LONG-TERM EXPOSURE TO AIR POLLUTION FROM ROAD TRAFFIC AND DEVELOPMENT OF AIRWAY AND ALLERGIC DISEASES IN CHILDREN

Olena Gruzieva

Stockholm 2012
To my parents
ABSTRACT

Allergic diseases are of great public health concern and constitute one of the most prevalent childhood illnesses. Air pollution exposure is related to several types of adverse health effects. There are, however, limited prospective data on long-term exposure to air pollution and effects on childhood respiratory and allergic morbidity, particularly concerning the role of timing of exposure, and susceptible groups. The aim of this thesis was to investigate effects of long-term exposure to air pollution on the development of airway disease in children, focusing on allergic sensitization, asthma-related symptoms and lung function. It is based on two epidemiologic materials, the Swedish birth cohort BAMSE and the ESCAPE study, which included five European birth cohorts.

Over 4000 children in the BAMSE cohort were followed with repeated questionnaires, blood samples and lung function tests until 12 years of age. Outdoor concentrations of nitrogen oxides (NO\textsubscript{x}), and particles with an aerodynamic diameter of <10 µm (PM\textsubscript{10}) from traffic, were assigned to residential, day care, and school addresses by dispersion models. Air pollution exposure during the first year of life was related to an increased risk of sensitization to pollen allergens at 4 years (OR, 1.83; 95% CI, 1.02–3.28) for a 46.7 µg/m\textsuperscript{3} increase in exposure to NO\textsubscript{x}, but no consistent association was seen at 8 years of age. Our results suggested possible associations between exposure to air pollution during infancy and asthma in children up to 12 years of age. Risks appeared particularly elevated in children aged 8-12 years (OR, 2.0; 95% CI, 1.1–3.5) and for nonallergic asthma (3.8; 0.9–16.2), for a 7.2 µg/m\textsuperscript{3} increase in PM\textsubscript{10}. Furthermore, early-life exposure to air pollution seemed to have long-term negative consequences on lung function, particularly in atopic children, with a reduction of forced expiratory volume in one second (FEV\textsubscript{1}) of -136.9 mL; 95% CI, -224.1 to -49.7, for a 7.0 µg/m\textsuperscript{3} increase in PM\textsubscript{10}. Exposure after the first year of life appeared to have less impact on the respiratory outcomes under study.

In conclusion, our results suggest that the infancy period might be critical for the influence of air pollution exposure on the development of asthma, allergy and lung function in children. Furthermore, our results suggest that current air quality standards do not fully protect children against adverse respiratory effects.
LIST OF PUBLICATIONS

The thesis are based on the four following papers, which will be referred to in the text by their Roman numbers

   Traffic-related air pollution and development of allergic sensitization in children during the first 8 years of life.
   *Journal of Allergy and Clinical Immunology* 2012;129(1):240-246

    Air pollution exposure and allergic sensitization in children: a meta-analysis of five European birth cohorts within the ESCAPE project.
    *Manuscript*

    Exposure to air pollution from traffic and childhood asthma until 12 years of age.
    *Epidemiology* 2012 (accepted for publication)

    Traffic-related air pollution and lung function in children at 8 years of age – a birth cohort study.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BAMSE</td>
<td>Children, Allergy, Milieu, Stockholm, Epidemiological Survey</td>
</tr>
<tr>
<td>CHS</td>
<td>Children's Health Study</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>DEP</td>
<td>Diesel exhaust particles</td>
</tr>
<tr>
<td>EC</td>
<td>Elemental carbon</td>
</tr>
<tr>
<td>ESCAPE</td>
<td>European Study of Cohorts for Air Pollution Effects</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>FEV₁</td>
<td>Forced expiratory volume during 1 second</td>
</tr>
<tr>
<td>FEV₀.₅</td>
<td>Forced expiratory volume during 0.5 second</td>
</tr>
<tr>
<td>GEE</td>
<td>Generalized estimating equations</td>
</tr>
<tr>
<td>GINI</td>
<td>German Infant Nutritional Intervention</td>
</tr>
<tr>
<td>GIS</td>
<td>Geographical information system</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>LISA</td>
<td>Lifestyle-related Factors on the Development of the Immune System and Allergic Disease</td>
</tr>
<tr>
<td>LUR</td>
<td>Land use regression</td>
</tr>
<tr>
<td>MAAS</td>
<td>Manchester Asthma and Allergy Study</td>
</tr>
<tr>
<td>MMEF</td>
<td>Maximum midexpiratory flow</td>
</tr>
<tr>
<td>NO₂</td>
<td>Nitrogen dioxide</td>
</tr>
<tr>
<td>NOₓ</td>
<td>Nitrogen oxides</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PAH</td>
<td>Polycyclic aromatic hydrocarbons</td>
</tr>
<tr>
<td>PEF</td>
<td>Peak expiratory flow</td>
</tr>
<tr>
<td>PIAMA</td>
<td>The Prevention and Incidence of Asthma and Mite Allergy</td>
</tr>
<tr>
<td>PM₂.₅</td>
<td>Particles with an aerodynamic diameter of less than 2.5 μm</td>
</tr>
<tr>
<td>PM₂.₅ absorbance</td>
<td>Measurement of the blackness of PM₂.₅ filters (soot)</td>
</tr>
<tr>
<td>PM₁₀</td>
<td>Particles with an aerodynamic diameter of less than 10 μm</td>
</tr>
<tr>
<td>PM\text{coarse}</td>
<td>Particles with an aerodynamic diameter between 2.5 and 10 μm</td>
</tr>
<tr>
<td>r</td>
<td>Pearson correlation coefficient</td>
</tr>
<tr>
<td>SES</td>
<td>Socioeconomic status</td>
</tr>
<tr>
<td>SO₂</td>
<td>Sulfur dioxide</td>
</tr>
<tr>
<td>SPT</td>
<td>Skin prick test</td>
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</table>
1 GENERAL INTRODUCTION

Respiratory and allergic disorders in children are a serious public health issue due to their high prevalence and negative impact on the quality of life.\(^1\) The recent temporal trends in the prevalence of asthma symptoms have shown a mixed picture of increases in low prevalence areas, and a plateau or even a decrease in high prevalence areas.\(^4,5\) The underlying reasons for the observed trends are complex and multifactorial, including primarily environmental and lifestyle factors.\(^6,7\) Although genetic factors undoubtedly play a role in the development of asthma and allergies, other factors must play a dominant role, particularly in light of the substantial geographic variation in both the base prevalence rates and time trends.\(^4,8\) Air pollution constitutes one environmental exposure that has been associated with asthma and allergy as well as with health care utilization and school absenteeism.\(^9\) A cost-benefit analysis conducted to assess the impact of criteria air pollutants on children’s health showed substantial health and economic benefits following a reduction in air pollution levels.\(^10\) Therefore, outdoor air pollution exposure has become a subject of increasing concern for child health professionals, epidemiologists, environmental policy makers, and families.

It has been suggested that environmental factors may influence the development of respiratory allergies differently at different times of child’s life,\(^8\) thus, making the issue of timing of exposure of particular significance. In addition, as the expression of airway disease may vary with age,\(^11,12\) it is important to study the effects of exposure over a sufficiently long period of time, and so far little has been done in the context of prospective studies.

1.1 OUTDOOR AIR POLLUTION

1.1.1 Definition and sources

Polluted ambient air contains many different components, e.g. particulates, semivolatiles (PAH) and gases (NO, NO\(_2\), CO, SO\(_2\)). Particulate matter (PM) is a general term used for a mixture of organic and inorganic solid and liquid particles (including sulfate and nitrate ions, acid condensates, soil and road dust, soot, ash, molds, and pollens) of different origin, size, shape, surface area, chemical composition, physical properties, solubility and sources, suspended in air. Penetration in the tracheobronchial tract is determined by particle size and the efficiency of airway defense mechanisms. Inhalable particulate matter that can reach the lower airways is subdivided into particulate matter or PM\(_{10}\) (PM with an aerodynamic diameter <10 \(\mu\)m), coarse particulate matter or PM\(_{\text{coarse}}\) (PM with an aerodynamic diameter between 2.5 and 10 \(\mu\)m), fine particulate matter or PM\(_{2.5}\) (PM with an aerodynamic diameter <2.5 \(\mu\)m), and ultrafine or PM\(_{0.1}\) (PM with an aerodynamic diameter <0.1 \(\mu\)m).\(^13\) PM\(_{10}\) are commonly referred to as “thoracic” and only reach the proximal airways, where they are removed by mucociliary clearance if the airway mucosa is intact,\(^14\) whereas PM\(_{2.5}\) are referred to as “respirable” and are able to access the lower regions of the human respiratory tract. The size of the particles also determines their lifetime in the atmosphere, which ranges between parts of a second to several hours for ultrafine particles and up to several weeks for particles of a size between 0.1 and 1 \(\mu\)m (known as
accumulation mode particles).\textsuperscript{15} Ultrafine particles tend to accumulate or coagulate in the atmosphere to form larger particles. Coarse PM is largely composed of crustal materials, in contrast to PM$_{2.5}$ and PM$_{0.1}$, which have larger fractions of elemental and organic carbon.\textsuperscript{16}

Most of the NO$_x$ (sum of NO and NO$_2$) is emitted as nitrogen monoxide (NO) which is rapidly oxidised in the atmosphere by reaction with ozone (O$_3$) to nitrogen dioxide (NO$_2$).\textsuperscript{17} The relative amounts of NO and NO$_2$ therefore, depend on the ozone level and also on incoming solar radiation and air temperature. Ground-level ozone results from the effect of ultraviolet light on volatile organic compounds and NO$_2$.

Nitrogen oxides (NO$_x$) are primarily derived from automobile exhaust. Fine and ultrafine PM comes mainly from chemical or combustion processes, but some also as secondary particles from semivolatile compounds or through chemical reactions of gaseous pollutants in the atmosphere.\textsuperscript{17} Coarse particulate matter is primarily derived from re-suspended ground or fugitive dust mainly due to the turbulence generated by the vehicles. Road dust may originate from mechanical wear of tyres and brake lining of the vehicles, road surface wear (particularly by studded tyres), debris from vehicle loads, inflow of soil material, and sanding/salting of roads. Different components are often correlated to each other, both in time and space, because they come from the same sources that may be local or distant. The degree of internal correlations varies from place to place and also from time to time, partly because of contributions from other sources, topographical features and meteorological conditions.

\subsection{1.1.2 Air pollution exposure assessment}

Since it is not practical or feasible to monitor each component of the air pollution mix, ‘surrogates’ and summary measures are used to assess air pollution exposure. The most abundant components of air pollution in urban settings are NO$_2$, O$_3$, EC and PM coming largely from road traffic. The concentration of these indicators is used to represent the ambient pollution mixture.\textsuperscript{18} They are, however, not equally good indicators for each study setting. The choice depends on the health outcomes under study, the spatial distribution of the study population, as well as considered averaging time for the concentration of the pollutants.

