Environmental Factors in Relation to Asthma and Respiratory Symptoms among Schoolchildren in Sweden and Korea

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Abstract


This thesis studied environmental factors in relation to asthma and respiratory symptoms among schoolchildren in two countries. In Sweden, 1014 pupils (5-14 year) in 8 schools participated. Wheeze was reported by 7.8%, current asthma by 5.9%, doctor-diagnosed asthma by 7.7%, cat allergy by 6.8% and dog allergy by 4.8%. Current asthma was less common among those consuming more fresh milk and fish. Doctor-diagnosed asthma was less common among those consuming olive oil. Cat, dog and horse allergens were common in settled dust and related to respiratory symptoms. Pupils consuming butter and fresh milk had less respiratory symptoms in relation to allergen exposure. In schools with increased levels of microbial volatile organic compounds and selected plasticizers (Texanol and TXIB) asthma and respiratory symptoms were more common.

In Korea, 2365 pupils (9-11 year) in 12 schools participated (96%). In total, wheeze was reported by 8.0%, current asthma by 5.7%, doctor-diagnosed asthma by 5.4%, cat allergy by 2.6% and dog allergy by 4.9%. Contamination of dog and mite (Dermatophagoides farinae) allergen was common while cat allergen was uncommon. Remodelling, changing floor and building dampness at home were positively associated with asthma and respiratory symptoms. The strongest associations were found for floor dampness. Indoor/outdoor concentration of NOx, formaldehyde and ultrafine particles (UFP) at schools were positively associated with asthma and respiratory symptoms.

When comparing Sweden and Korea, Korean pupils had more breathlessness and asthma but reported less cat and pollen allergy. Swedish schools had CO2-levels below 1000 ppm, while most Korean schools exceeded this standard. Since both home and school environment may affect pupil’s asthma and respiratory symptoms, air quality should be an important health issue. Moreover, changes in dietary habits may be beneficial to decrease asthma and allergies. Furthermore, interaction between diet and environment needs to be further investigated.

Keywords: Allergen, allergy, asthma, dampness, diet, environment, formaldehyde, nitrogen dioxide, plasticizer, schoolchildren, ultrafine particles

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“Life is like a box of chocolates.
You never know what you're gonna get.”

- From the movie ‘Forrest Gump’ -
List of papers

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


III  **Kim JL**, Elfman L, Norbäck D. Respiratory symptoms, asthma and allergen levels in schools – comparison between Korea and Sweden. Indoor Air (in press)

IV  **Kim JL**, Elfman L, Wieslander G, Ferm M, Norbäck D. Respiratory symptoms and asthma among Korean pupils in relation to home, school and outdoor environment. (Manuscript)

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Abbreviations

AQG  Air Quality Guidelines
Bla g 1  *Blatella germanica* major allergen 1
Can f 1  *Canis familiaris* major allergen 1
cfu  colony forming units
CI  confidence interval
CO₂  carbon dioxide
Der f 1  *Dermatophagoides farinae* major allergen 1
Der p 1  *Dermatophagoides pteronyssinus* major allergen 1
ECRHS  European Community Respiratory Health Survey
ELISA  enzyme linked immunosorbent assay
Equ cx  *Equus cabbalus* allergen x
ETS  environmental tobacco smoke
Fel d 1  *Felis domesticus* major allergen 1
IgE  immunoglobulin E
ISAAC  International Study of Asthma and Allergies in Childhood
MVOC  microbial volatile organic compounds
NO₂  nitrogen dioxide
PBS  phosphate buffered saline
PM  particulate matter
ppm  parts per million
O₂  ozone
OR  odds ratio
PVC  poly-vinyl-chloride
RH  relative air humidity
SO₂  sulphur dioxide
TMPD-DIB  2,2,4-trimethyl-1,3-pentanediol diisobutyrate, TXIB
TMPD-MIB  2,2,4-trimethyl-1,3-pentanediol monoisobutyrate, Texanol
UFP  ultrafine particles
VOC  volatile organic compound
WHO  World Health Organization
Introduction

Background

Given the rising prevalence of asthma and allergy worldwide (von Mutius et al., 2000; Sennhauser et al., 2005), especially in children, there is a need for evaluating the possible causes and to identify measures to reduce the incidence. There are many different candidate factors for the increase of asthma and allergy. However, considering the rapid increase of the prevalence of asthma and allergic diseases in recent decades the potential risk factors can be focused on environmental changes rather than genetic factors.

Research has therefore paid special attention to environmental components. Indoor air is a dominant exposure for humans and there is mounting evidence that exposure in the indoor environment is the cause of excessive morbidity and mortality (Sundell, 2004). Moreover, there is a growing concern about the school environment with respect to asthma and allergy (Daisey et al., 2003; Munir et al., 1993; Putus et al., 2004; Smedje and Norbäck, 2001a; Smedje et al., 1997; Taskinen et al., 1997) since school can be considered the most important indoor environment for children, besides homes.

Previous measurements in schools have shown a high concentration of carbon dioxide (CO$_2$) due to low air exchange rate (Meyer et al., 2004), abundant airborne particles (Fox et al., 2003; Lee and Chang, 2000; Smedje et al., 1997; Tortolero et al., 2002) and dampness (Taskinen et al., 1997). Other problems are high room temperature (Ruotsalainen et al., 1995; Smedje et al., 1997) and exposure to allergens (Almqvist et al., 2001; Munir et al., 1993; Perzanowski et al., 1999; Rönmark et al., 1998; Smedje et al., 1997; Tranter, 2005), microorganisms, volatile organic compounds (VOC), microbial VOC (MVO), and formaldehyde (Meyer et al., 2004; Smedje et al., 1997; Taskinen et al., 1997; Tortolero et al., 2002). Most school environment studies were published from western countries (Daisey et al., 2003; Mendell and Heath, 2005) whereas there are relatively few from Asian countries including Korea.
Trends in asthma and allergic diseases

Worldwide

The prevalence of asthma and allergic disease has increased remarkably among schoolchildren in most countries since 1960s (Burney et al., 1990; von Hertzen and Haahleta, 2004; von Mutius et al., 1998). According to the International Study of Asthma and Allergies in Childhood (ISAAC) Phase one (year 1992-1998), there were large variations in the worldwide prevalence of asthma, allergic rhinoconjunctivitis and eczema (ISAAC steering Committee, 1998, Strachan et al., 1997; Williams et al., 1999). The prevalence of wheeze was from 4.1% to 32.1% among 6-7 year old children and from 2.1% to 32.2% among 13-14 year old children, respectively. Since the variations were large within regions, even in genetically similar groups, the research has been more focused on environmental factors, which have been studied in ISAAC Phase three (year 1999-2004).

Recent epidemiological studies reported that, in some countries, increase of allergic disease has reached a plateau and might even decline (Ronchetti et al., 2001; Zollner et al., 2005). However, there are still a number of studies showing a continuous increase in prevalence of childhood asthma or allergic diseases and several studies indicated reversing trends according to age (Asher et al., 2006; Garcia-Marcos et al., 2004; Maziak et al., 2003), gender (Grize et al., 2006; Maziak et al., 2003) or atopic phenotype (Asher et al., 2006; Grize et al., 2006; Lee et al., 2004; von Mutius et al., 1998).

Sweden

The prevalence of allergic disease has increased over the decades (Almqvist et al., 2003; Åberg et al., 1995; Bräbäck et al., 2004). The most common furry pet allergy is against cat allergen among Swedish schoolchildren (Rönmark et al., 1998; Rönmark et al., 2003). One study reported that there is a moderate increase in asthma-like symptoms and doctor-diagnosed asthma during 1985-1995 (Bräbäck et al., 2000). There is another study, which is a 10-year repeat study comparing the prevalence of asthma and allergic diseases in 35 schools in one Swedish county (Smedje and Norbäck, 2005). The study reported that asthma diagnosis and medication have increased during this 10-year period (1993-2003), but not self-reported allergic diseases. According to the ISAAC Phase three, there was no change in asthma symptoms, a decrease in allergic rhinoconjunctivitis and an increase in eczema among 6-7 year old children. For the older age group (13-14 year old), there was an increase in asthma and eczema but no change in allergic rhinoconjunctivitis (Asher et al., 2006). However, the ISAAC Phase three survey in Sweden has been performed in only one city, Linköping. Thus, the
results may not be representative for children all over Sweden, but for children in that area.

Korea
The burden of allergic disease has been speculated to increase with industrialization. The ISAAC Phase one study reported that wheeze was reported by 13.6% among 6-7 year old children and 7.9% among 13-14 year old children (Lee et al., 2001). Lee and co-workers (2001) reported eczema by 9.2% and allergic conjunctivitis by 9.7% among 6-7 year old children while eczema by 4.0% and allergic conjunctivitis by 17.5% among 13-14 year old children. One 5-year follow-up study showed that wheeze during the last 12 months was reported by 8.5% in 1995 and 9.3% in 2000 (Hong et al., 2004). Moreover, the study reported doctor-diagnosed asthma by 2.7% in 1995 and by 5.3% in 2000. According to the ISAAC Phase three, there was no change in asthma symptoms in Korea, a decrease in allergic rhinoconjunctivitis and an increase in eczema among 6-7 year old children. For the older age group (13-14 year old) there was no change in asthma symptoms, but an increase in allergic rhinoconjunctivitis and eczema (Asher et al., 2006).

Personal factors
Age and gender
There is a general agreement about the influence of age and gender on asthma. Many studies reported that boys have more asthma related symptoms than girls in their early age due to smaller lungs (Becklake and Kauffmann, 1999; Forsberg et al., 1997; Morgan and Martinez, 1992; Rönmark et al., 1998; Zannolli and Morgese, 1997). Zannolli and Morgese (1997) reviewed a number of publications over 30 years to determine evidence for an interference of puberty with the occurrence of asthma. This review concluded that puberty did not seem to affect asthma outcome, but boys had more asthma under the age of 10 or the mid-teens. A five time follow-up study reported that lung function during childhood (age up to 18 years) was better among girls than boys, but the reverse was seen in adulthood (from the age of 20 years and above) (Gustafsson and Kjellman, 2000).

Family history of asthma/allergic diseases and genetic factors
It is well known that a family history might be a risk factor for children. Two recent review studies presented that a positive family history of asthma is a consistent predictor of asthma risk in both children and adults (King et al., 2004; Sears, 1998). One Swedish study also showed that an adult with a
family history of asthma or rhinitis has a risk of three- to fourfold for developing asthma and of two to sixfold for rhinitis over adults without a family history (Lundbäck, 1998).

