Indoor and Outdoor Air Pollution in Relation to Allergy and Asthma in Taiyuan, China

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Abstract

The aim was to study the prevalence of asthma, eczema, allergy and respiratory symptoms among pupils in Shanxi province, China, in relation to home and school environment and outdoor air pollution. In one study there was a low prevalence of self-reported asthma, eczema and pollen or pet allergy among pupils (9-20y). Rural childhood and consumption of fruit and fish were negatively associated with asthma or allergy, while current urban residency and consumption of hamburgers tended to be risk factors. In another study in junior high school pupils, similar low prevalence of asthma and allergy was found. Compared with pupils at the same age in Uppsala, Sweden, asthma and allergy were less common while daytime attacks of breathlessness were more common in Chinese pupils. Parental asthma or allergy was a predictor of asthma symptoms. Factors in the home environment such as new floor, new furniture and ETS exposure were risk factors for asthma symptoms. Crowdedness, dust amount, CO₂, temperature and air humidity were negatively associated with respiratory symptoms. Microbial chemical components like muramic acid and ergosterol, markers for bacteria and fungi, were negatively associated with wheeze or daytime attacks of breathlessness. The associations with endotoxin varied depending on the length of 3-hydroxy fatty acids of the lipopolysaccharides (LPS). Among outdoor air pollutants, SO₂ and formaldehyde were positively associated with asthma symptoms or respiratory infections. In addition, indoor SO₂, NO₂ and formaldehyde were positively associated with asthma symptoms and respiratory infections. In conclusion, rural childhood and dietary factors can be protective for asthma and allergy. ETS and chemical emissions from new material at home can be risk factors for asthmatic symptoms. In the school environment, factors of indoor origin seemed to be generally protective for respiratory symptoms while factors of outdoor origin seemed to be risk factors.

Keywords: Indoor air, China, School, Allergen, Dust, Microbial exposure, SO₂, Air pollutants, Epidemiology

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To my parents
List of Papers

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


III. Zhao, Z. H., Sebastian A., Larsson L, Wang Z. H., Zhang Z., Norbäck D. Asthmatic symptoms among pupils in relation to microbial dust exposure in schools in Taiyuan, China. (manuscript)

IV. Zhao, Z. H., Zhang Z., Wang Z. H., Martin F., Liang Y. L., Norbäck D. Asthmatic symptoms among pupils in relation to winter indoor and outdoor air pollution in schools in Taiyuan, China. (manuscript)

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## Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>3-OH FAs</td>
<td>3-hydroxy fatty acids</td>
</tr>
<tr>
<td>ac/h</td>
<td>air exchange rate</td>
</tr>
<tr>
<td>BHR</td>
<td>bronchial hyper-responsiveness</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CV</td>
<td>coefficient of variance</td>
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<tr>
<td>DW</td>
<td>dust weight</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>ETS</td>
<td>environmental tobacco smoking</td>
</tr>
<tr>
<td>Erg</td>
<td>ergosterol</td>
</tr>
<tr>
<td>FF</td>
<td>Fleece factor</td>
</tr>
<tr>
<td>GC-MS/MS</td>
<td>tandem gas chromatography mass spectrum</td>
</tr>
<tr>
<td>G⁺ bacteria</td>
<td>gram positive bacteria</td>
</tr>
<tr>
<td>G⁻ bacteria</td>
<td>gram negative bacteria</td>
</tr>
<tr>
<td>HSP</td>
<td>heat shock protein</td>
</tr>
<tr>
<td>I/O</td>
<td>ratio of indoor/outdoor air pollutant concentration</td>
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<tr>
<td>LAL</td>
<td>Limulus Amebocyte Lysate</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>MuA</td>
<td>muramic acid</td>
</tr>
<tr>
<td>Ns/m²</td>
<td>student number per m² of classroom floor (crowdedness)</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>R.H.</td>
<td>relative humidity</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>Temp</td>
<td>temperature</td>
</tr>
<tr>
<td>TLR</td>
<td>toll-like receptor</td>
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<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acid</td>
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</table>
Introduction

Asthma, a chronic respiratory disease, has become a major public health problem in the world, especially in western countries. It has aroused great attention from both academic researchers and the public due to the rapid increase of asthma and allergy in the young generation during the last decades. This has been documented by a large number of epidemiological studies (1-6), including two international studies, the European Committee of Respiratory Health Study (ECRHS) (adults aged 20 to 44 years) (4) and the International Study of Asthma and Allergy Phase I-III (ISAAC I-III) (children aged 6-7 years and 13-14 years) (5, 6). Besides showing the clear pattern of increasing prevalence of asthma and allergic diseases in many parts of the world, there are marked variations of asthma and allergies between countries. This indicates that the risk factors for asthma and allergy are related more with ‘environment’ than the ‘genes’, but there is a need for better understanding of the gene-environment interaction for asthma and allergy.

Environmental risk factors for asthma or allergic diseases include a wide range of factors, not only the chemical and biological exposures in indoor and outdoor environment, but also social factors, early life, lifestyle and psychological factors. It has been proposed that it is the changes of a number of related factors associated with modern and western life style, the so-called ‘westernization package’, that provides possibilities for the increase of asthma (7). These changes can include two trends—not mutually exclusive—risk factors that promote asthma have been added to the environment or factors that provide protection are lost (8).

It is particularly important to identify risk factors and protective factors in children since young people are more susceptible (9). For them, homes and schools are the most important places where they spend as much as 80-90% of their time (10). However, school environment has received less attention by researchers compared with home environment (11, 12). There are indications that schools have environmental deficiencies more often than other buildings due to lack of financial resources (13).
Asthma and allergy among children

In the beginning of 1990s, there were a number of studies on the morbidity and mortality of asthma in children (14). Later epidemiological studies concluded that there was an increase of wheezing illness in children (9). The large ISAAC study, beginning in 1991, was completed in 2003 with three phases of cross-sectional multicenter epidemiological surveys in two age groups of 6-7 and 13-14 years old. The first phase study demonstrated the presence of the wide variations of prevalence of asthma symptoms between countries (for example, wheeze symptom: 2.1-32.2% for 13-14y group and 4.1-32.1% for 6-7y group), with the highest prevalence in English speaking countries and Latin America. The third phase study showed that, after a mean of 7 years since the first study, most centers had a change of prevalence at least one standard deviation for at least one disorder (asthma, allergic rhinoconjunctivitis and eczema), with increases being twice as common as decreases, and increases being more common in the 6-7 year age group than in the 13-14 year age group. However, the asthma symptoms in the older age-group showed decreases more often in places with high prevalence which were usually in industrialized countries (6). Asia Pacific and India were the only regions where increases of all three disorders occurred more often in both age-groups. Factors that affect asthma and allergies might act in different ways in developed countries than in developing countries, and interaction with socio-economic status might be important (6). Since a large part of the global population lives in Asia, especially in China, it is important to identify the reasons for the current increase of asthma and allergy in these regions.

Personal factors for children’s asthma and allergy

Age

There are a large number of studies on the natural history of asthma (15). In early childhood, “transient early wheezing” predominates during the first 3 years of life, due to anatomical factors (e.g. small airways) and infections (e.g. respiratory syncytial virus). During the middle part of the first decade of life, wheezing can be a mix of infectious and allergic wheezing. And later persistent allergic wheezing predominates, usually associated with a diagnosis of asthma (16). Later childhood and adolescent is accompanied with less onset of wheeze but increased incidence of asthma, particularly in females. Generally, if there is a later onset of asthma in childhood, the risk for asthma relapse in adulthood is decreased (17).
Gender
In earlier childhood, boys have more asthma, but beginning at puberty more girls get asthma so that by adulthood the sex ratio is skewed towards females (18-21). There is a similar gender pattern for the severity of symptoms, as measured by hospitalization (22), level of BHR (23) and prevalence of symptoms (24). Some evidence of sex-specific genes has been found associated with asthma phenotypes (25, 26).

Parental asthma and allergy
Parental history is used as an indicator of genetic susceptibility to asthma: 80% of children with two asthmatic parents develop the disease compared with 40% of children with one asthmatic parent and 10% of children with no asthmatic parents (27). Also, children with atopic parents have an increased risk of developing allergic sensitization to common aeroallergens (28). However, the genetics behind asthma and atopy is complex and few genes are consistently associated with the same asthma phenotype in different populations (29). Asthma is a heterogenous condition and different forms of asthma may predominate in different geographic locations (7). A significant part of the genetic determination of asthma might depend upon environmental factors that trigger the disease (gene-environment interaction) (30).

Early life factors
In the late 1980s, Strachan postulated that the negative associations of large household size and hay fever could be explained by a preventive effect of infection in early childhood, transmitted by older siblings. This theory was later termed as the “hygiene hypothesis” (31). Later studies also found that attendance of daycare center early in infancy were inversely related with wheeze, asthma and atopy at school age (32, 33). Moreover, it has been consistently demonstrated that farm children have less atopy and sometimes less asthma, as compared to urban children or rural non-farm children (34, 35). The reason has been suggested to be the higher exposure to microbial compounds in farms, supported by a study in Switzerland showing that farm children had higher levels of endotoxin in mattress dust, and endotoxin levels in mattress dust were negatively associated with hay fever, atopic asthma and atopic sensitization (36). In contrast, other studies have showed that early infections of the lower respiratory tract can be risk factors for persistent wheeze and asthma (37, 38).

Pet ownership early in life has been shown negatively associated with atopy (33, 39), and having a cat before 18 years was protective against allergy to outdoor allergens, airway hyperresponsiveness, current wheeze and current
Later studies suggested that these data might be mediated by avoidance behavior (removal of pets in families with sensitized and/or symptomatic children) which contributed to the inverse association (41), especially for cats (42). This was further confirmed in a recent study that the selective avoidance is present but it appears to be of limited magnitude and most likely accounts for only a part of the described protective effects (42). Other research has proposed that the increased exposure to bacterial endotoxin, which is associated with pets, may explain the effects (43, 44).

**Parasite infection** has been found inversely associated with the development of atopy in Africa and Latin America (45, 46). A recent review on associations between parasite and asthma concluded that parasites do not generally protect against asthma, and the associations can be species-specific that hookworms may reduce the risk of asthma (47). One study in Anqing, China, found that *Ascaris lumbricoides* was a risk factor for childhood asthma and atopy (48), and this study also raised the possibility that immune responses to parasitization and the predisposition to atopic disease may share common mechanisms (48). Prospective and intervention studies are needed to elucidate the potential mechanisms of parasite infection in a more clear way.

**Breast feeding** has been found protective for the development of atopic dermatitis (49) and asthma (50, 51). But some other studies suggested a neutral effect or that breast-feeding may increase the risk for atopy sensitization (52, 53). However, considering the overall protective effects, breast-feeding should be encouraged for at least 4 to 6 months in infants at both high and low risk of atopy and irrespective a history of maternal asthma (54).