Exposure to different pollution components can vary by location, proximity to roads, time of the day, season, the rate at which chemical reactions occur, and other factors. Epidemiologic studies on outdoor air pollution apply different methodologies to assess exposure, and methodological variation may be the source of inconsistent results across the studies.

There have been many advances in the assessment of traffic-related air pollution exposure, including direct measures of traffic itself (such as distance to the nearest road and traffic volume within buffers), geostatistical interpolation from monitoring stations, personal monitoring, modeling of concentrations of pollutant indicators.\textsuperscript{19} Although proximity-based models are the easiest to apply, they are subject to error because they do not consider the parameters that affect the dispersion and physicochemical activity of the pollutants.\textsuperscript{20} Geostatistical interpolation models are best implemented in combination with dense, well-distributed monitoring.
networks. Their main limitations are the size of the network and the number of measurements needed to assess the spatial distribution of pollution surrogates precisely over time. Also, unlike proximity models, interpolation models do not specifically relate to the traffic source.

Fine spatial scale modeling (e.g., dispersion models, land use regression models, hybrid models) is becoming increasingly available for modern cities providing an exposure contrast that is dominated by traffic sources. Dispersion modeling is based on mathematical description of deterministic processes using data on emissions, meteorological conditions and topography to retrospectively calculate source-specific air pollution concentrations over time at relevant geographical locations. Dispersion models must be calibrated and validated against measurements. Land use regression uses the monitored levels of the respective pollutants as the dependent variable, as well as traffic, topography and other Geographic Information Systems (GIS) variables as the independent predictors in a multivariate regression model. Land use regression is attractive in that it can account for the diversity of sources that contribute to a surrogate; however, the true contribution (in terms of associated variance) of traffic to the regression is not always known or reported. Both models are data- and calculation-consuming and depend on the accuracy of the input data, as well as on the validity of the model assumptions.

Personal monitoring is the most direct way of measuring the exposure of subjects to air pollutants. The drawback of this approach, however, is the high cost of implementation, resulting in small number of observations, which tends to produce sample biases. Hybrid models that combine measurements of personal exposure to traffic surrogates or time-activity data with exposure models may have higher validity in epidemiological studies.

1.2 HEALTH EFFECTS OF AIR POLLUTION

1.2.1 Allergic sensitization

Sensitization is a process by which a subject acquires markedly increased allergic sensitivity to a substance through repeated exposure to this substance. As the allergy develops, the response becomes worse with even short exposures to low concentrations eliciting severe reactions. Sensitization is most often assessed through skin prick test (SPT) and measurement of specific IgE-antibodies in blood. Skin prick test assesses specific IgE attached to cells in the skin important in allergies called "mast" cells. Immunoglobulin E is a protein involved in allergic reactions. The allergen-specific IgE antibody test measures the amount of IgE antibody in the blood for the suspected allergen(s). A multitest for specific IgE to several common inhalant or food allergens is frequently used as a screening instrument with further testing of all sera that were positive in the test for each allergen. Many studies have used IgE measurements alone or in combination with SPT for definition of allergy.

There is some evidence from experimental studies that exposure to air pollution may increase the risk of developing allergic sensitization and promote allergic inflammation. It has been shown that exposure to air pollutants including diesel exhaust particles (DEPs), gases, and
metals cause lung injury, inflammation, suppression of key host defences, including mucociliary clearance in the airways, and may potentiate allergic airway responses. Furthermore, air pollutants not only have a direct or indirect effect upon the individual, but also upon aeroallergens themselves. Thus, air pollutants may transport the allergens through the airways, interact with antigenic proteins in a way that increases allergenicity of aeroallergens. It has been shown that pollen in heavily polluted areas expresses a larger amount of proteins described as being allergenic, compared to zones characterized by lower pollution.

The results of epidemiologic studies on air pollution and sensitization are, however, debatable and inconsistent, which may in part be attributed to methodological differences, including study design, study population (population-based or high-risk), measured pollutants, definition of the outcomes, choice of potential confounders and analytical methods. Thus, some cross-sectional studies have reported increased risks of allergic sensitization in relation to ambient air pollution exposure measured as nitrogen dioxide, particulate matter, or ozone, whereas others did not find any associations. To date, there are only a few prospective cohort studies on sensitization following children from birth to school age with detailed assessment of exposure to air pollution. The Dutch cohort PIAMA reported an increased risk for food allergy at 4 years of age, but not at 8 years. Statistically significant associations between particulate matter exposure and sensitization to inhalant allergens were shown in the German LISA/GINI cohort at the age of 6 years. Earlier findings from the Swedish cohort BAMSE indicated that exposure during infancy was related to pollen sensitization at 4 years of age. However, no association between life-time air pollution exposure and sensitization to any allergen was seen in the Oslo cohort.

Summing up, both cross-sectional studies with their well-known limitations for etiological inference, as well as prospective cohort studies are inconclusive, while experimental evidence suggests that certain air pollutants can enhance immunologic responses to allergens and elicit inflammatory reactions in the airways.

### 1.2.2 Asthma-related respiratory symptoms

Asthma is the most frequent chronic disease in children. The main features of bronchial asthma include the development of airway inflammation and bronchial hyperresponsiveness, reversible airflow obstruction, and airway remodeling due to hypersensitivity to environmental irritants. Hypersensitivity mediated by immunological mechanisms is called allergy. However, in some individuals with hypersensitivity no such immunological mechanism can be identified, in which case they are diagnosed with nonallergic hypersensitivity. In addition to the problem of the inherent biologic diversity of asthma phenotypes, the definition of this disease is complicated by the methods and criteria used to characterize it in epidemiologic studies.

Current research suggests a complex aetiologic pathway for asthma, with air pollution likely to play an important role. Chronic exposure to high levels of air pollution is associated with small airway remodeling with increase in fibrous tissue and smooth muscle in the bronchioles, resulting in chronic airflow obstruction. It has been reported earlier that genetic risk factors for
Asthma may be influenced by exposure to air pollution, when the latter may suppress the protective effects of certain alleles and increase the risk of asthma in a subgroup of children. Some studies report positive significant associations between traffic-related exposure to air pollution and asthma or associated symptoms, while others indicate null results. Children with asthma have an increased risk of exacerbations and more frequent visits to the emergency department and hospital admissions when exposed to higher concentrations of ambient air pollutants. In addition, the risk for asthma has been shown to increase proportionally with increased vehicular emission exposures, suggesting that chronic exposure to these pollutants may increase the risk of developing asthma. However, the link with developing new-onset asthma is less established as only a few studies investigated associations between air pollution and incidence of asthma and their results are inconsistent. European birth cohort studies following children until the age of 4 or 8 years suggest a positive relationship between traffic-related pollution and physician-diagnosed asthma. The findings from the Southern California Children’s Health Study (CHS) suggested that lifetime history of physician-diagnosed asthma, as well as the onset of asthma were related to outdoor residential exposure to NO\textsubscript{2}. The investigators showed that an overall measure of traffic-related pollution and estimated exposure to individual pollutants were all associated with a significant increased risk of asthma irrespective if the exposures occurred at home or at school. The results of two recent meta-analyses showed an association between NO\textsubscript{2} levels and asthma incidence, but not prevalence. Exposure to PM\textsubscript{2.5} levels, NO\textsubscript{2}, and soot was associated with a significant increase in the incidence and prevalence of asthma during the first 8 years of life in the PIAMA study. In contrast, no association between modeled air pollution exposure at the birth address and doctor diagnosed asthma was found in the two Scandinavian cohorts. Similarly, no overall evidence of associations between ambient NO\textsubscript{2} and doctor diagnosis of bronchitis, asthma or wheeze was found in a pooled analysis.

1.2.3 Lung function

Lung function in childhood is a strong predictor for lung function in early adulthood, which is, in turn, an important health predictor throughout the life. Spirometry and other methods of mechanical lung function tests have been used widely in both controlled studies of the effects of air pollution and epidemiologic studies. Commonly used parameters of lung function include forced vital capacity (FVC) - the total volume exhaled after a maximum inspiration; forced expiratory volume within 1 second (FEV\textsubscript{1}) - a marker of airway obstruction, measured as the maximum volume that can be exhaled during 1 second; peak expiratory flow (PEF) - the maximum flow generated during expiration performed with maximal force and started after a full inspiration, and forced expiratory flow between the 25\textsuperscript{th} and 75\textsuperscript{th} percentile of FVC (FEF\textsubscript{25–75}), also known as maximum midexpiratory flow (MMEF). Flow measures are markers of small-airway function. FVC is primarily a function of the pulmonary parenchyma, with differences in volume mainly attributable to differences in the number of alveoli and reduction in their growth.

Existing evidence suggests that air pollution has adverse long-term effects on lung function growth in children, resulting in deficits of lung function at the end of adolescence. No study has,
however, followed up adolescents until they reached the plateau phase of early adulthood, e.g. to about 20 years of age for women and 25 years of age for men. It is, therefore, unknown if growth deficits will be compensated by an extended growth phase, or if these individuals will enter the lung function decline phase of later adulthood with a reduced pulmonary function. Many studies have reported some statistically significant adverse effects of air pollution on lung function. A recent review of panel studies reported significant adverse effects of air pollution on children's pulmonary health, especially for asthmatics. Outdoor levels of PM$_{10}$ and PM$_{2.5}$ have been related to acute reductions in FEV$_1$ and FVC, observed either within the same day, a few hours after exposure, or days after exposure (lag response). In contrast, recent pooled analyses of cross-sectional studies in 12 countries did not reveal any association between PM$_{10}$ exposure and lung function in children.

Understanding the chronic respiratory health effects of outdoor air pollutants is more complex. The most relevant assessment of air pollution and lung function emerged from the Southern California Children Health Study (CHS) that enrolled children from 12 communities with different air pollution exposure levels across California. Results from this study show that children aged 10 to 18 years exposed to higher concentrations of nitrogen dioxide, acid vapor, and PM$_{2.5}$ and who lived in heavy traffic areas have reduced lung growth. In the CHS there was even some evidence that lung function can improve once exposure to air pollution is reduced, even after long-term exposure. Thus, children who moved to areas with higher PM$_{10}$ levels showed lower rates of annual growth in pulmonary function, whereas subjects who moved to cleaner places showed higher rates of annual pulmonary function growth. In a cohort from Mexico City that included over 3000 healthy 8-year-old children at baseline and followed during 3 years, exposure to ozone, PM$_{10}$, and NO$_2$ were associated with significant deficits in lung growth. Similarly, in a 3-year follow-up study conducted on school children in Austria, long-term exposure to PM$_{10}$ was negatively associated with lung function growth and linked to developmental deficits in the large and small airways. Thus, results from these studies suggest that an air pollution–related decrease in lung growth prevents many children from achieving their topmost lung volume. Findings of various population-based studies are supported by animal studies, indicating that exposure of neonatal rats to PM led to a decrease in the rate of cell proliferation in the alveolar region.
1.3 AIMS AND SPECIFIC RESEARCH QUESTIONS

The aim of this thesis was to investigate effects of long-term exposure to air pollution from road traffic on the development of airway and allergic diseases in children, focusing on allergic sensitization, asthma-related symptoms and pulmonary function. In particular, it set out to:

- investigate if air pollution exposure increases the risk of allergic sensitization in school age children
- examine if long-term exposure to traffic-related air pollution is associated with the risk of asthma symptoms in children up to 12 years of age
- investigate if long-term exposure to air pollution from road traffic is related to lung function in 8-year-old children
- explore at which ages air pollution exposure exerts its main effects in children
2 MATERIALS AND METHODS

The papers that constitute this thesis are based on the Swedish population-based birth cohort BAMSE (papers I, III, IV) and the European Study of Cohorts for Air Pollution Effects (ESCAPE) (paper II).