Besides the general evidence of hereditary aggregation of asthma and atopic diseases, genes may contribute to asthma or allergic disease (Bossé and Hudson, 2006; Heinzmann and Deichmann, 2001; Patino and Martinez, 2001; Holgate et al., 2006). The majority of these genes will be expected to be involved in the control of IgE and/or Th2 mediated inflammatory responses. Therefore, there has been great focus to identify candidate genes in the occurrence of asthma or allergic disease. Many reviews reported that there were a large number of genes potentially involved in asthma or atopic diseases (Hall, 1998; Holgate et al., 2006; Castro-Giner et al., 2006). However, it is unlikely that any single gene plays a major role in atopic disease etiology. Atopic diseases are a result of the interaction of environmental exposures in individuals with genetic susceptibility (Castro-Giner et al., 2006; Heinzmann and Deichmann, 2001; Kaiser, 2004; Koppelman, 2006; Patino and Martinez, 2001; Wiesch et al., 1999).

Dietary habits

There are a number of studies on the role of different dietary factors for asthma or allergic diseases. It has been suggested that a high intake of antioxidant vitamins (beta-carotene, vitamins A, C and E) and mineral cofactors (selenium, zinc and copper) may be essential for antioxidant defence mechanisms (Baker and Ayres, 2000; McKeever and Britton, 2004). Consumption of fruits or vegetables can be beneficial for pulmonary diseases, asthma or allergy (Baker and Ayres, 2000; Denny et al., 2003; Devereuz, 2006; McKeever and Britton, 2004; Romieu and Trenga, 2001; Smit, 2001) and there is possible evidence that dietary lipids may influence asthma and allergy (Devereuz and Seaton, 2005; Romieu and Trenga, 2001). A high intake of omega-3 essential fatty acids, mainly from fat fish, can be protective for various diseases including asthma and lung diseases (Mickleborough and Rundell, 2005; Romieu and Trenga, 2001; Schwartz, 2000; Simopoulos, 2002). Moreover, some studies suggested a protective effect of higher consumption of milk (Wickens et al., 2002; Woods et al., 2003), milk fat/butter (Wijga et al., 2003) and yoghurt (Wickens et al., 2002) on asthma and allergy. On the other hand, a high consumption of fast food (Hijazi et al., 2000; Norbäck et al., 2006; Wickens et al., 2005) has been associated with an increased risk for asthma.

There are only few investigations on interaction between dietary factors and environmental pollution. It has been suggested that smokers or subjects living in areas with high ambient air pollution levels need a higher intake of dietary antioxidants (Romieu and Trenga 2001). In one study from Scotland,
subjects with seasonal allergy had more allergic symptoms during pollen season if they had a lower intake of zinc. In addition, those with the lowest intake of vitamin C, manganese and magnesium had more increased bronchial hyper-responsiveness (Soutar et al., 1997). These limited data suggest that there could be an interaction between dietary factors and different types of pollutants with respect to respiratory health effects.

Environmental factors
The review has mainly focused on environmental factors and settings investigated in this thesis.

Environmental tobacco smoke
Maternal smoking during pregnancy and childhood environmental tobacco smoke (ETS) exposure have been recognised as risk factors for respiratory infection, respiratory symptoms, decreasing lung function, bronchial hyper-responsiveness, exacerbation/severity of asthma or increasing levels of total IgE (Chan-Yeung, 1999; Cook and Strachan, 1997; Strachan and Cook, 1998). Weiss and co-workers (1994) presented that maternal cigarette smoking is a significant risk factor for the development of asthma or asthma related symptoms in the first year of life. Moreover, according to one review study (King et al., 2004), six studies have shown a modest increase in the incidence of asthma related to ETS exposure, with odds ratios (ORs) ranging from 1.3 to 2.3. Three of the 6 studies reported adverse respiratory effects under the age of 7. This review suggested ETS avoidance as measure for the primary prevention of childhood asthma.

Ventilation and carbon dioxide
Several studies have been performed to evaluate ventilation effects on health aspects. A review study (Seppänen et al., 1999) reported consistent relationships between ventilation rates and occupant’s health in 20 studies, mostly among adults. The review concluded that ventilation rates below 10 l/s per person is associated with increased health complaints. Another recent review on ventilation and health suggested that outdoor air supply rates below 25 l/s per person increased the risk of adverse health symptoms (Wargocki et al., 2002). Another review study (Daisey et al., 2003) pointed out that there were only two school studies from Europe, as of 1999, on relations between prevalence of adverse health effects and ventilation rate or CO₂ concentration. One of the studies investigated 22 classrooms in 5 newly renovated Norwegian schools and found significant correlation between high CO₂ levels (1500-4000 ppm) and symptoms of headaches, dizziness, heavy headed,
difficulties concentrating and unpleasant odour (Myrvold et al., 1996). The other study assessed association between symptoms related to sick building syndrome among children and CO₂ levels and indoor climate in 14 classrooms (Potting et al., 1987). The Swedish two year follow-up study showed that incidence of asthmatic symptoms was reduced in schools with new ventilation systems with increased air exchange (Smedje and Norbäck, 2000).

Allergens

Allergen inhalation is an important cause of asthma in especially predisposed children, mediated by an IgE mechanism (Peat et al., 1996; Platts-Mills et al., 1997; Custovic et al., 1998). First the exposure to an allergen gives rise to sensitisation, with the formation of specific IgE. At a later contact mediators are released with inflammatory response of the airways and with a subsequent increase in bronchial hyperresponsiveness. At prolonged allergen exposure, inflammation may lead to irreversible damage to the bronchial wall with chronic deterioration of respiratory function.

In general, homes with pets present greater allergen contaminations than schools. However, school is a major public environment, where pupils keeping pets at home actually transfer allergens into this environment. Previous studies have demonstrated contamination with cat and dog allergens in the classrooms, which could cause symptoms of asthma and allergy (Almqvist et al., 2001; Munir et al., 1993; Perzanowski et al., 1999; Smedje et al., 1997; Smedje and Norbäck, 2001a; Tranter, 2005). Some studies also reported presence of allergens from house dust mites *Dermatophagoides pteronyssinus* and *D. farinae* (Der p 1/ Der f 1), cockroach (*Blatella germanica*, Bla g 1) and mouse (*Mus m 1*) (Amr et al., 2003; Bates et al., 1996; Tortolero et al., 2002) in schools.

Dampness, microorganisms and MVOC

Since the concept of the hygiene hypothesis was introduced (Strachan, 1989), there has been a focus on protective effects by exposure to endotoxins and microbial compounds in early life with respect to asthma and atopy (Braun-Fahrlander, 2003; Douwes et al., 2004; Holt et al., 1999; Martinez and Holt, 1999; von Mutius et al., 2000; von Mutius, 2002; Riedler et al., 2001). In contrast, several studies have demonstrated a high prevalence of respiratory symptoms among children living in moisture-damaged or moldy buildings (Billings and Howard, 1998; Bornhag et al., 2004; Bornhag et al., 2005a; Garrett et al., 1998; Mommers et al., 2005; Peat et al., 1998; Simon et al., 2005; Venn et al., 2003). Moreover, some studies have reported that microbial exposures in schools could be a risk factor for respiratory symptoms, asthma and allergy (Meklin et al., 2005; Meyer et al., 2004; Putus et al., 2004; Smedje et al., 1997; Smedje et al., 1996).
Molds and bacteria may produce specific volatile organic compounds (MVOC) (Claeson et al., 2002; Fischer and Dott, 2003; Wessén and Schoeps, 1996) but the specificity of MVOC as an indicator of microbial exposure has been questioned (Pasanen et al., 1998). In addition, it has been claimed that the indoor levels of MVOC are much lower than the sensory irritation thresholds (Pasanen et al., 1998; Korpi et al., 1999). We found only two epidemiological studies on MVOC, both reporting positive associations between indoor MVOC and asthma or allergy (Elke et al., 1999; Smedje et al., 1996). In some countries, MVOC measurements are routinely used by building consultants for investigation of suspected “sick buildings” (Wessén and Schoeps, 1996, Schleibinger et al., 2005).

Chemical exposure

Exposure to air pollution in relation to human health is of great concern. Many studies have shown associations between air pollution and decrease of pulmonary function (Bedi et al., 1984), increased mortality (Schwartz, 1994; Touloumi et al., 1994; Ostro et al., 1999; Gauderman et al., 2000) and increased respiratory symptoms (Timonen and Pekkanen, 1997; McConnell et al., 1999; Hwang and Chan, 2002).

Sulphur dioxide (SO_2)

SO_2 air pollution is produced mainly from combustion of fossil fuel, and is usually a complex mixture with particulate matter (PM) (WHO, 2000). Recent epidemiological studies reported that day-to-day changes in mortality, morbidity or lung function can be related to outdoor air concentrations of SO_2 in the generally exposed population (WHO, 2005). The Hong Kong study (Hedley et al., 2002) reported that a major reduction in sulphur content in fuels over a very short period of time was associated with positive health effects, less childhood respiratory disease and all age mortality. Other studies also showed that SO_2 concentration was significantly associated with increased mortality or hospital admissions (D’Amato et al., 2000; Heinrich, 2003; Sunyer et al., 1997). In many industrialized countries ambient SO_2 levels have been decreased.

Nitrogen dioxide (NO_2)

NO_2 is a major constituent of combustion-generated air pollution. It mainly derives from traffic related exhaust and gas-cooking (WHO, 2005). The toxicology of NO_2 exposure suggests a potential risk in relation to respiratory symptoms and lung function (Samet et al., 1990; Utell et al., 1997). Research has therefore focused on susceptible persons such as the elderly, children and persons with chronic respiratory diseases.

There have been several studies about children’s respiratory adverse health effects in relation to NO_2 (Belanger et al., 2006; Lee et al., 2005; van...
Strien et al., 2004). However, there is relatively little information on health effects by NO₂ exposure at school (Nitschke et al., 2006; Smedje et al., 1997).

**Ozone (O₃)**
Ambient O₃ is a compound formed by the action of sunlight on NO₂ and reactive hydrocarbons, both of which are emitted by motor vehicles and industrial sources. O₃ is a powerful oxidant and respiratory tract irritant in both children and adults, causing shortness of breath, chest pain when inhaling deeply, wheezing and cough (D’Amato G et al., 2002; Holz et al., 1999; Kinney et al., 1996; Leikauf, 2002; Ostro et al., 1999; Weschler, 2006).

At concentrations exceeding 240 μg/m³, adverse health effects among both asthmatic and healthy adults have been reported in many studies of clinical inhalation or field studies (WHO, 2005). Most clinical or field studies on O₃ exposure were performed among healthy adults, and showed significant adverse health outcomes. Thus, effects could be even more pronounced in children who are more vulnerable.

