , when compared with dwellings without these markers (*paper II*), figure 6.

The data of the BAMSE study were more complex in terms of data analysis and interpretation, as parents answered the question on windowpane condensation on four different occasions. The more often the parents reported WPC, the higher were the measured indoor humidity and lower the ACH (*paper IV*), figure 6.

Figure 6. Reported windowpane condensation in the homes of the children in the BAMSE case-control study, in relation to measured air exchange rate and absolute indoor humidity





An increased risk of recurrent wheezing was demonstrated for children in homes where windowpane condensation was consistently reported over a span of years, OR 2.2 (1.1-4.5), table 3.

Data from the BAMSE study further show that air change rates were significantly lower in homes with an indoor vapour contribution  $\geq 3 \text{ g/m}^3$ , compared with homes with a lower indoor vapour contribution: mean ACH 0.47 vs 0.78,  $p < 0.0001$ , for the entire group of homes, and mean ACH 0.35 vs 0.58,  $p = 0.027$ , for single-family homes with concrete slab/crawl space foundation. In addition indoor humidity levels were higher, although the difference was not statistically significant - neither for the entire group of BAMSE homes nor for the subgroup of single-family houses with crawl space/concrete slab foundation.

In Study 1, windowpane condensation and indoor vapour contribution  $\geq 3 \text{ g/m}^3$  were used as indicators of poor ventilation ( $< 0.5$  ACH) and high indoor humidity ( $\geq 7 \text{ g/kg}$ ) (*paper II*). The report of no window pane condensation on the interior surface of double-glazed windows in winter and a measurement of a vapour contribution below  $3 \text{ g/m}^3$ , indicated (90-100%) low indoor air humidity and low mite allergen concentrations in mattress dust, table 5.

**Table 5.** Sensitivity, specificity, predictive values and the accuracy of condensation and indoors vapour contribution  $\geq 3 \text{ g/m}^3$ , as markers for different indoor humidity variables

		Sensitivity %	Specificity %	Pos. predictive value		Neg. predictive value		Accuracy %
				N	%	N	%	
<b>Condensation*</b>								
<i>Study 1</i>	/ ACH $< 0.5$	75	70	27/34	<b>80</b>	16/25	<b>64</b>	73
	/ AIH $\geq 7 \text{ g/kg}$	100	51	10/34	<b>29</b>	25/25	<b>100</b>	59
	/ AIH $\geq 5.8 \text{ g/kg}$	82	73	27/34	<b>79</b>	19/25	<b>76</b>	78
<i>BAMSE</i>	/ ACH $< 0.5$	46	71	77/183	<b>42</b>	258/347	<b>74</b>	63
	/ AIH $\geq 7 \text{ g/kg}$	58	70	49/182	<b>27</b>	312/348	<b>90</b>	68
	/ AIH $\geq 5.8 \text{ g/kg}$	46	76	119/182	<b>65</b>	203/348	<b>58</b>	61
<i>BAMSE**</i>	/ ACH $< 0.5$	57	61	31/47	<b>66</b>	25/48	<b>52</b>	59
	/ AIH $\geq 7 \text{ g/kg}$	82	60	18/47	<b>38</b>	44/48	<b>92</b>	65
	/ AIH $\geq 5.8 \text{ g/kg}$	67	76	38/47	<b>80</b>	29/48	<b>60</b>	71
<b>Vapour contribution <math>\geq 3 \text{ g/m}^3</math></b>								
<i>Study 1</i>	/ ACH $< 0.5$	56	91	20/22	<b>91</b>	21/37	<b>57</b>	70
	/ AIH $\geq 7 \text{ g/kg}$	100	76	10/22	<b>45</b>	37/37	<b>100</b>	80
	/ AIH $\geq 5.8 \text{ g/kg}$	67	100	22/22	<b>100</b>	26/37	<b>70</b>	81
<i>BAMSE</i>	/ ACH $< 0.5$	62	82	99/162	<b>61</b>	294/355	<b>83</b>	76
	/ AIH $\geq 7 \text{ g/kg}$	76	77	60/162	<b>37</b>	337/356	<b>95</b>	77
	/ AIH $\geq 5.8 \text{ g/kg}$	54	92	141/162	<b>87</b>	237/356	<b>67</b>	73
<i>BAMSE**</i>	/ ACH $< 0.5$	65	86	33/38	<b>86</b>	32/50	<b>64</b>	74
	/ AIH $\geq 7 \text{ g/kg}$	84	68	16/38	<b>42</b>	47/50	<b>94</b>	72
	/ AIH $\geq 5.8 \text{ g/kg}$	62	83	32/38	<b>84</b>	30/50	<b>60</b>	70

\* Window pane condensation on the interior side of double-glazed windows (*inspection data*)

\*\* Subgroup of BAMSE with single-family homes built on crawl space or concrete slab foundation

When applied to the BAMSE case-control study, WPC reported by the parents at the time of inspection was found to be a somewhat weaker indicator of an ACH  $< 0.5$ , compared with results of paper II, even though 63% of the entire group of homes were correctly classified vs 73% in Study 1. The accuracy of WPC as an indicator of AIH  $\geq 7 \text{ g/kg}$ , was on the other hand slightly stronger, 68% vs 59%. The usefulness of indoor vapour contribution as an indicator of deficient indoor air quality was of similar magnitude for the BAMSE study as for Study 1 (*paper II*). Further, in the BAMSE study, window pane condensation and indoor vapour contribution, as indicators of indoor humidity above the median of

BAMSE, AIH 5.8 g/kg, showed high specificity and 65-100% positive predictive values (*these data are only presented in this thesis and not in any separate paper*).