2.1 THE BAMSE STUDY

2.1.1 Study design and study population

The BAMSE (Children, Allergy, Milieu, Stockholm, Epidemiological Survey) is an ongoing prospective birth cohort study. The parents of all children born between February 1994 and November 1996 in 4 municipalities of Stockholm County were invited to participate in the study. The recruitment areas were selected to represent both urban and suburban environments (Figure 1). In total, 7221 infants were born during the time period. After excluding 3132 children due to wrong address information, unwillingness to participate, parental insufficient knowledge of Swedish, planning to move outside of Stockholm within the coming 12 months, a sibling already enrolled in the study or severe illness of the child, the final cohort comprised 4089 children, representing 75% of all eligible children.

Figure 1. Catchment areas for enrollment of children in the BAMSE cohort
When the children were 2 months of age the parents received a postal questionnaire on parental allergies and environmental exposures (e.g., housing conditions, lifestyle factors, socio-economic status etc). When the children were 1, 2, 4, 8 and 12 years old, the parents received repeated postal questionnaires focusing mainly on symptoms of allergic disease in their children, as well as various risk factors (Figure 2). In addition, all children with completed questionnaires at 4 and 8 years of age were invited to a clinical examination, including lung function testing and blood sampling. Blood was drawn from 2614 (64%) and 2480 (61%) of the children at 4 and 8 years of age, respectively. The examinations were performed at the Department of Occupational and Environmental Health, Stockholm County Council, by paediatric nurses.

![Figure 2. Timeframe of the BAMSE cohort follow-ups (1994 - 2012)](image)

In 1996 a survey of non-responders and actively excluded families was performed with a short version of the questionnaire on home environment, smoking habits, allergic heredity. Response rates were 58% or 83%, respectively. Neither heredity nor pet keeping at home was different between the non-responders and actively excluded families and those included in the study population. However, parental smoking was significantly more prevalent among the non-responders and actively excluded families compared to the participating families (smoking among mothers: 18% in non-responders and excluded families combined versus 9% for those included in the BAMSE cohort; smoking among fathers: 23% versus 17%, respectively). 79
2.2 THE ESCAPE STUDY

The ESCAPE project (European Study of Cohorts for Air Pollution Effects, www.escapeproject.eu) is a multicenter study designed to investigate the effects of long-term air pollution exposure on various health endpoints, making use of health data from existing cohort studies.

For the aim of paper II, five European birth cohorts participating in the ESCAPE project were selected: BAMSE (Sweden), LISA and GINI (Germany), MAAS (Great Britain) and PIAMA (The Netherlands). All of these cohorts were originally initiated to study risk factors for development of asthma and allergies. They all started in the mid-nineties with subsequent follow-up on several occasions (Table 1). At the time of recruitment parents completed a screening questionnaire on their history of asthma and allergic diseases, lifestyle and socio-economic factors, home environment, etc. At the age of 4 years (5 - for the MAAS cohort, 6 – for the LISA/GINI cohort) and 8 years (10 - for the LISA/GINI cohort) children were invited to clinical examinations including blood tests. Follow-up questionnaires were concomitantly sent out to the parents to collect information about health and various environmental exposures for the children. All children who provided blood samples comprised the study population.

Table 1. Description of five European birth cohorts included in the meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Recruitment</th>
<th>Follow-up</th>
<th>N of subjects</th>
<th>Blood test for specific serum IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>At baseline</td>
<td>at 4 years*</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>PIAMA, nationwide, NL</td>
<td>1996-1997</td>
<td>1997-2005</td>
<td>3963</td>
<td>747 (19)</td>
</tr>
<tr>
<td>MAAS, Manchester, UK</td>
<td>1995-1997</td>
<td>1996-2009</td>
<td>1185</td>
<td>484 (41)</td>
</tr>
</tbody>
</table>

*GINI/LISA - data at 6 years; MAAS - data at 5 years
** GINI/LISA - data at 10 years
2.3 AIR POLLUTION EXPOSURE ASSESSMENT

2.3.1 Dispersion modeling

The assessment of air pollution exposure in papers I, III and IV was done by dispersion modeling.\textsuperscript{19,22} Information on residential, day care and school addresses was retrieved from the postal questionnaires and translated into geographical coordinates. Missing address data was complemented with information from tax authorities. Temporally and spatially resolved emission calculations were performed by SLB-analys at the Stockholm Environment and Health Protection Administration. Only emissions from road traffic were included, using NO\textsubscript{x} as a marker of exposure to exhaust particles, and PM\textsubscript{10} as a marker for road dust.

The local contribution to the annual mean concentrations of NO\textsubscript{x} and PM\textsubscript{10} were calculated using a Gaussian air quality dispersion model and a wind model, both part of the Airviro Air Quality Management System (http://airviro.smhi.se). The calculations were performed on a 25 m resolution grid for addresses in the more densely populated areas, such as urban areas, and 100 m or a 500 m grid in less densely populated areas. Addresses located outside the counties of Stockholm and Uppsala were excluded from exposure assessment. To compensate for the coarse resolution of the dispersion calculations in rural areas, adjustments were made based on the concentration gradient from roads with more than 10 000 vehicles per day. Emission databases for NO\textsubscript{x} were available for the years 1990, 1995, 2000, 2002, 2003 and 2004 (Figure 3). To obtain NO\textsubscript{x} concentrations for all years during the period of interest the model calculations were interpolated. PM\textsubscript{10} model calculations were only performed for the year 2004, when the most complete database was available, and applied to all years during the observation period. In addition, a street canyon contribution was added for addresses in the most polluted street segments in the inner city of Stockholm with multistorey houses on both sides, using the Airviro street canyon model (http://airviro.smhi.se). To define which streets should be considered as highly polluted we used already existing street canyon model calculations of NO\textsubscript{2} for the year 2006. Streets where the 98\textsuperscript{th} percentile of the daily mean value for NO\textsubscript{2} at two meters above street level was higher than 48 μg/m\textsuperscript{3} were classified as polluted street segments. For both PM\textsubscript{10} and NO\textsubscript{x} the calculated concentrations at half-height level in the street canyons (about 10 m above the ground level) were assigned to addresses situated closer than 40 meters from the polluted street segments. In order to obtain air pollution levels that are comparable to measured levels of PM\textsubscript{10} and NO\textsubscript{x}, the regional background of 10 μg/m\textsuperscript{3} PM\textsubscript{10} and 3 μg/m\textsuperscript{3} NO\textsubscript{x}, respectively should be added to our estimates.\textsuperscript{83}

Age- and municipality specific information on the time children were at daycare and schools was used to estimate time-weighted average exposure to NO\textsubscript{x} and PM\textsubscript{10}, respectively. In case parents provided two residential addresses for the same time period (e.g. related to divorce), the child was assumed to have spent 50% of the time at each place. For the first year of life only residential addresses were considered, since children in Sweden rarely start day care before 12 months of age.
2.3.2 Land use regression modeling

For the purpose of paper II, exposure to various air pollutants was estimated through land use regression models developed for each study area within the framework of the ESCAPE project. A total of 20 PM and 40 NOₓ sampling sites were selected in each study area to characterize the spatial distribution of the addresses of the cohort participants, including regional background, urban background and traffic sites. Each site was measured 3 times during 2 weeks, in the cold, warm and intermediate seasons and the results were averaged to estimate the annual average, adjusting for temporal variation using a centrally located background reference site. LUR models were developed for PM₂.₅, PM₂.₅ absorbance, PM₁₀, PM coarse, NO₂, NOₓ based on measured annual average concentrations using a set of geographic variables from both European and local datasets, included according to a supervised stepwise selection procedure. In addition, two traffic variables were specified: traffic intensity on the nearest street and traffic load on all major roads in a 100m buffer. Modeling was done locally at each centre, following a common exposure assessment manual (http://www.escapeproject.eu/manuals/). The models were then used to estimate air pollution concentrations at the birth addresses, as well as addresses at the time of the 4 and 8 years follow-ups for each study participant. In addition, the modeled concentrations for each baseline address were extrapolated back in time considering temporal trends in air pollution levels using routine air pollution monitoring data.
2.3.3 Short-term exposure assessment

Additionally, for the aims of paper IV we included information on short-term exposure to PM$_{10}$ and ozone. Hourly mean values for PM$_{10}$ were measured on two streets in the Stockholm city center - at the rooftop of one building and at 3 m above street level. Measurements were also performed at a rural station located 70 km southwest of Stockholm (Aspvreten). Approximately 1 to 3% of the observations were missing each year. Missing values for PM$_{10}$ at one of the city center measuring stations were imputed using predictions from a linear regression model based on PM$_{10}$ for the same point in time from the other two stations. If data from the rural station were lacking it was excluded from the imputation model. After imputation, less than 0.1% of the values were missing.

Ozone was measured at a rural station situated 70 km northeast of Stockholm (Norr Malma). To predict missing values at this station observations from another rural station located 20 km southwest of Stockholm (Alby) were used. Before imputation the yearly number of missing values was up to 1.6%. After imputation no ozone observations were missing.

2.4 DEFINITION OF HEALTH OUTCOMES

2.4.1 Allergic sensitization

In the BAMSE cohort blood samples obtained during the clinical examinations at 4 and 8 years of age were tested with Phadiatop (a mixture of common airborne allergens: birch pollen, timothy grass pollen, mugwort pollen, cat, dog, horse dander, mold [Cladosporium herbarum], house dust mite [Dermatophagoides pteronyssinus]) and fx5 (a mixture of common food allergens: cow’s milk, egg white, soy bean, peanut, cod fish and wheat; ImmunoCAP System; Phadia AB, Uppsala, Sweden) at a certified laboratory (Department of Medicine, Karolinska University Hospital). Sera that were scored positive for Phadiatop or fx5, defined as an IgE concentration of $\geq$0.35 kilounits of antibody per litre (kU/L), were further analyzed against allergen-specific IgE antibodies to the single inhalant and food allergens.