**Formaldehyde**
Formaldehyde is a flammable, colourless reactive and readily polymerized gas at normal room temperature and pressure. It is known to cause irritation of the eyes, nose and throat. The most common indoor sources of formaldehyde are products containing resins, glues, insulating materials, chipboard, plywood and fabrics. Other sources can be cigarette smoke, heating and cooking (WHO, 2000).

There are several studies on formaldehyde and health effects such as asthma, respiratory and atopic disease (Garrett et al., 1999; Norbäck et al., 1990; Norbäck et al., 1995a; Smedje et al., 1996; Smedje et al., 1997; Rumchev et al., 2002; Venn et al., 2003; WHO, 2000). A recent review concluded that formaldehyde is primarily a hazardous air pollutant in respect to exacerbating asthma (Leikauf, 2002).

**Volatile organic compounds (VOC)**
Indoor air contains many different kinds of VOC which are mainly emitted from building materials, consumer products and human activities. Besides direct emissions, dampness in buildings may stimulate emission of VOC, due to degradation of building materials or microbial activity (Norbäck et al., 1993). Moreover, Wieslander and co-workers (1997) postulated that newly painted indoor surfaces may stimulate emission of VOC.

A review study emphasized that the concept total VOC (TVOC) should not be used as a risk indicator to draw conclusion since TVOC consists of different mixtures of chemical substances, with varying biological effects. When using TVOC for health outcome, the toxicologically potent substances can be underestimated while low-toxicity substances can be overestimated.
However, one study investigated associations in asthmatic subjects to inhalation of a mixture of VOC. Lung function was reduced among those subjects at a concentration of 25 mg/m³, which is not an unusual level of VOC in work environments (Harving et al., 1991). Pappas and co-workers (2000) reported that persons with asthma were found to have decrease in forced expiratory flow rates after a 4-hour VOC mixture exposure to 50 mg/m³. Moreover, a recent review concluded that some specific VOC may be a primary cause or aggravate existing asthma (Leikauf, 2002). Another recent review summarized that avoiding VOC around conception and during pregnancy as well as in the first years of life is important to reduce or at least control the development of asthma (van den Hazel et al., 2006). Therefore, continued research is needed to establish a risk index for health and comfort effect for VOC (Andersson et al., 1997).

**Plasticizers**

Plasticizers are organic compounds that are added to a rigid polymer in order to make it more flexible and easier to work with. Poly-vinyl-chloride (PVC) materials are common sources for indoor plasticizer contamination (Bornehag et al., 2005b). In damp and alkaline indoor environments, 2-ethyl-1-hexanol may be formed by degradation of di-ethyl-hexyl-phthalate in floor materials or water based glue (Norbäck et al., 2000a). Moreover, certain bacteria and fungi may produce 2-ethyl-1-hexanol (Nalli et al., 2005). Some studies found associations between childhood asthma and amounts of plastic materials in dwellings (Jaakkola et al., 2000; Oie et al., 1997; Oie et al., 1999). Other studies also reported that there were associations between asthma and allergic symptoms and PVC floor coatings with dampness (Bornehag et al., 2005a) as well as dust concentration of certain phthalate esters (Bornehag et al., 2005b). An association between 2-ethyl-1-hexanol and sick building syndrome (Kamijima et al., 2002), nasal symptoms, inflammation (Wieslander et al., 1999) and asthma (Norbäck et al., 2000a) have been reported.

TMPD-MIB (2,2,4-trimethyl-1,3-pentanediol monoisobutyrate, Texanol) and TMPD-DIB (2,2,4-trimethyl-1,3-pentanediol diisobutyrate, TXIB) are plasticizers, commonly used in water based paints (Wieslander et al., 1997), PVC-materials and other plastic materials such as urethanes wallpaper and artificial leather products (Cain et al., 2005). In a recent review, it was concluded that TMPD-DIB could contribute to indoor odour and irritation (Cain et al., 2005), and it could also be an ambient environmental pollutant (Dsi-kowitzky et al., 2004). According to my knowledge, there are no epidemiological studies available on asthma or allergy in relation to these two compounds.
Particles

Ambient aerosols are suspensions of stable solid or liquid particles in ambient air. Ambient particulate matter (PM) has been classified in three size distributions (Hind, 1999). PM\(_{10}\), which consists of particles <10 µm in aerodynamic diameter, are able to reach the respiratory tract below the larynx. Particles smaller than 2.5 µm (PM\(_{2.5}\)) can penetrate into the gas-exchange region (alveoli) of the lung. In urban or industrialized areas PM\(_{2.5}\) comprise 60-70% of the PM\(_{10}\) fraction and consist to a high degree of elemental carbon derived from stationary or mobile combustion sources. Ultrafine particles (UFP) are approximately less than 100 nm in diameter and are a component of air pollution, derived mainly from primary combustion sources.

Many toxicological and epidemiological studies have been performed, especially in relation to pulmonary/cardiovascular effects of inhaled ambient particles (Brunekreef et al., 2005; Moshammer et al., 2006; Oberdörster, 2001; Penn et al., 2005; Pope and Dockery, 2006; Schlesinger et al., 2006; Schulz et al., 2005; Sioutas et al., 2005). A recent review summarized that long-term PM exposure is associated with elevated total, cardiovascular and infant mortality and morbidity of respiratory symptoms, lung growth and function of the immune system (Kappos et al., 2004). The review study also reported that short-term exposure has been consistently associated with mortality or morbidity, especially in patients with asthma or respiratory disease. Another recent review concluded that an independent coarse PM showed stronger adverse effects or as strong short-term effects as fine PM, on asthma, chronic obstructive pulmonary disease and respiratory admissions (Brunekreef et al., 2005).

Among the inhaled particles, UFP are considered important for adverse health aspects since they can be transported and deposited in the lung. Because of their high deposition efficiency, they can migrate from there into the systemic circulation and to the heart (Penn et al., 2005). One experimental study also presented that UFP made of low-solubility, low-toxicity materials are more inflammogenic in the rat airways than fine respirable particles at similar mass concentrations (Donaldson et al., 2002; de Haar et al., 2006).

Most studies on particles have been on outdoor particles and there are few studies on indoor particles in relation to health (Schneider et al., 2003). Moreover, relatively few epidemiologic studies so far have been performed on the health effects of indoor or outdoor UFP exposure.
School environment in relation to asthma and allergy

Sweden

In Sweden, researchers have paid special attention to the school environment in relation to children’s health. Smedje and co-workers (1997) showed abundant levels of cat and dog allergens from 96 classrooms in 38 randomly selected schools. Another study also found common contamination of cat and dog allergens in Swedish schools, while dust mite and cockroach allergens were generally immeasurable (Perzanowski et al., 1999). A study by Almqvist and co-workers (1999) indicated that there was significant cat allergen exposure at schools and allergen contamination derived from transfer through clothing from homes with cats.

A few publications reported adverse effects on asthma or allergy due to allergen contaminations in schools (Almqvist et al., 2001; Munir et al., 1993; Smedje and Norbäck, 2001a; Smedje et al., 1997). Munir and co-workers (1993) concluded that both cat and dog allergen levels present in the 29 classrooms (4 schools) in southern Sweden were sufficient to sensitise children and to induce asthma in those with cat or dog allergy.

Smedje and co-workers (1997) also reported associations between current asthma and different environmental factors in schools such as more open shelves, lower room temperature, higher relative humidity (RH), high formaldehyde concentration, volatile organic compounds and microbial exposure. Another study showed that high formaldehyde concentration and molds in classroom may affect the four year incidence of asthma and sensitivity to furry pets among schoolchildren (Smedje and Norbäck, 2001a). Moreover, one study reported a lower degree of nasal patency among adults at high concentrations of respirable dust, NO₂, formaldehyde, total molds and presence of *Aspergillus spp.* in the classrooms (Norbäck et al., 2000b). Although the study was performed among adults, similar effects could be considered for children.

Korea

Despite the increase of prevalence of allergic disease in Korea (Hong et al., 2004), there is no publication available on school environment in relation to respiratory symptoms. Only one study measured indoor and outdoor bioaerosol levels in 44 classrooms at 11 elementary schools as well as at 83 recreation facilities and 20 homes (Jo and Seo, 2005). However, the study only examined the bioaerosol levels to evaluate indoor and outdoor environment without analysing health effects.

Since there was no school environmental study in relation to health aspects, existing publications on Korean indoor and outdoor environment including
homes have been reviewed. There are few studies so far published on allergen levels in the Korean home environment. One study from various parts of Korea showed that Der f 1 was found in 20.6% and Der p 1 in 6.5% of homes, while other house dust mite species were rare (Ree et al., 1997). Another study in Busan and Seoul showed that B. germanica was the dominant cockroach specie found in residences (Lee et al., 2003). Lately, allergen levels of Der f 1 and Bla g 1 have been studied in Korean homes, both in bedroom dust and air (Park et al., 2002), and levels of Der f 1 have been compared in buckwheat pillows and synthetic pillows (Nam et al., 2004). Sensitisation to house dust mite, but not to German cockroach, seemed to play a considerable role in reduced pulmonary function among asthmatic children (Choi et al., 2005).

Moreover, many studies have been published on outdoor air pollution in Korea, such as respirable suspended particulate, SO₂, NO₂, O₃, carbon monoxide and VOCs, in relation to prevalence of asthma, respiratory symptoms or pulmonary function among adults (Bae et al., 2004; Hong et al., 2005) and schoolchildren (Choi et al., 2000; Jang et al., 2003; Lee et al., 2002; Lee et al., 2005; Kim et al., 2005a; Song, 2001). However, there are only a few Korean studies on indoor air pollution in relation to effects of respiratory symptoms or pulmonary function. One study evaluated the traffic-related air pollution and indoor source of pollution for workers (polishers and repairmen) in shoe stalls (Bae et al., 2004). The other one investigated the relationship between daily personal NO₂ exposure and pulmonary function among university students (Hong et al., 2005). Both studies were performed among adults.

On the whole, there is no epidemiological study available on Korean indoor school environment in relation to pupils’ respiratory symptoms.
Objectives

The main objective of this thesis was to study the associations between selected environmental factors and selected health factors such as asthma, respiratory symptoms and self-reported pollen and pet allergy among pupils.