### **House dust mites**

In *Study I*, designed to study mite infestation in single-family homes in relation to ventilation differences, mite allergens were detected in mattress dust in 73% of the homes. Concentrations of mite allergen in mattress dust  $\geq 2$   $\mu\text{g/g}$  occurred in six of the 34 houses where the owner reported windowpane condensation, compared with none of the 25 houses without such condensation ( $p=0.03$ ). For vapour contribution the corresponding figure was 1 of 37 houses with vapour contribution below 3  $\text{g/m}^3$ , compared with 5 out of 22 houses exceeding 3  $\text{g/m}^3$ ,  $p=0.02$  (*paper II*). In those houses with levels of HDM allergen exceeding 0.1  $\mu\text{g/g}$  dust, *Dermatophagoides pteronyssinus* (Der p 1) was present in 73% of the dust samples, *Dermatophagoides farinae* (Der f 1) in 79% and *Dermatophagoides microceras* (Der m 1) in 68% of the samples. Occurrence of Der m 1 only, was found in 17% of the samples positive for HDM. In homes with HDM levels  $\geq 1$   $\mu\text{g/g}$  all three species were equally frequent (6/8, 5/8 and 5/8 samples, respectively).

In contrast to Study 1, we found no associations between ventilation rate/indoor humidity and house dust mite allergen in study IV. However, very few homes seemed to be infested by house dust mites and only 9 homes held allergen levels of HDM  $>0.5$   $\mu\text{g/g}$  (*paper IV*).

Unfortunately, we did not have the possibility to analyse the presence of *Dermatophagoides microceras* (Der m 1) in BAMSE (*paper IV*), since extract containing Der m 1, was no longer available at the time of the study. In addition, due to change of laboratory the detection limit for the other mite allergens had risen from 0.007  $\mu\text{g/g}$  in Study 1 to 0.055  $\mu\text{g/g}$  in the BAMSE study.

### **Moisture and mould**

In the BAMSE study (*paper V*), there was strong and consistent evidence for an association between recurrent wheezing and indicators of dampness, both as reported prospectively and/or as observed on site during inspections the winter season after the children with recurrent wheeze had been identified. The association was further strengthened when exposure to an increased indoor humidity was included as an indicator of dampness, expressed as an absolute indoor humidity above the median concentration of 5.8 g/kg, or window pane condensation reported in at least three of the four consecutive questionnaires, table 6, next page.

Further, a trend was found between the risk of recurrent wheezing in relation to number of indicators of dampness in combination, with a higher risk for children exposed to several such indicators. Three or more signs of dampness were associated with an OR of 2.7 (1.2-5.4) The consistent association between indicators of dampness and recurrent wheezing was also shown for data for the whole cohort.

In the BAMSE study, it was possible to compare the parents' prospective reports of home dampness with signs of dampness observed by the inspectors. Parents of the children with single or double allergic heredity more often reported problems with moisture, mould or windowpane condensation (WPC), figure 7, next page. Looking at the same data for the entire cohort such a trend could be confirmed. The coherence between parental reports vs inspector notes of any damp or mould as well as parental reports vs inspector notes of WPC was rather weak, 34% and 63%, respectively, even though significantly associated with recurrent wheezing ( $p<0.001$ , both).

**Table 6.** Case-control data obtained within the BAMSE birth cohort study, presenting OR and 95% CI for recurrent wheezing in children at the age of two, in relation to various indoor exposures related to dampness during the first two years of life

Exposure	Number (%)		Adjusted <sup>(1)</sup>	
	of cases N=181	of controls N=359	OR	95% CI
Any dampness prospectively reported <sup>(2)</sup>	54 (29.8)	86 (24.0)	1.4	0.9- 2.2
Mould odour prospectively reported <sup>(2)</sup> -yes vs no	20 (11.0)	23 (6.4)	2.0	1.0- 3.9
Dampness observed at home inspection:				
- mould spots on surface material/ tile joints in wet areas (shower/ bath room)	22 (12.2)	52 (14.5)	1.0	0.5- 1.7
- any sign of dampness <sup>(3)</sup>	53 (29.3)	81 (22.6)	1.6	1.0- 2.5
-Any signs of dampness <sup>(3)</sup> reported WPC <sup>(4)</sup> >2/AIH>5.8 <sup>(5)</sup>	43 (23.9)	51 (14.6)	2.0	1.2- 3.4
Prospectively reported dampness <sup>(2)</sup> or currently at inspection	69 (38.1)	109 (30.3)	1.5	1.0- 2.3
Signs of dampness <sup>(3)</sup> both prospectively <sup>(2)</sup> and currently at inspection	19 (10.5)	29 (8.1)	1.8	0.9- 3.5
WPC <sup>(4)</sup> in all 3 questionnaires + inspection form	26 (14.4)	26 (7.2)	2.2	1.1- 4.5
Repainting – in dwelling but not in child’s room <sup>(6)</sup>	30 (16.6)	75 (20.9)	1.1	0.7- 1.9
“ – in dwelling, including child’s room <sup>(6)</sup>	74 (40.9)	110 (30.6)	1.7	1.1- 2.6

(1)Analyses performed with unconditional logistic regression; adjusted for gender, heredity, maternal smoking, breast feeding and building age