In paper II, apart from the BAMSE cohort four other European birth cohorts were included, where IgE sensitization was measured with either Phadiatop and fx5 (MAAS cohort, UK), or with alternative test systems (i.e., radioallergosorbent test-like method used at the Sanquin Laboratories (Amsterdam, The Netherlands), the CAP-RAST FEIA (Pharmacia Diagnostics, Freiburg, Germany)). Sensitization was defined as a IgE antibody level of $\geq$0.35 kU/L to any of the allergens tested. The reference group comprised subjects without sensitization to any allergen.

2.4.2 Asthma symptoms

Definitions were based on the parental reporting in the questionnaires when children were 1 year, 2 years, 4 years, 8 years and 12 years as following:
Wheeze: at least 1 episode and at least 3 episodes of wheeze during the last 12 months prior to the questionnaire at the respective age.

Asthma: at least 3 episodes of wheeze combined with treatment with inhaled corticosteroids and/or signs of bronchial hyperreactivity (wheezing or severe coughing at exaltation and cold weather, or disturbing cough at night) without ongoing respiratory infection were required to fulfill asthma definition at the age of 1 and 2 years. At 4, 8 and 12 years, children experiencing at least 4 episodes of wheeze in the last 12 months or at least 1 episode in combination with prescription of inhaled corticosteroids were classified as asthmatics.

Incident asthma was defined as first time asthma at the corresponding age without having it previously. Incident wheeze was defined accordingly.

Considering concomitant sensitization status, we defined asthma as allergic if the child fulfilled the criteria of asthma at 4 and/or 8 years and was also sensitized to any common inhalant allergen at the respective age, and as nonallergic when the child had asthma without being sensitized.

2.4.3 Lung function

As a part of medical examination at 8 years children underwent maximum expiratory flow volume tests, performed with a spirometer (2200 Pulmonary Function Laboratory; Sensormedics, Anaheim, CA, USA). Each child performed several slow and forced vital capacity expirations using nose clip. The highest values of forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV₁), and forced expiratory volume in 0.5 sec (FEV₀.₅) were extracted and used for analysis, provided that the child’s effort was coded as maximal by the test leader, the curve passed visual quality inspection, and that the two highest readings were reproducible according to the American Thoracic Society and European Respiratory Society (ATS/ERS) criteria. Additionally, we calculated forced expiratory volumes below 80% and 85% of the predicted value based on the BAMSE study population using age, gender, height and weight as predictor variables.

2.5 STATISTICAL ANALYSES

In the BAMSE study (papers I, III and IV) all the results are presented for a 5th to 95th percentile difference in the estimated NOₓ and PM₁₀ levels. Exposure time windows were defined as average concentrations during the first year of life, 1 to 2 years (only in paper III), 2 to 4 years, 4 to 8 years and 8 to 12 years (the latter only in paper III). Additionally, current exposure (during preceding 12 months) was considered in paper III. NOₓ and PM₁₀ concentrations were highly correlated (e.g., r = 0.96 during the first year of life), thus, we only assessed one pollutant model. We also explored the inclusion of several exposure time windows simultaneously into the models, but because of substantial collinearity the main analyses shown use models unadjusted for the other time windows.
In paper I the associations between air pollution and allergic sensitization were analyzed by multiple logistic regression modeling, and the results are presented as odds ratios (ORs) and 95% confidence intervals (CIs). The final models were adjusted for covariates identified in an earlier study and included known or suspected risk factors for sensitization or allergy, as well as variables that had influence on the effect estimates (i.e., municipality, socioeconomic status, heredity [mother and/or father with a doctor’s diagnosis of asthma and asthma medication and/or doctor-diagnosed hay fever in combination with furred animals and/or pollen allergy]), mother’s smoking during pregnancy and infancy (>1 cigarette per day), year that the house was built, damp or mold in the home at birth, and the child’s sex.

Paper II constitutes a meta-analysis of the effects of air pollution exposure on allergic sensitization in five European birth cohorts. The results are presented as odds ratios (ORs) and 95% CIs for increments of 10 µg/m³ (NO₂, PM₁₀), 1×10⁻⁵ m⁻¹ (PM₂.₅ absorbance), 5 µg/m³ (PM₉₅ and PM₂.₅), 20 µg/m³ (NOₓ), 4,000,000 v/day* m (traffic load on all major roads in a 100m buffer), 5,000 v/day (traffic intensity on the nearest street), where the latter two were combined with background NO₂ in the analyses. A common set of potential individual-level and area-level confounders were defined a priori and included: sex, age at review, maternal smoking during pregnancy, smoking in child's home, ≥12 weeks of breastfeeding, atopic mother and/or father, maternal and paternal education, mold at home, furred pets at home, older siblings, gas cooking, study region (the latter only for the BAMSE cohort). For time-varying confounders such as environmental tobacco smoke, use of gas for cooking, furred pets at home etc. current information was used to coincide with the outcome assessment. Contextual neighbourhood (SAMS) socioeconomic factors obtained from Statistics Sweden (SCB; http://www.scb.se) were also linked to the study population. In addition, we expanded the main model by adding variables that could also be in the etiologic pathway from air pollution to sensitization, such as birth weight. Stratified analyses were performed in order to assess if the associations between air pollution and sensitization were influenced by gender or by change of residence since birth. Additional analyses were also performed with back-extrapolated exposure taking into account changes in air pollution levels over time. Each center performed analyses locally according to the common study protocol and cohort-specific OR estimates were subsequently combined using a random effects model. Statistical heterogeneity among studies was evaluated using the $I^²$ statistics.

In paper III multinomial logistic regression was applied to investigate the relationship between air pollution exposure and asthma outcomes combined with sensitization and the results are presented as ORs and 95% CIs. Longitudinal associations between air pollution levels and repeated questionnaire reports of binary asthma outcomes were analyzed using logistic regression models applying generalized estimating equations (GEE) with stationary correlation structure to account for correlations between repeated observations in the same subject. The models incorporated interaction terms between time indicator variable and exposure to evaluate the effect of exposure over time. To assess effect modification by gender and by allergic heredity, variables representing the product of air pollutant exposure and potential modifiers were entered into the models. Adjustments in the multivariate analyses were made for covariates which were selected based on previous literature and were shown to lead to more than 5%
change in odds ratio. Only municipality, socioeconomic status, heredity and year the house was built fulfilled these criteria.

In paper IV linear regression analysis was used to assess associations between air pollution and lung function parameters, and the results are presented as β-values and 95% CIs. Selection of potential confounding variables was based on earlier literature, as well as on their influence on the effect estimates with more than 10% change in the β-coefficient. These included municipality, sex, age, height and heredity for asthma and/or allergy. To account for possible influence by short-term effects of air pollution, we fitted a model that adjusted for the average ozone and PM$_{10}$ levels, temperature, and relative humidity for lags of 1 to 3 and 1 to 7 days before each child’s lung function test. Furthermore, we evaluated gender, concomitant asthma and sensitization as potential effect modifiers for the relationship between air pollution and lung function through stratified analyses and introducing the appropriate interaction term in the model.

All statistical analyses were performed with STATA Statistical Software (Release 11.1; StataCorp, College Station, Texas, USA).
2.6 ETHICAL CONSIDERATIONS

The BAMSE study was approved by the Ethics Committee of Karolinska Institutet, Stockholm, Sweden. The cohort studies included in paper II were approved by local ethics committees. The parents of all participating children received written information about the purpose of the study and provided informed consent. Families were informed about the procedures and were free to withdraw at any stage.
3 RESULTS

3.1 AIR POLLUTION AND DEVELOPMENT OF ALLERGIC SENSITIZATION

3.1.1 BAMSE study

There were 2545 children with blood samples at 4 years and 2174 children with blood samples at 8 years who had complete exposure and confounder information and thus, were included in the analysis. A total of 614 (24%) children were sensitized to inhalant and/or food allergens at age 4, and 765 children (35%) were sensitized at 8 years of age. A total of 11% and 21% of the children were sensitized to any pollen (9% and 16% to birch pollen), and 7.5% and 16% were sensitized to furred animals at 4 and 8 years, respectively. Sensitization to cow’s milk dominated among the food allergens and occurred in 8% of 4-year-olds and 10% of 8-year-olds.

There was no overall risk of sensitization at 4 years of age associated with traffic-related air pollution exposure (Table 2). However, exposure during the first year of life was associated with an increased risk of sensitization to pollen (OR, 1.83; 95% CI, 1.02 - 3.28) for traffic-related NO$_x$ and 2.07 (95% CI, 0.98 - 4.39) for traffic-related PM$_{10}$. On the other hand, there was no apparent effect of exposure to air pollution after the first year of life on the development of sensitization.

At 8 years of age, there was also no clear increase in the risk of overall sensitization associated with exposure to traffic-related air pollution (Table 3). However, a tendency to an increased risk of sensitization against food allergens was indicated, with an OR of 1.50 (95% CI, 0.89 - 2.53) for NO$_x$ exposure during the first year of life and an OR of 1.77 (95% CI, 0.93 - 3.37) for PM$_{10}$ exposure during the first year of life.
Table 2. Air pollution exposure during the first year of life and from 1 to 4 years in relation to allergic sensitization at 4 years of age

<table>
<thead>
<tr>
<th>IgE sensitization at 4 years</th>
<th>Traffic-NO$_x$</th>
<th>Traffic-PM$_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR$^a$ (95% CI)</td>
<td>OR$^a$ (95% CI)</td>
</tr>
<tr>
<td></td>
<td>1$^{st}$ year</td>
<td>1-4 years</td>
</tr>
<tr>
<td>No sensitization$^b$</td>
<td>1931</td>
<td>1.00</td>
</tr>
<tr>
<td>Food or inhalant sensitization$^c$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Food sensitization</td>
<td>614</td>
<td>1.29 (0.83 – 2.01)</td>
</tr>
<tr>
<td>- Inhalant sensitization:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Pollen$^d$</td>
<td>406</td>
<td>1.24 (0.73 – 2.11)</td>
</tr>
<tr>
<td>• Furred animals$^e$</td>
<td>389</td>
<td>1.43 (0.84 – 2.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>280</td>
<td>1.83 (1.02 – 3.28)</td>
</tr>
<tr>
<td></td>
<td>189</td>
<td>1.05 (0.48 – 2.34)</td>
</tr>
</tbody>
</table>

$^a$Odds ratios are calculated for a difference in the air pollution level from the 5$^{th}$ to 95$^{th}$ percentile, corresponding to 7.2 and 6.7 µg/m$^3$ for PM$_{10}$, 46.7 and 34.3 µg/m$^3$ for NO$_x$ (during 1$^{st}$ year of life and 1-4 years, correspondingly).
Adjusted for municipality, socioeconomic status, heredity, mother’s smoking during pregnancy and infancy, year that house was built, damp or mold in the home at birth, sex of the child