- To investigate associations between formaldehyde, allergens and indoor climate in Swedish schools and selected health factors among primary schoolchildren.
- To investigate associations between dietary factors and selected health factors among Swedish primary schoolchildren.
- To study possible interaction between selected dietary factors and allergens with respect to selected health factors in Swedish schoolchildren.
- To study associations between airborne microorganisms, MVOC, selected plasticizers and selected health factors in Swedish schoolchildren.
- To evaluate allergen levels in the Korean schools and compare them with the Swedish study.
- To compare selected health factors among pupils between Korea and Sweden.
- To investigate associations between ambient chemical pollutants or ultrafine particles and selected health factors in Korean schoolchildren.

The studies were approved by the Ethics Committee of Uppsala University.
Material and methods

Study design

Paper I and II
All eight primary schools (1-6th form), belonging to Knivsta municipality, a part of the rural outskirts of Uppsala city, Sweden were included in the study. In year 2000, the headmasters of these schools were contacted and they all agreed to participate. All pupils in the eight primary schools (n=1482) received a questionnaire in Swedish during April-May 2000. The questionnaire was mailed to the parents, with instructions to answer the questions in co-operation with the child. For building inspections and indoor measurements, three home classrooms were arbitrarily chosen from each school. The only exception was one school, which only had two classrooms and both were included.

Paper III and IV
Korea has a 6 year primary school system and the children start at the age of seven and attend school 6 days/week. In year 2004, 12 schools in Guri, Namyangju and Chuncheon city in Korea were arbitrarily chosen for this study. The school administrators in these schools were contacted and agreed to participate. Among these three cities, Guri is the closest and Chuncheon is the farthest from Seoul, the capital of Korea, and Namyangju is located between the two cities. All pupils in 4th grade (9-11 years) in the selected schools were included for the questionnaire study. The questions were translated from English to Korean by two persons, and back-translated by another person. For building inspections and indoor measurements, three home classrooms were arbitrarily selected from each school, the same as in Paper I and II. The only exception was two schools, which only had two classrooms and both were included.

For comparison between Sweden and Korea in Paper III, pupils aged 9-11 years were selected from the Swedish schools (Paper I and II). Figure 1 illustrates season, countries and exposure factors involved.
The questionnaire
Asthma and respiratory symptoms (Paper I, II, III and IV)

The questionnaire contained a set of questions asking about asthma, which included doctor-diagnosed asthma, current asthma medication or asthma attacks during the last 12 months. Current asthma was defined as having either current asthma medication or having had an asthma attack during the last 12 months, a definition previously used in the ECRHS study (Plaschke et al., 2000). Another set of questions asked about airway symptoms related to asthma during the last 12 months, without using the phrase “asthma”. These symptoms included: 1) wheezing or whistling in the chest 2) at least one daytime attack of shortness of breath during exercise or while resting 3) at least one night-time awakening with attacks of breathlessness or tightness in the chest (Norbäck et al., 1999). Moreover, the questionnaire contained questions about allergy, including allergy to pollen and furry pets, which have been used in previous school investigations (Smedje and Norbäck, 2001a).

Dietary habits (Paper I)

There were seven questions about current consumption of meat, fish, fruit, vegetables, fresh milk, fermented milk products and fast food such as hamburgers. There were five alternatives given for each type of food item, intended to measure the frequency of consumption. In the statistical calculations, a consumption score was used as follows; 0= never, 1= less than once
Home environmental factors (Paper IV)

There were questions about current home environment including remodelling and indoor painting or changing floor material during the last 12 months, house age, ETS (The frequency was coded as 0=never, 1=1~3 times a week, 2=3~4 times a week and 3=every day) and indoor dampness and mold growth during the last 12 months. Indoor dampness and mold growth was defined as if any of the following four types had been observed within the home during the last 12 months: 1) water leakage or water damage indoors on walls, floors or ceilings 2) bubbles or yellow discoloration on plastic floor covering or black discoloration of parquet floor 3) visible mold growth indoors on walls, floor or ceilings 4) moldy odour indoors except in basement. Subjects reporting one or more signs of dampness are referred to as reporting any sign of indoor dampness and mold growth during the last 12 months (Norbäck et al., 1999).

Measurement

General building inspection (Paper I, II, III and IV)

The school buildings were inspected and all samples were collected during May–June and November–December, 2000 (Paper I), May-June, 2000 (Paper II) and November–December, 2004 (Paper III and IV). Pupils of this age spend most of their time in a home-classroom in both Sweden and Korea. We arbitrarily selected 3 home-classrooms from each school (n= 8 in Paper I and II, n=12 in Paper III and IV). The only exception was one school in Paper I and II and two schools in Paper III and IV, which only had two classrooms and both were included.

The school buildings were inspected and details on construction, materials, type of ventilation system, cleaning routines, amount of open shelves and textiles and number of pupils were noted. The room volume (m$^3$), shelf factor (m/m$^3$), textile factor (m$^2$/m$^3$) and population density (number of students/ m$^3$) were calculated in each classroom.

Temperature, RH and concentration of CO$_2$ were measured in each classroom during normal activities with closed windows and doors. These factors were measured over time (45-60 min) with Q-Trak™ IAQ Monitor (TSI Incorporated, St Paul, Minnesota, USA), by logging average values over one minute. The instruments were regularly calibrated by Comfort Control, the
Swedish service laboratory for TSI equipment. The average concentration for each school was calculated.

**Indoor allergens (Paper I and III)**

Each classroom was divided into two halves and one settled dust sample was collected from the corridor-side and one from the window-side. Vacuum cleaning with a special dust collector and an ALK-filter (ALK Abello, Copenhagen, Denmark) was performed for 4 minutes per sample equally divided between floor and desks/chairs (Smedje et al., 1997). The filters were sealed with a lid and stored in plastic bags at –20 °C until extraction. The same sampling procedure and instruments were used in both countries.

In Paper III, airborne samples were collected during 6-9 days by placing two Petri dishes in each classroom (1-1.5 m height) with base and lid facing upward on horizontal surfaces on the corridor side (Karlsson et al., 2002). Petri dishes were closed and stored at room temperature until extraction. This method was not available in our laboratory when the Swedish study was performed.

The total amount of dust in each settled dust sample was weighed and 100 mg was extracted in 2 ml of phosphate buffered saline containing 0.05% Tween 20 (PBS-T) for two hours at room temperature with rotation. Samples were centrifuged at 4,500 rpm (3,500 g value) for 10 min followed by another at 10,000 rpm (7,000 g value) for 10 minutes. Supernatants were stored at –20 °C until analysis.

The base and lid of the Petri dish for airborne allergen were extracted with 3 mL PBS-T containing 1% BSA. The buffer was mixed for 1 hour at room temperature on the base and then transferred to the lid and continued for another hour. Evaporation was minimized by keeping the Petri dishes closed during the rotation. The extract was centrifuged at 10,000 rpm for 10 minutes. Supernatants were stored at –20°C until analysis.

Allergen levels were determined using two-site sandwich ELISA for cat (Fel d 1), dog (Can f 1), cockroach (Bla g 1), dust mites (Der f 1 and Der p 1) (INDOOR Biotechnologies Ltd., Manchester, UK) and horse allergen (Equ c x) (MABTECH, Stockholm, Sweden) (Emenius et al., 2001) using monoclonal antibodies. The assays were basically performed according to the protocols provided by the manufacturer except for the dog assay, where the horseradish peroxidase labelled goat anti-rabbit immunoglobulin was from DakoCytomation (Norden AB, Stockholm, Sweden). Allergen concentrations were expressed as ng/g dust, except for horse allergen which was expressed as Unit/g dust, where 1 Unit is equal to 1 ng protein of a horsehair and dander extract used as standard (Allergon, Valinge, Sweden and NIBSC, Hertfordshire, UK). Protein determination on the standard was performed
with the micro-BCA method (Pierce, Rockford, IL, USA) using BSA as standard. Samples with intra-assay CV>10% were re-assayed. The detection limit in settled dust samples was 200 ng/g dust for cat, dog and dust mite allergens and 200 U/g dust for horse allergen.

Airborne cat allergen samples were analysed using a commercial signal amplification kit (Ampak™, DakoCytomation) basically following the manufacturer’s recommendations (Karlsson et al., 2002). The standard curve range was 2.5 to 160 pg Fel d 1/ml. Detection limit for airborne allergens were 0.005 ng/ml for Fel d 1 and 0.39 ng/ml for Can f 1 in solutions. Airborne allergen levels were expressed as ng/(m²day) calculated on a total surface of 0.024 m² of the Petri dish. This corresponds to a detection limit of 0.10 ng/(m²day) for Fel d 1 and 7.86 ng/(m²day) for Can f 1.

Microorganisms, MVOC, plasticizers & formaldehyde (Paper II)
Airborne microorganisms, MVOC, selected plasticizer compounds and formaldehyde were sampled simultaneously in the classrooms (3 samples/school) and outside the building (1 sample/school) during May. Airborne microorganisms were sampled with airflow of 2.0 l/min for 4 hours on 25 mm nucleopore filters (pore size 0.4 µm). The total concentration of airborne molds and bacteria were determined by the CAMNEA (collection of airborne microorganisms on nucleopore filters, estimation and analysis) (Palmgren et al., 1986) performed by Pegasus Lab, Uppsala, Sweden. This method is based on acridine orange staining and epifluorescence microscopy. Selected viable molds and bacteria species were determined by incubation on two different media; Tryptone glycose agar (TGEA) and malt extract agar (DG18) at 22 °C (±1 °C). The incubation time was 7 days for both media and all microorganisms except for Streptomyces sp., where the incubation time was 21 days. The detection limit for viable organisms was 30 colony forming units (cfu) per m³ of air and 11,000 cfu/m³ for total bacteria and molds.

Airborne MVOC and plasticizers were sampled on a charcoal tube (Anasorb 747, SKC Inc. Eighty Four, PA, USA) with a pump with a flow rate of 0.25 l/min for 4 hours. The tubes were desorbed with 2 ml of methylene chloride and analysed by selective ion monitoring gas chromatography mass spectrometry (GC-MS) (Wessén and Schoeps, 1996) by Pegasus Lab, Uppsala, Sweden. The detection limit was 1 ng/m³ for all MVOC and 0.1 µg/m³ for 2-ethyl-1-hexanol, TMPD-MIB and TMPD-DIB. The total concentration of the selected MVOC (total MVOC) was defined as mass summation excluding the butanols, 2-ethyl-1-hexanol, TMPD-MIB and TMPD-DIB. Indoor concentrations of formaldehyde were measured with glass fibre filters impregnated with 2,4-dinitro-phenylhydrazine (Andersson et al., 1981) with an air sampling flow of 0.2 l/min during six hours. The filters were analysed by liquid chromatography at the Department of Occupational and Environ-
mental Medicine, Örebro, Sweden. The detection limit for formaldehyde was 6 µg/m³.