(2)Exposure questionnaire, answered when child was at a median age of two months

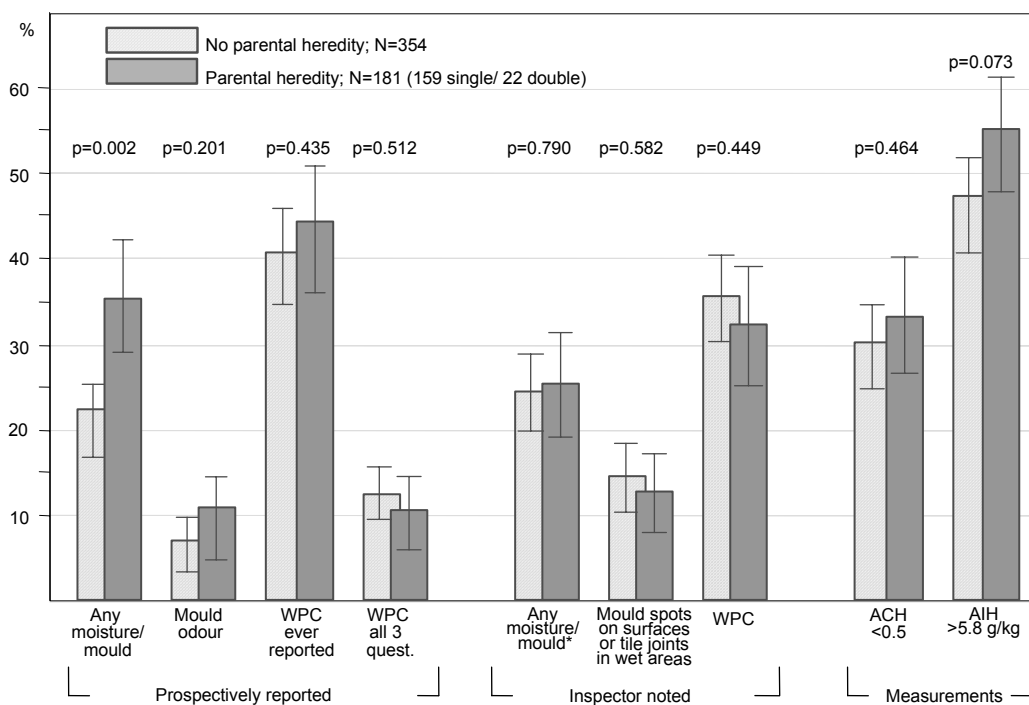
(3)Damp stain/mould-mildew-spots/mould odor etc; not included: mould spots on surfaces in wet areas and WPC

(4)WPC = windowpane condensation on the interior side of double glazed windows

(5)Absolute indoor humidity (AIH) above median level 5.8 g/kg

(6)Reported at the time of inspection

**Figure 7.** Prospectively reported and currently noted and measured by inspector: presence of various indicators of indoor dampness including indoor humidity in homes of families with or without parental allergic disease. Data given in percentages



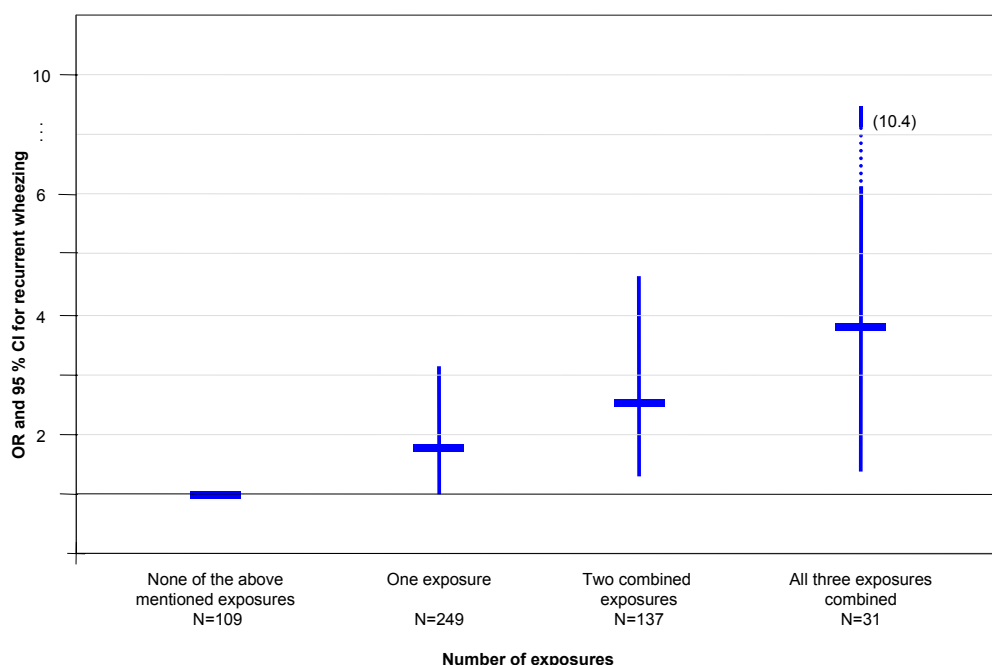
\* not including mould spots on surfaces in wet areas and window pane condensation as signs of dampness

## VOLATILE ORGANIC COMPOUNDS (VOC)

In study I, all houses were originally constructed with a garage connected to the house, but for 10 of the houses the garage was used for other purposes than in the original planning. Petrol fumes could be detected in eight of the 49 houses where the garage was used for parking cars, compared with none of the 10 houses where the garage was used for other purposes (ns). No correlation between petrol fumes and ACH levels was found ( $p=0.2$ ) (*paper I*).

In BAMSE, more than half of the families reported that some part of the interior of their home had been painted during the year before their child's birth or first year of life. Fifty-two percent of the families had used water-based latex paint only, and in total 88% had used such paint to some degree. Repainted surfaces in the child's bedroom was associated with an increased OR for recurrent wheezing, 1.7 (1.3-2.6) (*paper IV*). In addition, a positive trend was suggested for combined exposure to different indicators of dampness, exposure to environmental tobacco smoke (ETS) and repainting of the child's bedroom, reaching an odds ratio of 4.0 (1.4-10.4), figure 8.