$^b$Reference category
$^c$Food allergens according to fx5, inhalant allergens according to Phadiatop
$^d$Birch, timothy or mugwort
$^e$Cat, dog or horse

In children who were free from sensitization at 4 years, we found an excess risk of food sensitization at 8 years in relation to NO$_x$ exposure in the first year of life (OR, 2.30; 95% CI, 1.10 - 4.82) and PM$_{10}$ exposure in the first year of life (OR, 2.56; 95% CI, 0.96 - 6.86; Table 4). When investigating the food allergens separately, the risk increase was statistically significant only for peanut allergens (OR, 2.01; 95% CI, 1.00 - 4.05). The risks appeared less increased for the subsequent exposure periods.
Table 3. Air pollution exposure during the first year of life, as well as from 1 to 4 and 4 to 8 years in relation to allergic sensitization at 8 years of age

<table>
<thead>
<tr>
<th>IgE sensitization at 8 years</th>
<th>1\textsuperscript{st} year</th>
<th>1-4 years</th>
<th>4-8 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO\textsubscript{x}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No sensitization\textsuperscript{b}</td>
<td>1409</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Food or inhalant sensitization\textsuperscript{c,d}</td>
<td>765</td>
<td>1.14 (0.73 – 1.78)</td>
<td>0.95 (0.65 – 1.38)</td>
</tr>
<tr>
<td>- Food sensitization</td>
<td>440</td>
<td>1.50 (0.89 – 2.53)</td>
<td>1.31 (0.84 – 2.03)</td>
</tr>
<tr>
<td>- Inhalant sensitization:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Pollen\textsuperscript{d}</td>
<td>454</td>
<td>1.30 (0.76 – 2.22)</td>
<td>0.80 (0.50 – 1.29)</td>
</tr>
<tr>
<td>• Furred animals\textsuperscript{e}</td>
<td>349</td>
<td>1.15 (0.62 – 2.14)</td>
<td>0.98 (0.59 – 1.63)</td>
</tr>
<tr>
<td>PM\textsubscript{10}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food or inhalant sensitization\textsuperscript{c,d}</td>
<td>765</td>
<td>1.35 (0.79 – 2.31)</td>
<td>0.98 (0.65 – 1.48)</td>
</tr>
<tr>
<td>- Food sensitization</td>
<td>440</td>
<td>1.77 (0.93 – 3.37)</td>
<td>1.37 (0.83 – 2.25)</td>
</tr>
<tr>
<td>- Inhalant sensitization:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Pollen\textsuperscript{d}</td>
<td>454</td>
<td>1.53 (0.79 – 2.96)</td>
<td>0.76 (0.46 – 1.25)</td>
</tr>
<tr>
<td>• Furred animals\textsuperscript{e}</td>
<td>349</td>
<td>1.43 (0.68 – 3.03)</td>
<td>0.89 (0.51 – 1.56)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Odds ratios are calculated for a difference in the air pollution level from the 5\textsuperscript{th} to 95\textsuperscript{th} percentile, corresponding to 7.2, 6.7 and 6.6 \textmu g/m\textsuperscript{3} for PM\textsubscript{10}, 46.7, 34.3 and 25.7 \textmu g/m\textsuperscript{3} for NO\textsubscript{x} (during 1\textsuperscript{st} year of life and 1-4 and 4-8 years, correspondingly). Adjusted for municipality, socioeconomic status, heredity, mother’s smoking during pregnancy and infancy, year that house was built, damp or mold in the home at birth, sex of the child.

\textsuperscript{b}Reference category

\textsuperscript{c}Food allergens according to fx5, inhalant allergens according to Phadiatop

\textsuperscript{d}Birch, timothy or mugwort

\textsuperscript{e}Cat, dog or horse
Table 4. Air pollution exposure during the first year of life, as well as from 1 to 4 and 4 to 8 years in relation to allergic sensitization at 8 years of age in children not sensitized at 4 years

| IgE sensitization at 8 years | NO\textsubscript{x} | \multicolumn{3}{c}{OR\textsuperscript{a} (95% CI)} |
|-----------------------------|------------------|----------------|----------------|----------------|
|                             | no               | 1\textsuperscript{st} year | 1-4 years | 4-8 years |
| NO\textsubscript{x}         |                   |                             |           |           |
| No sensitization\textsuperscript{b} | 1197             | 1.00                        |           |           |
| Food or inhalant sensitization\textsuperscript{c}: | | | | |
| - Food sensitization        | 131              | 2.30 (1.10 – 4.82)          | 1.55 (0.81 – 2.97) | 1.38 (0.74 – 2.57) |
| - Inhalant sensitization:   | 180              | 1.05 (0.49 – 2.24)          | 1.08 (0.59 – 1.99) | 0.96 (0.54 – 1.70) |
|   • Pollen\textsuperscript{d} | 125              | 1.34 (0.59 – 3.05)          | 1.07 (0.53 – 2.15) | 0.88 (0.45 – 1.71) |
|   • Furred animals\textsuperscript{e} | 77                | 1.12 (0.37 – 3.38)          | 1.58 (0.71 – 3.51) | 1.22 (0.54 – 2.72) |
| PM\textsubscript{10}        |                   |                             |           |           |
| Food or inhalant sensitization\textsuperscript{c}: | | | | |
| - Food sensitization        | 131              | 2.56 (0.96 – 6.86)          | 1.39 (0.63 – 3.06) | 1.20 (0.60 – 2.39) |
| - Inhalant sensitization:   | 180              | 1.30 (0.52 – 3.24)          | 1.19 (0.60 – 2.38) | 1.20 (0.65 – 2.19) |
|   • Pollen\textsuperscript{d} | 125              | 1.44 (0.51 – 4.09)          | 1.05 (0.47 – 2.34) | 0.98 (0.49 – 1.98) |
|   • Furred animals\textsuperscript{e} | 77                | 1.57 (0.42 – 5.95)          | 1.61 (0.59 – 4.39) | 1.44 (0.59 – 3.52) |

\textsuperscript{a}Odds ratios are calculated for a difference in the air pollution level from the 5\textsuperscript{th} to 95\textsuperscript{th} percentile, corresponding to 7.2, 6.7 and 6.6 \mu g/m\textsuperscript{3} for PM\textsubscript{10}, 46.7, 34.3 and 25.7 \mu g/m\textsuperscript{3} for NO\textsubscript{x} (during 1\textsuperscript{st} year of life and 1-4 and 4-8 years, correspondingly). Adjusted for municipality, socioeconomic status, heredity, mother’s smoking during pregnancy and infancy, year that house was built, damp or mold in the home at birth, sex of the child

\textsuperscript{b}Reference category

\textsuperscript{c}Food allergens according to fx5, inhalant allergens according to Phadiatop

\textsuperscript{d}Birch, timothy or mugwort

\textsuperscript{e}Cat, dog or horse

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3.1.2 ESCAPE study

Children who were included in the analyses of paper II came from five European birth cohorts participating in the ESCAPE project who have been followed up to 8 years of age, provided blood samples at 2 follow-up occasions and had complete data on exposure and covariates. The prevalence of sensitization to any inhalant or food allergens within the five cohorts ranged from 24.1% to 40.4% at the age of 4 years (the lowest rate in the BAMSE cohort and highest in PIAMA), and from 34.8% to 47.9% at the age of 8 years (in BAMSE and LISA/GINI South, respectively).

All but one estimate of the associations between exposure to air pollution and sensitization to any common food and/or inhalant allergen at 4 years of age were positive in direction but none was statistically significant (Figure 4). The combined adjusted odds ratios for air pollution exposure at birth ranged from 0.94 (95% CI, 0.63 – 1.40) for 1×10^{-5} m^{-1} increase in PM_{2.5} absorbance to 1.26 (0.90 - 1.77) for 5 µg/m^3 increase in PM_{2.5} exposure. For current exposure, the combined ORs results were compatible with an absence of effect. Similarly, no clear evidence of a negative impact of air pollution on the development of sensitization at 8 years was found, neither with baseline exposure, nor with current.

We additionally performed a number of sensitivity analyses. First, we expanded our analyses to additional adjustment for an extended set of potential confounders, including birth weight. However, this did not change the results of the main models. Second, we computed summary estimates across gender strata. Although the results did not reveal any convincing gender-specific pattern, somewhat stronger associations between air pollution exposure at birth and sensitization at 4 and 8 years were suggested among boys. Thus, the risk estimates for the effects of 5 µg/m^3 increase in PM_{2.5} exposure at birth on sensitization at 8 years of age were OR = 1.62 (95% CI, 1.05 - 2.50) and 1.00 (0.63 - 1.58) for boys and girls, respectively. Third, the results of stratified analysis by moving status also displayed some variation. A tendency to higher risks was seen in subjects who did not change their baseline address up to the age of 8 years, with OR = 1.68 (95% CI, 1.03 – 2.75) compared to movers: 1.02 (0.72 - 1.46) for the increment of 1×10^{-5} m^{-1} PM_{2.5} absorbance exposure at birth address. Furthermore, the models were overall not sensitive to additional adjustment for area-level characteristics. No significant indication of area clustering was observed. Repeating the analysis using the backextrapolated NOx, NO2 and PM_{10} concentrations did not alter the results.
Figure 4. Meta-analyses of the associations between early life or current air pollution exposure and sensitization to any common allergens at 4 years in five European birth cohorts.
3.2 AIR POLLUTION AND ASTHMA

To be included in the longitudinal analyses of paper III, information on exposure, confounders and outcomes under consideration at a minimum 3 out of 5 time points was required. A total of 3633 children were eligible for analyses of the effects of exposure during infancy (89%), as well as 3477 children (85%) - for analyses of the effects of average air pollution exposure since the previous follow-up. Furthermore, 2518 and 2378 children were included in the analyses of asthma in combination with IgE sensitization at the age of 4 and 8 years, respectively. A total of 3.9%, 5.9%, 7.0%, 6.2%, and 6.7% of the children included in the analyses met the asthma definition criteria at 1, 2, 4, 8, and 12 years of age, respectively. Among asthma cases, 34% were sensitized to common inhalant allergens at the age of 4, and 58% at the age of 8 years. At least 1 episode of wheezing during the last 12 months was reported by about 15% during the first 4 years of life, with a decreasing trend to the age of 12. The incidence of wheeze symptoms appeared to be highest during the first 2 years of life. All symptoms were more prevalent in boys than girls.