Chemical air pollutants and ultrafine particles (Paper IV)
Passive sampling of SO₂, NO₂, O₃ (samplers from IVL Swedish Environmental Research Institute, Göteborg, Sweden) and formaldehyde (samplers from SKC Inc., PA, USA) were performed during 7 days for both indoor and outdoor environment. Indoor samplers were placed on a wall in each classroom (approximately 1.5 m above the floor) while outdoor samplers were placed inside a plastic box hanging outside a classroom window protected from rain. Both indoor and outdoor UFP were measured with a P-Trak™ (model 8525 ultrafine particle counter, TSI), measuring particles in the size range of 0.02-1.00 µm (Norbäck et al., 2006).

The samplers for SO₂, NO₂ and O₃ were analysed at an accredited laboratory (IVL Swedish Environmental Research Institute, Göteborg, Sweden) and the samplers for formaldehyde were analysed by another accredited laboratory (Occupational and Environmental Medicine, University Hospital, Örebro, Sweden).

Statistical methods
Differences in prevalence were analysed by χ²-test. To analyse the associations between symptoms and different environmental factors, multiple logistic and linear regression analyses were performed with general adjustments for age and gender, and additional adjustment for different confounders in each study (See respective paper). The results are presented as ORs with a 95% confidence interval (CI).

For the statistical calculations, exposure levels below the detection limit were assigned a value of half of the detection limit. Mean exposure levels were calculated for each school and used in the regression models (Paper I, II, and III). In Paper IV, two different mean exposure levels were calculated. Mean exposure levels for each classroom were used for analysis of association between indoor exposure and symptoms, and for the rest of the analyses the mean exposure levels for each school were used (see respective table). Correlations between different factors were performed by Kendall’s Tau-beta rank test. In all statistical analyses, two-tailed tests and a 5% level of significance were applied.

Paper I
Unpaired t-test was used to study age trends, by comparing mean age in children with and without a particular symptom. Median and inter-quartile
range (IQR) was calculated for allergens, as they were not normally distributed.

The statistical analyses in Paper I were mainly performed by the SPIDA statistical package from the Statistical Laboratory, Macquarie University, Australia (Gebski et al., 1992). Interaction terms in logistic regression analysis were tested statistically, using the STATA program (Stata Corporation, College Station, TX, USA).

Paper II, III and IV

All statistical analyses were performed with Statistical Package for Social Sciences (SPSS) for Windows, version 12.0.1.

Arithmetic mean value with standard deviation and min-max value were calculated for indoor climate and ventilation (Paper II). Interaction terms in logistic regression analysis were tested by STATA, the same as in Paper I.

Median and IQR was used for allergens as in Paper I. Differences in environmental factors between Korea and Sweden were analysed by Mann-Whitney test (Paper III).

In Paper IV, for association analysis between indoor measurements and pupils’ symptoms, the population was selected as pupils in classrooms where environmental measurements were performed (pupils=1223, classes=34). For the outdoor association, the total numbers of pupils (N=2365) was used.
Results

Paper I

In total, 1482 Swedish pupils received a questionnaire, of which 1014 (68%) answered. The median age was 9 years (range 5-14 year, S.D: 2.0) and 51% were girls. Generally, boys reported more symptoms than girls (Table 1). At increased age, pupils reported more daytime breathlessness ($P<0.01$) and atopic sensitisation ($P<0.05$).

Table 1. Reports on current airway symptom, asthma and atopic sensitisation

<table>
<thead>
<tr>
<th>Questions on current airway symptoms</th>
<th>Boys (%) (N=492)</th>
<th>Girls (%) (N=522)</th>
<th>Total (%) (N=1014)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheeze</td>
<td>8.6</td>
<td>7.1</td>
<td>7.8</td>
</tr>
<tr>
<td>Daytime breathlessness</td>
<td>4.4</td>
<td>4.6</td>
<td>4.5</td>
</tr>
<tr>
<td>Nocturnal breathlessness</td>
<td>2.3</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Questions on asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doctor-diagnosed asthma</td>
<td>10.2</td>
<td>5.4**</td>
<td>7.7</td>
</tr>
<tr>
<td>Current asthma</td>
<td>7.9</td>
<td>4.1*</td>
<td>5.9</td>
</tr>
<tr>
<td>Questions on atopic sensitisation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat allergy</td>
<td>8.0</td>
<td>5.6</td>
<td>6.8</td>
</tr>
<tr>
<td>Dog allergy</td>
<td>6.1</td>
<td>3.5*</td>
<td>4.8</td>
</tr>
<tr>
<td>Pollen allergy</td>
<td>10.7</td>
<td>7.0*</td>
<td>8.8</td>
</tr>
</tbody>
</table>

*$P<0.05$, **$P<0.01$

Current asthma was less common among those consuming more milk ($P<0.05$) and fish ($P<0.01$). Poly-unsaturated fatty acids was associated with more wheeze ($P<0.05$) while olive oil was associated with less doctor-diagnosed asthma ($P<0.05$).

Totally, 74% of the classrooms had mean CO₂-levels below 1000 ppm. The median allergen concentration per gram dust was 860 ng/g Fel d 1 (cat allergen), 750 ng/g Can f 1 (dog allergen) and 945 U/g Equ cx (horse allergen). Equ cx was associated with more wheeze ($P<0.05$), daytime breathlessness ($P<0.05$) and current asthma ($P<0.05$). Can f 1 was associated with wheeze ($P<0.05$) and daytime breathlessness ($P<0.05$). The associations between allergens and respiratory symptoms were more pronounced among those not consuming butter and with a low intake of milk (Table 2).
Table 2. OR with 95% CI for relationships between selected symptoms and allergens, stratified for frequency of consumption of milk and butter.

<table>
<thead>
<tr>
<th></th>
<th>Wheeze</th>
<th>Daytime breathlessness</th>
<th>Current asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Less than daily milk consumption (n=146)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fel d 1</td>
<td>2.18(0.43-10.1)</td>
<td>16.67(0.58-476)</td>
<td>6.51(0.77-55.0)</td>
</tr>
<tr>
<td>Can f 1</td>
<td>2.64(0.69-10.1)</td>
<td>18.31(0.77-436)</td>
<td>7.74(1.09-55.1)*</td>
</tr>
<tr>
<td>Equ cx</td>
<td>1.46(0.88-2.41)</td>
<td>2.46(0.93-6.54)</td>
<td>2.51(1.19-5.30)*</td>
</tr>
<tr>
<td><strong>Daily milk consumption (n=860)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fel d 1</td>
<td>1.21(0.43-3.42)</td>
<td>2.26(0.62-8.26)</td>
<td>1.45(0.44-4.79)</td>
</tr>
<tr>
<td>Can f 1</td>
<td>1.46(0.68-3.14)</td>
<td>2.02(0.82-4.98)</td>
<td>1.41(0.59-3.37)</td>
</tr>
<tr>
<td>Equ cx</td>
<td>1.15(0.89-1.49)</td>
<td>1.26(0.95-1.66)</td>
<td>1.14(0.85-1.52)</td>
</tr>
<tr>
<td><strong>No consumption of butter (n=824)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fel d 1</td>
<td>2.68(1.05-6.84)*</td>
<td>3.38(0.96-119)</td>
<td>2.42(0.83-7.04)</td>
</tr>
<tr>
<td>Can f 1</td>
<td>2.65(1.28-5.51)**</td>
<td>2.66(1.07-6.60)*</td>
<td>2.16(0.96-4.85)</td>
</tr>
<tr>
<td>Equ cx</td>
<td>1.44(1.12-1.84)**</td>
<td>1.38(1.04-1.83)*</td>
<td>1.36(1.04-1.80)*</td>
</tr>
<tr>
<td><strong>Consumption of butter (n=186)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fel d 1</td>
<td>0.17(0.02-1.73)</td>
<td>1.59(0.06-45.9)</td>
<td>1.27(0.04-45.7)</td>
</tr>
<tr>
<td>Can f 1</td>
<td>0.44(0.08-2.33)</td>
<td>1.73(0.17-17.6)</td>
<td>1.38(0.10-19.7)</td>
</tr>
<tr>
<td>Equ cx</td>
<td>0.61(0.31-1.22)</td>
<td>1.00(0.50-1.97)</td>
<td>0.83(0.34-2.05)</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01

Paper II

This study was based on the same schools and pupils as in Paper I.

None of the classrooms had visible mold growth or dampness. Total bacteria and total molds were below the detection limit in 13 of 23 indoor samples (57%). Mean viable mold concentration was 360 cfu/m³ indoors and 980 cfu/m³ outdoors. Mean total MVOC concentration was 423 ng/m³ indoors and 123 ng/m³ outdoors. Indoor mean concentration of TMPD-MIB and TMPD-DIB, two common plasticizers, were 0.89 and 1.64 µg/m³, respectively.

At increased indoor concentrations of total MVOC, nocturnal breathlessness (P<0.01) and doctor-diagnosed asthma (P<0.05) were more common. Moreover, there were positive associations between nocturnal breathlessness and 3-methylfuruan (P<0.01), 3-methyl-1-butanol (P<0.05), dimethyldisulfphide (P<0.01), 2-heptanone (P<0.01), 1-octen-3-ol (P<0.05), 3-octanone (P<0.05), TMPD-MIB (P<0.05) and TMPD-DIB (P<0.01). Indoor concentration of TMPD-DIB was positively associated with wheeze (P<0.05), daytime breathlessness (P<0.05), nocturnal breathlessness (P<0.01) and doctor-diagnosed asthma (P<0.05). There were no associations between any symptoms and formaldehyde concentration.
Table 3. OR with 95% CI for associations between respiratory symptoms and exposure in the schools

<table>
<thead>
<tr>
<th></th>
<th>Wheeze</th>
<th>Daytime breathlessness</th>
<th>Nocturnal breathlessness</th>
<th>Doctor-diagnosed asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total MVOCa</td>
<td>1.46(0.77-2.79)</td>
<td>1.82(0.74-4.47)</td>
<td>5.25(1.82-15.2)**</td>
<td>2.07(1.09-3.93)*</td>
</tr>
<tr>
<td>TMPD-MIBa</td>
<td>1.15(0.94-1.42)</td>
<td>1.24(0.94-1.65)</td>
<td>1.41(1.00-1.98)*</td>
<td>1.17(0.94-1.44)</td>
</tr>
<tr>
<td>TMPD-DIBa</td>
<td>1.85(1.08-3.17)*</td>
<td>2.39(1.15-4.96)*</td>
<td>5.71(2.04-16.0)**</td>
<td>1.97(1.14-3.42)*</td>
</tr>
</tbody>
</table>

*OR expressed as change of coefficient per 1 μg/m³

*p < 0.05, **p < 0.01

Moreover, there were correlations between Total MVOC and the two plasticizers, TMPD-MIB (r=0.48; P<0.01) and TMPD-DIB (r=0.53; P<0.01).