**Figure 8.** OR and 95% CI for recurrent wheeze in relation to joint effects of (1) interior painting of the child's room before or after birth, (2) current ETS and (3) current measured increased indoor humidity\*



\* absolute indoor humidity above median (5.8 g/kg) or presence of WPC according to at least 3 of 4 possible reports

Neither exposure to a new PVC floor coating, nor the use of wall-to-wall carpets was associated with an increased risk of infant recurrent wheezing (data not presented).

## AIR POLLUTION INCLUDING NO<sub>2</sub>

The mean NO<sub>2</sub> level for all outdoor measurements was 21.8 µg/m<sup>3</sup> (SD 6.3). Levels were highest in urban areas and decreased in areas less exposed to traffic, and this was true both for outdoor and indoor levels, figure 3, page 28. Mean indoor NO<sub>2</sub> exposure was 12.6 µg/m<sup>3</sup> (SD 8.5). Indoor NO<sub>2</sub> levels were affected by outdoor generated NO<sub>2</sub>, with a clear correlation between outdoor and indoor NO<sub>2</sub> levels in homes where gas was not used and where no family member smoked,  $r=0.69$ ,  $p<0.001$ , and highly increased for homes where a gas stoves was used for cooking. Gas stoves (46 homes) were mainly present in buildings erected before 1940, which predominated in the urban area. Within this area, mean levels of NO<sub>2</sub> in homes where gas stoves were used was 22.6 µg/m<sup>3</sup>, compared with 16.4 µg/m<sup>3</sup> in homes with-

out gas. In contrast, in homes without gas stoves, mean indoor levels of NO<sub>2</sub> were only slightly different for homes of the children with smoking parents compared with homes of non-smokers, 12.3 µg/m<sup>3</sup> (SD 4.9) and 11.5 µg/m<sup>3</sup> (SD 5.5), respectively.

An interesting finding was the inverse relation between NO<sub>2</sub> exposure and building age, which became important for the confounding control in the risk assessments, figure 3, page 28, table II, page 29. When building age was included in the model, an increased risk of recurrent wheezing was suggested, though not statistically significantly different from one, table 7, and this was particularly evident for children who fulfilled the criterion of recurrent wheezing at two years of age but not at one.

**Table 7.** Odds ratios and 95% confidence intervals for recurrent wheezing in children up to the age of two, in the BAMSE case-control study, in relation to measured and calculated annual outdoor and indoor NO<sub>2</sub> exposure

		Concentration of NO <sub>2</sub> (µg/m <sup>3</sup> )							
		<25 perc.		25-50 percentile		51-75 percentile		>75 percentile	
Controls	Cases	OR	OR*	95%CI	OR*	95%CI	OR*	95%CI	
<b>INDOOR EXPOSURE<sup>1</sup></b>									
<i>Based on measurements</i>									
-in quartiles	330	170	1	0.96	0.52-1.77	1.08	0.57-2.03	1.51	0.81-2.82
- exposed to >75 perc. vs. lower			1					1.48	0.91-2.42
<b>OUTDOOR EXPOSURE<sup>2</sup></b>									
<i>Based on measurements</i>									
- in quartiles	332	168	1	0.76	0.42-1.36	0.80	0.43-1.47	1.60	0.78-3.26
- exposed to >75 perc. vs. lower			1					1.92	1.05-3.53
<i>Based on calculated annual average exposure</i>									
- in quartiles	332	171	1	0.73	0.41-1.31	0.71	0.38-1.34	1.74	0.87-3.49
- exposed to >75 perc. vs. lower			1					1.92	1.05-3.53

\* Adjusted for gender, heredity, maternal age and smoking, any breast feeding and building age

<sup>1</sup> Indoor NO<sub>2</sub> exposure, range within different quartiles: <8.4 µg/m<sup>3</sup>; 8.4-11.6 µg/m<sup>3</sup>; 11.7- 15.6 µg/m<sup>3</sup> and >15.6 µg/m<sup>3</sup>, respectively

<sup>2</sup> Outdoor NO<sub>2</sub> exposure, range within different quartiles: <14.8 µg/m<sup>3</sup>; 14.8-21.3 µg/m<sup>3</sup>; 21.4-28.4 µg/m<sup>3</sup> and >28.4 µg/m<sup>3</sup>, respectively

The adjusted OR for recurrent wheezing associated with the use of a gas stove was 1.72 (0.77-3.87), without any apparent gender differences.

Another intriguing finding in this study was an interaction between NO<sub>2</sub> exposure and exposure to environmental tobacco smoke, in this paper defined as “any smoking family member” at the time of inspection. When NO<sub>2</sub> exposure was divided on the 4<sup>th</sup> quartile, OR for exposure to both NO<sub>2</sub> and ETS was 3.10 (1.32-7.30), compared with children unexposed to ETS and in the lower quartile of NO<sub>2</sub>. The corresponding interaction for outdoor NO<sub>2</sub> levels did not reach statistical significance (p=0.15), but the OR for exposure both to outdoor NO<sub>2</sub> and ETS was 3.60 (1.37-9.45), compared with children in the lowest exposure category for both agents, table 8, next page.

No consistent indications of gender related effects were found.