Exposure during the first year of life tended to have positive overall effects on both prevalent and incident wheeze and asthma symptoms during the first 12 years of life (Figure 5). The OR for a 5th to 95th difference in traffic-PM$_{10}$ exposure and prevalence of asthma was 1.3 (95% CI, 0.7 – 2.2) and for at least 1 episode of wheeze was 1.2 (0.8 – 1.6). The association appeared strongest for asthma at 12 years of age (2.0; 1.1 – 3.5). The same pattern was seen for asthma incidence.
O wheeze ≥ 1 episode; ● wheeze ≥ 3 episodes; ● asthma

**Figure 5.** Associations between exposure to PM$_{10}$ and NO$_x$ from road traffic during the first year of life, and prevalent or incident asthma, and wheeze during the first 12 years of life among children in the BAMSE birth cohort in Stockholm

Air pollution exposure during infancy appeared to be associated with primarily nonallergic asthma at 8 years of age (ORs for 5$^{th}$–95$^{th}$ percentile difference in traffic-PM$_{10}$ = 3.8 (95% CI, 0.9 – 16.2) (Table 5). A suggestion of increased risk was observed at the age of 4 years, which was statistically significant using traffic-NO$_x$ as indicator of exposure (2.4; 95% CI, 1.0 – 5.6). No significant interactions with air pollution were seen for sex (p = 0.21) or heredity (p = 0.57 and 0.48, for one and two affected parents, respectively).
Table 5. Associations between exposure to PM$_{10}$ as well as NO$_x$ during the 1st year of life and asthma, in relation to sensitization, at 4 and 8 years of age in the BAMSE cohort in Stockholm

<table>
<thead>
<tr>
<th></th>
<th>no.</th>
<th>PM$_{10}$</th>
<th></th>
<th>NO$_x$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>adjusted$^b$</td>
<td>OR (95% CI)</td>
<td>adjusted$^b$</td>
</tr>
<tr>
<td>Crude$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At age 4 years:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No asthma &amp; no</td>
<td>2008</td>
<td>1.0</td>
<td>1.0</td>
<td>2.1</td>
<td>2.4</td>
</tr>
<tr>
<td>sensitization$^c$</td>
<td></td>
<td>(0.5 – 4.8)</td>
<td>(0.5 – 5.3)</td>
<td>(0.9 – 5.0)</td>
<td>(1.0 – 5.6)</td>
</tr>
<tr>
<td>Nonallergic asthma</td>
<td>126</td>
<td>1.5 (0.3 – 5.9)</td>
<td>1.4 (0.3 – 6.8)</td>
<td>1.3 (0.4 – 4.4)</td>
<td>1.5 (0.4 – 5.1)</td>
</tr>
<tr>
<td>Allergic asthma$^d$</td>
<td>65</td>
<td>1.0 (0.3 – 5.9)</td>
<td>1.0 (0.3 – 5.9)</td>
<td>1.5 (0.4 – 6.8)</td>
<td>1.3 (0.3 – 6.8)</td>
</tr>
<tr>
<td>At age 8 years:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No asthma &amp; no</td>
<td>1692</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>sensitization$^c$</td>
<td></td>
<td>(0.5 – 4.8)</td>
<td>(0.5 – 5.3)</td>
<td>1.3 (0.4 – 4.4)</td>
<td>1.5 (0.4 – 5.1)</td>
</tr>
<tr>
<td>Nonallergic asthma</td>
<td>73</td>
<td>3.1 (0.7 – 13.1)</td>
<td>3.0 (0.9 – 16.2)</td>
<td>2.3 (0.7 – 7.1)</td>
<td>2.6 (0.9 – 8.1)</td>
</tr>
<tr>
<td>Allergic asthma$^d$</td>
<td>100</td>
<td>1.0 (0.3 – 5.9)</td>
<td>1.1 (0.3 – 3.8)</td>
<td>0.7 (0.2 – 2.2)</td>
<td>0.8 (0.2 – 2.4)</td>
</tr>
</tbody>
</table>

$^a$Odds ratios are calculated with adjustment for municipality only (design variable) for a 5th to 95th percentile difference in the air pollution exposure levels during the first year of life, corresponding to 7.2 µg/m$^3$ for PM$_{10}$ and 46.8 µg/m$^3$ for NO$_x$.

$^b$Odds ratios adjusted for municipality, socioeconomic status, heredity, and year the house was built.

$^c$Reference category.

$^d$Sensitization to inhaled allergens is defined as an IgE (immunoglobulin E) antibody level of $\geq0.35$ kUA/l.

No overall effect was observed with average air pollution exposure since the date of previous follow-up (ORs for a 5th to 95th difference in traffic-PM$_{10}$ exposure = 0.9 (95% CI, 0.6 – 1.3 and 0.9 (0.6 - 1.4) for prevalent and incident asthma, respectively). A similar absence of effects was seen in relation to exposure during the preceding 12 months.
3.3 AIR POLLUTION AND LUNG FUNCTION DEVELOPMENT

Maximum expiratory flow volume tests were performed in 2113 8-year-old children. There were 1924 subjects (47%) with information on air pollution exposure, confounders and lung function measurements. A total of 6.8 and 10.5% of subjects with spirometric measurements had less than 85% predicted FEV₁ and FEV₀.₅ levels, respectively, and approximately half of these had less than 80% predicted levels.

Exposure to traffic-PM₁₀ during the first year of life was associated with forced expiratory volume deficits of -59.3 mL (-113.0 to -5.6) in FEV₁ and -62.4 mL (-113.7 to -11.1) in FEV₀.₅ for a 5th to 95th percentile difference in time-weighted exposure. Similar effects were seen for FVC, but not statistically significant. On the other hand, no clear effects on lung function were seen in relation to air pollution exposure after infancy (Figure 6).

Further analyses suggested stronger effects in boys, in those sensitized against any common inhalant and/or food allergen, and in those with asthma, with deficits in FEV₁ of -79.6 mL (-155.7 to -3.5), -136.9 mL (-224.1 to -49.7), and -90.6 mL (-293.4 to 112.3), respectively (Table 6). However, the apparent effect modification was not statistically significant (p = 0.35, 0.13 and 0.69, respectively).

Figure 6. Lung function measurements in relation to traffic-PM₁₀ exposure in different time periods of life

- ¶ first year of life exposure; ◇ 1-4th years exposure; ◇ 4-8th years exposure
Table 6. Association between exposure to traffic-PM$_{10}$ during the first year of life and FEV$_1$ at 8 years of age

<table>
<thead>
<tr>
<th>Traffic-PM$_{10}$</th>
<th>No</th>
<th>Point Estimates in ml (95% CI)*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>1851</td>
<td>-59.3 (-113.0 to -5.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>Girls</td>
<td>902</td>
<td>-37.1 (-112.7 to 38.4)</td>
<td>0.34</td>
</tr>
<tr>
<td>Boys</td>
<td>949</td>
<td>-79.6 (-155.7 to -3.5)</td>
<td>0.04</td>
</tr>
<tr>
<td>Sensitized at 8 yrs†</td>
<td>606</td>
<td>-136.9 (-224.1 to -49.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Not sensitized at 8 yrs †</td>
<td>1119</td>
<td>-44.8 (-116.6 to 26.9)</td>
<td>0.22</td>
</tr>
<tr>
<td>Asthma at 8 yrs‡</td>
<td>144</td>
<td>-90.6 (-293.4 to 112.3)</td>
<td>0.38</td>
</tr>
<tr>
<td>No asthma at 8 yrs</td>
<td>1696</td>
<td>-55.4 (-111.2 to 0.3)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Results are presented in ml for a difference in PM$_{10}$ level from 5$^{th}$ to 95$^{th}$ percentile, corresponding to 7 µg/m$^3$.

Adjusted for municipality, sex, age, height and heredity

†Defined as IgE values for Phadiatop ≥0.35 kU/l and/or IgE-value for food-mix ≥ 0.35 kU/l

‡Defined as at least 4 episodes of wheeze in the last 12 months or at least 1 episode in combination with prescription of inhaled corticosteroids

We also analyzed effects at less than 80%, as well as 85% of predicted FEV$_1$ and FEV$_{0.5}$ to determine whether exposure to air pollution was associated with clinically important lung function deficits. Strong associations were indicated between exposure to traffic-PM$_{10}$ during the first year of life and forced expiratory volumes below 80% and 85% of predicted. Corresponding odds ratios of 4.1; (95% CI, 0.8 - 20.3), and 6.1 (2.3 –16.5) as well as 4.0 (1.2 - 13.1) and 2.5 (1.0 - 6.3) were seen for FEV$_1$ and FEV$_{0.5}$, respectively (Figure 7).

Additional adjustment for temperature, relative humidity, ozone and PM$_{10}$ levels during three-to-seven days before each child’s pulmonary function test showed little effect on the estimates of long-term effects of air pollution.
Figure 7. Association between first year of life exposure to traffic- PM$_{10}$ and forced expiratory volumes below 85% and 80% of predicted
4 DISCUSSION

4.1 MAIN FINDINGS

We found no clear associations between estimated ambient exposure to traffic-related air pollution and overall sensitization to common inhalant and food allergens in 8-year-old children. However, the results suggest increased risks of sensitization to certain allergens related to exposure during infancy. Our findings are in line with other cohort studies that have evaluated effects of exposure to air pollution on the development of sensitization in young children. A causal association is also supported by experimental evidence indicating that exhaust particles can enhance immunologic responses to allergens and inflammatory reactions in the airways. We observed stronger risks for sensitization against pollen allergens at 4 years, whereas at 8 years the elevated risks were primarily driven by food allergens. Cross-reactions between pollen and certain food allergens might contribute to the observed associations between air pollution exposure and food allergy at the later age. Thus, looking at separate food items, we observed the clearest risk for peanut allergens, which are known to cross-react with pollen. On the other hand, no apparent association between air pollution exposure and allergic sensitization in the meta-analysis of the five European birth cohorts was seen. Similar findings were reported earlier from the Oslo cohort, as well as from the 8-year follow-up of the Dutch cohort PIAMA. One of the possible explanations may lie in the different exposure assessment approach, discussed below.

Our results suggested positive associations between exposure to traffic-related air pollution during infancy and overall risks of respiratory symptoms in children over the first 12 years of life. The evidence regarding effects of long-term exposure to air pollution on respiratory symptoms is limited, as only a few studies have investigated the development of disease up to school age, with estimates of outdoor air pollution exposure based on small spatial scales, and by timing exposure prior to the development of symptoms. A Dutch cohort demonstrated positive associations between levels of traffic-related air pollution at the birth address, and incidence as well as prevalence of asthma in children who were followed from birth until 8 years of age. In contrast, a Norwegian study did not find any association of either early-life or lifetime average traffic-related exposures with asthma onset or with current respiratory symptoms in 9- to 10-year-old children in Oslo.

We found that exposure to traffic-related air pollution during infancy was associated with a decreased lung function in children at 8 years of age. The strongest effects were suggested for FEV$_1$ and FEV$_{0.5}$, which reflect the mechanical properties of the large and medium size airways and not as much on FVC, representing lung size. This is consistent with the Californian CHS and the Oslo cohort findings, even though the Californian study found the largest effect on midexpiratory flow, MMEF, possibly more representing the bronchioles. Differences in effects on lung function variables from air pollution might partly be explained by the mixture of components in traffic related emission. We have in our study focused on PM$_{10}$ as exposure estimate, which in Stockholm is primarily influenced by coarse particles (>2.5 µm), although it also contains fine and ultrafine particles. Our results are in general agreement with the other
studies considering that levels of smaller particles, such as PM$_{2.5}$, correlate to PM$_{10}$ and are also supported by our findings for traffic-NO$_x$, which correlate with fine particulate emissions from motor vehicles.