Paper III

All pupils in 4th grade in 12 Korean schools (Guri, Namyangju and Chuncheon) received a questionnaire (N=2453) and 96% completed it (N=2365). For a comparison between Korea and Sweden questionnaire studies, only pupils aged 9-11 years (N=448) were included from the Swedish schools (see Paper I and II).

In both Sweden and Korea, boys reported more symptoms. The prevalence of wheeze was similar, while daytime (OR =14.0, 95% CI = 9.0-21.9) and nocturnal breathlessness (OR =3.1, 95% CI = 1.5-6.4) were much higher among Korean students.

Table 4. Current airway symptoms, asthma and atopic sensitisation

<table>
<thead>
<tr>
<th></th>
<th>Sweden (%)</th>
<th>Korea (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n=448)</td>
<td>Total (n=2365)</td>
</tr>
<tr>
<td>Questions on current airway symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheeze</td>
<td>7.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Daytime breathlessness</td>
<td>4.8</td>
<td>41.2***</td>
</tr>
<tr>
<td>Nocturnal breathlessness</td>
<td>1.8</td>
<td>5.4**</td>
</tr>
<tr>
<td>Questions on asthma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever had asthma</td>
<td>8.1</td>
<td>11.8*</td>
</tr>
<tr>
<td>Doctor-diagnosed asthma</td>
<td>7.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Current asthma</td>
<td>5.2</td>
<td>5.7</td>
</tr>
<tr>
<td>Questions on atopic sensitisation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat allergy</td>
<td>7.0</td>
<td>2.6***</td>
</tr>
<tr>
<td>Dog allergy</td>
<td>5.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Pollen allergy</td>
<td>9.4</td>
<td>4.0***</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01

The mean temperature in the classrooms was 21 °C in both countries. The Korean schools had no mechanical ventilation system and CO₂-levels exceeded 1000 ppm in all except one of the classrooms while most Swedish
classrooms had lower than 1000 ppm. Indoor RH and population density were higher in the Korean classrooms than Swedish. On the contrary, open shelves and textiles were more common in the Swedish schools.

There was a different pattern regarding pet-keeping at home between the two countries.

![Figure 2. Reports of pet-keeping in the Korean and Swedish schools](image)

In the Korean schools, dog allergen (Can f 1) was most common, whereas cat (Fel d 1), dog and horse allergen (Equ cx) were abundant in the Swedish schools (Figure 3).

![Figure 3. Median allergen levels in the Korean and Swedish schools](image)

Fel d 1 was detected in 91% and Can f 1 in 61% of all air samples in the Korean classrooms. The geometric mean values were 0.88 ng/(m²·day) for
Fel d 1 and 20.91 ng/(m²·day) for Can f 1. Moreover, there was an association between allergen levels in dust and air samples, and number of pet-keepers in the classrooms.

Figure 4. Associations between number of pupils with pets and airborne allergen levels at Korean schools

**Paper IV**

This study is based on the same Korean material as used in Paper III. Remodelling and indoor painting (11.5 %) or changing floors (18.9%) during the last 12 months, ETS (50.0%) and indoor dampness and mold growth (21.9%) during the last 12 months were relatively common.

The mean of chemical exposure with min-max values are presented in Table 5. The indoor/outdoor ratio of SO₂, NO₂, O₃, formaldehyde and ultrafine particles (UFP) were 0.3, 0.6, 0.6, 7.2 and 1.7, respectively.

Table 5. *Indoor and outdoor chemical exposure in 12 Korean schools*

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>M (S.D.)</th>
<th>Min-max</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO₂ (µg/m³)</td>
<td></td>
<td>Indoor 34 0.6(0.2)</td>
<td>0.4-1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outdoor 12 3.1(2.0)</td>
<td>0.3-7.1</td>
</tr>
<tr>
<td>NO₂ (µg/m³)</td>
<td></td>
<td>Indoor 34 18.8(7.7)</td>
<td>7.2-37.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outdoor 12 30.7(9.8)</td>
<td>16.5-48.6</td>
</tr>
<tr>
<td>O₃ (µg/m³)</td>
<td></td>
<td>Indoor 34 7.5(3.3)</td>
<td>2.5-57.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outdoor 12 15.5(5.3)</td>
<td>6.4-25.9</td>
</tr>
<tr>
<td>Formaldehyde (µg/m³)</td>
<td></td>
<td>Indoor 34 27.7(8.3)</td>
<td>16-47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outdoor 12 4.3(1.8)</td>
<td>2-9</td>
</tr>
<tr>
<td>UFP (10³ pt/cm³)</td>
<td></td>
<td>Indoor 34 18.2(17.3)</td>
<td>3.7-52.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outdoor 12 16.5(12.5)</td>
<td>3.3-45.3</td>
</tr>
</tbody>
</table>

M (S.D.) = arithmetic mean with standard deviation
There were positive associations between respiratory symptoms and particular home environmental factors (remodelling and indoor painting or changing floor material during the last 12 months, house age, ETS and indoor dampness and mold growth during the last 12 months) (Table 6). However, there was no association for ETS and house age (data not shown). We also found associations between respiratory symptoms and indoor/outdoor NO$_2$, outdoor formaldehyde and UFP, when adjusting for age, gender and home environmental factors (Table 7). Moreover, in old school buildings, pupil reported less ever had asthma (OR = 0.83, 95 CI = 0.69-1.00) and less current asthma (OR = 0.75, 95 CI = 0.57-0.98). Adjusted OR were calculated per 10 year of building age.
Table 6. OR with 95% CI for associations between respiratory symptoms and home environmental factors (n=2365)

<table>
<thead>
<tr>
<th></th>
<th>Wheeze</th>
<th>Daytime breathlessness</th>
<th>Nocturnal breathlessness</th>
<th>Ever asthma</th>
<th>Atopic sensitisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remodelling</td>
<td>1.46(0.96-2.22)</td>
<td>1.70(1.30-2.17)***</td>
<td>1.39(0.84-2.31)</td>
<td>1.24(0.85-1.82)</td>
<td>1.68(1.14-2.48)**</td>
</tr>
<tr>
<td>Changing floor</td>
<td>1.38(0.97-1.97)</td>
<td>1.52(1.23-1.88)***</td>
<td>1.65(1.09-2.48)*</td>
<td>1.48(1.09-2.00)*</td>
<td>2.04(1.48-2.79)***</td>
</tr>
<tr>
<td>Indoor dampness</td>
<td>1.91(1.39-2.63)***</td>
<td>1.92(1.57-2.34)***</td>
<td>1.75(1.18-2.59)**</td>
<td>1.36(1.02-1.82)*</td>
<td>1.18(0.85-1.65)</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001

Table 7. OR with 95% CI for associations between respiratory symptoms and indoor/outdoor environment

<table>
<thead>
<tr>
<th></th>
<th>Wheeze</th>
<th>Daytime breathlessness</th>
<th>Nocturnal breathlessness</th>
<th>Ever asthma</th>
<th>Atopic sensitisation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outdoor environment (n=2365)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.28(1.07-1.53)**</td>
<td>1.04(0.93-1.15)</td>
<td>0.95(0.76-1.18)</td>
<td>0.91(0.78-1.07)</td>
<td>1.05(0.89-1.24)</td>
</tr>
<tr>
<td>Formaldehyde&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.07(0.40-2.87)</td>
<td>1.34(0.76-2.35)</td>
<td>4.47(1.58-12.7)**</td>
<td>3.90(1.81-8.42)**</td>
<td>0.94(0.37-2.40)</td>
</tr>
<tr>
<td>UFP&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.01(1.00-1.02)</td>
<td>1.00(0.99-1.01)</td>
<td>0.99(0.97-1.01)</td>
<td>1.02(1.01-1.03)**</td>
<td>0.99(0.98-1.00)</td>
</tr>
</tbody>
</table>

| **Indoor environment (n=1223)** |
| NO2<sup>a</sup>        | 1.40(1.05-1.86)*      | 0.96(0.82-1.13)        | 1.05(0.74-1.49)          | 1.18(0.93-1.50) | 1.68(1.29-2.19)***   |
| Formaldehyde<sup>a</sup> | 1.14(0.87-1.49)       | 0.95(0.82-1.11)        | 0.83(0.59-1.17)          | 0.91(0.72-1.15) | 0.94(0.72-1.22)      |
| UFP<sup>b</sup>         | 1.00(0.98-1.03)       | 1.00(0.99-1.01)        | 0.98(0.96-1.01)          | 1.02(1.00-1.03) | 0.99(0.97-1.02)      |

<sup>a</sup>OR expressed as change of coefficient per 10 µg/m³
<sup>b</sup>OR expressed as change of coefficient per 10<sup>3</sup> pt/cm³

*P < 0.05, **P < 0.01, ***P < 0.001
Discussion

The results indicate that environmental and dietary factors are associated with increased prevalence of asthma and respiratory symptoms among schoolchildren.

In the Swedish schools, the ventilation in the classrooms was generally good, but allergen contamination was abundant and MVOC and plasticizer exposure were common. At increased levels of allergens, MVOC and plasticizers, pupils reported more asthma and respiratory symptoms. Moreover, specific dietary factors such as consuming milk and fish were significantly related with less respiratory symptoms. We also found interactions between dietary factors and allergen exposure, particularly in subjects with consumption of fresh milk and milk fat, with respect to respiratory symptoms.

In the Korean schools, CO$_2$-levels were higher and pattern of allergen contamination was different when comparing with Swedish schools. Moreover, there was an association between allergen levels in dust and air samples, and number of pet-keepers in the Korean classrooms. Home environmental factors, indoor/outdoor airborne chemical pollutants and UFP were associated with respiratory symptoms.

Methodological considerations

Internal validity

Epidemiological studies can be affected by selection bias. The response rate was relatively good, 68% in Paper I and II and 96% in Paper III and IV, making severe selection bias less probable. Moreover, the proportion of girls was 51% in the Swedish schools (Paper I and II), and 49% in the Korean schools (Paper III and IV), indicating no gender selection. We had no background information on non-responders, so it was not possible to analyse selection effects on an individual level. For Paper I and II, we included all pupils in all primary schools in Knivsta, one suburban municipality outside of Uppsala city and for Paper III and IV we contacted arbitrarily 12 school administrators in three different cities, Guri, Namyangju and Chuncheon city in Korea. The hygienic measurements were performed in three arbitrarily chosen classrooms in all schools. For a comparison between Sweden and Korea (Paper III), allergen levels were measured at the same season (winter)
and the samplings were performed with the same method and equipment. Therefore, we consider that there was no serious selection bias in either of the school studies performed in the two countries.

