Adverse effects of exposure to air pollutants during fetal development and early life with focus on pre-eclampsia, preterm delivery, and childhood asthma

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He få väl va he vejl

Sankte Per – Skapelseberättelsen, burträskarens uppkomst
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table of Contents</td>
<td>i</td>
</tr>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>iv</td>
</tr>
<tr>
<td>Enkel sammanfattning på svenska</td>
<td>v</td>
</tr>
<tr>
<td>Original papers</td>
<td>vi</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td></td>
</tr>
<tr>
<td>Air pollution exposure</td>
<td>2</td>
</tr>
<tr>
<td>Air pollution and birth outcomes</td>
<td>2</td>
</tr>
<tr>
<td>Air pollution and asthma</td>
<td>3</td>
</tr>
<tr>
<td><strong>Aims of the thesis</strong></td>
<td>5</td>
</tr>
<tr>
<td>Overall aims</td>
<td>5</td>
</tr>
<tr>
<td>Specific aims</td>
<td>5</td>
</tr>
<tr>
<td><strong>Materials and methods</strong></td>
<td>6</td>
</tr>
<tr>
<td>Study populations</td>
<td>6</td>
</tr>
<tr>
<td>Outcome definitions</td>
<td>6</td>
</tr>
<tr>
<td>Covariate definitions</td>
<td>7</td>
</tr>
<tr>
<td>Exposure assessment</td>
<td>8</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>8</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>13</td>
</tr>
<tr>
<td>Paper I</td>
<td>13</td>
</tr>
<tr>
<td>Paper II</td>
<td>14</td>
</tr>
<tr>
<td>Paper III</td>
<td>14</td>
</tr>
<tr>
<td>Paper IV</td>
<td>15</td>
</tr>
<tr>
<td><strong>Discussion</strong></td>
<td>17</td>
</tr>
<tr>
<td>Exposure assessment</td>
<td>17</td>
</tr>
<tr>
<td>Statistical modeling</td>
<td>17</td>
</tr>
<tr>
<td>Mechanism</td>
<td>18</td>
</tr>
<tr>
<td>Comparison of findings</td>
<td>18</td>
</tr>
<tr>
<td>Policy implications</td>
<td>20</td>
</tr>
<tr>
<td>Summary of findings</td>
<td>20</td>
</tr>
<tr>
<td>Future research</td>
<td>21</td>
</tr>
<tr>
<td>Conclusions</td>
<td>22</td>
</tr>
<tr>
<td><strong>Acknowledgements</strong></td>
<td>23</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>25</td>
</tr>
</tbody>
</table>
Abstract

**Background** Air pollution exposure has been shown to have adverse effects on several health outcomes, and numerous studies have reported associations with cardiovascular morbidity, respiratory disease, and mortality. Over the last decade, an increasing number of studies have investigated possible associations with pregnancy outcomes, including preterm delivery. High levels of vehicle exhaust in residential neighborhoods have been associated with respiratory effects, including childhood asthma, and preterm birth is also associated with childhood asthma.

The first aim of this thesis was to investigate possible associations between air pollution exposure and pregnancy outcomes – primarily preterm delivery but also small for gestational age (SGA) and pre-eclampsia – in a large Swedish population (Papers I–III). The second aim was to study any association between exposure to high levels of vehicle exhaust during pregnancy and infancy and prescribed asthma medication in childhood (Paper IV).

**Methods** The study cohorts were constructed by matching other individual data to the Swedish Medical Birth Register. In the first two studies, air pollution data from monitoring stations were used, and in the third and fourth studies traffic intensity and dispersion model data were used.

Preterm delivery was defined as giving birth before 37 weeks of gestation. SGA was defined as having a birth weight below the 10th percentile for a given duration of gestation. Pre-eclampsia was defined as having any of the ICD-10 diagnosis codes O11 (pre-existing hypertension with pre-eclampsia), O13 (gestational hypertension without significant proteinuria), O14 (gestational hypertension with significant proteinuria), or O15 (eclampsia). Childhood asthma medication was defined as having been prescribed asthma medication between the ages of five and six years.

**Results** We observed an association between ozone exposure during the first trimester and preterm delivery. First trimester ozone exposure was also associated with pre-eclampsia. The modeled concentration of nitrogen oxides at the home address was associated with pre-eclampsia, but critical time windows were not possible to investigate due to high correlations between time windows. We did not observe any association between air pollution exposure and SGA. High levels of vehicle exhaust at the home address, estimated by nitrogen oxides and traffic intensity, were associated with a lower risk of asthma medication.
**Conclusion** Air pollution exposure during pregnancy was associated with preterm delivery and pre-eclampsia. We did not observe any association between air pollution levels and intrauterine growth measured as SGA. No harmful effect of air pollution exposure during pregnancy or infancy on the risk of being prescribed asthma medication between five and six years of age was observed.
Abbreviations

ATC – Anatomical therapeutic chemical
BMI – Body mass index
CO – Carbon monoxide
ICD-10 – International Statistical Classification of Diseases, version 10
LBW – Low birth weight
NO₂ – Nitrogen dioxide
NOx – Nitrogen oxides
O₃ – Ozone
OR – Odds ratio
PM₁₀ – Particulate matter with a diameter <10 µm
SGA – Small for gestational age
SMHI – Swedish meteorological and hydrological institute
SO₂ – Sulfur dioxide
WHO – World health organization
Enkel sammanfattning på svenska

Ogynnsamma födelseutfall, exempelvis förtida födsel, har visats öka risken för sämre studieresultat och mer sjuklighet genom hela livet. Astma under barndomen kan leda till en lägre fysisk aktivitet eller ett lägre psykosocialt välbefinnande.