**Table 8.** Odds ratios and 95% confidence intervals for recurrent wheezing in children exposed both to the upper quartile of NO<sub>2</sub> levels and environmental tobacco smoke, compared with double unexposed children

		Exposure to INDOOR NO <sub>2</sub>											
		Below 4 <sup>th</sup> quartile				Above 4 <sup>th</sup> quartile				Exposure to ETS - independent of NO <sub>2</sub>			
Exposure to ETS		Ca <sup>1</sup>	Co <sup>1</sup>	OR <sup>2</sup>	95 % CI	Ca <sup>1</sup>	Co <sup>1</sup>	OR <sup>2</sup>	95 % CI	Ca <sup>1</sup>	Co <sup>1</sup>	OR <sup>2</sup>	95 % CI
Not exposed		90	188	1		28	63	1.09	0.62-1.91	118	251	1	
Exposed		33	63	0.96	0.51-1.83	19	15	3.10	1.32-7.30	52	78	1.31	0.75-2.30
Exposure to NO <sub>2</sub> independent of ETS		123	251	1		47	78	1.47	0.91-2.37				
		Exposure to OUTDOOR NO <sub>2</sub>											
		Below 4 <sup>th</sup> quartile				Above 4 <sup>th</sup> quartile				Exposure to ETS - independent of NO <sub>2</sub>			
Exposure to ETS		Ca <sup>1</sup>	Co <sup>1</sup>	OR <sup>2</sup>	95 % CI	Ca <sup>1</sup>	Co <sup>1</sup>	OR <sup>2</sup>	95 % CI	Ca <sup>1</sup>	Co <sup>1</sup>	OR <sup>2</sup>	95 % CI
Not exposed		87	184	1		30	67	1.58	0.84-2.96	117	251	1	
Exposed		38	63	1.08	0.58-2.02	13	15	3.60	1.37-9.45	51	78	1.31	0.74-2.30
Exposure to NO <sub>2</sub> independent of ETS		125	247	1		43	82	1.86	1.0-3.34				

<sup>1</sup> Cases and Controls, respectively

<sup>2</sup> Adjusted for gender, heredity, maternal age and smoking during pregnancy, breast feeding and building age



## DISCUSSION

### **Building age**

In most western countries, building technology has changed dramatically in the last few decades of the 20<sup>th</sup> century, and the housing standard has improved considerably. The question is whether adverse effects on health may follow in the wake of the new technology. In the BAMSE study (*paper III-V*), we found a clear association between outcome and the age of the buildings, as a proxy for construction differences, though the casual mechanisms are not clear. In addition, an increased risk of infant recurrent wheezing was found for children living in single-family homes with crawl space/concrete slab foundation. It has been difficult to evaluate our results on building age in relation to other studies on children's respiratory health. However, our results support studies on adults conducted in Sweden: one in northern Sweden, focusing on the office environment, and the other within the municipality of Stockholm, the area of the BAMSE cohort study, focusing on sick building symptoms (SBS) in adults. Strikingly, these two studies demonstrate similarly increased risks of negative health outcomes (SBS), associated with buildings of same generations as those of the BAMSE study (*paper IV*).<sup>80,82</sup> Sundell et al further suggested an elevated risk of SBS as well as "skin symptoms" for office workers in buildings with foundations consisting of a concrete slab on the ground or with crawl space; a risk elevation similar in magnitude to that found in the BAMSE case-control study (*paper I*).<sup>80</sup> In addition, Norbäck et al found an increased risk of asthma in adults, related to increased humidity in concrete floor constructions and to emissions of 2-ethyl-1-hexanol as an indicator of dampness-related alkaline degradation of the plasticiser DEHP.<sup>180</sup>

There are few epidemiological studies on this topic, but it may be suggested that building construction and materials influence the prevalence of infant recurrent wheezing, although life style factors, such as smoking, use of gas stoves, indoor humidity production and probably also various types of furnishings, etc, might contribute to the effect.<sup>94,154</sup>

### **Ventilation systems and air change rate**

In several studies, mechanical ventilation has been shown to provide buildings with adequate ventilation and protect homes from increased humidity and a consequent mite growth, also indicating a potentially healthier indoor environment.<sup>97,193</sup> In the BAMSE study we measured higher ventilation flows in mechanically ventilated homes; however, like other authors, we failed to demonstrate any relationship between any certain type of ventilation system and infant recurrent wheezing.<sup>82,89</sup> One likely explanation may be that the effect of the ventilation system is strongly dependent on the building itself. A natural ventilation system installed in an old apartment building may have the entire requirement for efficient ventilation – during the wintertime. In a highly insulated modern single-family house, the conditions may be quite different. In addition, to function adequately, mechanical ventilation systems are dependent on maintenance and adjustments of the air flows, including a balance between supply and exhaust air, and therefore do not necessarily guarantee an adequate ventilation. However, the results still indicate that in single-family homes in cold temperate regions, mechanical ventilation increases the possibility of reaching an ACH of  $\geq 0.5$ , which protects against indoor humidity levels that would contribute to mite survival in the winter.

The ventilation rate was not found to be associated with infant recurrent wheezing. Again, this lack of association has been demonstrated by others, using the same measurements as we used for the BAMSE study.<sup>89</sup> This negative result may also have a variety of explanations. Firstly, exhaust ventilation without sufficient supply air, or a poorly balanced supply and exhaust ventilation system, may create conditions

of either a negative or positive indoor air pressure, that theoretically may generate unwanted effects on the indoor air quality.<sup>201</sup> An indoor air pressurisation will increase the risk of driving humid indoor air into the building envelope, with a subsequent risk of condensation within the walls, creating favourable conditions for microbial growth.<sup>114;202</sup> An indoor depressurisation, on the other hand, may result in increased leakage into the room through the building envelope including the foundation.<sup>203;204</sup> We also found that petrol fumes could be detected in single-family houses where the garage was used for parking cars, whereas such compounds were not detected in homes where the garage was used for other purposes (*paper I*). In addition, in areas with high levels of outdoor pollutants, excessive ventilation may also result in increased indoor levels of outdoor-generated, potentially health-related air pollutants,<sup>86</sup> as also demonstrated in *paper III*.