From an individual perspective the estimated effect on lung function seen in our study is quite small (-3.3% for FEV$_1$ and -4.7% for FEV$_{0.5}$), but even a minor shift in the population distribution of lung function can substantially increase the prevalence of subjects exhibiting respiratory function below clinical thresholds. In our study this is indicated by the sharply increased risks of having a lung function below 80 and 85% of predicted.

**Timing of exposure**

Children constitute a potentially vulnerable subgroup with regard to the detrimental impacts of air pollution.\cite{91,92,93} We investigated several time aspects of long-term exposure, including early-life exposure and more recent exposure. In general, the strongest effect was observed in relation to exposure during infancy, which is in line with previous studies indicating that prenatal and early-life periods represent critical windows for the effects of exposure on development of childhood respiratory morbidity.\cite{7,94}

Children inhale a higher volume of air per body weight compared with adults, delivering higher doses of different components that may remain in the lung for a longer period.\cite{95} Another source of increased sensitivity of children to air pollution may be differences in the respiratory, immune, endocrine, and nervous systems during phases of rapid growth and development.\cite{96} The developing lung may be highly susceptible to damage from exposure to environmental toxicants due to the prolonged maturation of the respiratory system, extending from the embryonic stage of development in utero through to adolescence.\cite{97} Furthermore, the exposure pattern differs between children and adults in several aspects. Children spend more time outdoors and their physical activity outdoors might increase ventilation rates as compared to adults.\cite{98,99} This may result in higher risks due to increased exposure to certain air pollutants of outdoor origin that may have lower indoor concentrations. Moreover, the outdoor breathing zone of children is closer to the ground than that of adults. As ambient pollutants derived from motor-vehicle traffic are emitted close to the ground and are dispersed afterwards, the concentrations are higher close to the ground at least near busy roads.\cite{15} Children’s walking or playing close to busy roads would in addition increase their exposure to ambient air pollutants. Also, children’s breathing through the mouth increases the inhalation and deposition of pollutants in the lungs and the airways.

With regard to the effects of air pollution exposure after the first year of life, our results are consistent with those of several previous studies, in which no overall associations between traffic-related air pollutants and sensitization were found.\cite{39,41,44} Noteworthy, in our study higher concentrations of combustion-related air pollutants during the infancy period than later in life might also have contributed to the higher risks associated with exposure during this period. The mean local contribution above regional background to the children’s outdoor air pollution exposure levels was 4.2, 3.9, 3.6, 3.5 and 4.6 µg/m$^3$ for traffic-PM$_{10}$, and 21.4, 18.6, 13.4, 10.6 and 7.8 µg/m$^3$ for traffic-NO$_x$ during the first year of life, 1 to 2, 2 to 4, 4 to 8, and 8 to 12 life years, correspondingly. The main explanation for the observed trends in traffic-related NO$_x$
concentrations over the study period is that the recruitment of our cohort occurred shortly after the introduction of catalytic converters in private cars. This led to a reduction in the emissions for several air pollutants, which continued to decrease with the replacement of older vehicles. In a Norwegian study with fairly similar levels of outdoor air pollution to those in Sweden, it was suggested that if an association between air pollution exposure and allergic outcomes exists, it might not be identified because of comparatively low exposure levels.

The current air quality standard in Sweden for PM$_{10}$ is 40 µg/m$^3$, as an annual average and the same for NO$_2$ (Swedish EPA; http://www.naturvardsverket.se/Start/Lagar-och-styrning/Miljokvalitetsnormer/Utomhusluft-miljokvalitetsnormer/). In the BAMSE cohort, the total PM$_{10}$ exposure levels during the first year of life ranged between 10.1 to 27.7 µg/m$^3$ (including a background level of 10 µg/m$^3$), thus not exceeding the legal limits. Exposure to NO$_2$ for the same time period was up to 54.3 µg/m$^3$ based on the calculation from NO$_x$ levels, using the formula NO$_2$ = NO$_x$ $(0.66 + 34/(NO_x + 100))$, and with the addition of a 3 µg/m$^3$ background level. These levels were above the legal limits only in 2% of the children. Our findings indicate that current air quality standards do not fully protect children against adverse respiratory effects from air pollution.

**Air pollution exposure assessment**

Dispersion modeling and land use regression (LUR) modeling are two of the approaches that are currently used for modeling fine-scale spatial variations in air pollution concentrations. In paper I, III and IV the individual exposure estimates for each cohort member were derived from a time- and space-resolved dispersion model with addition of street canyon contribution for addresses in the most polluted street segments. Model calculations of air pollution concentrations agree well with measurements. Measurements in Stockholm also show that ambient levels of NO$_2$ or NO$_x$ are related to both soot levels and particle number concentrations, indicating that NO$_x$ might be a good indicator for combustion-related particles.

One of the limitations is that calculations of air pollutant concentrations could only be performed for the years when emission databases were available (for NO$_x$: 1990, 1995, 2000, 2002-2004, 2006, and 2010). For PM$_{10}$, only the emissions data for 2004 were used. However, the constant percentage of studded tires in the Stockholm vehicle fleet, as well as the relatively stable traffic work in the inner city during the last decades, suggest that the emissions of PM$_{10}$ have not changed much during the period from 1994 to 2004. Another weak point lies in the lack of meteorological adjustment for PM$_{10}$ concentrations. Contrary to NO$_x$ (and exhaust particles), street moisture has a crucial influence on PM$_{10}$ concentrations. Unfortunately, relevant data were not available to adjust for road moisture in estimating the yearly PM$_{10}$ concentrations.

To increase the precision in the exposure assessment, we took time-activity patterns into account, considering the time children spent at home, day care, and/or school, which has not been done in earlier birth cohort studies on the health effects of air pollution. Our results indicate that home addresses well represent exposure to air pollution in young children of Stockholm. This is probably because the children’s kindergartens and schools are often located in the
vicinity of their residential areas. Furthermore, air pollution levels at the home address are highly relevant as exposure estimates in infancy since children in Sweden usually do not start day care before 12 months of age.

In paper II air pollution exposure was assessed by means of LUR modeling. A limitation of this approach was that the LUR models were based on measurement campaigns carried out in 2008/2009, but the relevant exposure time periods were between 1994 and 2008. However, a recent study in the Netherlands found that the spatial contrasts of NO$_2$ at 35 measurements sites were relatively stable from 1999 to 2007. Moreover, a sensitivity analysis using back-extrapolated NO$_2$, NO$_x$ and PM$_{10}$ concentrations provided similar results as the models using exposure taken directly from LUR models. Furthermore, it was found that a LUR model developed from 2008/2009 showed substantial improvement in the explained variance for certain pollutants in certain areas compared to the models from 1999/2000. However, despite that ESCAPE LUR models showed on average moderate to good explained variance, model performance varied between different areas, as well as between pollutants (i.e., $R^2$ for PM$_{2.5}$ ranged from 35% in Manchester to 94% in Stockholm County). Thus, if early life exposure plays an important role for later development of sensitization, recent LUR models may not be ideal for estimating relevant exposure. Additional limitation was lack of information on the street configuration (e.g. canyons). It should also be recognized that the modeled individual concentrations did not account for time activity patterns and indoor/outdoor differences and are, therefore, not equivalent to personal exposure.

**Effect modification**

There is a growing body of evidence on sex-specific differences in the associations between air pollution exposure and respiratory morbidity. However, the literature is far from consistent as some of the studies reported stronger effects among girls, while others showed the opposite. Disentangling of sex effects among children is complicated, given that they may follow age-related patterns, as well as be outcome-specific. We observed a tendency for a higher risk of decreased lung function (paper IV) in boys compared to girls which provides some support to earlier evidence of boys being more sensitive to air pollution in early life. Although the potential mechanisms are not understood yet, age differences in pulmonary physiology have been suggested to contribute to the observed sex-specific susceptibility (e.g. young boys have lower respiratory volumes and greater airway resistance, their growth spurt starts later and peak velocity reaches at an older age compared to girls). In addition to lung-specific factors, immune factors (boys are more sensitized) in early life might also be important. Alternatively, boys might experience higher levels of exposures because they are relatively active and spend more time outdoors. Data regarding the role of allergic sensitization as a potential modifying factor for respiratory symptoms and lung function loss in relation to air pollution exposure in children are limited. Our results suggest that traffic-related air pollution primarily increases the risk of nonallergic asthma symptoms (paper III), which is in line with the earlier evidence provided by experimental and epidemiologic studies. It has been hypothesised that air pollutants induce nonspecific irritative changes in the airways through changes in the formation of reactive oxygen species,
alterations in antioxidant defence, and increased nonallergic inflammation. Nonallergic asthmatics have a higher sensitivity of the nasal and bronchial epithelia to nonallergic stimuli such as air pollutants, strong smells, cold air, respiratory viruses etc. compared to allergic asthmatics. Alternatively, children with an allergic predisposition may spend more time indoors compared with those without an allergic predisposition, thus, experiencing different exposure profiles.

Several cross-sectional studies have reported larger effects of air pollution exposure on lung function in atopic children. Although the exact mechanisms are unclear, it has been suggested that both air pollution and sensitization might be independently involved in the induction of Th2 immune response and that sensitized children experience subclinical asthma-like changes in their pulmonary function. Thus, air pollution exposure in allergic children may exert a synergistic effect on the allergic inflammation response to specific allergens.

It should be noted, that in all studies included in this thesis interactions between air pollution exposure and potential modifying factors were not statistically significant, and results have to be interpreted with caution.

4.2 METHODOLOGICAL ASPECTS

Epidemiologic studies are potentially subject to two broad categories of errors that may affect their precision and validity: random error and systematic error (bias).

4.2.1 Random error

Random error results from fluctuations around a true value because of sampling variability. It is inversely related to precision and is expressed by the width of confidence interval. The precision in the measure of association mainly depends on the sample size, but also on the prevalence of exposure and outcome and the quality of exposure and outcome specification. Confidence intervals were computed in all papers included in this thesis to demonstrate the precision of the risk estimates. The sample sizes in our studies varied between 1900 subjects in paper IV and 6500 in paper II, which gave relatively narrow confidence intervals in our main analyses. The subgroup analyses, however, included lower number of subjects in each stratum, which leads to a higher statistical uncertainty around the estimates, and difficulties in correctly assessing the risk.