Denna avhandling har två huvudspår: (1) Att studera samband mellan luftförroreningshalter under graviditeten och ogynnsamma utfall, främst havandesksapsförgiftning, för tidigt födda barn samt tillväxthämning. (2) Att studera samband mellan luftförroreningshalter under graviditeten eller spädbranstiden och astmamedicinering under barndomen.

De viktigaste fynden är att förhöjda ozonhalter i ett tidigt skede av graviditeten ledde till en ökad risk för barnet att bli för tidigt fött och för mamman att drabbas av havandesksapsförgiftning. Bodde mamman i ett område med högre avgashalter hade hon en högre risk att drabbas av havandesksapsförgiftning, och mamnor som bodde i områden med lägst avgashalter hade en mindre risk att föda ett tillväxthämmat barn.

Det fanns inga tecken på att bo i områden med högre avgashalter eller mer trafikerade områden under graviditeten eller spädbranstiden ökade risken för att barnet skulle behöva medicineras för astma före skolåldern.


De luftförroreningar som studerades var ozon och kväveoxider, där kväveoxider användes som ett mått på trafikavgaser. Som ett ytterligare mått på trafik studerades det genomsnittliga antalet fordon som passerade hemadressen.
Original papers


Introduction

Adverse pregnancy outcomes such as a shorter period of gestation and intrauterine growth restriction have been shown to have an effect on morbidity throughout childhood, adolescence, and adult life\(^1\)-\(^2\). A shorter period of gestation is also associated with decreased school performance and is an important predictor of lower socioeconomic status in adulthood\(^2\)-\(^4\).

Common proxies for intrauterine growth restriction in the scientific literature over the years have been low birth weight (LBW) at term and small for gestational age (SGA)\(^5\)-\(^6\). LBW is defined as having a birth weight lower than 2,500 g, and SGA is commonly defined as having a birth weight lower than either the 10\(^{th}\) percentile for a given gestational age or lower than 2 standard deviations below the average birth weight for a given gestational age. LBW was the more common metric in earlier studies, but as estimates of gestational age have been increasingly reliable and more readily available SGA has become more common. Similarly, preterm delivery has also been more commonly studied as the reliability of gestational age estimates have improved, and preterm delivery is defined as being born before the 37\(^{th}\) week of gestation\(^7\)-\(^9\). Known risk factors for preterm delivery and LBW are smoking and pre-eclampsia.

Approximately 3\%-8\% of all pregnancies in Western countries are complicated by pre-eclampsia\(^10\)-\(^11\). The main symptoms of pre-eclampsia are hypertension and proteinuria\(^12\). Pre-eclampsia is more common among nulliparous women and women with previous chronic hypertension\(^11\).

A shorter period of gestation in particular is associated with a higher rate of asthma, possibly because the lung tissue might not be fully developed at the time of delivery\(^13\).

Childhood asthma and wheezing are heterogeneous conditions with multiple causes\(^14\), including environmental tobacco smoke and dampness in the home\(^15\). Wheezing in infants is often a transient condition that is closely associated with respiratory infections, but the causes of persistent wheezing remain unclear. Asthma is the most common chronic disease in childhood and can affect both physical fitness and psychosocial wellbeing\(^16\).
Air pollution exposure

Nitrogen oxides (NOx) consist of nitrogen dioxide (NO₂, an oxidant with inflammatory effects⁷) and nitrogen oxide (NO). NOx are formed at high temperature in combustion engines and are, therefore, a marker of vehicle exhaust and traffic pollution.

Ground level ozone (O₃, an oxidant with short-term effects on lung function and airway inflammation⁷) is formed along with NO through a chemical reaction between oxygen and NO₂ under the influence of sunlight. In areas with high NO emissions, more O₃ is consumed to oxidize NO to NO₂.

Long-term exposure to air pollution has been associated with increased mortality and incidence of chronic diseases, and there is stronger evidence for the negative effects of particulate matter than for NO₂ and O₃¹⁷-¹⁸. Primary combustion particles, including soot particles and elemental carbon, are likely to be more harmful than particle mass in general. However, black carbon and elemental carbon are seldom measured by typical monitoring stations.

Exposure misclassification is always a potential problem in epidemiological studies using measured or modeled outdoor concentrations in the area or at home. This is because study subjects have different time-activity patterns and might have a true exposure that is lower or higher than what the exposure variable suggests. This type of exposure misclassification will likely dilute any association between exposure and outcome.