Another problem in epidemiological studies is information bias (recall bias), due to an awareness of exposure and this may lead to over-reporting of symptoms. In the studies, information on symptoms (Paper I-IV), dietary habits (Paper I) and home environmental factors (IV) were collected by the same questionnaire before exposure measurements were performed.

Self-reported asthma has a high specificity, but the sensitivity is not as high, when using a verified doctor-diagnosed asthma as golden standard. Thus, a majority of those reporting asthma in our study could be expected to actually have asthma, but some underestimation of the true prevalence could be expected. On the contrary, validation studies on allergy among Swedish schoolchildren have shown that the prevalence of self-reported pollen and pet allergy is higher than the prevalence of positive skin prick tests (Hattevig et al., 1987; Bråbäck et al., 1994). This indicates that self-reported allergy from questionnaire data may lead to an overestimation, when a positive allergy test is used as the golden standard. Unfortunately, we could not find any validation study on asthma or self-reported allergy among Korean schoolchildren. One previous Korean study showed similar prevalence of pollen allergy but higher of cat allergy, which was verified by skin prick test (Kim et al., 1997). Thus, there could be some under-reporting of cat allergy in our study. We found no available population data about dog allergy in Korea.

The exposure measurements were all carried out by professional environmental engineers shortly after the questionnaires had been answered. The same calibrated direct-reading instruments were used in all schools, and all environmental samples were analysed in the same batch in the laboratories.

When performing a number of statistical tests, there is a risk for mass significance. In the studies, a number of statistical tests were performed, but we found mostly similar results using different types of statistical models. Finally, a cross-sectional study has certain limitations with respect to causality, e.g. if there is selection effects over time in relation to exposure or dietary factors. Selection effects in relation to exposure are less likely in the school environment. In Paper I, we found some indications of health-related avoidance behaviour in relation to milk allergy/lactose intolerance, but not fish and fruit consumption.

Therefore, we consider the study to have sufficient internal validity, and the results are less likely to be seriously biased by information or selection bias, or due to chance findings.
External validity

All primary schools in Knivsta, Uppsala were included for the study (Paper I and II). In Paper I and II, the results of building characteristics and formaldehyde level were similar but air levels of microorganisms were lower than in other previous studies in Sweden (Norbäck et al., 2000b; Smedje et al., 1997).

Primary schools in three different cities in Korea were arbitrarily chosen for the study in Paper III and IV. For Paper III and IV, there are no comparable data on allergen or chemical pollutant levels in Korean school environments. However, we arbitrarily chose the schools located in three different regions, to find generalized exposure levels among similar aged pupils.

Since we mostly measured different environmental exposure in two countries, Sweden and Korea, it is unclear if the associations are similar in both countries. Moreover, the studies were limited to specific geographic areas but schools were arbitrarily selected. Thus, the results could be valid for children with similar age who are exposed to the particular environment in the same areas.

Asthma and respiratory symptoms

We found more asthma and more dog and pollen allergy among boys in the Swedish school study (Paper I and II). Totally 10.2% of the boys and 5.4% of the girls had ever had doctor-diagnosed asthma and current asthma was reported by 7.9% of the boys and by 4.1% of the girls. Moreover, there was no significant age trend on prevalence of self-reported cat and dog allergy.

Among the Korean school study (Paper III and IV), boys also reported more ever had doctor-diagnosed asthma (6.5%) and current asthma (6.9%).

The gender difference on prevalence of asthma, in both Swedish and Korean school study, is in agreement with previous studies in younger children (Forsberg et al., 1997; Rönmark et al., 1998; Morgan and Martinez, 1992). However, in the Korean school study, there was no significant gender difference in other respiratory symptoms except doctor-diagnosed asthma and current asthma, suggesting that there might be a gender bias in asthma diagnosis and treatment.

School environment

Aspects of indoor climate

Mean room temperature was 23.2 °C in May and 21.4 °C in December in the Swedish schools (Paper I and II), and 21.7 °C (December) in the Korean schools (Paper III and IV). This is in agreement with other studies in Swe-
den, showing room temperature above 22 °C in the classrooms, particularly in the warm part of the year (Smedje et al., 1997; Ruotsalainen et al., 1995), whereas the levels of temperature and RH in the Korean schools were slightly higher than in a previous environment study (Jo et al., 2005).

Most classrooms (74 %) had sufficient ventilation during both seasons, with CO₂-levels below the hygienic level of 1000 ppm in the Swedish schools, while CO₂-levels in the Korean schools exceeded the hygienic level in all classrooms except one. In the Swedish schools most classrooms had the new type of displacement ventilation system, a type of ventilation that reduces the CO₂-levels in the classrooms (Smedje and Norbäck, 2000). However, there was no mechanical ventilation system in the Korean schools. Thus, the concept of mechanical ventilation needs to be introduced in the future.

Allergen contamination in relation to pet-keeping

Pet allergen levels in the Korean schools were correlated to the number of furry pets kept at home. This is reasonable since the main source of allergen contamination in classrooms is from the clothes of the children (Smedje and Norbäck, 2001a; Perzanowski et al., 1999; Patchett et al., 1997). Textile materials and fittings may also serve as reservoirs of deposited allergens (Smedje and Norbäck, 2001b).

Keeping pets at home in Sweden is not restricted to the countryside and a majority of families with children keep furry pets, also when living in towns. Data from the county of Uppsala have shown that 56% of compulsory schoolchildren had furry pets at home, of which 34% had a cat and 25% had a dog (Smedje and Norbäck, 2001a).

In Korea, pet-keeping is generally becoming more popular and keeping small dogs are relatively common, which was also seen in our findings. However, there was no information on pet-keeping rates or types of pets in the general population in Korea.

The high levels of cat and dog allergens in the Swedish classrooms are in agreement with data from previous studies in Sweden (Munir et al., 1993; Perzanowski et al., 1999; Smedje et al., 1997), but results are not directly comparable due to methodological differences such as different type of filter, extract buffer or extraction time. The presence of high levels of horse allergen in the Swedish schools is a new (but not unexpected) finding since Knivsta is a rural part of the municipality of Uppsala, with a lot of the children keeping pets at home and riding a horse. The absence of house dust mite allergens in the Swedish schools is in agreement with previous findings (Smedje et al., 1997).
In the Korean schools, dog allergen was the most common followed by mite allergen (Der f 1). Since the climate in Korea is warmer and more humid, the level of mite allergens at home is probably higher in Korea, and consequently more mite allergens can be transported to the school environment.

However, cat and horse allergen levels in the classrooms were relatively low, which is logical since cat ownership or horse riding was not common among Korean pupils. We have found no publications on measurements of furry pet allergen levels in Korean indoor environments such as dwellings and schools. Previous studies from Korea have shown that the house dust mites (Der f 1 and Der p 1) are relatively common in Korean homes (Ree et al., 1997) and Der f 1 can be detected in settled dust and in air samples from Korean dwellings (Park et al., 2002; Nam et al., 2004). In addition, the German cockroach is common in Korean homes (Lee et al., 2003) and Bla g 1 allergen has been detected in bedrooms in Korean homes, both in dust and in the air (Park et al., 2002).

Particular factors in relation to symptoms

Dietary factors

Our data in Paper I suggested a protective effect of more frequent fish consumption with respect to asthma symptoms, which was consistent even when excluding subjects reporting food allergy/intolerance. A high intake of omega-3 essential fatty acids, a fatty acid mainly from fat fish, has been shown to be protective for asthma (Romieu and Trenga, 2001; Simopoulos, 2002). In Sweden, fat fish such as salmon and herring is a significant part of the total fish consumption, but we have no detailed information on how much fat fish the schoolchildren consume, since we only asked about the frequency of consumption. A protective effect of fish consumption has been demonstrated in previous studies with respect to asthma (Hodge et al., 1996; Fluge et al., 1998; Nafstad et al., 2003) and bronchial hyper-responsiveness (Peat et al., 1992). In contrast, a Japanese study showed a higher consumption of fish among those with asthmatic symptoms (Takemura et al., 2002).

Moreover, we found an association between fast food consumption and asthma symptoms in subjects with no history of food allergy/intolerance. This is in agreement with previous studies (Hijazi et al., 2000; Norbäck et al., 2006; Wickens et al., 2005). Unexpectedly, we found a positive association between frequent fruit consumption and asthma, which is in contrast to most other studies showing a protective effect of fruit consumption (Denny et al., 2003; Norbäck et al., 2006; Romieu and Trenga, 2001). This may be explained by avoidance habits among subjects with food allergy. Initially we
found that frequent consumption of milk was protective for asthma, which is in agreement with some previous studies (Wickens et al., 2002; Woods et al., 2003). The association was less pronounced when excluding subjects with food allergy/intolerance. Thus, the association could partly be due to milk avoidance among those with milk allergy/intolerance.

The majority of the families consumed margarine (81%) and olive oil (55%) but butter was rarely used (18%). This illustrates the internationalization of dietary habits, since olive oil is not a part of traditional Swedish cooking. When restricting the analysis to subjects without food allergy/intolerance, we found a negative association between margarine consumption and wheeze, and between olive oil consumption and asthma. Two previous studies have shown that margarine consumption can be a risk factor for atopic sensitisation (Bolte et al., 2001) and wheeze (Farchi et al., 2003). We found a protective effect of olive oil consumption, with respect to asthma, among those without any food allergy/intolerance. Finally, we found that consumption of poly-unsaturated oils was a risk factor for asthma. This illustrates the need to further study the effects of consumption of different types of oils in relation to respiratory symptoms.

Allergens

In the Swedish study (Paper I), we found frequent contamination of cat, dog and horse allergens in settled dust in classrooms. There was an association between dog and horse allergen contamination in the schools and current asthma and respiratory symptoms. To my knowledge, there are no previous studies showing associations between airway symptoms and dog and horse allergens in classrooms. Some previous studies have indicated that cat allergen (Smedje et al., 1997; Smedje and Norbäck, 2001a) and cockroach allergen contamination, specially in USA (Amr et al., 2003), could be risk factors for asthma in schoolchildren.

In addition, we found less association between allergen levels and respiratory symptoms among children with consumption of butter and with a more frequent intake of fresh milk. In contrast, children consuming margarine had a more pronounced association between allergen levels and respiratory symptoms. Thus, it is possible that there is a protective effect linked to intake of milk fat. To my knowledge, our study is the first publication on interaction between indoor allergens and dietary factors.