**Current dietary factors**

There are global changes of dietary habits, with increased consumption of fast food and soft drinks (55). Frequent fast food and soft drink consumption has been associated with a higher prevalence of childhood asthma in Saudi Arabia (56) and New Zealand (57). Decreasing antioxidant intake (fruit and vegetables), increased n-6 polyunsaturated fatty acid intake (PUFA; margarine, vegetable oil), and decreased n-3 PUFA (oily fish) intake may have contributed to the ongoing increase in asthma and atopic diseases (58). However, intervention studies mainly with supplementation with vitamin C have found insignificant or small beneficial effects (59). Since dietary habits are established early in life (60) and fetal and early life factors influence the development of asthma and atopic diseases, the focus has shifted towards dietary habits in pregnancy and early childhood (61).
Chemical and biological environment for children

Indoor environment

It is estimated that the average individual born today will spend over 95% of their life indoors (62). The home environment, in particular, represents an important source of fetal and early childhood exposures to many biological, chemical, and physical agents (63). Up till now, indoor exposure to the following substances has been reported to be associated with children’s respiratory health, especially with asthma or allergy: (i) dampness (12, 64-66); (ii) biological agents including moulds (67, 68), bacteria (35, 69), fungi (70) and allergens from cockroach (71), dust mites (72), pets (73) and rodent animals (74); (iii) textile, carpet and chemical floor materials (75); (iv) air pollutants including environmental tobacco smoke (ETS) (76), NO₂ (gas cooking) (77), SO₂ (78) and O₃ (mainly of outdoor source) (79), and Volatile organic substances (VOC) including formaldehyde (furniture, painting) (80).

Indoor climate

It has been suggested that modern buildings with better insulation may result in warmer, more humid houses with a poorer availability of fresh air (81). Poor ventilation has been associated with several health outcomes including sick building syndrome symptoms (SBS) (82), perceived air quality (PAQ) (83) and respiratory allergies and asthma. However, studies also found neither current asthma nor new onset of asthma was related to the type of ventilations system, but related with different pollutants (84, 85). Building dampness, due to high indoor humidity causing condensation, poor building design or structure deficiencies, has been recognized as potential problems for respiratory health, by being a breeding ground for moulds, fungi, bacteria and dust mites (86). In schools, thermal conditions (temperature and humidity) can affect pupils’ school performance or attendance (87).

Biological contaminants

Moulds, particularly their spores, produce allergenic and toxic material in the form of mycotoxins and glucans. In a meta-analysis by Fung and Hughson (88), it was concluded that there was an association between mould exposure and both allergy and respiratory symptoms. The increase of risk for children having cough and wheeze is generally in the range of 1.5-3.5 if the home has mould (12). For asthmatics sensitized to fungi, mouldy homes may adversely influence asthma (89). Ergosterol (Erg) and glucan (1-3-β-D-glucan) are two components used as indicators of fungal biomass (90). Epidemiological studies on glucan in relation to atopy and respiratory symptoms are quite inconsistent with some positive associations (91), but the mechanisms of the effect of fungi or mould remain unclear.
Bacteria are categorized as gram-negative bacteria (G− bacteria) and gram-positive bacteria (G+ bacteria). Microbes are currently viewed as important immunoregulators in addition to their roles as pathogens (74). Many studies have demonstrated a reduction in allergen sensitization in children of farmers, as well as in children with pets in their homes, and in children raised in day-care centers from an early age (36, 39, 92, 93). This effect has been suggested due to the microbial stimulation of the immune system. On the other hand, in adults, occupational exposure to endotoxin may cause airflow obstruction and neutrophil inflammation (94). The typical Monday asthma of cotton workers (i.e., byssinosis) is caused by endotoxin exposure (95). In fact, among others, endotoxin (lipopolysaccharide, LPS) and muramic acid (MuA) are two important bacterial components.

Endotoxin is found in the outer layer of the outer cell membrane of all G− bacteria. It has been reported to be both harmful and beneficial in the context of allergy and asthma. Studies on the immune response to endotoxin have indicated the importance of exposure timing, dosage, environmental cofactors and genetics for the direction of the endotoxin effects (96). Generally, the earlier exposure and lower level of endotoxin are in favor of the beneficial effects of endotoxin. Combined exposure to environmental cofactors, for example, heat shock protein (HSP) (97) and bacterial DNA (98), may enhance the immune modulatory effect. Different genetic pattern in the promoter region for CD14 may also modify response (99). The LPS recognition system is showed as follows (Figure 1).

By taking the asthma phenotypes into consideration, Radon has summarized the effects of endotoxin with respect to asthma (100),

"the risk of atopic asthma, mainly dominated by eosinophilic response, is decreased in those exposed to endotoxins. In contrast, the risk of non-atopic asthma, characterized by neutrophilic response, is enhanced in subjects with higher endotoxin exposure"
Endotoxin has been measured by the kinetic limulus amebocyte lysate (LAL) method since 1980s. However, large variations between laboratories have been observed in calibration test. Alternatively, tandem gas-chromatography mass spectrum (GC-MS/MS) can be used to measure the level by using internal standards for calibration (90). This method is capable to identify the different types of LPS with different lengths of carbon atoms.

Other microbial components, such as muramic acid (MuA), can also act as an immuno-modulator. As a component of peptidoglycan, MuA is present in both G- and G+ bacteria. But since the cell wall of G+ bacteria is thicker, MuA is mainly a marker for G+ bacteria. MuA can be recognized by TLR-2 receptor, and this receptor also reacts to compounds in the parasite cell walls. Despite relatively less related studied, MuA has been found inversely associated with wheezing and asthma regardless of farming and endotoxin exposure (101).

Allergens in indoor environment include dust mite, mold, pet, cockroach, rodent allergens. Studies have found that 80% of the allergenic material from dogs (Can f 1) is found in non-respirable particles >5µm whereas the majority of allergenic material from cats (Fel d 1) is in respirable particles <5 µm (102). Current reviews conclude that cat ownership shows an inconsistent effect for asthma and rhinitis while dog ownership generally shows no effect or a protective effect against sensitization (73). Mite allergens are the most common cause of indoor environment related with asthma (103). In some areas, cockroach or rodent allergy may also be important (104, 105).

VOC and formaldehyde

VOC and formaldehyde are emitted from many materials in modern homes. Common sources include solvents, floor adhesive, particle boards, wood stain, paint, cleaning products, polishers and room fresheners (106). Formaldehyde is a strong irritant, but the irritative properties differ in different VOCs. A standard mixture of 22 VOC with concentration of 25 to 50 mg/m³ has been found to induce airway inflammation and irritation (107-109). For formaldehyde, the WHO guideline is 100 µg/m³ as 30-minute mean value (110). Norback et al. examined the associations between asthmatic symptoms and VOCs and formaldehyde in the dwellings of 88 Swedish subjects and found positive associations between the prevalence of reported nocturnal breathlessness and concentrations of both substances (111). Later Wieslander et al. found recently painted indoor surfaces were a risk factor for respiratory symptoms (Odds ratio 1.5, 95% CI 1.0-2.4) and particularly for newly painted wood details and kitchens (112). Formaldehyde emission from new furniture is an important issue in Asia countries, and high levels of indoor formaldehyde have been measured, both in China (113), Korea (114) and Singapore (86).
Environmental tobacco smoke

There is consistent evidence that exposure to environmental tobacco smoke is a risk factor for the development of asthma, as well as for asthma attacks in asthmatic children. Exposure to tobacco smoke products in utero has been shown a risk factor for wheezing in the first year of life (115). Asthmatic children with smoking parents have more frequent asthma attacks and more severe symptoms (116, 117). Maternal smoking seems to be associated with a higher risk than paternal smoking (118).

Outdoor air pollutants

Outdoor air pollution includes both gaseous and particulate pollution. Primary pollutants are emitted directly out of exhaust pipes and stacks (NOx, SO2, PM, soot etc.). Secondary pollutants are formed from the primary pollutants in the co-presence of sunlight, moisture, or both (O3 and secondary particles, like sulfate). In developing countries, like in China, the pollution can be a mixture of emissions from coal-fired power plants, industrial companies and traffic exhaust. In industrialized countries, the main source of air pollution is traffic exhaust and photochemical air pollution, like ozone (119). Children are affected more by outdoor air pollution than adults since they spend more time outdoors and more often do outdoor exercises. Also their bodies are growing and can be more affected by pollutants that impair. In addition, children may have a higher uptake of air pollutants as well.

There is accumulating evidence that outdoor air pollutants aggravate the existing respiratory diseases (120-123). But there is less evidence on the causal effects on the incidence of asthma (124, 125). Classic air pollutants include SO2, NO2, O3 and particular matter (PM).

Experimental exposure to SO2 has showed that people with asthma are particularly susceptible to SO2 and may react with airway obstruction. The effect is enhanced with physical exercise (126). A study from London demonstrated increased exposure to SO2 and ozone was associated with increased attendance in pediatric emergency department for acute wheeze (127). There are also other studies showing no associations between SO2 and asthma related symptoms (128, 129). The WHO air quality guideline for SO2 is 20µg/m3 for 24-h mean SO2 and 500µg/m3 for 10-minute mean value (130).

NO2 has been found to be associated with slight decrement in lung function in children at short-term exposure. At long-term exposure, children exhibit increased respiratory symptoms, decreased lung function, and increased incidence of chronic cough and bronchitis. NO2 is marker of traffic air pollution, but a causal relationship between NO2 exposure and respiratory effects
has not yet been established. The WHO air quality guideline for NO$_2$ is 40µg/m$^3$ as an annual mean, and 200µg/m$^3$ for 1-hour mean value (130).

O$_3$ has been reported to be associated with worsening of athletic performance, reducing the lung function, wheezing, coughing, and asthma exacerbations among asthmatics (131). The WHO air quality guideline for O$_3$ is 100µg/m$^3$ as 8-hour mean value (130).

Despite observed harmful effects of air pollutants, the classic study comparing the former East and West Germany documented that asthma, hay fever and bronchial hyperresponsiveness were far more common in former West Germany than in the more polluted East Germany (132). Moreover, the ISAAC study revealed that Chinese children from Hong Kong had a three-fold higher prevalence of asthma than children living in the far more polluted cities of Mainland China (133, 134). It has been pointed out that increases in asthma prevalence have occurred at the same time as general improvements in air quality in western countries (135). Asthma is a complex disease with multi-factors and the effects of air pollutants alone apparently cannot explain the prevalence pattern of this disease.

School environment and asthma and allergy

School is an important public environment for children. Studies on school environment can be found from Nordic countries and other places in the world (136-141). Schools can be contaminated by different chemical and biological substances, and impaired indoor climate, poor ventilation, as well as noise, light, and odor.