The association between air pollution exposure and health outcome might be confounded by risk factors that are correlated with both exposure and outcome. Potential confounders must vary together in the same dimension(s) as the exposure, for example, if the exposure varies over time, then potential confounders must also vary over time.

Air pollution and birth outcomes

Early studies on the effects of air pollution on birth outcomes focused mainly on long-term average exposures in different districts within the study area. The main outcome of interest was LBW⁵-⁶,¹⁹-²², but preterm delivery and SGA were also studied²³. The air pollutants investigated in those studies were mainly carbon monoxide (CO), sulfur dioxide (SO₂), NOx, and total suspended particles. Most studies reported that elevated levels of SO₂ were associated with increased odds of LBW⁵,²⁰-²³.
Later studies focused on preterm delivery as the outcome of interest to a much larger extent than the earlier studies, although LBW was still frequently studied\textsuperscript{7-9, 24-33}. Long-term air pollution averages were still the most common exposure metric used, but the exposure assessment shifted from area averages to using the nearest monitoring station and including only subjects living within a certain distance from the monitoring station. There were some studies that used modeling approaches in the exposure assessment, including kriging\textsuperscript{32}, dispersion modelling\textsuperscript{29} and land-use regression\textsuperscript{25}. Particulate matter with an average diameter less than 10 µm (PM\textsubscript{10}) or 2.5 µm (PM\textsubscript{2.5}) were the most commonly studied air pollutants, although others such as NO\textsubscript{2}, NO\textsubscript{x}, SO\textsubscript{2}, O\textsubscript{3}, and CO were also studied. Most studies showed positive associations between air pollution exposure and the studied birth outcome. However, the critical timing of the exposure varied across the different study populations.

In more recent studies, preterm delivery, SGA, and LBW have been the most commonly studied outcomes, although a few studies have used pre-eclampsia as the outcome\textsuperscript{34-44}. The exposure assessments were similar to those of the earlier studies, i.e., measuring exposure levels from the nearest monitoring station within a certain radius or using a dispersion model to estimate the level of exposure at the home address. As in earlier studies, higher levels of air pollution were positively associated with preterm delivery, LBW, and SGA. Higher levels of PM\textsubscript{10} and NO\textsubscript{2} have been associated with an increased risk of pre-eclampsia\textsuperscript{37, 40}, but higher levels of CO were associated with a decreased risk of pre-eclampsia\textsuperscript{44}.

A potential pathway through which ambient air pollution could lead to adverse pregnancy outcomes is through systemic inflammation\textsuperscript{45}. Proinflammatory cytokines could disrupt trophoblastic invasion during placentation leading to suboptimal function of the placenta\textsuperscript{46-47}. This could in turn lead to pre-eclampsia, preterm delivery, or intrauterine growth restriction\textsuperscript{48}.

**Air pollution and asthma**

Air pollution exposure and proximity to traffic have been linked to asthma or asthma symptoms in several studies\textsuperscript{49-55}, and air pollution exposure has also been linked to hospital admissions and emergency room visits for asthma\textsuperscript{56-57}. Associations between early life exposure to air pollution and childhood asthma have been reported in several studies\textsuperscript{58-63}, and there is some evidence that exposure to air pollution during gestation might be associated with an increased risk of developing asthma during childhood\textsuperscript{64}. These findings are
inconsistent\textsuperscript{52}, however, and an association between air pollution exposure during childhood and asthma has not always been found\textsuperscript{65-66}.

Air pollution exposure might lead to epigenetic reprogramming that could potentially lead to an elevated risk of developing respiratory diseases in general\textsuperscript{67}. Alternatively, air pollution exposure during periods of rapid development of lung tissue, such as during the last stage of pregnancy or during infancy, might cause disruption in the development of the respiratory organs that could lead to a higher risk for asthma\textsuperscript{59,67}. 
Aims of the thesis

Overall aims

The overall aims of this thesis were (1) to assess any possible association between air pollution exposure during pregnancy and important adverse pregnancy outcomes and (2) to assess any possible associations between air pollution exposure during pregnancy or infancy and asthma medication during childhood.

Specific aims

In Paper I, the aim was to study any association between $O_3$ and $NO_2$ exposure during the first and second trimesters and the last week of gestation and preterm delivery and duration of gestation.

Paper II aimed to assess the association between first trimester levels of $O_3$ and NOx and preterm delivery in a different cohort as well as to study pre-eclampsia and SGA as outcomes.

The aim of Paper III was to study any association between traffic pollution at the home address during pregnancy, as indicated by NOx and traffic intensity, and preterm delivery, pre-eclampsia, and SGA.