However, the risk of a non-differential misclassification of the exposure cannot be excluded, as measurements were performed during separate, but consecutive, winter periods and during one four-week period only for each child. Such a misclassification of exposure could have occurred since outdoor air temperature is one driving force especially for natural ventilation systems,<sup>201</sup> and in addition the outdoor temperature probably influences the families' readiness to air out their homes, etc.<sup>205</sup> However, that is likely to dilute any relationship between the exposure and outcome.

### **Indoor humidity**

In line with many other surveys,<sup>108;109</sup> we found an association between an absolute indoor humidity and infant recurrent wheezing in BAMSE (*paper IV*). Exposure to increased indoor humidity further strengthened the association between signs of dampness, such as moisture/mould, and infant recurrent wheezing. However, *paper IV* also demonstrates a strong correlation between outdoor and indoor humidity levels during the winter months when measurements were performed. Thus, indoor air humidity is clearly influenced by outdoor air humidity. As for the air change rate, measurements were performed during separate but consecutive winter periods. Despite the adjustment of outdoor humidity levels, the risk estimates for wheezing associated with indoor humidity may reflect not only the exposure differences between different homes, but also differences due to the actual outdoor humidity at the time when measurements were performed. Again, this possible source of misclassification of the true long-term exposure of the indoor air humidity, as a consequence of differences in the actual time of measurements in individual buildings, is likely to weaken the association between indoor air humidity and recurrent wheezing in the BAMSE study.

The problem with what the measured humidity value actually reflects, may also have affected other studies in countries with a temperate climate, where measurements of indoor humidity have been limited to one relatively short time period. For a careful assessment, this influence of the outdoor humidity on indoor air is also important to bear in mind for professionals assessing the quality of indoor air in homes and other non-industrial indoor environments.

By measuring the indoor vapour contribution, i.e. the difference between outdoor and indoor humidity, one might be able to overcome the problems caused by short measurement times and differences in weather conditions. The lack of association between the indoor vapour contribution and recurrent infant wheezing in the present study may be a result of a methodological weakness, as we could only calculate the indoor vapour contribution limited to a mean of the measured four-week period. Calculations based on simultaneously logged outdoor and indoor humidity levels might have improved the assessment (*paper IV*).



### **Signs of dampness**

In the BAMSE study (*paper IV*), recurrent wheezing was found to be more common in children exposed to various indicators of dampness in the home, such as damage by damp, mould odour and visible mould, compared with non-exposed children. A consistent association between indicators of dampness and recurrent wheezing in the case-control study was also shown for the whole cohort. Again, our findings are in line with findings presented in numerous papers that report an increased prevalence of respiratory symptoms in damp or mouldy homes.<sup>108;109</sup> We also found a trend in the risk of recurrent wheeze in relation to the number of indicators of dampness, with a higher risk for children exposed to several indicators. In adults, such a dose-response relationship, between the number of indicators of dampness and SBS symptoms was also demonstrated by Engvall et al.<sup>206</sup> Our results further indicate that children whose homes showed signs of dampness during a prolonged period, i.e. from the child's birth until the time of inspection were at higher odds of having recurrent wheezing than children exposed during a more limited time period.

In study I, we assessed wintertime windowpane condensation on the interior side of double-glazed windows, and indoor vapour contribution as indicators of poor ventilation (<0.5 ACH) and high indoor humidity ( $\geq 7$  g/kg and  $\geq 45$  % RH). Applied to the BAMSE study, the two indicators showed similar overall accuracy as for study I. Absence of windowpane condensation on double-glazed windows and low indoor vapour contribution ( $< 3$  g/m<sup>3</sup>) during the winter seem to be adequate indicators of a home without indoor air humidity level exceeding 7 g/kg. For both studies, the presence of the two indicators was associated with a less than 45% increased risk of an indoor humidity  $\geq 7$  g/kg (in Study 1 associated with an increased risk of high mite allergen levels in mattress dust). If the cut-off for "increased humidity" was set at 5.8 g/kg (which was the median indoor humidity exposure in the BAMSE study and associated with recurrent wheezing), however, the risk was 65-100%. Thus, the presence of the indicators seems to be associated with an increased indoor humidity that might be related to adverse health effects (infant recurrent wheezing) even though not necessarily with humidity levels that promote house dust mite infestation.

### **House dust mite allergen**

In study I, analyses of allergens from three dust mite species (Der p 1, Der f 1 and Der m 1) showed that levels of house dust mite allergen were significantly higher in homes with windowpane condensation and/or a indoor vapour contribution  $\geq 3$  g m<sup>3</sup>. This expected association could not be demonstrated in the BAMSE study, where only two species (Der p 1 and Der f 1) were analysed, and it must be emphasised that there may be several reasons for this. One reason for the lack of association is that the BAMSE study was not primarily designed to study the role of mite infestation and health effects, and that the prevalence of mite infestation in the dwellings was very low. Another important reason for the differences may be the differences in methods of analysing mite allergen. Just as with allergens from furred pets, humans can transport mite allergen passively with their clothing into different indoor environments.<sup>134;207</sup> Thus, low levels of mite allergen may result from contamination rather than true mite infestation and this fact makes the difference in detection limits in the two studies (0.007 vs 0.055  $\mu$ g/g dust) less important. A non-detectable infestation of *Dermatophagoides microceras* (Der m 1) could be a more serious problem when one tries to compare the results of Study 1 and the BAMSE study (*paper IV*) and evaluate the impact of HDM allergen exposure on health.