Allergens in schools are commonly found and have been indicated as a problem for children’s respiratory health (142, 143). Cat (Fel d 1) and dog (Can f 1) allergens are the most frequently detected allergens (143), but other allergens such as horse (Equ c x), house dust mite (Der f 1, Der p 1) and cockroach (Bla g 1 and 2) have also been found in school dust. Factors influencing the allergen concentration include type of surface, cleaning frequency, number of occupants, classroom humidity, season and geography area (143).

Epidemiological studies in Swedish schools have found that current asthma was more common among pupils in schools with more open shelves, lower room temperature, higher relative air humidity, higher concentrations of formaldehyde or other VOC and viable moulds or bacteria or more cat allergen (139). Improved school ventilation system was associated with lower report of asthmatic symptoms (84). In areas with heavy outdoor air pollution, increased ventilation could introduce more outdoor air pollutants in the
classrooms. Besides the effects on respiratory health, poor indoor environmental quality is associated with reduced mental health and school performance in the children (87).

Risk factors for asthma and allergy in Chinese children

China is a large developing country with rapid changes of lifestyles accompanying the economic development. Consistent with the general pattern of asthma and allergy in developing countries, there is a low prevalence of asthma among Chinese school children. This is illustrated in the Chinese part of the large ISAAC questionnaire study I to III (6, 144). Within China, the highest prevalence rate of asthma among children has been reported from Hongkong, which is a westernized city in subtropical climate (145). There is currently a concern that the asthma and allergies are becoming important public-health issue in China.

Atopy has been demonstrated at a relatively high level of prevalence by skin prick tests among children in Hongkong (41.2%), Beijing (23.9%) and Guangzhou (30.8%) (146). In this study, atopy was defined by having at least one positive skin prick test of cat, house dust mite, mixed grass pollen, mixed tree pollen, cockroach, or Alternaria tenuis. House dust mite is one of the most common types of sensitization in the world, especially in warm climate areas. In a comparative study between urban and suburban areas of Beijing, the prevalence of sensitization to 13 common allergens was higher in the urban students than suburban ones (147).

While different risk factors for asthma and allergy have been extensively investigated in industrialized countries, less is known on the significance of environmental risk factors in China. However, some environmental exposure factors have been shown in relation to asthma and allergy in China, such as pet allergen exposure or animal contact (148, 149), environmental tobacco smoke (149, 150), particle exposure when using coal or gas for home heating or cooking, or using wood for cooking without ventilation (149, 151), outdoor air pollution (152) and dampness or molds in the dwellings (148, 149).

Epidemiological studies on asthma and allergy among Chinese pupils showed that air pollution from coal burning for heating was associated with wheeze and asthma in mainland China (153). Outdoor concentrations of a mixture of air pollutants (PM$_{2.5}$, PM$_{10-2.5}$, SO$_2$, NOx) were associated with the prevalence of cough with phlegm and wheeze (153). In a comparative study including Hongkong, Beijing and Guangzhou (146), sensitization to house dust mites and cats was associated with current wheeze and BHR in Chinese school children. However, the author concluded that the difference
Helminthic infection is a common public health problem indicated by a Chinese nationwide survey. And *Ascaris lumbricoides* was the most common helminthic infection, among which children aged between 5 to 19 years old are the most prevalent group (154). With strict definition of asthma including both airway responses to methacholine and questionnaire, it was found that the children infected with *Ascaris* had a higher prevalence of asthma, independently of sensitization to aeroallergens. The same study also suggested that the discordant relationship between the high prevalence of atopy and the low prevalence of asthma in the Chinese population could be due to *Ascaris* infection as one of the adjuvant factors (48).

There is comparatively less information on genetic heterogeneity of genes related to asthma and atopy in the Chinese population. One Chinese study reported a familial clustering of bronchial hyperresponsiveness (BHR), with the strength of relationship in the order of father-offspring < mother-offspring < offspring-offspring (155).

Generally, there are few epidemiological studies on childhood asthma and allergy in China, using environmental exposure assessment, based on the objective measurement. Moreover, very few studies have been dealing with the school environment which is an important place for children. One recent school study have been performed in junior high schools in Shanghai in the South of China (156, 157), but we found no such studies from the Northern part of China. Shanxi province is the main area for coal mining in China, providing 2/3 of the total domestic coal production. Taiyuan is the capital city of Shanxi province, with a heavy burden of air pollution. In 2000, the average annual concentration of SO$_2$ was 200µg/m$^3$ and annual TSP was 400µg/m$^3$ (158). Some studies have been performed on the air pollution in Shanxi province, including some studies on health effects (159). However, little knowledge is known on children’s asthma and asthmatic symptoms in this area, especially in relation to dietary factors, home environment and school environment both indoor and outdoor.
Aims of present investigations

The principle aim was to study reports on asthma, allergy and asthmatic symptoms in Chinese pupils, in relation to dietary factors, home environment and school environment. Specifically, the thesis had the following three aims:

1. To study the prevalence of asthma, eczema and allergy in pupils in relation to current dietary and selected residential factors in Taiyuan, Shanxi province, China.

2. To compare the prevalence of asthma, allergy and asthmatic symptoms among pupils in Taiyuan, China and Uppsala, Sweden, as well as to compare the school environment.

3. To investigate if microbial exposure could be of significance in the school environment, with respect to asthmatic symptoms among pupils in Taiyuan, China.

4. To investigate asthmatic symptoms, airway infections and a history of atopy among pupils in relation to indoor and outdoor air pollution and indoor climate in schools in Taiyuan, China.

5. To investigate asthmatic symptoms, airway infections and a history of atopy among pupils in relation to selected indoor exposure in the dwellings, such as environmental tobacco smoke (ETS) and new building materials with possible chemical emissions.

The studies were approved by the Ethics Committee of the Uppsala University.
Summary of Study Design

Taiyuan (urban & rural)

- 2360 (2 primary, 2 secondary) → 90% → 2116 (9-20y)
- Paper I
  - Health parameters (asthma, allergy, eczema)
  - Age, gender, childhood (urban vs. rural)
  - Diet (food item frequency)
  - Recent home new painting, floor and ETS

Uppsala

- (1482) (8 primary) → 68% → (1014) (1014)
- Paper II
  - Health parameters (incl. asthma symptoms)
  - School Allergens

Taiyuan (urban)

- 2209 (10 secondary) → 90% → 1993 (11-15y)
- Paper III
  - Age, gender, parental asthma or allergy
  - School dust microbial components

- Paper IV
  - Recent home new furniture, floor, painting and ETS
  - Air pollutants

Figure 2. Graph for the study design
Methods and Materials

Basically, two cross-sectional studies are included in this thesis (Figure 2). The first study (paper I) (2116 subjects) was performed in both urban and rural areas, aiming to investigate the prevalence of asthma, eczema and allergy among school pupils with urban and rural childhood in Taiyuan, China, in relation to current dietary and selected residential factors. The second study (paper II, III, IV) (1993 subjects) was performed only in urban schools, aiming to describe the prevalence of asthma, allergy and asthmatic symptoms, in relation to school environment including microbial exposure, air pollutants, and selected exposure in the home environment.

Questionnaires were used to collect data on pupils’ health parameters, including asthma, eczema, allergy and asthmatic symptoms in both studies. Selected residential factors and current dietary information were also collected by questionnaire in the first study (paper I), as well as certain home environmental factors in the second study (paper III, IV). Objective measurements were performed for the school environment assessment (II, III, IV), including indoor climates, allergens, microbial exposure and air pollutants etc.

Study population

The first study (paper I)

Totally four schools were arbitrarily selected in urban and rural areas in Taiyuan city, two primary schools and two secondary schools. One was within the urban Taiyuan city (around 3 million inhabitants) and the other three were in Qingxu county, a rural area 30km outside of Taiyuan. All classes were selected except 1st-3rd classes. The pupils were at age of 9-20 years old.

The second study (paper II, III, IV)

The second study was performed within the urban areas in Taiyuan city. Ten junior high schools were arbitrarily selected without knowing any previous complaints of health problems. In each school, if there were more than five first-year classes, five classes were selected from various floors and different
parts of the building; if there were five or less than five first-year classes, all were selected. In total, 46 first-year classes were included containing 2209 pupils aged between 11-15 years old.

For the comparison with Swedish pupils in paper II, we used data from a previous school study in Uppsala (160). It comprised eight primary schools (1st–6th form) in Knivsta, Uppsala county, situated in mid-Sweden. In each school, all pupils were invited to participate but only three classes were selected for environmental measurements, except in one school which only had two classes. In order to get children of comparable age groups, only children aged between 12-14 years old were included in this study.

Health parameters
A self-administered questionnaire on pupils’ respiratory health was distributed to the pupils. The same questionnaire was used in China and Sweden, translated from Swedish to Chinese, and back translated to Swedish by another person. Age, gender and standardized questions on pupils’ respiratory health were adapted from the ISAAC (144), ECRHS (161) and Swedish school studies (139).

The first study (paper I)
A questionnaire mainly adapted from previous studies in Swedish schools was used (139). It was distributed in classes by teachers and collected back the same day. The key questions on pupils’ health in this questionnaire were as follows:

1. Have you ever had asthma? (你是否曾经患过哮喘?)
2. Have you ever had eczema? (你曾经患过湿疹(藓)吗?)
3. Do you have allergy to cat? (你对猫过敏吗?)
4. Do you have allergy to dog? (你对狗过敏吗?)
5. Do you have allergy to pollen? (你对花粉过敏吗?)

In addition, questions on type of childhood (urban vs. rural childhood), current diet and current indoor environment in dwellings were included. For questions on diet, food frequency was asked on eight kinds of food: meat, fish, fruit, fruit juice, raw vegetables, cooked vegetables, hamburgers, and carbonated soft drinks. There were no questions on animal keeping, parental allergy or asthma or parental education.
The second study (paper II, III, IV)

The questionnaire used in this study included more information on pupils' health. In addition to ‘ever asthma’ ‘doctor’s diagnosed asthma’ and ‘allergy’, questions on respiratory symptoms were included in this study: current wheeze, daytime and nocturnal attacks of breathlessness, and respiratory infections as well. The main questions were asked as follows:

1. Have you ever had wheeze or whistling in the chest in the last 12 months?
   (在最近十二个月里，你是否出现过呼吸有杂声或哮鸣音(尖哨声)?)

2. Have you ever had attacks of breathlessness at rest (without exercise) in the daytime in the last 12 months?
   (在最近的十二个月里，你是否在运动之后感到呼吸困难?)

3. Have you ever had attacks of breathlessness at rest (after exercise) in the daytime in the last 12 months?
   (在最近的十二个月里，你是否在白天休息(未运动)的情况下感到呼吸困难?)