Paper IV studied the association between traffic pollution during pregnancy and from birth to the first birthday, as indicated by NOx and traffic intensity, and being prescribed asthma medication between five and six years of age.
Materials and methods

Study populations

All four studies were performed on study populations in the greater Stockholm area. Paper I was based on all singleton births conceived between 1988 and 1995. Papers II and III were based on all singleton births conceived between August 1997 and February 2006 and delivered at one of the five hospitals in the greater Stockholm area (Danderyd, Huddinge, Karolinska, Södersjukhuset, and Södertälje). The third study was further restricted to include only women who did not change addresses during pregnancy and who had a delivery that started spontaneously. The fourth study was based on all singleton births between July 1, 2000, and October 30, 2005. Depending on the timing of the exposure of interest in the fourth study, the inclusion criteria were altered. When studying exposure during pregnancy, only children born to women who did not change addresses during pregnancy were included. When studying exposure during infancy, those who did not change addresses between birth and their first birthday were included in one dataset and those who did not change address during infancy but whose mothers changed addresses during pregnancy were included in a separate dataset.

Outcome definitions

Duration of gestation was based on ultrasound examination or the date of the last menstrual period (Paper I). Preterm delivery was defined as having a gestational period shorter than 37 weeks (Papers I–III). SGA was defined as having a birth weight below the 10th percentile for the given duration of gestation in days (Papers II and III) and according to sex (Paper I). Pre-eclampsia was defined as having any of the ICD-10 (International Statistical Classification of Diseases, version 10) diagnoses of O11 (pre-existing hypertension with pre-eclampsia), O13 (gestational hypertension without significant proteinuria), O14 (gestational hypertension with significant proteinuria), or O15 (eclampsia) (Papers II and III). Two different definitions of childhood asthma medication were used. The first included any prescribed asthma medication between the ages 5 and 6 years (Anatomical Therapeutical Chemical (ATC) codes R03AC, R03AK, R03BA, R03BC, R03CC, or R03DC), and the second included being prescribed anti-inflammatory medicines or leukotriene antagonists on two or more occasions between the ages of 5 and 6 years (ATC codes R03AK, R03BA or R03DC) (Paper IV).
Covariate definitions

The Swedish Medical Birth Register contains data on a number of different covariates such as smoking habits, family situation, and parity.

In Paper I we included maternal smoking habits (non-smoker, moderate smoker (1–9 cigarettes/day) and heavy smoker (10 or more cigarettes/day)), maternal parity (0, 1, 2, 3, or more para), and infant sex (male, female). The date of conception was used to adjust for possible confounding from temporal and seasonal trends, and data on temperature (°C) and relative humidity (%) were used in the modeling.

Paper II used the same covariates as Paper I, but it also included data on maternal age, maternal asthma status, highest level of maternal education, BMI (kg/m²) at antenatal care registration, family situation, and maternal region of origin in the statistical modeling. Maternal age was a continuous variable. Maternal asthma status was a dichotomous factor defined as a case if the mother was prescribed any asthma medication between July 2005 and December 2011 (ATC codes R03AC, R03AK, R03BA, R03BC, R03CC, or R03DC) or had ever received hospital care for asthma bronchiale since 1997. Highest level of education had five category levels (pre-upper secondary school (<9 years), upper secondary school (2 years), post-upper secondary school (<3 years), post-secondary school (3 years), and postgraduate education). BMI was categorized according to the WHO classification; underweight (less than 18.5), normal weight (between 18.5 and 25.0), overweight (between 25.0 and 30.0), and obese (more than 30.0). Family situation was recorded as single, cohabiting with the father, or other. Maternal region of origin was crudely divided into Africa, the Americas, Asia and Oceania (except the Middle East), Europe (except the Nordic countries), the Middle East, and the Nordic countries.

The same set of explanatory variables and potential confounders were used in Paper III as in Paper II.

In Paper IV, the date of birth and birth month were used instead of date of conception to account for temporal and seasonal trends. Maternal age at delivery, maternal region of origin, and highest level of maternal education were defined as in the previous studies. Parity was used as an indicator variable for having older siblings. In addition, maternal and paternal asthma medication was included and was defined using the same set of ATC codes as in Papers II and III. The duration of gestation and the occurrence of pre-eclampsia and SGA were all included in the modeling approach. The number
of times the child changed addresses up to the age of four was followed yearly resulting in 5 possible discrete states of 0–4 address changes.

**Exposure assessment**

The City of Stockholm Environment and Health Administration provided time-series of air pollution measurements for all studies. The modeled annual averages for the 100 m × 100 m squares where the home addresses of the study population were located were provided for Papers III and IV. NO\textsubscript{2} (1-hour maxima, Paper I) and NO\textsubscript{x} (daily mean, Paper II) were measured daily at rooftop level at three monitoring stations. O\textsubscript{3} (8-hour maxima, Papers I and II) was measured at rooftop level at two monitoring stations, Figure 1. In the case where one station had a missing value, the data were imputed from the other stations by linear regression. The time-series from the monitoring stations were used to create citywide averages that were used to calculate time-dependent averages over weeks and trimesters (12 weeks). If there were more than 19 days of missing data, no trimester average was calculated.

Annual average NO\textsubscript{x} levels were estimated at the location of the home address using the SMHI-Airviro Gauss dispersion model\textsuperscript{68}, Figure 2. The input to the model was provided by the emission inventory of the City of Stockholm Environment and Health Administration and the Uppsala Air Quality Management System, which consist of road traffic NO\textsubscript{x} emissions (i.e. traffic flow, vehicle fleet composition, emission factors and meteorology). The ratio between the estimated annual averages at the location of the home address and the annual city average from the monitoring stations were used to estimate trimester, pregnancy, and first-year exposures for each study subject in the third and fourth studies.