In Study 1 (*paper I and II*), Der m 1 was present in 68% of the samples of levels exceeding 0.1  $\mu$ g/g dust, and in 17% of the dust samples it was the only mite allergen found. In a study by Wickman et al, Der m 1, was found to be the most common of the assayed house dust mite allergens, likewise prevalent

in the most highly infested homes.<sup>92</sup> This result has been further supported by findings of Warner et al, who found that Der m 1 was the major HDM allergen in homes of asthmatic children.<sup>208</sup>

With this in mind, our data on mite infestation in the homes of the children of the BAMSE case-control study should be interpreted as an underestimation of the occurrence of dust mites and possibly any associations to ACH/indoor humidity. However, since mite infestation in the homes of the Stockholm area is rare (approximately 10% of houses),<sup>92</sup> it is unlikely that analyses including detection of *Dermatophagoides microceras* (Der m 1) would have resulted in any dramatic changes of the results, even though there may have been an association between mite allergen exposure and health effects.

### **Indoor painting**

The children whose bedroom was painted during the year before the child was born, or during the child's first year of life, were at higher odds of having recurrent wheezing compared with those children whose bedroom was not painted. We also found a joint effect between paint and other indoor exposures on wheezing. There are a few epidemiological studies that elucidate the influence of newly painted surfaces in the home environment. Åberg et al found that repainting, or new wallpaper, in the bedroom of the child after birth caused a moderately increased risk of allergic disease,<sup>90</sup> and Diez et al demonstrated an increased risk of pulmonary infections in six-week old infants if restorations including painting had occurred during the pregnancy period.<sup>172</sup> Further, Wieslander et al showed that asthma in adults was associated with domestic exposure to painted surfaces and in addition that blood eosinophil concentrations were significantly elevated among subjects living in newly painted dwellings.<sup>168</sup> Even though there seems to be a rapid decrease in emissions from fresh paint, one may speculate that also long-lasting low-levels emissions may be detrimental to health.

In the BAMSE case-control study, new furnishings and other effects of building renovation may constitute potential confounding factors in relation to use of paint, as emissions from other building components and home furnishing materials may contribute to similar exposures.<sup>84;157;174;175</sup> Unfortunately, we were not able to control for other exposures than renovation with PVC flooring, that was not found associated with recurrent wheezing in our study. Hence, our results on health effects in relation to paint need to be interpreted with caution, in particular since the data on interior painting were collected after onset of symptoms.

### **Air pollutants including nitrogen dioxide (NO<sub>2</sub>)**

The epidemiological evidence on the relationship between NO<sub>2</sub> exposure and recurrent wheezing is inconsistent. In contrast to two recent Nordic studies,<sup>21;76</sup> but in line with a collaborative European study,<sup>67</sup> an association between NO<sub>2</sub> exposure and recurrent wheezing was suggested in the BAMSE study. The reasons for the apparent inconsistency between different studies remain unclear. However, one notable finding in our study was the confounding effect of the age of the children's homes on the risk estimates of NO<sub>2</sub> exposure. Most children exposed to increased levels of NO<sub>2</sub> lived in old brick-built apartment blocks, erected before 1940. Children less exposed to NO<sub>2</sub> lived more often in buildings erected after 1940 with other design and construction, as demonstrated in *paper III*, associated with an increased odds of having recurrent wheezing. The impact of building age in association with NO<sub>2</sub> has also been discussed by Krämer et al, who found an increased OR for the association between NO<sub>2</sub> and some atopic diseases, when building age was included in the analyses.<sup>83</sup> The findings indicate that health effects associated with building age, or more likely building-related factors, should be taken into consideration when evaluating the association between NO<sub>2</sub> exposure and health.

We also found that NO<sub>2</sub> particularly increased the risk in combination with exposure to ETS, indicating a combined risk of various indoor exposures, as demonstrated by others.<sup>94;154</sup> In view of the small num-

ber of children with a combined exposure to increased NO<sub>2</sub> levels, parental smoking and indoor signs of moisture and mould, the effect of such combined exposure was not assessed in the BAMSE study.

As for ventilation rate and indoor air humidity, the limited measuring period could result in substantial misclassification of long-term exposure to NO<sub>2</sub>. However, analyses of the estimated annual average exposure levels confirmed the results obtained with the measured data. Further, some studies indicate that exposure estimated by personal sampling is better correlated to indoor NO<sub>2</sub> levels than to outdoor levels.<sup>209;210</sup> We measured NO<sub>2</sub> at fixed sites, in and outside the dwellings, respectively. Because of this, imprecise assessment of a child's exposure could have occurred due to the position of the child's room in relation to the street and the living room, where NO<sub>2</sub> had been measured. It may thus be expected that exposure estimates based on outdoor measurements will contribute to a dilution of any relationship between NO<sub>2</sub> exposure and recurrent wheezing, especially for children living in homes equipped with a gas stove.<sup>211</sup>

Children exposed to combustion products from gas appliances have in some surveys shown an increased risk of respiratory symptoms.<sup>69;145;146</sup> We found no clear association between the use of gas stoves and recurrent wheezing. Our risk estimate for use of gas stove was of the same order of magnitude as for those exposed to increased levels of outdoor and indoor NO<sub>2</sub>, but the number of gas stoves was low. Therefore, the present results on gas stoves and recurrent wheezing should be interpreted carefully.