4. Have you ever waked up at night due to breathlessness in the last 12 months?
   (在最近的十二个月里，你是否由于呼吸困难而晚间醒来?)

5. Have you ever had upper respiratory infection, cold or middle-ear infection in the last 3 months?
   (在最近的三个月里，你是否有过上呼吸道感染，感冒或中耳炎?)

In addition, questions on parental asthma or allergy were asked as follows:

6. Please check (✓) if your parents have ever had the following diseases:
   (如你家人有过以下症状(即便现在已经痊愈)，请打勾)
   
<table>
<thead>
<tr>
<th></th>
<th>Asthma (哮喘)</th>
<th>Allergy (过敏)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father (父亲)</td>
<td>(            )</td>
<td>(            )</td>
</tr>
<tr>
<td>Mother (母亲)</td>
<td>(            )</td>
<td>(            )</td>
</tr>
</tbody>
</table>

There were no questions on pupils’ pet ownership, parental education and type of home cooking (gas, coal or electricity etc.).

Assessment of Environmental Exposure

Environmental assessment includes both home environment and school environment. Home environment was evaluated by questionnaire in two studies, including current home painting, new floor, new furniture and ETS at home. The first study also contained information on childhood environment (rural
vs. urban) and building materials as well. The questions were asked as follows:

**Paper I**

1. Did you grow up in the countryside or in a city? (你在农村长大还是在城市长大？)

**Paper I, III, IV**

1. Was there any new painting at home in the last 12 months? (在最近一年里，你家里是否油漆过？)
2. Was there any new floor material used at home in the last 12 months? (在最近一年里，你家里是否换过新的地板？)
3. Was there any new furniture at home in the last 12 months? (III, IV) (在最近一年里，你家里是否添置新家具？)
4. Is there anybody smoking at home? (有人在你家里吸烟吗？
   Every day ( ) 1~4 times/week ( ) 1~3 times/month ( ) Never ( )
   每天有 ( ) 1-4 次/星期( ) 1~3次/月( ) 从未有过 ( )

School environment measurements were performed around one week after the questionnaire was completed. It included the following aspects: classroom inspection, indoor and outdoor climate (CO₂, Temp, RH), dust amount, allergens in the settled dust (cat, dog, horse, cockroach, mite) and airborne dust (cat and dog), dust microbial components including MuA, Erg and LPS (endotoxin), and chemical air pollutants (SO₂, NO₂, O₃ and formaldehyde) indoor and outdoor in schools.

**Classroom inspection and indoor and outdoor climate**

Each classroom was inspected by a trained environmental hygiene researcher during December 2004-January 2005. The basic information included the number of students, room volume (m³), types of heating and ventilation system and signs of mould growth or dampness. Crowdedness (Ns/m²) was calculated, which was defined as the average person per m² of the classroom floor area. Moreover, shelf factor (m/m³) and fleece factor (m²/m³) were calculated in each classroom, of which shelf factor (m/m³) was defined as the length of open shelves in relation to the room volume, and fleece factor (m²/m³) was defined as the surface area of fabrics in relation to the room volume (Skov et al., 1990).

For indoor climate measurements, 3 classrooms in each school were selected (total 30 classrooms). However, due to practical reasons, 24 classrooms were
able to get measurements by a direct-reading instrument with in-built data logger. Air exchange rate per hour (ac/h) of the classrooms was calculated based on the equilibrium CO₂ concentrations, room volume and number of students (162). Simultaneously, the corresponding outdoor climate was monitored by using the same instrument.

**Dust collection**

Two samples of settled dust were collected for each classroom. One was from the corridor half and the other from the window. Dust was collected by a vacuum cleaner equipped with a special dust collector (ALK Abello, Denmark) fitted with a Millipore filter (pore size 6 µm). Vacuum cleaning was performed for 4 min for each sample, 2 min on the floor and 2 min on the desks and chairs (139). The filters were stored at 20°C until extraction.

Airborne dust was collected on one Petri-dish in each classroom, placed on the top of the blackboard (about 2 m height) in the front of the classroom and kept open for 7 days (163). After buffer extraction of allergens, the liquid was transferred to an Eppendorf tube and centrifuged. The supernatants were stored at 20°C until analysis.

**Allergens in the settled and airborne dust**

Basically, Enzyme-Linked Immunosorbent Assay (ELISA) and amplified ELISA were applied to determine the allergen levels in the dust samples. Airborne allergens were measured only in Chinese schools.

Two-site sandwich ELISA was applied to determine the allergen levels of cat (Fel d 1), dog (Can f 1), house dust-mite (Der p 1 and Der f 1), cockroach (Bla g 2) and horse (Equ c x) (164) for both settled and airborne dust samples by using monoclonal antibodies. The dog allergen assay is a monoclonal/polyclonal ELISA, using anti-Can f 1 mAb 6E9 for allergen capture and the polyclonal rabbit anti-Can f 1 for detection. The procedures basically followed the methods provided by the manufacturers with the exception for the dog allergen assay where the horseradish peroxidase-labelled goat anti-rabbit immunoglobulin was from DakoCytomation Norden AB, Stockholm, Sweden. The allergen level is expressed as ng/g dust, except for horse allergen concentration which is expressed as U/g dust, where 1 Unit equals to 1 ng protein of horse hair and dander extract used as standard.

Amplified ELISA was used for Petri-dish samples only for cat allergen with levels lower than 1.0 ng/ml by the conventional ELISA. It was completed with a commercial signal amplification kit basically by following the manufacturer’s protocol (163). The amount of the allergens per filter was calcu-
lated by multiplying the concentration values by the total dust weight per filter.

**Microbial components in the settled dust**

GC-MS/MS, a sensitive and specific method, was applied to determine the microbial components in the settled dust. They were MuA, 3-OH FAs and ergosterol, three chemical markers for bacteria (mostly gram positive/G⁺), G⁻ bacteria (endotoxin, LPS) and fungi, respectively.

Hereto, 3-OH FAs is a component of lipid A in the molecule of lipopolysaccharide (LPS). By GC-MS/MS method, different types of LPS can be differentiated between different lengths of 3-OH FAs carbon atoms: C10, C12, C14, C16 and C18 (165). Total LPS concentration was calculated from the sum of the concentration of C10, C12, C14 and C16, divided by a factor of four, since each LPS molecule has four molecules of 3-OH FAs (166). A hydrolysate of 13 C-labeled cyanobacterial cells was used as internal standards for LPS and MuA and dehydrocholesterol was used for ergosterol measurement. The microbial amounts per sample for three microbial markers were calculated by multiplying the concentration with the total weight of dust sample.

**Chemical air pollutants indoor and outdoor in schools**

Chemical air pollutants were measured both indoors and outdoors for SO₂, NO₂, O₃ (by IVL diffusive samplers from Swedish Environmental Research Institute L.t.d., Göteborg, Sweden, Fig. 2) and formaldehyde (by SKC UMEx 100 diffusive samplers from SKC, PA, USA). For the formaldehyde samplers, the uptake rate of 20.4 ml/min was used in calculations, as suggested by the laboratory. All these samplers are small, silent, and have a light weight without need of electricity. The sampling technique is based on molecular diffusion of gases which are quantitatively collected on an impregnated filter or an adsorbent material, giving a concentration value integrated over time.

The final concentrations were calculated by credited laboratories specializing in these sampler analyses, reported as the average value across the 7-day measurement period. In addition, in order to evaluate how the indoor air were affected by outdoor air pollution, the ratios (I/O) between indoor and outdoor air pollutant concentrations were calculated.
Statistical Methods

Statistical calculations were mainly performed by Statistical Package for the Social Sciences (SPSS) and partly by SPIDA statistical package. In all analyses, two-tailed test and a 5% level of confidence were applied. The major statistical methods used in this study are listed as follows:

**Chi-square test**
- Health parameters and diet comparing pupils with rural and urban childhood
- Current home environment comparing pupils with rural and urban childhood
- Health parameters comparing pupils in China and Sweden
- Health parameters comparing boys and girls in Chinese schools
- Health parameters comparing Chinese pupils with and without parental asthma or allergy

**Mann-Whitney U-test**
- Differences in allergen levels and indoor environmental factors between China and Sweden

**Kendal Tau-β rank correlation test**
- Correlations between different school indoor climatic factors
- Correlations between different indoor school environmental factors
- Correlations between indoor climatic factors and other environmental measurements.
- Correlations between age and allergen levels in dust in Swedish schools

According to our measurement schedule, three classrooms in each school were selected to monitor the indoor air pollutant concentrations with one sample of each kind in each classroom for 7 days (around 2 m high from floor level). Simultaneously, one sampler of each kind was put in each school outside the classrooms to measure the outdoor air pollutant levels (around 2.5-3.5 m high above ground).

*Figure 3. The diffusive (passive) samplers used in this study for SO₂, NO₂ and O₃ (from Martin F. and Henning R., 1997)*
Multiple logistic regressions

- Associations between health parameters and environmental factors, such as microbial exposure and air pollutants, controlling for age, gender, parental asthma or allergy, and indoor painting, new floor and current ETS at home. Other associations were performed by different models.
Results

Paper I

Totally, 2116 out of 2360 pupils (90%, aged 9-20 y) participated in the questionnaire study, among which 1.7% ever had asthma, 0.8% had doctor’s diagnosed asthma, 2.6% eczema and 2.7% pollen or cat allergy. No gender differences of asthma or allergy were detected, except for eczema which was reported more by girls than boys.

*Childhood (urban vs. rural) and asthma, eczema and allergy*

In total, 60% of pupils reported a rural childhood and 37% an urban childhood. Further, 54% of pupils with urban childhood still live in urban places (Taiyuan city), and 53% of pupils with rural childhood have lived in the same dwellings since birth. By multiple logistic regression analysis, rural childhood consistently showed negative associations with all health parameters (asthma, eczema, allergy) while current urban residency was numerically positively associated with all health parameters but only significantly with eczema (Table 1).

Table 1. Associations (OR 95% CI) between asthma, eczema, allergy and childhood (urban vs. rural) and current residency (urban vs. rural)†

<table>
<thead>
<tr>
<th></th>
<th>Ever asthma</th>
<th>Doc.D-asthma</th>
<th>Eczema</th>
<th>Atopy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rural childhood</strong></td>
<td>0.17</td>
<td>0.15</td>
<td>0.29</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Current urban residency</strong></td>
<td>(0.05-0.60)**</td>
<td>(0.03-0.81)*</td>
<td>(0.13-0.66)**</td>
<td>(0.25-0.99)*</td>
</tr>
</tbody>
</table>

Doc.D-asthma: doctor’s diagnosed asthma; Atopy: pollen or cat allergy;
P<0.05, ** P<0.01; *** P<0.001

† controlling for age, gender, indoor painting, new floor material and ETS in the dwellings.
Diet and asthma, eczema and allergy

Diet habit showed significant differences between pupils with urban and rural childhood. Except for consumption of raw vegetable (not an eating habit for Chinese culture), pupils with urban childhood consumed more frequently a variety of food including meat, fish, fruit, cooked vegetables, hamburgers, fruit juice and carbonated soft drinks ($P<0.001$ for all these items except cooked vegetables).