Annual daily average traffic flow at the location of the home address was used as an alternative vehicle exhaust exposure metric in Papers III and IV. These data were also provided by the City of Stockholm Environment and Health Administration.

**Statistical analysis**

Logistic regression was used for the main analyses in Papers I, II, and IV. Linear regression was used for the continuous outcomes in Paper I. Mixed-model logistic regression was used in Paper III. All analyses were performed in R programming software\textsuperscript{69}.
Figure 1. Monthly average NO$_2$ and O$_3$ levels.

In Paper I, each time window was studied separately (first trimester, second trimester, last week of gestation, and last week at risk for preterm delivery). For each outcome, the estimated air pollution effect was modeled in three steps. First, unadjusted models were fitted. Next, the models were adjusted for maternal smoking, parity, sex of the infant, temperature, relative humidity, season, and long-term trend. The last model estimated the effects of the air pollutants simultaneously. The seasonal patterns were accounted for by fitting the day of the year of conception as a cyclic spline function. A cubic regression spline was fitted to the conception year to adjust for long-term trends.

Given the results in Paper I, only exposures to NOx and O$_3$ during the first trimester were studied in Paper II. The modeling was performed in six steps. First, unadjusted models were fitted for each pollutant separately. Then maternal age (as a cubic regression spline), parity, highest level of education,
region of origin, maternal asthma, and seasonal trend (as a cyclic spline) were added to the model. In the third step, a cubic regression spline to account for any long-term trend was added. The fourth step was to study the exposures simultaneously. First-trimester temperature and relative humidity were added in the fifth step. Finally, maternal smoking habits, family situation, and BMI at the first antenatal visit were added. Additionally, we explored if maternal asthma acted as an effect modifier for O$_3$ by adding an interaction term in the modeling procedure.

The modeling approach in Paper III was similar to that of the previous papers. The initial model consisted of home address NOx levels or traffic flow and a random intercept for home municipality. In the second modeling step, a set of maternal variables was added (asthma status, level of education, region of origin, age and parity), along with conception date, first trimester O$_3$, and temperature. In the third and final step, maternal smoking status, family situation, and BMI at the first antenatal visit were included in
the model. Additional analysis was performed on the subset of the study population that had complete information for all explanatory variables in order to investigate if changes in estimates were due to adjustment for additional factors or due to restriction of the study population.

Similarly to the other studies, the statistical analyses in Paper IV were performed in a forward stepwise fashion. Initially, a crude model including only outcome and exposure was fitted. In the second step, older siblings, parental asthma, maternal region of origin, maternal age and level of education at the time of delivery, number of address changes, birth month, and date of birth were added to the model. Pregnancy outcomes (duration of gestation (cubic regression spline), pre-eclampsia, and SGA) were added in the third step. In the last step, maternal BMI at the first antenatal visit was added. The modeling approach for those who changed address differed in step 2 in that older siblings, level of education, and number of address changes were excluded.
### Table 1. Outcome frequencies and selected mean exposure levels across studies.

<table>
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<tr>
<th>Paper I&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Number (%)</th>
<th>Mean first trimester NO&lt;sub&gt;2&lt;/sub&gt; (sd), µg/m&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Mean first trimester O&lt;sub&gt;3&lt;/sub&gt; (sd), µg/m&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm delivery, Yes</td>
<td>6,154 (5.3)</td>
<td>38.4 (5.4)</td>
<td>57.6 (13.4)</td>
</tr>
<tr>
<td>No</td>
<td>109,434</td>
<td>38.5 (5.4)</td>
<td>57.1 (13.1)</td>
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<tr>
<th>Paper II&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Number (%)</th>
<th>Mean first trimester NOx (sd), µg/m&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Mean first trimester O&lt;sub&gt;3&lt;/sub&gt; (sd), µg/m&lt;sup&gt;3&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Pre-eclampsia delivery, Yes</td>
<td>3,239 (2.7)</td>
<td>96.6 (16.3)</td>
<td>68.6 (13.3)</td>
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<tr>
<td>No</td>
<td>117,516</td>
<td>96.9 (16.3)</td>
<td>68.4 (13.2)</td>
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<tr>
<td>Preterm delivery, Yes</td>
<td>5,341 (4.4)</td>
<td>96.7 (16.3)</td>
<td>67.8 (13.3)</td>
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<td>No</td>
<td>115,404</td>
<td>96.9 (16.3)</td>
<td>67.6 (13.2)</td>
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<tr>
<td>SGA&lt;sup&gt;b&lt;/sup&gt;, Yes</td>
<td>11,334 (9.4)</td>
<td>97.0 (16.2)</td>
<td>67.9 (13.3)</td>
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<tr>
<td>No</td>
<td>109,216</td>
<td>96.9 (16.3)</td>
<td>67.9 (13.3)</td>
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<th>Paper III&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Number (%)</th>
<th>Mean first trimester NOx (sd), µg/m&lt;sup&gt;3&lt;/sup&gt;</th>
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<td>Pre-eclampsia delivery, Yes</td>
<td>2,774 (2.7)</td>
<td>15.4 (7.6)</td>
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<td>No</td>
<td>99,994</td>
<td>15.1 (7.4)</td>
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<td>Preterm delivery, Yes</td>
<td>2,823 (2.8)</td>
<td>15.3 (7.5)</td>
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<tr>
<td>No</td>
<td>98,220</td>
<td>15.1 (7.4)</td>
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<td>SGA&lt;sup&gt;b&lt;/sup&gt;, Yes</td>
<td>9,977 (9.8)</td>
<td>14.9 (7.2)</td>
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<td>No</td>
<td>91,960</td>
<td>15.1 (7.4)</td>
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<th>Paper IV&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Number (%)</th>
<th>Mean first year NOx (sd), µg/m&lt;sup&gt;3&lt;/sup&gt;</th>
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<td>Two prescriptions of anti-inflammatory medicine or leukotriene antagonists</td>
<td>2,354 (3.0)</td>
<td>13.2 (6.2)</td>
</tr>
<tr>
<td>Any prescribed asthma medication</td>
<td>7,614 (9.7)</td>
<td>13.1 (6.1)</td>
</tr>
<tr>
<td>No prescribed asthma medication</td>
<td>70,526</td>
<td>13.4 (6.4)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Air pollution data from monitoring stations. <sup>b</sup>Small for gestational age. <sup>c</sup>Air pollution data from dispersion model.
Results