### **Selection bias**

The BAMSE case-control study (*paper III-V*) was conducted within the BAMSE cohort study. For both cases and controls it was required that they should still be living in the same home as when they were new born. Thus, of 321 identified cases, only 181 were recruited to the case-control part of the study, which could have introduced a bias. When we compared "the movers" in the entire cohort, with those children still living in the same home as when they were born, no difference was found in the prevalence of recurrent wheezing, indicating that "having moved" during the first two years of life was not associated with recurrent wheezing.

The effect of *the matching* of cases and controls needs to be discussed. The purpose of this design was partly to bridge the necessary effect of measuring the environmental exposures during different seasons, and in addition during different years. Another aspect was to minimise the effect of being born during different seasons, leading to exposure differences that potentially could be associated with the development of asthma symptoms/allergy. The matching of cases and controls in the BAMSE study was made by date of birth. However, the use of matching may in turn introduce a negative effect due to loss of observations, especially in connection with stratification into subgroups. When stratifying for gender and other variables, we had to dissolve the matching. In general, however, comparisons of conditional and unconditional logistic regression in the full group of children resulted in slightly higher odds ratios and narrower CIs for the unconditional analyses. Therefore, it is unlikely that a statistically significant difference between stratified groups (as for gender) would have been found, if we had been able to perform conditional logistic regression. However, matching by age made it impossible to assess whether children born during certain seasons were at different odds of having recurrent wheezing related to the indoor exposures. With regard to the discussed aspects, we do not think that the results of the BAMSE study are influenced by selection bias or matching design.

### **Information bias**

A methodological problem could arise if there is a recall bias that is related to the awareness of an exposure and the potential impact of this exposure on health. This could lead to stronger propensity to report

environmental exposures among parents who themselves suffer from allergic diseases or who have children with respiratory symptoms. Most exposure data used for the BAMSE case-control study (*papers III-V*) had either been gathered prospectively at the child's median age of two months, i.e. before onset of disease, or been verified by home inspections and assessed by use of measurements. However, data on interior painting were not obtained until after the time of onset of disease. Data on windowpane condensation (WPC) were reported by the parents at four time points (at child's mean age two months, 1 and 2 years and at the time of inspection), and there were more reports of WPC for each successive opportunity to answer the question. However this increased reporting of WPC was similar for the parents of the cases and for the parents of the control children. The reasons remain unclear. The influence of being involved in a longitudinal study on children's health cannot be neglected.

Further, parents suffering from asthma/allergy more often reported problems with moisture and mould than did families free from such disease, and there was a relatively weak coherence between parental reports vs inspector notes of various signs of dampness. This rather weak coherence might have several explanations. The parental reports and the investigator notations refer to exposures at two different time points; moisture/mould damages may have been taken care of after the parental report, but before the inspection, or new damages may have arisen. It could also imply that in families with allergic diseases, an awareness of various environmental exposures might result in a difference in perception of such exposure compared with families without allergic diseases. Our results from the questionnaire on any moisture/mould indicate that this may be the case. On the other hand, it is not unreasonable that moisture/mould give rise to symptoms in adults as well as in children.<sup>109,212</sup>

It should be stressed that the associations between signs of dampness and infant recurrent wheezing in the BAMSE study remain consistent both for parent-reported and inspector-observed dampness, with a slightly stronger association for the inspector-observed dampness. Thus, we do not think that the results of the BAMSE case-control study are severely affected by information bias.

### **Confounding**

To identify potential confounders we ran several regression models with a number of covariates and the final model used for the assessments was mainly based on strength of association with outcome. In some instances we used variables for the adjustments that were not significant in the case-control study, but that were so in the cohort analyses. We have chosen six months of breast-feeding, since this was used in the cohort analyses of the BAMSE study, although using a longer mean period of breast-feeding (9 months as cut-off) slightly strengthens the association between the assessed exposures and recurrent wheezing in the case-control part of the study. Further, the adjustments for maternal smoking is made for a similar reason; it was found to be a significant risk factor for recurrent wheezing in the whole cohort, and besides, we think it would be questionable to present analyses without any adjustment for maternal smoking. We found that nitrogen dioxide exposure and building age had strong confounding effects on each other and this effect on the analyses is accounted for. Thus, we do not think that the results are strongly affected by inadequate control of cofounders, even though effects of unknown exposures never can be totally excluded.

## CONCLUSIONS

Based on data from presented studies, following conclusions can be drawn:

- ◇ Building age, or more likely certain types of building constructions, seems to be associated with recurrent wheezing in children up to the age of two, and this is not primarily explained by differences in ventilation systems, air exchange rate or mite infestation
- ◇ Mechanical ventilation is likely to increase the possibility of reaching a ventilation rate  $\geq 0.5$  air changes per hour in one storey single-family houses in cold temperate regions, which protects against indoor humidity levels  $\geq 7\text{g/kg}$  that may contribute to mite survival
- ◇ On the other hand, an excessive air exchange in rate probably under certain conditions have a negative effect on indoor air quality and may increase the exposure to air pollutants generated outdoors
- ◇ Signs of dampness in buildings, such as increased indoor humidity, damage by damp, mould odour or visible mould, appear to increase the risk of recurrent wheezing in young children. It is probably not the moisture itself that cause the effect, but moisture is an indicator of exposures associated with various chemical and microbial processes within the building that may result in adverse health effects
- ◇ Recently painted surfaces in the child's bedroom appeared associated with infant recurrent wheezing, but our results must be interpreted carefully
- ◇ Exposure to air pollution including  $\text{NO}_2$ , particularly in combination with exposure to ETS, may increase the risk of infant recurrent wheezing

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# APPENDIX







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