Two models were applied to analyze the associations between dietary factors and health parameters (Table 2). The most consistent results were the protective effect of fruit consumption for both asthma and allergy. Frequent fish consumption was associated with a lower cumulative incidence of asthma, doctor’s diagnosed asthma but more allergy. Frequent consumption of hamburgers, a proxy variable for consumption of fast food, was associated with more asthma and eczema.

Table 2. Associations (OR 95% CI) between asthma, eczema, allergy and dietary factors

<table>
<thead>
<tr>
<th></th>
<th>Ever asthma</th>
<th>Doc.D-asthma</th>
<th>Eczema</th>
<th>Atopy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fruit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model I</td>
<td>0.53</td>
<td>0.96</td>
<td>0.96</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>(0.30-0.92)*</td>
<td>(0.37-2.51)</td>
<td>(0.58-1.58)</td>
<td>(0.40-0.98)*</td>
</tr>
<tr>
<td>Model II</td>
<td>0.40</td>
<td>0.90</td>
<td>0.88</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>(0.19-0.82)*</td>
<td>(0.31-2.60)</td>
<td>(0.50-1.54)</td>
<td>(0.29-0.84)**</td>
</tr>
<tr>
<td><strong>Fish</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model I</td>
<td>0.94</td>
<td>0.55</td>
<td>1.04</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td>(0.43-2.02)</td>
<td>(0.16-1.92)</td>
<td>(0.57-1.90)</td>
<td>(1.21-3.73)**</td>
</tr>
<tr>
<td>Model II</td>
<td>0.32</td>
<td>0.15</td>
<td>0.55</td>
<td>2.15</td>
</tr>
<tr>
<td></td>
<td>(0.11-0.97)*</td>
<td>(0.03-0.80)*</td>
<td>(0.26-1.17)</td>
<td>(1.07-4.32)*</td>
</tr>
<tr>
<td><strong>Hamburgers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model I</td>
<td>2.05</td>
<td>1.50</td>
<td>1.84</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>(1.09-3.87)*</td>
<td>(0.59-3.85)</td>
<td>(1.12-3.04)*</td>
<td>(0.92-2.65)</td>
</tr>
<tr>
<td>Model II</td>
<td>2.28</td>
<td>0.97</td>
<td>1.41</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>(0.89-5.80)</td>
<td>(0.23-4.17)</td>
<td>(0.70-2.85)</td>
<td>(0.33-1.64)</td>
</tr>
</tbody>
</table>

Doc.D-asthma: doctor’s diagnosed asthma; Atopy: pollen or cat allergy;  
* $P<0.05$, ** $P<0.01$; *** $P<0.001$  
*Model I adjustment for age and gender only, analyzing each dietary variable separately; Model II adjustment for age, gender, rural childhood, current urban residency, indoor painting, new floor materials, ETS in the dwellings and all other dietary variables at the same time.
Paper II

Prevalence of asthma, allergy and asthmatic symptoms

Totally, 1993 out of 2209 Chinese pupils (90%, mean 13y) participated in the second questionnaire study in Taiyuan. Compared with the same age group in Uppsala, Sweden (200 pupils), Chinese pupils had significantly lower prevalence of ‘ever asthma’, ‘doctor’s diagnosed asthma’, ‘current asthma’ and ‘furry pet or pollen allergy’ ($P<0.001$) (Table 3). On the other hand, Chinese pupils had significantly higher reports of daytime attacks of breathlessness than Swedish pupils.

Table 3. Prevalence of asthma, allergy, and asthmatic symptoms in Taiyuan, China and Uppsala, Sweden

<table>
<thead>
<tr>
<th></th>
<th>China (n=1993)</th>
<th>Sweden (n=200)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asthma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever asthma</td>
<td>1.8</td>
<td>9.5</td>
<td>0.18 (0.10-0.32)***</td>
</tr>
<tr>
<td>Doctor’s diagnosed asthma</td>
<td>1.2</td>
<td>9.0</td>
<td>0.12 (0.06-0.23)***</td>
</tr>
<tr>
<td>Current asthma $^a$</td>
<td>0.7</td>
<td>7.2</td>
<td>0.10 (0.04-0.20)***</td>
</tr>
<tr>
<td><strong>Current airway symptoms (during the last 12 months)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheezing or whistling in the chest</td>
<td>8.4</td>
<td>8.0</td>
<td>1.08 (0.63-1.84) NS</td>
</tr>
<tr>
<td>Daytime attacks of breathlessness</td>
<td>29.8</td>
<td>7.1</td>
<td>5.71 (3.29-9.92)***</td>
</tr>
<tr>
<td>Nocturnal attacks of breathlessness</td>
<td>2.1</td>
<td>1.0</td>
<td>2.23 (0.54-9.28) NS</td>
</tr>
<tr>
<td>At least one airway symptom $^b$</td>
<td>33.9</td>
<td>9.7</td>
<td>5.36 (3.31-8.68)***</td>
</tr>
<tr>
<td><strong>Allergies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat allergy</td>
<td>1.2</td>
<td>6.6</td>
<td>0.17 (0.08-0.33)***</td>
</tr>
<tr>
<td>Dog allergy</td>
<td>1.3</td>
<td>4.0</td>
<td>0.30 (0.13-0.68)**</td>
</tr>
<tr>
<td>Pollen allergy</td>
<td>2.1</td>
<td>12.7</td>
<td>0.17 (0.10-0.26)***</td>
</tr>
<tr>
<td>Furry pet or pollen allergy</td>
<td>3.8</td>
<td>16.2</td>
<td>0.24 (0.16-0.37)***</td>
</tr>
</tbody>
</table>

NS, not significant; ** $P=0.002$; *** $P<0.001$

$^a$ current asthma is defined as either having current asthma medication or having had an asthma attack during the last 12 months;

$^b$ symptoms defined as either wheeze or whistling in the chest, daytime attacks of breathlessness or nocturnal attacks of breathlessness during the last 12 months.
Classroom inspection, indoor and outdoor climate

Classrooms in Taiyuan, China were relatively crowded with an average of one student per m\(^2\). The CO\(_2\) level was high (average 2211 ppm, the recommended standard is 1000 ppm) with low personal outdoor air supply rate (mean 3.6 l/s). Compared with Swedish schools, the classroom temperature was lower in Taiyuan (average 21.4\(^\circ\)C vs. 14.7\(^\circ\)C) and the relative humidity was slightly higher (31% vs. 42%). No shelves were used in Chinese classrooms (shelf factor 0 m/m\(^3\)). Curtains or desk upholsteries were present in some Chinese classrooms (fleece factor 0.03 m\(^2\)/m\(^3\)). The comparison of all indoor and outdoor climatic factors is showed in Table 4.

Table 4. Classroom inspection, indoor and outdoor climate in Taiyuan, China and Uppsala, Sweden

<table>
<thead>
<tr>
<th></th>
<th>China</th>
<th>Sweden</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of students</td>
<td>48 (33-60)</td>
<td>20 (8-43)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Room volume (m(^3))</td>
<td>193 (161-225)</td>
<td>202 (82-470)</td>
<td>0.16</td>
</tr>
<tr>
<td>CO(_2) (ppm)</td>
<td>2211 (789-4170)</td>
<td>761 (400-1170)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Outdoor air supply rate l/(s. person)</td>
<td>3.6 (1.3-10.4)</td>
<td>14.8 (6.2-31.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature ((^\circ)C)</td>
<td>14.7 (11.2-18.4)</td>
<td>21.4 (20.2-22.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td>42 (31-62)</td>
<td>31 (20-46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fleece factor (1/m)</td>
<td>0.03 (0-0.14)</td>
<td>0.08 (0.01-0.36)</td>
<td>0.001</td>
</tr>
<tr>
<td>Shelf factor (1/m(^2))</td>
<td>0 (0-0)</td>
<td>0.10 (0-0.22)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Outdoor climate

<table>
<thead>
<tr>
<th></th>
<th>China</th>
<th>Sweden</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO(_2) (ppm)</td>
<td>522 (480-559)</td>
<td>368 (345-395)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature ((^\circ)C)</td>
<td>-1.8 (-5.5-2.6)</td>
<td>5.0 (0-13.7)</td>
<td>0.002</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td>52 (30-64)</td>
<td>82 (34-97)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

\(^{a}\) Calculated from the estimated equilibrium concentration of CO\(_2\).

Allergens

Totally, 79 samples of settled dust from 39 classrooms were collected in Chinese classrooms for allergen assay (Table 5). Results showed that the allergens levels were below the detection limit in almost all samples. Only 3% of samples contained traces of cat or dog allergens and they were all from different classrooms. In comparison, the dust samples in Swedish classrooms contained higher levels of both cat (Fel d 1), dog (Can f 1) and horse (Equ c x) (P<0.001) allergens. No house dust mite (Der f 1, Der p 1) or cockroach (Bla g 2) allergens were detected in any Swedish or Chinese classrooms.
Airborne allergen analysis was carried out for 44 samples, collected on Petri-dishes in the Chinese classrooms. Fel d 1 and Can f 1 were detected in 93% (41/44) (>0.01 ng/ml) and 55% (24/44) (>0.39 ng/ml) of all samples, respectively. The GM values across 10 schools were 16.82 and 17.74 ng/m²/day for cat and dog allergens. Mite (Der f 1, Der p 1), horse (Equ c x) and cockroach allergens (Bla g 1) were not detected in any air sample (<0.39 ng/ml) by conventional ELISA. Two schools had GM values of cat allergen more than 50 ng/m²/day, and one had dog allergen more than 50 ng/m²/day. In addition, there was a positive correlation between airborne cat and dog allergen in the Chinese classrooms (Tau $\beta$ 0.36; $P=0.001$).