The proportion of preterm deliveries was higher in the earlier cohort (all singleton children who were conceived between 1988 and 1995, Paper I) than in the later cohort (all singleton children conceived between August 1997 and February 2006, Paper II) (Table 1). Almost 3% of the pregnant women suffered from pre-eclampsia and pregnancy-induced hypertension. The average O₃ levels increased from the first study cohort to the second, but the average difference in first trimester O₃ levels between preterm and term deliveries were consistently 0.5–0.6 µg/m³.

Figure 3. Annual average O₃ and proportion of preterm delivery between 1988 and 1995.

Paper I

Higher first trimester O₃ levels were associated with slightly increased odds of preterm delivery and a shorter period of gestation in both the unadjusted and adjusted models. The second trimester and last week of gestation O₃ levels were not associated with an altered risk of preterm delivery, but were
weakly associated with a shortened period of gestation. Figure 3 indicates a co-variation between O₃ levels and preterm delivery during the study period, in this case without any adjustment.

Elevated NO₂ levels during the first and second trimester were not associated with an increased risk of preterm delivery, but they were associated with a slightly longer period of gestation. Elevated NO₂ levels during the late stage of pregnancy were associated with an increased risk of preterm delivery.

**Paper II**

Higher levels of first trimester O₃ were again associated with higher odds of preterm delivery in a new cohort. The association was stronger when elective Caesarean sections were excluded from the analysis. There was some evidence of effect modification by maternal asthma status where the association between O₃ and preterm delivery was stronger among asthmatic mothers than non-asthmatic mothers. Higher levels of NOx did not appear to have an effect on the risk of having a preterm delivery.

There was no evidence of any increased risk of SGA by having elevated first trimester levels of O₃ or NOx, in fact, nearly all estimated associations indicated a slightly decreased risk of SGA.

Increased first trimester O₃ was associated with higher odds of pre-eclampsia. The association was slightly weaker when excluding elective Caesarean sections; however, having an elective Caesarean section is a likely outcome of having pre-eclampsia. Increased levels of first trimester NOx did not exhibit any association with pre-eclampsia.

**Paper III**

Due to high correlation between the NOx levels at the home location through all periods of pregnancy, it was difficult to identify periods of increased susceptibility. The NOx level at the home address was positively correlated with a slight, but not statistically significant, increased risk of preterm delivery. Higher levels of NOx were associated with an increased risk of SGA when comparing any of quartiles 2, 3, or 4 with the first quartile. There was, however, no difference in estimated effect among quartiles 2, 3, and 4. There was a marked association between home address NOx and pre-eclampsia with an OR of 1.17 per 10 µg/m³ increase in NOx in the fully adjusted model.
There were no associations between average traffic intensity and preterm delivery or SGA. Traffic intensity was associated with slightly increased odds of pre-eclampsia, but the association was close to the null.

**Figure 4.** Main findings of the studies in terms of ORs per 10 µg/m³ increase in air pollution level. Exposure periods were the first trimester in Papers I, II and III and the pregnancy period in Paper IV. The results for Paper I are from the multi-pollutant model in Table 4 of Paper I. For Paper II, the results were collected from Model 4 in Paper II as shown in Tables 1, 3, and 4 of Paper II for the non-restricted study population. The results from Papers III and IV were collected from Model 2 in Table 2 of Papers III and IV.

**Paper IV**

Higher levels of NOx during pregnancy or during infancy were associated with a lower risk of using asthma medication between the ages five and six.
years. When restricting the analysis to only those who changed addresses prior to delivery, the protective association remained but were not as marked as for those who remained at the same address throughout their pregnancy.