Microbial volatile organic compounds

Totally, 10 out of 14 MVOC were significantly associated with nocturnal attacks of breathlessness, of which 5 with significant p-values below 0.01
Associations between indoor VOC in dwellings and attacks of nocturnal breathlessness have previously been reported (Norbäck et al., 1995a). We have found only two previous epidemiological studies on MVOC in relation to asthma and asthmatic symptoms, both reporting significant positive associations between MVOC and asthma (Elke et al., 1999; Smedje et al., 1996). In addition, one experimental exposure chamber study showed that 3-methylfuran, one common MVOC, can cause increase of inflammatory biomarkers in nasal lavage and decreased forced vital capacity of the lungs (Wålinder et al., 2005). Moreover, one atopic subject with previous occupational exposure to molds had an acute airway obstruction and delayed pulmonary reactions with influenza-like symptoms after being exposed to 3-methylfuran (Wålinder et al., 1998). This indicates that delayed respiratory reactions to MVOC may occur, but the concentration of 3-methylfuran in the exposure chamber study was much higher than in our schools. Generally, the concentration of MVOC in our study was similar to another Swedish school study (Smedje et al., 1996) using the same analytical laboratory as we have used. In contrast, higher levels of MVOC were measured in a German study (Elke et al., 1999). However, that study was performed in dwellings and used a less specific analytical method (dual-column gas chromatography with flame ionisation detection), while we used GC-MS with selective ion monitoring (Wessén and Schoeps, 1996). Despite no visible signs of dampness and molds in any classroom, there were large variations of MVOC concentrations, which was not related to air exchange rate, population density, shelf or textile factor, but associated with air concentrations of plasticizers. Laboratory measurements of material samples have shown some increase of MVOC when the material is moldy, but emission of MVOC may also occur from reference materials (Pasanen et al., 1998). Therefore, it is unclear to what extent MVOC is a specific indicator of indoor microbial exposure. In order to clarify the usefulness of MVOC as an indicator of hidden mold growth, further field studies are needed.

**Plasticizers**

Besides the association with MVOC in Paper II, we found positive associations between TMPD-MIB and TMPD-DIB, two common plasticizers, and asthma and asthmatic symptoms. The higher levels of TMPD-MIB and TMPD-DIB were found in two schools, which were newly constructed/reconstructed, suggesting that one source could be emissions from new building materials. We have found no other epidemiological studies on associations between these two compounds and asthma. TMPD-MIB was associated with only nocturnal attacks of breathlessness, while TMPD-DIB was associated with both asthma and various types of asthmatic symptoms. However, there was no association between 2-ethyl-1-hexanol and asthma or respiratory symptoms. The levels of 2-ethyl-1-hexanol were low (0.2-2.0
µg/m³) in the classrooms and were equal to what is normally found indoors. Levels above 5-10 µg/m³ are found in buildings with floor dampness, causing excessive emission of 2-ethyl-1-hexanol formed by alkaline degradation of di-ethyl-hexyl-phtalate in PVC-floor materials (Wieslander et al., 1999). TMPD-MIB is found in water based paints (Wieslander et al., 1997; Sparks et al., 1999), and levels in the range of 30-100 µg/m³ are generally found in recently painted indoor environments (Sparks et al., 1999). Our indoor levels (0.07-4.4 µg/m³) were much lower, since none of the schools had been painted during the last year. TMPD-DIB is mainly emitted from PVC-materials but is also used as plasticizer in other polymer products (Cain et al., 2005). Our levels (0.7-3.4 µg/m³) were similar to what is normally found in dwellings and were much lower than the levels detected in recently painted dwellings (Wieslander et al., 1997). One experimental study concluded that TMPD-DIB can contribute to odour at ppb levels, but irritation occurs only at higher levels (Cain et al., 2005). On the other hand, it has been demonstrated that TMPD-DIB is found in higher levels in dwellings with health problems (‘sick houses’) as compared to normal houses (Kostiainen, 1995). Moreover, an association between TMPD-DIB-levels in dwellings and clinical signs of eosinohilic inflammation has been demonstrated (Norbäck et al., 1995b). Since TMPD-DIB and phthalate esters are both used as plasticizers in PVC-materials, it is likely that these compounds are associated in indoor environment. We did not measure phthalates in our study and other studies on health effects of phthalate plasticizers (Bornehag et al., 2004; Shea et al., 2003) usually have not measured TMPD-DIB. Thus, TMPD-DIB levels could be a proxy-variable for phthalate exposure, and vice versa, and in future studies exposure to different types of plasticizer compounds should be evaluated.

Home environmental factors

Home environmental factors were questioned in the same questionnaire, which was used to report symptoms. In Paper IV, there were strong associations between both respiratory symptoms and reports on atopic sensitisations on one side and the following factors; remodelling and indoor painting, changing floor materials and indoor dampness and molds. Adverse effects on indoor dampness were in agreement with many previous studies (Billings and Howard, 1998; Bornehag et al., 2005a; Gunnbjörnsdottir et al., 2003; Norbäck et al., 1999; Venn et al., 2003). In our study, we found particularly high ORs for signs of dampness in the floor construction, which is in agreement with some Swedish studies (Bornehag et al., 2005a, Gunnbjörnsdottir et al., 2006; Norbäck et al., 1999). Moreover, adverse asthmatic symptoms have previously been shown for recent renovation (Jaakkola et al., 2004) and newly painted surfaces (Jaakkola et al., 2004; Wieslander et al., 1997). There are also Asian home environmental studies on airway/respiratory symptoms
and asthma (Lee et al., 2006; Saijo et al., 2004; Yang et al., 1997). One Japanese study showed that there were significant relationships between both VOCs and building dampness and throat and respiratory symptoms (Saijo et al., 2004). Chinese studies reported associations between respiratory symptoms and molds or indications of home dampness (Salo et al., 2004; Yang et al., 1997; Zheng et al., 2002).

In Korea, there are studies performing characterization or measurement of indoor pollution of dwellings (Kim et al., 1996; Kim et al., 2005b; Son et al., 2003; Yang et al., 2004), but we found no available publication on home environmental exposure in relation to health effects.

Airborne chemical exposures
There were associations between more wheeze and atopic sensitisation at increased levels of indoor NO₂ (Paper IV). Previous studies showed that NO₂ can be a risk factor for respiratory health among children (Pilotto et al., 1997; Mi et al., 2006). We also found positive associations between indoor O₃ and both daytime breathlessness and asthma. Adverse effects of O₃ on respiratory health have been reported in a review article (Sundell and Zuber, 1996). However, these studies are based on outdoor O₃. There are few studies on indoor O₃ in relation to respiratory health. In contrast to our finding, one Shanghai school study found negative association between both indoor/outdoor O₃ and daytime breathlessness (Mi et al., 2006).

Outdoor levels of O₃ in our study were well below 100 µg/m³ for daily maximum 8 hours mean, but outdoor NO₂ was sometimes above 40 µg/m³ for annual mean (WHO standard) (WHO, 2005).

We found consistent associations between outdoor formaldehyde and both nocturnal breathlessness and asthma, although average outdoor formaldehyde levels were much lower than 100 µg/m³ for a 30-minute average, the recommended value by WHO AQG (WHO, 2000). Despite higher levels of indoor formaldehyde there was no health association. One possible explanation could be that outdoor formaldehyde is an indicator of reactive chemistry where other irritative compounds can be formed (Sundell and Zuber, 1996; Weschler, 2006) while indoor formaldehyde in our study was mainly from the building materials.

In addition, we found that pupils in the new schools (less than 10 year old) reported more symptoms. This could be explained by effects from emissions of new materials, which was seen in new dwellings or different building design between schools.

Ultrapine particles
In Paper IV, we measured UFP both indoors and outdoors. Since it was the first study to measure UFP in the school environment, the levels could not be
compared with any other school study. The results showed associations between ever had asthma and indoor/outdoor UFP exposure.

Over the years, there have been many concerns on UFP in relation to health (Donaldson et al., 2002; Sioutas et al., 2005; Penn et al., 2005). One review article summarized that inhalation of low doses of carbonaceous UFP can cause mild pulmonary inflammation in rodents. Moreover, a sensitised respiratory tract can increase the susceptibility to adverse health effects due to UFP. Furthermore, UFP effects can be significantly enhanced by a gaseous co-pollutant such as ozone (Oberförster, 2001). Therefore, there are needs of future studies on UFP in relation to health, especially for vulnerable subject such as elderly, children or persons with chronic respiratory symptoms.
Conclusions and future implications

There is a need for international studies comparing indoor and outdoor environments in different parts of the world by using standardised methods. This work was performed in the two developed countries, Sweden and Korea, the latter with rapid changes of diets, life styles and environmental pollution. This thesis contributes to the knowledge on environmental risk factors, particularly in the school environment.

Allergens, dietary factors and chemical exposure can be of importance for asthma and atopic sensitisation in children. A more frequent consumption of fish and olive oil could be beneficial, whereas consumption of fast food and poly-unsaturated oils could be risk factors. Since we found less association between allergen levels and respiratory symptoms among children consuming butter and fresh milk, it is possible that there is a protective effect linked to the intake of milk fat.

The ventilation measurements confirmed that use of the new type of displacement ventilation enables the Swedish schools to keep mean CO₂-levels below 1000 ppm, which is in accordance with current Swedish ventilation standards. However, in most Korean schools CO₂-levels exceeded this standard. Therefore, the concept of mechanical ventilation system should be considered in Korean schools as well.

The pattern of allergen exposure differed between the two countries; Dog and one type of mite (Der f 1) allergen were the dominating allergens in the Korean schools, while cat, dog and horse allergens were more common in the Swedish schools. Future intervention studies in different parts of the world, may contribute to establish standards for indoor allergen levels.

Contamination of specific allergens, microbial compounds and chemical pollutants was common in school environments. At increased exposure to allergens, MVOC, NO₂, formaldehyde and ultrafine particles pupils were more likely to have asthma and respiratory symptoms. Since different allergens and microbial/chemical compounds may affect pupils' respiratory symptoms, air quality ought to be an important public issue and more research should be put into this issue. Changes in dietary habits as well as improvements of the indoor environment can be relevant to counteract the in-
crease of asthma and allergies in the society. In particular, since allergens are transported from other environments, mainly the home environment, the best method should be to minimize transfer of allergens. Increased cleaning in the schools may also reduce allergen levels, but the efficiency of this measure must be evaluated in intervention studies. Furthermore, interaction between dietary habits and environmental exposure needs to be further investigated in the future.
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