Table 5. Allergen levels in the settled dust in schools in Taiyuan, China and Uppsala, Sweden †

<table>
<thead>
<tr>
<th></th>
<th>China (n=78)</th>
<th>Sweden (n=46)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cat allergen (Fel d 1)</strong> concentration (ng/g dust)</td>
<td>&lt;100 (&lt;100-&lt;100)</td>
<td>1300 (868-2325)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Dog allergen (Can f 1)</strong> concentration (ng/g dust)</td>
<td>&lt;200 (&lt;200-&lt;200)</td>
<td>1650 (785-2300)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Horse allergen (Equ cx)</strong> concentration (U/g dust)</td>
<td>&lt;200 (&lt;200-&lt;200)</td>
<td>1250 (695-2800)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Cat allergen amount</strong> (ng/filter)</td>
<td>60 (28-83)</td>
<td>982 (633-1907)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Dog allergen amount</strong> (ng/filter)</td>
<td>120 (57-165)</td>
<td>986 (625-1863)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Horse allergen amount</strong> (U/filter)</td>
<td>110 (57-160)</td>
<td>930 (448-2506)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Total dust (mg/filter)</strong></td>
<td>1108 (565-1604)</td>
<td>794 (562-1201)</td>
<td>0.102</td>
</tr>
</tbody>
</table>

† For values less than the detection limit, half of the detection limit value was used for calculation of the amount per filter values and other statistical tests.

Paper III

Microbial components

The levels of microbial components in the settled dust are showed in Table 6, in the unit of concentration (per gram of dust) and amount values (per dust sample). Among different types of LPS, higher chain lengths of LPS were found with higher concentrations.
The mutual correlations between three microbial components were tested by Kendal Tau-β correlation analysis. Significant correlations between concentrations values were found between MuA and LPS (tau-β 0.40, \( P < 0.01 \)) and MuA and Erg (tau-β 0.24, \( P < 0.01 \)), while no significant correlations were detected for Erg and LPS. For amount per sample values, all three components were higher correlated with each other (\( P < 0.001 \)).

Table 6. Average levels of microbial components in the settled dust in 39 classrooms in Taiyuan, China.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Amount per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
</tr>
<tr>
<td></td>
<td>M (SD)</td>
</tr>
<tr>
<td>LPS a, b</td>
<td>18.64 (6.19)</td>
</tr>
<tr>
<td>C10 b</td>
<td>1.70 (1.62)</td>
</tr>
<tr>
<td>C12 b</td>
<td>10.00 (4.13)</td>
</tr>
<tr>
<td>C14 b</td>
<td>21.47 (6.55)</td>
</tr>
<tr>
<td>C16 b</td>
<td>41.39 (18.95)</td>
</tr>
<tr>
<td>C18 b</td>
<td>35.52 (13.05)</td>
</tr>
<tr>
<td>MuA c</td>
<td>9.53 (4.60)</td>
</tr>
<tr>
<td>Erg c</td>
<td>0.69 (0.40)</td>
</tr>
</tbody>
</table>

M (SD): arithmetic mean (standard deviation);
\( a \) LPS is calculated by \( (C10+C12+C14+C16)/4 \) since 1 mole of LPS molecules carrying 4 moles of 3-OH FAs;
\( b \) The concentration values are in the unit of nmol/g dust and the amount values in unit of nmol/sample;
\( c \) The concentration values are in the unit of µg/g dust and the amount values in unit of µg/sample.

Paper IV

*Chemical Air pollutants*

The average levels of chemical air pollutants including SO\(_2\), NO\(_2\), O\(_3\) and formaldehyde both indoor and outdoor are shown in Table 7. Among them, SO\(_2\) was extremely high (average indoor level of 264.8 µg/m\(^3\) and outdoor of 712.8 µg/m\(^3\)) and three outdoor samples were even above the upper detection limit. The average ratios between indoor and outdoor levels (I/O) of SO\(_2\), NO\(_2\), O\(_3\) and formaldehyde were 0.38, 0.79, 0.93 and 0.38, respectively.
Table 7. Average levels of chemical air pollutants both indoor and outdoor in 10 schools during the measuring period in Taiyuan, China.

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Indoors</th>
<th>Outdoors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indoor climate, ventilation and air pollutants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SO₂(µg/m³)</td>
<td>34</td>
<td>10</td>
</tr>
<tr>
<td>M (SD)</td>
<td>264.8 (139.0)</td>
<td>712.8 (189.3)</td>
</tr>
<tr>
<td>Min-Max</td>
<td>60.0-641.1</td>
<td>476.0-1015.0</td>
</tr>
<tr>
<td>NO₂(µg/m³)</td>
<td>34</td>
<td>10</td>
</tr>
<tr>
<td>M (SD)</td>
<td>39.4 (9.5)</td>
<td>52.3 (9.5)</td>
</tr>
<tr>
<td>Min-Max</td>
<td>15.5-61.6</td>
<td>37.9-65.2</td>
</tr>
<tr>
<td>O₃(µg/m³)</td>
<td>34</td>
<td>10</td>
</tr>
<tr>
<td>M (SD)</td>
<td>10.1 (10.4)</td>
<td>12.4 (3.3)</td>
</tr>
<tr>
<td>Min-Max</td>
<td>3.0-61.2</td>
<td>7.1-17.5</td>
</tr>
<tr>
<td>Formaldehyde (µg/m³)</td>
<td>31</td>
<td>9</td>
</tr>
<tr>
<td>M (SD)</td>
<td>2.3 (1.1)</td>
<td>5.8 (0.6)</td>
</tr>
<tr>
<td>Min-Max</td>
<td>1.0-5.0</td>
<td>5.0-7.0</td>
</tr>
</tbody>
</table>

* number of classrooms and schools available for indoor and outdoor air pollutant measurements, respectively.

Paper III-IV

**Parental asthma or allergy and pupils’ respiratory health**

Positive associations were found between parental asthma or allergy and ever asthma, allergy, wheeze and daytime attacks of breathlessness (Fig. 4).

![Figure 4](image-url)

*Figure 4. Associations between parental asthma or allergy and pupils’ respiratory health, controlling for age and gender.*
**Current home environment and pupils’ respiratory health**

Controlling for age, gender and parental asthma or allergy, regression analysis showed that new floor material, new furniture and ETS at home were positively associated with wheeze or daytime attacks of breathlessness (Fig. 5). ETS at home showed a dose-response trend by an increasing odds ratio with more frequent ETS exposure at home.

![Wheeze or whistling in the chest](image)

![Daytime attacks of breathlessness](image)

*Figure 5. Associations between current home environment and pupils’ respiratory health, controlling for age, gender and parental asthma or allergy. * P<0.05; ** P<0.01; *** P<0.001

**School environment and pupils’ respiratory health**

Controlling for age, gender, parental asthma or allergy and home environment (new furniture, new floor, new painting and ETS), all school environmental factors were analyzed for their associations with pupils’ reported respiratory symptoms. The symptoms tested included ‘wheeze’ ‘daytime attacks of breathlessness’ ‘nocturnal attacks of breathlessness’ ‘a history of allergy’ and ‘respiratory infections’.

Results showed that indoor environmental factors (in the sequence of Y-axis variables in Fig. 6) including crowdedness, indoor CO₂, RH and temp, dust weight, Erg, MuA, total LPS and shorter lengths of LPS (C10, C12, C14) (microbial components are all shown in amount per sample values) tended to be negatively associated with symptoms of wheeze, daytime attacks of breathlessness and nocturnal attacks of breathlessness. Associations with daytime attacks of breathlessness were most pronounced (Fig.6). On the other hand, these factors were numerically positively associated with respiratory infections among which associations with crowdedness, dust weight, total LPS and longer lengths of LPS (C14, C16, C18) were statistically significant.
Airborne cat (Fel d 1) and dog (Can f 1) allergens were not significantly associated with any symptoms except that air cat allergen was positively associated with respiratory infections.

MuA concentration was consistently negatively associated with wheeze and daytime attacks of breathlessness (data not shown). Total LPS concentration was positively associated with daytime attacks of breathlessness, but concentrations of C10 and C12 LPS were negatively associated with wheeze and daytime attacks of breathlessness. Ergosterol concentration was negatively associated with respiratory infections even by mutual adjustment.

Among chemical air pollutants, indoor air concentrations of SO2, NO2 and formaldehyde were positively associated with nocturnal attacks of breathlessness, as well as formaldehyde with wheeze and NO2 with respiratory infections (Fig. 6). Among outdoor pollutants (data not shown), SO2 was positively associated with respiratory infections and formaldehyde was positively associated with wheeze, daytime attacks of breathlessness and respiratory infections.

Correlations between different environmental factors

CO2, Temp and RH were inter-correlated with each other (P<0.05; tau-β0.31-0.70). Both CO2 and RH were positively correlated with classroom crowdedness (Ns/m2). Temp and RH were negatively correlated with air exchange rate (ac/h).

In addition, crowdedness was positively correlated with airborne cat allergen, dust amount (dust weight) and amount of microbial compounds for all three markers and for all types of 3-OH FAs.

Airborne cat allergen (Fel d 1) but not dog allergen (Can f 1) was correlated with dust amount (tau-β 0.40, P<0.001) and amounts of microbial compounds for all three markers. Neither of airborne allergens were correlated with fleece factor.

Microbial components in amount per sample value were also correlated with RH (except for C16) and CO2 (only for MuA, Erg and total LPS) (Table 8). On the other hand, microbial components in concentration values had no correlation with dust amount, but crowdedness was positively correlated with total LPS concentration and two LPS types of C16 and C18.

Indoor SO2, NO2 and O3 were not correlated with any indoor climatic factors while formaldehyde was positively correlated with temperature (tau-β 0.41, P<0.05). All four air pollutants were numerically negatively related with
ac/h, despite of no statistical significance. None of the outdoor chemical pollutants were significantly correlated with each other. Indoor concentration of SO₂, NO₂ and O₃ were significantly positively correlated with each other while SO₂ was negatively correlated with formaldehyde.

Table 8. Correlations between climatic factors, crowdedness, allergens and dust weight, with microbial amounts per sample values

<table>
<thead>
<tr>
<th>Amount per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>MuA</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Temp</td>
</tr>
<tr>
<td>RH</td>
</tr>
<tr>
<td>CO₂</td>
</tr>
<tr>
<td>Ns/m²</td>
</tr>
<tr>
<td>ac/h</td>
</tr>
<tr>
<td>Fel d 1</td>
</tr>
<tr>
<td>Can f1</td>
</tr>
<tr>
<td>DW</td>
</tr>
</tbody>
</table>
Figure 6. Associations between indoor school environment and pupils’ respiratory symptoms. * P<0.05; ** P<0.01; *** P<0.001
General Discussion

We found there was a low prevalence of self-reported asthma, eczema, and allergy (pollen or cat allergy) in pupils (9-20 y) in Taiyuan, Shanxi province, China. Rural childhood was negatively associated with pupils’ all these diseases and more consumption of fruit and fish was associated with less asthma. On the other hand, current living in urban areas and consumption of hamburgers were instead risk factors.