Traffic intensity showed a similar pattern of association with asthma medication as NOx, although for those who changed address there was neither a protective nor a harmful effect.
Discussion

Exposure assessment
Papers I and II used city-wide fluctuations in urban background air pollution values over time to estimate individual exposure. In other words, individual exposure was a function of conception date only, and two women who conceived on the same day would have been assigned the same levels of exposure regardless of where they lived. This can lead to exposure misclassification, particularly for pollutants with important local sources, such as NO₂ and NOx, which vary both in time and strongly in space because they are formed primarily through combustion in vehicle engines. This is less of an issue for O₃ that, although it is consumed by locally emitted NO, depends mainly on incoming air masses and varies seasonally over time. This means that there was a greater chance to detect an association between O₃ and pregnancy outcomes than between NO₂ or NOx and pregnancy outcomes in Papers I and II. Papers III and IV were better designed to estimate associations between spatially varying traffic air pollution and the studied outcome. However, due to the exposure assessment it was possible to separate between effects of exposure during different time windows.

Statistical modeling
The statistical models in the four papers include several covariates, some of which might be considered confounders and some of which might be important determinants but unlikely confounders. For example, parity would not be considered a confounder in the papers that focus on the correlation between the temporal variation of exposure and pregnancy outcomes unless one suspects that families with different numbers of children are differentially prone to plan for a child at a certain time during the year and that parity is associated with the outcome. In the papers focusing on spatial variation, parity could be a confounder if families of different sizes have different probabilities of living in environments with certain air pollution concentrations.

In the first two papers, the potential confounders of interest were time-varying variables – such as season of conception and the meteorological variables – because they could affect the outcome and because O₃ varies with season. In Papers III and IV, the confounders that were identified were the proxies for socioeconomic status, i.e. level of education and maternal region of origin. The reason why they could be confounders is that people with a higher level of education tend to live in the central areas in Stockholm where traffic air pollution levels are higher; therefore, neglecting to adjust for socioeconomic status might lead to the conclusion that living in more
polluted areas might have a beneficial effect on pregnancy outcomes and childhood asthma medication.

**Mechanisms**

In the studies of preterm delivery, pre-eclampsia was thought of as a possible intermediate step along the causal pathway between air pollution exposure and preterm delivery. In Paper II this was explored by adjusting the models in the main analysis for pre-eclampsia. This proposed that the previously observed association would be masked by adding pre-eclampsia as a factor in the model. This did not change the estimated association indicating that pre-eclampsia is not an important intermediate step between air pollution exposure and preterm delivery.

In a similar manner, the adverse pregnancy outcomes studied in the first three papers could be possible pathways through which air pollution exposure during pregnancy could lead to childhood asthma. Being born after a shorter gestational period or being growth restricted were associated with an increased risk of asthma medication (Paper IV, Table 1). If air pollution levels during pregnancy were associated with both pregnancy outcomes and the need to use asthma medication, it is possible that the relationship between air pollution exposure and childhood asthma medication would be masked by adjusting for pregnancy outcomes. However, no adverse association between traffic pollution during pregnancy (or infancy) and asthma medication was apparent, and no changes in the estimated association were observed after adjusting for birth outcomes.

**Comparison of findings**

There was an association between elevated O₃ levels during the first trimester of pregnancy and preterm birth in Paper I and Paper II. This association has been reported previously in a few other studies⁹,36-37,47,71, but other studies have found no such association²⁴,33,72. In one of the studies, however, the association did not remain after adjustment for possible confounders⁷¹. The differing results might be explained in part by different timing and intensity of the seasonal O₃ peaks, for example, in Stockholm and Shanghai⁹ the highest levels of O₃ are found during spring⁷³, while the highest levels of O₃ in the US and London are found during summer³⁶,⁷⁴-⁷⁵.

Vitamin D status varies with the season – with a trough in the spring⁷⁶-⁸⁰ – and a lower level of vitamin D status is associated with increased sensitivity to inflammation⁸¹. Higher O₃ levels have been reported to be associated with inflammation in early pregnancy⁴⁵, and inflammation during early
pregnancy has been associated with a higher prevalence of preterm delivery\(^8\).

High NO\(_2\) levels late in pregnancy were associated with preterm delivery in Paper I, but no association was observed in the other stages of pregnancy. The association between elevated levels of late-pregnancy NO\(_2\) and preterm delivery is in agreement with previous studies\(^{33,47,71-72,83}\). Higher levels of NO\(_x\) early in pregnancy were not associated with preterm delivery in Paper II. In Paper III, only the results for the average levels of NO\(_x\) during the first trimester were reported because the NO\(_x\) levels were highly correlated between different pregnancy time windows. No statistically significant association between elevated NO\(_x\) levels and preterm delivery was observed. The problem with correlated exposure estimates over different time windows have been reported elsewhere\(^{26,29}\). Although no statistically significant associations between early pregnancy or pregnancy NO\(_x\) levels and preterm delivery were observed in Papers II and III, the observed tendencies are in the same direction as previously published results\(^{25,29,84}\).