4.2.2 Systematic error

Systematic errors (or bias) do not depend on study size or chance, but may occur from factors involved in the choice or recruitment of a study population or in the definition and measurement of study variables. The most common types of such errors are selection bias, information bias and confounding. An estimate that has little systematic error is regarded as valid.
Selection bias

Selection bias results from procedures of selecting subjects into a study that lead to results different from what would have been obtained from the entire population targeted for study. At the stage of recruitment of participants selection bias refers to the concept of external validity (e.g. the extent to which findings can be generalized to populations other than the one being investigated). The BAMSE cohort is a population-based study which facilitates generalization of our findings to the population of respective ages, at least for the areas under study. The original cohort included 75% of those eligible. A survey of the non-responder-excluded group did not reveal significant differences in most background characteristics (e.g. allergic heredity, pet keeping). However, parental smoking was more prevalent in the non-participating families, which means that the cohort was not fully representative regarding this exposure.

Maintaining a high response rate during follow-up is crucial for avoiding selection bias. In the BAMSE cohort the response rates were high throughout the whole study period, with 82% of the original cohort still remaining at 12 years of age. Subjects, who remained in the study and were followed with questionnaires over 12 years, did not differ significantly from those in the original cohort. Similarly, background data of children with lung function tests and blood samples did not differ significantly on key characteristics, such as sex, parental allergy etc. from those in the original BAMSE cohort. Taken together, this indicates that selection bias resulting from loss to follow-up was of minor importance.

Similarly, the cohorts participating in ESCAPE project are population-based studies, designed to study the development of asthma and allergies during childhood. The response rates at 8-10 years varied between 90% (of the original study population) in the MAAS cohort to 60% in the LISA/GINI South cohort. Overall, there were no substantial differences between the subjects remaining and those in the respective original cohorts. It should be noted, however, that in the subset of children with IgE measurements in the PIAMA and LISA/GINI cohorts, the proportion of children with allergic parents was somewhat higher than in the baseline population.

Information bias

Information bias denotes any systematic error related to assessment of exposure and/or outcome. Misclassification of exposure could be differential if it is related to disease status or any bias in collected disease status, and otherwise non-differential. Non-differential misclassification generally tends to dilute the results (OR→1), while differential misclassification can either cause an increased or decreased risk estimate compared to the true value. Similarly, misclassification of outcome is differential if it is related to exposure, and non-differential otherwise. To get reliable data on allergic sensitization, the IgE concentrations were measured using standardized methods. Lung function was measured by a trained staff of paediatric nurses in accordance with the American Thoracic Society and European Respiratory Society (ATS/ERS) criteria. Furthermore, a quality control was performed by manually reviewing the curves for technical acceptability. Both IgE and lung function were properly measured without knowledge of air pollution exposure.
Misclassification of the outcome is often of concern in studies where asthma and wheeze are assessed by parental questionnaires, particularly in young children who have less distinctive symptoms. Furthermore, it could be influenced by different diagnostic practices among doctors. Childhood asthma is not a homogeneous disease and different phenotypes of wheeze and asthma have been defined based on prognosis, allergic status and pulmonary function. Because of the difficulties involved in diagnosis in young children due to a lack of understanding of the ultimate physiopathological mechanisms of the disease in childhood, the term asthma is often replaced by wheezing, which also may take symptoms over time into consideration, such as transient wheeze, late-onset wheeze and persistent wheeze. \textsuperscript{8,127} Wheeze in early infancy has been well characterized, often with decreased flow rates at birth, onset of wheeze within the first year and resolution by mid-childhood with no lasting effects on lung function. “Nonatopic wheezers” have more frequent symptoms during the first year of life and may continue wheezing through childhood, but usually episodes become less common by early adolescence. Most of the children with persistent wheeze, who will subsequently be diagnosed with asthma, experience their first symptoms before 3 years of age. By age 3, they can have abnormal pulmonary function that persists to adulthood, and by adolescence the majority will become sensitized to Aeroallergens, based on symptoms and/or results of allergen challenge or detection of allergen-specific IgE.\textsuperscript{128-130} Distinguishing among the different phenotypes in early childhood is, therefore, crucial to understanding the role of risk factors and their timing.

Since many children who experience wheeze early in life will outgrow their symptoms, asthma is difficult to define before school age.\textsuperscript{130} We observed the highest risk of asthma related to air pollution exposure in older children (i.e. 8 or 12 years) when asthma presumably can be defined more reliably than in younger children with “asthma” who often suffer from wheezing triggered by respiratory tract infections.

Some misclassification of air pollution exposure is likely to have occurred, partly because modeling of air pollution may give error in the exposure estimates and because outdoor exposure may not reflect true personal exposure. Since exposure was estimated independently from IgE tests, asthma symptoms and lung function and potential confounders, bias in exposure related to outcomes in unlikely, and random error will probably weaken the associations between exposure and outcomes.

\textit{Confounding}

The effects of other factors may confound the estimated associations between exposure and outcome. A confounder must be associated with the outcome, but not as an effect of the outcome, and it must be associated with the exposure, but not as an effect of the exposure (effect mediator).\textsuperscript{122} Depending on the direction of the associations that the confounding factor has with exposure and outcome confounding can cause either overestimation or underestimation of the true effect of the exposure. We have addressed confounding by adjusting for a number of known or suspected risk factors for respiratory allergies, as well as for variables that had influence on the effect estimates.
An important source of bias that deserves particular consideration when evaluating the effects of traffic air pollution is socioeconomic status (SES). Disentangling the extent of such bias in air pollution studies can be quite difficult, as different SES definitions have been used, including parental occupation, household income, ethnic group, type of dwelling, etc. Interestingly, the relation between SES position and air pollution exposure varies between the countries. For example, in the US studies low SES is frequently correlated with living near busy roadways and is associated with asthma incidence and severity.\cite{131,132} Similarly, associations between pollutant concentrations and physician visits in children in Toronto, Canada are higher in children from low-SES families.\cite{133} In Stockholm, in contrast, children with the highest exposure levels tended to come from families of high socioeconomic status, largely because they more often lived in the inner city where air pollution levels are higher. Therefore, it is critically important to properly control for and interpret this potential confounder in environmental studies where associations are attributed to air pollution exposure.

In addition to the individual SES characteristics, it has also been suggested that neighborhood socioeconomic position and similar area structural factors may be powerful sources of confounding in studies of area related environmental factors and health.\cite{134} It has been argued that air pollution may act as intermediate factor for contextual SES, which may be a distal cause.\cite{135} Alternatively, if the effects of air pollution are non-causal, including area-level SES indicators may detect important confounding which may explain the relationship.\cite{136} In order to test for possible contextual confounding, we added neighborhood SES indicators in the combined analysis of ESCAPE cohorts (paper II). However, no significant impact on the main results was observed in any of the included cohorts.

Short-term exposure to outdoor air pollution has been shown to influence lung function impairment in children.\cite{137} We, therefore, included both short- and long-term air pollution exposures in the analysis in paper IV, to exclude possible confounding or decreased precision of the long-term exposure estimates by short-term exposure. Additional adjustment for temperature, relative humidity, as well as short-term exposures (previous days’ concentrations of $O_3$ and $PM_{10}$) showed, however, little influence of short-term exposure on the effect estimates for long-term exposure on lung function. Similar findings were reported from the Children’s Health Study and the Oslo cohort.\cite{89,90}

An influence by other unknown or unmeasured factors (residual confounders) cannot be excluded.\cite{138,139} However, from a methodological point of view, the assessment of health effects of air pollution in children should be less subject to confounding than in adults’ studies by concomitant factors like smoking, occupational exposures, exposures while commuting and travelling, etc.\cite{15}
5 CONCLUSIONS

Based on the presented studies, it may be concluded that long-term exposure to air pollution from road traffic adversely affects the respiratory system in children. More specifically:

➢ There was no overall increase in the risk of allergic sensitization in children up to school age related to air pollution exposure. However, sensitization to certain allergens, such as pollen, may be associated with exposure during infancy.

➢ Air pollution exposure during the first year of life tended to be associated with asthma in children over the first 12 years of life, particularly in the oldest children and for those with nonallergic asthma. Further follow-up will determine whether these effects persist into higher ages.

➢ Exposure to traffic-related air pollution during infancy was associated with decreased lung function in children up to 8 years of age, particularly in those with atopy. Thus, early life exposure seems to have long-term effects on lung function, which may be of importance also later in life.

➢ Taken together, our findings indicate that the infancy period may be critical for the influence of air pollution on asthma, allergy and lung function in children up to school age.

➢ Our results suggest that current air quality standards do not fully protect children against adverse respiratory effects.
6 SVENSK SAMMANFATTNING


Syftet med denna avhandling var att undersöka långtidseffekterna av exponering för luftföroreningar från vägtrafiken på utvecklingen av luftvägs- och allergiska sjukdomar hos barn med fokus på allergisk sensibilisering, astmarelaterade symtom och lungfunktion. Delarbetena baseras på två epidemiologiska studier, den svenska födelsekohorten BAMSE och ESCAPE studien som inkluderade fem europeiska födelsekohorter.

Över 4000 barn i BAMSE-kohorten följdes med upprepade enkäter, blodprover och lungfunktionsundersökningar upp till 12 års ålder. Utomhuskoncentrationer av kväveoxider (NOₓ) och inandningsbara partiklar från vägtrafiken (PM₁₀) beräknades för samtliga adresser till bostäder, daghem och skolor hos barnen med hjälp av spridningsmodeller. Exponering för luftföroreningar från vägtrafiken under det första levnadsåret var relatad till en ökad risk för sensibilisering mot pollen vid 4 års ålder, men inga tydliga samband sågs vid 8 år. Våra resultat antyder samband mellan exponering för trafikrelaterade luftföroreningar under spädbarnstiden och astma hos barn upp till 12 års ålder. Riskerna föreföll särskilt uttalade hos barn i åldersgruppen 8-12 år och för icke-allergisk astma. Dessutom tycktes exponering för luftföroreningar tidigt i livet ha långsiktiga negativa konsekvenser på lungfunktionen, särskilt hos barn som var allergiska mot vanliga luftburna och födoämnesallergen. Exponering för luftföroreningar efter det första levnadsåret tycktes dock ha mindre inverkan på de studerade respiratoriska utfallen.

I fem europeiska barnkohorter som deltar i ESCAPE-projektet, användes land-use regressionsmodeller för att bedöma exponering för en rad luftföroreningsexponenter vid hemadresserna. Blodprov från mer än 6500 barn vid 4 och/eller 8 års ålder analyserades för specifika IgE mot vanliga luftburna och födoämnesallergen. En kombinerad analys av de olika kohorterna visade inte något klart samband mellan luftföroreningsexponering och utvecklingen av allergisk sensibilisering hos barn upp till 10 års ålder.

Sammanfattningsvis tyder våra resultat på att exponering för luftföroreningar under spädbarnstiden kan påverka utvecklingen av astma, allergi och lungfunktion hos barn upp till skolåldern. Resultaten talar även för att aktuella gränsvärder för luftförorening inte helt skyddar mot skadliga luftvägseffekter hos barn.
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