In the two Chinese populations, similar lower prevalence of asthma and allergy (pollen, cat or dog allergy) was found among pupils (11-15 y) in Taiyuan city, compared with pupils in Uppsala, Sweden. Daytime attacks of breathlessness were reported by higher proportions of Chinese pupils. Parental asthma or allergy was shown to be an indicator of pupils’ asthma symptoms. In the home environment, recent new floor, new furniture and ETS exposure were positively associated with pupils’ asthmatic symptoms. In the school environment, classroom crowdedness, dust amount, CO₂, temperature and air humidity were negatively associated with respiratory symptoms. Microbial chemical compounds such as muramic acid and ergosterol, markers for bacteria and fungi, were negatively associated with wheeze or daytime attacks of breathlessness. The associations with endotoxin varied depending on the length of 3-hydroxy fatty acids of the LPS. Among outdoor pollutants, SO₂ and formaldehyde were positively associated with respiratory infections or asthma symptoms. In addition, indoor SO₂, NO₂ and formaldehyde were positively associated with asthma symptoms and respiratory infections.

Comments on internal validity

In the two Chinese studies, the schools were arbitrarily selected and questionnaire surveys were completed with high response rates (90%), so selection bias is less likely. Information bias can be a potential problem in questionnaire studies without clinical diagnosis or objective exposure assessment. A strong association between self-reported physician diagnosed asthma and airway hyperresponsiveness, measured by methacholine challenge test, has been demonstrated in Chinese children (167). The authors concluded that it was appropriate to ask on self-reported doctor’s diagnosed asthma in rural
China. Our question on eczema was similar as the question on eczema in the ISAAC study, which has been validated in Chinese children, using clinical examination as golden standard. Specificity and sensitivity were similar and fairly good, 67 and 71%, respectively (168). We have found no validation studies on self-reported atopy in Chinese children, but our prevalence was similar or lower than cat allergy prevalence detected by skin-prick test in urban children in Beijing (5.6–10.8%), in suburban children outside Beijing (3.7%), Guangzhou (4.3%), and Hong Kong (3.7%) (147, 169). Thus, a majority of those reporting asthma, eczema, and allergy in our study could be expected to actually have asthma, eczema, or allergic sensitization, but some underestimation of the true prevalence could be expected. Moreover, questionnaire assessment of allergy does not detect subject with increased levels of IgE antibodies who don’t have any allergic symptoms.

Food frequency questionnaire (FFQ) has been validated for fish and fruit consumption against dietary records (170, 171), omega-3-fatty acid measurements in serum (172), or spouse versus husbands’ report on fish consumption (173), with acceptable agreement. Validation of questionnaires can be culture dependent and we have found no diet validation studies from China. Some authors have concluded that as the associations between FFQ and dietary records are moderate and in general, only strong associations between diet and health can be detected by using FFQ.

Both in Sweden and China, the same questionnaire was used, and the Chinese version was translated from Swedish and back to Chinese by another person. All indoor measurements were performed with the same methods and instruments, and sampling was done in winter time in both countries. The allergen analyses were done in the same laboratory in Sweden. Thus, both questionnaire data and environmental data should be comparable from a methodological point of view.

In the second Chinese study, the medical data was collected one week before the environmental measurements, thus exposure levels were unknown when they answered the questionnaires, and there were no visible signs of microbial growth in any classroom. The statistical analyses were performed by controlling for potential confounders with available data information, including both parental asthma or allergy and pupils’ home environment. It was not possible to control for other types of home air pollution from burning of gas or coal which can bring harmful effects on children’s respiratory health. However, since the study was performed in one city, we do not expect large variation of cooking and heating habits within the area, and no major associations between cooking or heating-related exposure and ETS, new building materials at home or measured school exposures. In conclusion we do not believe that our conclusions are seriously affected by selection or informa-
tion bias. However, the cross-sectional study design limits the possibility to draw conclusions on causal relationships.

Comments on external validity
The two Chinese studies were performed in 2002 and 2004, respectively. China is a large country with rapid changes of the economy and the indoor and outdoor environment. Since there are no other similar school studies from other areas of China, except in Shanghai, it is hard to comment on the issue of external validity. The results, however, may be representative for the prevalence of asthma, allergic and respiratory symptoms among teenagers at the similar age in Taiyuan, Shanxi province, China.

Environmental measurements were performed by standardized methods, by calibrated instruments, and samples were analyzed by well known laboratories. The diffusion samplers were selected to have little influence by wind speed and other climatological factors that may disturb some other types of samplers. For the measurements of microbial components, a relative new method of GC-MS/MS was used. This method should be more valid than the commonly used Limulus test for LPS, but results are not directly comparable since the Limulus test is a biological test method. Moreover, our method for LPS has the advantage that it can differentiate between different types of LPS.

Comments on the prevalence of asthma, allergy and respiratory symptoms
Reports on asthma, eczema and allergy to pollen or furry pets were uncommon among Chinese school children in Taiyuan area. The prevalences were similar in the first and second questionnaire survey. By comparison with the same age group of children in Uppsala, Sweden, cumulative incidence of asthma, doctor’s diagnosed asthma and allergy were much less common in Taiyuan city.

No gender difference was found in symptoms among Chinese pupils except that girls reported more daytime attacks of breathlessness after exercise. However, age was a significant predictor, with a higher prevalence of asthma and allergies among Chinese children born in the period 1990–1992, as compared with those born earlier. The interpretation of this as a cohort effect suggested an increase of asthma and allergies in Shanxi province. This interpretation is supported by data from a large national survey in children (0–14
years) in 27 cities in China. They reported an increase of the prevalence of asthma from 0.51% in 1990 to 1.02% in 2000 in Taiyuan city (174).

The cumulative incidence of asthma in our study was comparable with the ISAAC study performed in five Chinese cities among pupils aged 13–14 years old, but the ISAAC study reported a relatively higher prevalence. This could be partly explained by the different economic development levels and life styles, since most cities in the ISAAC study in China were performed in the most developed areas. For the Swedish pupils in our study, data is comparable with a previous school study from Uppsala (139).

In contrast, when asking about symptoms without using the word ‘asthma’, Chinese pupils had much higher prevalence of daytime attacks of breathlessness after exercise. One explanation to this finding could be the heavy outdoor air pollution in Shanxi province (the mean levels of SO₂ in winter of 700 µg/m³, and the annual mean levels of PM₁₀ of 252 µg/m³ according to measurements from our research group and the local monitoring station (unpublished data). Since we did not perform any clinical investigation, we have no information on the prevalence of bronchial hyper-responsiveness (BHR) in the pupils. One previous Chinese study showed that BHR decreased substantially in Hongkong children when SO₂-levels were reduced to 20–30 µg/m³ after an environmental intervention (175).

There is little data on pollen allergy in China, but our data are comparable with previous data from Hong Kong where 1.2% of children (6–7 years) reported pollen allergy in 1994–1995 and 1.4% of the same age reporting in 2000–2001 (176). In addition, our data on self-reported pollen and pet allergy are comparable with other studies from China measuring allergic sensitization by skin prick test. Cat allergy was somewhat higher in these studies as compared to our data, 3.7%, 5.6%, and 4.3% in Hong Kong, Beijing and Guangzhou, respectively (146). Another study reported that the prevalence of cat allergy was 10.8% in urban children in Beijing, but only 3.7% in suburban children outside Beijing (147). Thus, our data on pollen and cat allergy are in line with what could be expected, as Taiyuan is a less developed area than Beijing. However, there may be an underestimation of the true prevalence in Taiyuan, but it is unlikely that recall bias could explain the large difference in pet allergy between China and Sweden. Moreover, sensitized subjects with no allergic symptoms may not report allergy in a questionnaire study.
Comments on rural childhood, diet and asthma or allergy

A rural childhood was associated with less asthma, eczema and atopic sensitization, while current urban residency was a significant risk factor for eczema only. A protective effect of a rural childhood with respect to the development of asthma and atopic sensitization has been reported from other parts of the world (34, 35), as well as from China (177). We did not ask specifically about growing up on a farm. Since a majority of children in rural Shanxi live in families involved in agriculture, it is likely that many with a rural childhood are exposed to farm animals. A study on urban children (14–16 years) in Wuhan, an industrial city in mid-China, reported that 51.4% of children have had early childhood (0–6 years) animal exposure (148). This indicates that even urban children in China can have frequent animal exposure, and it could be expected that contact with animals, especially farm animals, is even higher in the countryside.

We found that children with an urban childhood had a higher current consumption of meat, fish, fruit, hamburgers, fruit juice, and carbonated soft drinks. Thus, moving to the city may involve dietary changes that can have both positive and negative effects, with respect to asthma and allergy development. When analyzing dietary risk factors in our study, the most consistent result was the beneficial effect of high fruit consumption. This is in agreement with some previous studies, mainly focusing on obstructive lung disease (178). Those with a more frequent consumption of fish reported less asthma, but more pollen and cat allergy. Evidence for a beneficial effect of fish consumption on asthma has been reported from western countries (179, 180), including one study in Swedish school children using the same FFQ as in our study (160). The effect has been suggested to be related to a higher intake of omega-3-fatty acids from deep-sea fish (178). Shanxi province is situated far away from the sea, the fish consumption was relatively low, and mostly not deep-sea fish, suggesting that there may be other beneficial components in fish. The positive association between fish consumption and atopic sensitization remains unclear. Children may have immunoglobulin E (IgE)-mediated allergy to fish proteins, but we have no information on such allergies in this study. Finally, consumption of hamburgers, a proxy variable for consumption of fast food, was associated with more asthma and eczema. In China, western fast food (e.g. hamburgers and pizza) is fashionable, and more expensive than ordinary restaurant food. Fast food consumption is linked to obesity (181), and has been reported to be linked to an increase of childhood asthma in Saudi Arabia (56), New Zealand (57), and Sweden (160). Frequent soft drink consumption was associated with a lower prevalence of asthma but a higher prevalence of atopic sensitization. The reasons remain unclear. Adverse health effects of soft drinks have been reviewed,
and may include allergic reactions to additives, for example, representatives or aspartame (182). As fast food consumption is increasing in the world, further studies on possible effects of these dietary changes on asthma and allergy development are needed.

Comments on allergens in schools

We found large differences in allergen levels in settled dust between China and Sweden. Settled dust from Taiyuan had only trace amounts of cat and dog allergen and no horse allergen. This is in agreement with a previous study from Shanghai schools (156). Most of the Swedish classrooms contained high levels of both cat, dog and horse allergen, which is consistent with previous school studies (143). A correlation between the number of pet owners and allergen level in the classroom has been demonstrated (183). We did not have information on pet keeping in our Chinese study and there are a few publications on pet keeping in China. In an epidemiological study among 15-year-old pupils in Wuhan, China, 19.9% kept cats and 21.3% kept dogs at home (148).