Similarly to Paper II, a study from Pennsylvania reported a tendency that higher levels of first trimester O\(_3\) led to an increased risk of pre-eclampsia\(^{37}\). A study from California reported similar associations as in Paper II for O\(_3\) and pre-eclampsia in Orange County but not in Los Angeles\(^{85}\). The differing results for Los Angeles might be because they reported entire pregnancy exposure associations only. A few studies have reported associations between vehicle exhaust and pre-eclampsia, and these associations were of similar magnitude as reported in Paper III\(^{37,85-87}\).

The association between late pregnancy levels of NO\(_2\) (Paper I) and preterm delivery and between pregnancy average levels of NO\(_x\) and pre-eclampsia (Paper III) suggest that vehicle exhaust exposure in Stockholm could be high enough to cause adverse effects on pregnancy outcomes.

The observed association between NO\(_x\) and SGA in Paper III – where mothers who resided in areas with NO\(_x\) levels exceeding the 1st quartile were more likely to give birth to an SGA infant – supports the conclusion of small or no effect in a review by Glinianaia et al., although that review focused on particulate matter\(^{88}\). No association between O\(_3\) or NO\(_x\) and SGA was observed in Paper II, which might be because only the exposure in the first trimester was studied and effects on fetal growth are likely to occur later in pregnancy\(^{89}\).

No positive association between NO\(_x\) levels at home and being prescribed asthma medication was found, and similar studies have reported
inconsistent results\textsuperscript{59,60}. Several smaller studies have, however, reported an association between traffic air pollution and incident or new-onset asthma\textsuperscript{61-63}. The differing results might be because a more accurate exposure assessment is possible in a smaller study population\textsuperscript{62} or because the studied population was likely to be more sensitive to airway insults than the general population\textsuperscript{61}. A possible explanation for the negative associations observed in Paper IV and in the other Swedish study by Lindgren et al.\textsuperscript{91} is that in populations living in a relatively clean environment there is some beneficial adaptation among children living in neighborhoods with moderately higher levels of air pollution.

**Policy implications**

In the recent WHO REVIHAAP (2013) report, O\textsubscript{3} was “upgraded” as a health problem\textsuperscript{18}. Climate change will increase O\textsubscript{3} exposure assuming that everything else stays the same. The increased proportion of diesel cars in countries like Sweden is changing the ratio between NO and NO\textsubscript{2} in local emissions and is increasing O\textsubscript{3} levels in the cities\textsuperscript{92}. The local regulations for O\textsubscript{3} could be stricter, but large-scale measures would have to be undertaken in order to effectively reduce concentrations.

In Paper I it was estimated that 129 cases (7.8%) of preterm delivery could be attributed to being exposed to the highest quartile of O\textsubscript{3} instead of the lowest quartile. Similarly, 138 cases (5.5%) of pre-eclampsia and 211 cases (5.2%) of preterm delivery could be attributed to being exposed to O\textsubscript{3} levels exceeding the 25\textsuperscript{th} percentile in Paper II. Being exposed to the highest quartile of NO\textsubscript{2} was associated with 85 cases (5.2%) of preterm delivery in Paper I. Stronger regulations of air pollution might lead to substantial monetary savings from a public point of view partly because preterm infants are more likely to spend a prolonged time in the hospital before being discharged and women suffering from pre-eclampsia need more medical attention prior to delivery, and partly because preterm children have increased morbidity. Supporting this it was shown that preterm children were prescribed more asthma medication than term children (Table 1 in Paper IV).

**Summary of findings**

Papers I and II showed a consistent association between first trimester O\textsubscript{3} exposure and preterm delivery. These results support previous findings\textsuperscript{9,47,71} and have been repeated in later publications\textsuperscript{36-37}. Paper II was one of the first papers reporting an association between first trimester O\textsubscript{3} and pre-eclampsia\textsuperscript{37,85,87}. The strong observed association between traffic-based air pollution and pre-eclampsia in Paper III adds additional strength to the few previously published findings\textsuperscript{37,85-87}.
Future research
Future research should strive to explore if the spatial pattern of O$_3$ is associated with birth outcomes or childhood morbidity. Spatiotemporal models should be implemented in order to improve the exposure assessment of traffic-related air pollution and O$_3$. Instead of using proxies for traffic pollution, such as NO$_2$ and NOx, more biologically active substances, such as soot and elemental carbon, should be used. In order to further improve the exposure assessment, occupational exposure and exposure levels during commuting should be accounted for.
Conclusions

- First trimester $O_3$ was associated with an increased likelihood of preterm delivery. There was some evidence of an association between higher early pregnancy $O_3$ levels and pre-eclampsia.

- Traffic pollution at the home address during gestation was strongly associated with pre-eclampsia.

- There was an indication that traffic pollution at the home address during gestation could lead to an increased risk of preterm delivery.

- Living in a neighborhood with levels of traffic pollution above the 1st quartile was associated with SGA.

- There was no increased likelihood of being prescribed asthma medication between five and six years of age among children living in more polluted areas during their first year or among children having a mother who lived in a more polluted area during pregnancy.
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