Allergen measurements in settled dust are commonly used as a proxy-variable for indoor allergen exposure in schools (143). However, measurements of airborne allergens should be a better predictor for personal exposure, particularly for allergens with small particle size like cat and dog allergen (102, 184, 185). Surprisingly, we found high allergen levels in the air in Chinese classrooms, measured by the Petri-dish method (163). Unfortunately, we did not use the Petri-dish method in the Swedish schools, but other studies have demonstrated that cat, dog and horse allergens can be detected in Swedish classrooms by this method (163, 186). If we compare our geometric mean levels of cat allergen in Taiyuan schools (16 ng/m²/day) with previous data from Stockholm, Sweden (163), they are comparable. The highest levels in Chinese schools were comparable with the level in the Swedish schools with frequent pet ownership (>20%). This illustrates that exposure to cat and dog allergen in Chinese schools should not be neglected, and that allergen analysis in settled dust may not be a suitable proxy-variable for personal allergen exposure in some indoor environments.

We can only speculate on the discrepancy between airborne and settled dust measurements. We have found no data in the literature on matrix effects of the ELISA method for cat and dog allergen. The dust seemed to have different composition. If the Chinese settled dust contains a large proportion of fine particles originating from outdoor air pollution, it might be more easily re-dispersed into the air. This means that low allergen levels in settled dust, even below the detection limit, may generate significant airborne concentra-
The effects of the dust composition on the allergen analysis results, as well as on the airborne allergen exposure, need to be further investigated.

Schools may be the main site of exposure for children without pets at home since pet allergens are easily transported to the school environment. During a 4-year follow-up period it was shown that children in schools with a higher concentration of cat allergen also had a higher risk for asthma diagnosis (85). Moreover, cross-sectional studies have shown association between asthmatic symptoms and cat allergen (139), dog and horse allergen (160) in settled dust in schools. Finally, pet allergen exposure at school can cause acute asthma attacks in sensitized children (187, 188). Thus, allergen contamination in schools can be a significant public health issue, both in Sweden and China.

Comments on parental asthma or allergy with pupils’ respiratory health

Totally 11% of Chinese pupils in our second school study had parents with asthma or allergy, and parental asthma/allergy was a strong predictor of pupils’ asthma, allergy and respiratory symptoms such as wheeze and daytime attacks of breathlessness. This is in agreement with data from other countries and illustrates the significance of genetic factors for asthmatic symptoms also in Chinese children (155).

Comments on home environmental factors

Presence of new floor materials and new furniture in the homes in Taiyuan area in China was positively associated with wheeze and daytime attacks of breathlessness in the second study. Associations between chemical emissions in the home environment and asthma have been previously reported, including formaldehyde and other chemical emissions from painting, furniture polishers or adhesive chemicals in the furniture or floors (106, 111-113). We do not have detailed information on the type of floor material in each home in our study, but the main types of new floor materials in this area are tiles, wood or plastic material (e.g. poly-vinyl-chloride) or marble. Thus, it is not unlikely that some of these materials might give chemical emissions when they are new (113) which may cause airway irritation. To our knowledge, there are no previous international publications on associations on respiratory effects of new materials in Chinese dwellings.

ETS is a well known domestic risk factor for respiratory health (117). In our study, it was positively associated with wheeze and daytime attacks of
breathlessness, with a dose-response trend. Exposure to ETS at home is still common in China, and there hasn’t been particular rules banning the indoor smoking in public areas in China (149, 150). In contrast, we found a negative association between ETS and a history of atopy. One possible explanation could be social-class factors. It is well known from other countries that atopy is more common in higher social class (189, 190), and we could expect the higher ETS exposure might indicate a lower socio-economic status also in China.

Comments on microbial components and asthma symptoms

In the second Chinese school study, we found associations between microbial chemical markers in school dust and both airway symptoms and respiratory infections. The most consistent finding was for muramic acid, a marker mainly for gram-positive bacteria, which was negatively associated with wheeze and daytime attacks of breathlessness, even when mutually adjusting for the concentration of microbial compounds. This is in agreement with a previous school study from Shanghai (191), where a negative association between muramic acid and daytime attacks of breathlessness was found. Moreover, a study in dwellings, using the similar GC-MS/MS method found a negative association between muramic acid concentration in dust and wheeze, still significant after controlling for endotoxin concentration (101). Thus, our findings support the view that protective effects of other bacterial components than endotoxin should be considered. It should be noted that the innate immune system has receptors for muramic acid, different from those recognising endotoxin. Toll like receptor 2 (TLR2) recognizes peptidoglycan of gram-positive bacteria, while toll like receptor TLR4 recognizes endotoxin (192).

Ergosterol was negatively associated with respiratory infections, even after mutual adjusting for the concentration of other microbial compounds in the dust. Most other studies on fungal exposure have shown positive associations with respiratory health, including asthmatic symptoms and airway infections (64, 193). The Shanghai school study found a positive association between ergosterol in classroom dust, and airway infections (191). In contrast to most other studies, one recent study has reported that fungal exposure, measured as β(1,3)-glucan concentration in household dust, was protective for atopic wheeze (194). Ergosterol is a non-specific marker of fungal exposure, and is produced by both fungi and less harmful yeasts. Therefore, ergosterol per se may not be a health-relevant marker in a general sense.
Associations for endotoxin were more complex in our study. By measuring the 3-OH FAs of different carbon chain lengths, we could distinguish between different types of LPS, which is not possible in the conventional Limulus test. Shorter chain lengths of 3-hydroxy fatty acids from LPS (C10, C12 and C14) were negatively associated with wheeze and daytime attacks of breathlessness. Longer chain lengths (C14, C16, C18) were positively associated with respiratory infections. The analysis of different types of LPS could provide new information to further disentangle the paradoxical nature of endotoxin, reported to be both friend and foe to allergy and asthma (96, 100, 195). Different types of LPS could reflect different gram-negative species. Several Pseudomonas species that are ubiquitous in the environment produce C10 and C12 3-OH FAs, whereas Actinobacteria commonly found in indoor environments produce C18 3-OH FAs. To our knowledge, our study is the first epidemiological study on respiratory effects of different types of LPS.

We used both concentration in the dust and amount per sample as a proxy-variable for microbial exposure. In general, the associations were more pronounced for the amount per sample values. The advantage with measuring amount is that it is not influenced by dilution effects by inert fine dust. In addition, we found that total amount of dust per sample was associated with respiratory symptoms in a similar way as total LPS amount, supporting the view that total dust load can be a general marker of some types of microbial stimulation, as previously suggested (196). We suggest that in future studies both amount and concentration are evaluated, and moreover, bacterial species associated with the different chain lengths of 3-OH FAs should be further investigated.

Comments on chemical air pollutants and asthma symptoms
Indoor and outdoor air pollutants at school (SO2, NO2, formaldehyde) were found to be risk factors for either wheeze, attacks of breathlessness or respiratory infections. A similar association between NO2 and respiratory symptoms and current asthma has been reported from a previous school study from Shanghai (157). Moreover, respiratory effects of outdoor SO2 have been demonstrated in other studies from China (197). To our knowledge, however, there are no previous studies on respiratory effects of SO2 exposure inside schools. The literature on respiratory effects of SO2 is not very consistent, as a number of studies have failed to demonstrate any association between SO2 and asthmatic symptoms (128, 129). In these studies, however, exposure levels were much lower than in our study from the Taiyuan area. Compared with SO2 and NO2, formaldehyde contributed more consistently
for respiratory symptoms, including wheeze, daytime and nocturnal attacks of breathlessness, despite a level well below the WHO air quality guidelines. The associations were stronger for outdoor than for indoor formaldehyde. It is possible that outdoor formaldehyde is an indicator of reactive chemistry, and an indicator of other even stronger irritants formed in the chemical reactions (198, 199).

Associations for SO₂, NO₂ and formaldehyde were more pronounced for nocturnal attacks of breathlessness, as compared to daytime attacks. This could indicate delayed respiratory effects of these air pollutants. Such delayed effects have previously been suggested in a longitudinal study of winter air pollution in China (200). In our study, we found no associations between ozone and respiratory health, but the levels were low, possibly due to the high levels of particle pollutants in the Taiyuan area.

Comments on indoor climate in the schools

The level of CO₂ was well above the recommended limit value of 1000 ppm (201) in most classrooms, and closely related to crowdedness in the classroom and negatively associated with air exchange rate. Surprisingly, we found negative associations for wheeze and attacks of breathlessness and either CO₂, room temperature or relative air humidity. Moreover, crowdedness was negatively associated with daytime attacks of breathlessness. These findings contrast to the general knowledge on health effects of ventilation, where increased air exchange or reduced CO₂ levels are associated with less symptoms (83). One possible explanation could be that in Taiyuan city, the outdoor air pollution levels are so high in wintertime that they may counteract the beneficial effects of increased ventilation in the classrooms. An alternative explanation, more speculative, could be that there is some beneficial effects of crowdedness as such on respiratory health, related to microbial exposure. One previous study has indicated a beneficial effect of crowdedness in dwellings on lower respiratory diseases (202). Interestingly, we found that crowdedness was associated with increased prevalence of respiratory infections. This is understandable since closer contact between people leads to easier spread of infections, particularly in a very crowded indoor environment like Chinese schools.

When comparing Uppsala, Sweden with a heavily polluted city like Taiyuan, the contrast in outdoor air pollution levels is very large to one or two orders of magnitude (203, 204). Despite the very heavy air pollution load in Taiyuan (152, 205), the children had a low prevalence of self-reported asthma, asthma diagnosis, asthma medication, and allergy. This illustrates that there could be some protective factors in the Taiyuan area, such as dietary factors.
or exposure to microbial components (48). On the other hand, lower awareness of asthma among the Chinese public and different standards of asthma diagnosis may also explain our findings, at least partly.
Conclusions and implications

The increase of asthma and allergies is an important global issue. This thesis has contributed to the knowledge on environmental risk factors for asthma and allergy in Chinese school children. Further studies on health effects of indoor and outdoor environmental exposure are needed, both in China and other developing countries. A combination of environmental measurements with questionnaire data on medical symptoms and environmental exposure can give useful information on environmental risk and protective factors. Moreover, multi-center studies are needed using a combination of standardized environmental measurements and questionnaires, to identify both local and general risk factors for asthma and allergies. There is a need to further study the school environment, both in Asia and other parts of the world. Moreover, measurements and control of chemical emissions from new building materials used in Chinese dwellings seems to be an important issue. The role of microbial stimulation needs to be further evaluated by more sophisticated methods than previously have been the case, and chemical analysis of microbial markers by tandem-GC-MS seems to be a promising method. In addition, the immunological effects of microbial stimulation after the first years of life, both in childhood, adolescence and adulthood needs to be further investigated. A major challenge in the future is to combine the global demand for a better life and a higher living standard in the developing countries with a sustainable development with better indoor and outdoor environment and better health, including a decline of the global increase of asthma, allergy and respiratory disease.
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