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Title: Association between preeclampsia and locally derived traffic-related air pollution: A retrospective cohort study

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Running title: Preeclampsia and traffic-related air pollution

Keywords: air pollution, vehicle emissions, preeclampsia, pregnancy outcome, land-use regression

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What is already known

Despite a number of plausible mechanisms by which air pollutants might contribute to this process, few studies have investigated the association between preeclampsia and traffic emissions, a major contributor to air pollution in urban areas.

What this study adds

Elevated exposure to traffic-related air pollution in pregnancy was associated with increased risk of preeclampsia. Effect sizes were highest among Aboriginal women and women with diabetes.

ABSTRACT

Background: Preeclampsia is a common complication of pregnancy and is a major cause of fetal-maternal mortality and morbidity. Despite a number of plausible mechanisms by which air pollutants might contribute to this process, few studies have investigated the association between preeclampsia and traffic emissions, a major contributor to air pollution in urban areas.

Objective: We investigated the association between traffic-related air pollution and risk of preeclampsia in a maternal population in the urban centre of Perth, Western Australia.

Method: We estimated maternal residential exposure to a marker for traffic-related air pollution (nitrogen dioxide, NO_2) during pregnancy for 23,452 births using temporally adjusted land-use regression (LUR). Logistic regression was used to investigate associations with preeclampsia.

Results: Each interquartile range (IQR) increase in levels of traffic-related air pollution in whole pregnancy and third trimester was associated with a 12% (1%, 25%) and 30% (7%, 58%) increased risk of preeclampsia, respectively. The largest effect sizes were observed for women aged under 20 or over 40 years, Aboriginal women and women with pre-existing and gestational diabetes, for whom an IQR increase in traffic-related air pollution in whole pregnancy was associated with a 34% (5%, 72%), 35% (0%, 82%), and 53% (7%, 219%) increase in risk of preeclampsia respectively.

Conclusion: Elevated exposure to traffic-related air pollution in pregnancy was associated with increased risk of preeclampsia. Effect sizes were highest for elevated exposures in third trimester, and among younger and older women, Aboriginal women, and women with diabetes.

INTRODUCTION

Preeclampsia is a common complication of pregnancy. It is characterised by new-onset gestational hypertension and proteinuria[1] and is a major cause of maternal and perinatal mortality in developing countries and a leading cause of morbidity worldwide[2]. It is diagnosed after 20 weeks gestation and complicates up to 8% of pregnancies. A recent study reported long-term declines in the rate of preeclampsia,[3] despite a rise in many of the associated risk factors, such as maternal age, body mass index (BMI) and pre-existing hypertension[4]. Interventions such as low doses of antiplatelet agents and early delivery[3] may provide a partial explanation for this secular trend, but such interventions do not explain the seasonal variations in rates of preeclampsia that have been reported in some studies[5] [6]. Ambient air pollution exposures may provide another explanation for these cyclical patterns.

Motor vehicle traffic is one of the greatest contributors to ambient air pollution in developed countries[7] and has been associated with adverse effects on cardiovascular, respiratory and pregnancy outcomes[8-14]. The biological mechanisms by which traffic-related air pollution may relate to preeclampsia are not yet well understood, partially because the condition is a multisystem disorder of unknown aetiology[15]. However, it is known that particulate matter air pollution is capable of augmenting the development and progression of atherosclerosis, and may potentially contribute to hypertension[16]. Preeclampsia and vascular atherosclerosis may share common pathways in relation to pollutants[4, 17]. Traffic-related air pollution has been directly correlated with endothelial dysfunction[18], a pre-cursor associated with preeclampsia[1]. Emissions may also contribute to an anti-angiogenic state[19] that may in turn contribute to the development of preeclampsia[20].

Few studies have evaluated the association between preeclampsia and traffic-related air pollution, and none have used field measurements of an air pollutant [9, 13, 21]. The aim of this study was to investigate whether exposure to traffic-related air pollution was associated with risk of preeclampsia, and whether this association was stronger among various higher risk groups, including the socioeconomically disadvantaged, women of Aboriginal origin and women with circulatory or respiratory morbidity.

METHODS

Study design and setting

A retrospective cohort study was conducted in the south-west area of Perth, Western Australia for singleton births to women resident in this region between 1st January 2000 and 31st December 2006 (N=23,452).

Data sources

Midwives' notifications, birth registrations and death registrations were obtained from the Western Australian Department of Health for the period between 1st January 2000 and 31st December 2006. Hospital morbidty records were obtained for the period between 1st January 1995 and 31st December 2006.

Meteorological information was included in the study with temperature, humidity, rainfall, hours of sunshine, evaporation, wind speed, atmospheric pressure obtained from the Australian Bureau of Meteorology (BOM) for the Perth metropolitan site (Site No. 9225) for the period 1st January 2000 and 31st December 2006.

The socioeconomic index for areas (SEIFA) score is an index of relative socioeconomic advantage and disadvantage and was obtained from the Australian Bureau of Statistics for each census collection district for each census year in the study period. Census collection districts contain on average 225 dwellings. The SEIFA score assigned to participants were the closest to the time of birth. Lower SEIFA scores indicate relative socioeconomic disadvantage and higher scores indicate relative advantage. The SEIFA index has a national mean of 1000 and a standard deviation of 100.

Outcome assessment

Data on the clinical diagnosis of preeclampsia in the maternal population were obtained from the Midwives Notifications System based on diagnosis by the attending clinician. During the study period, preeclampsia was defined as the onset of hypertension (systolic blood pressure >=140 mm Hg and/or diastolic blood pressure >=90 mm Hg) from 20 weeks' gestation onwards accompanied by proteinuria[22].

Exposure assessment

Levels of traffic-related air pollution at the residential addresses were estimated by a temporally adjusted land-use regression (LUR) model for NO_2. The LUR method was applied because it performs better than monitoring station methods for estimating NO_2 in urban areas and performs at least as well as geostatistical methods and dispersion models[23].

Outdoor air sampling campaign: Ogawa passive samplers were used to measure NO_2 for 1 to 2-week periods over 4 sampling sessions (seasons) in 2010. The outdoor NO_2 samplers were co-located with pressure tube and infra-red traffic counters operated by the local governments and the state main roads department all sites. Sites were selected to ensure representation of sub-regions and high (>40,000 vehicles/day), moderate (20,000–40,000 vehicles/day) and low (<20,000 vehicles/day) traffic roads. There were 22 sites across a study area of 238km^2 (1 site per 10.8km^2). The summer and winter seasons were over-sampled as highest and lowest NO_2 concentrations were expected in these seasons(Table 1). This approach also offset the cost of sampling in autumn and spring due to fewer sites in these seasons. There were also more samples taken in the summer sampling session as this session was used to confirm that similar measurements were obtained using one-week and two-week sampling periods. Two-week sampling periods were conducted thereafter. Having a different number of samples in each season had little impact on the LUR model as there was negligible difference between the variances of the effect estimates for each season. A total of 67 NO_2 samples were obtained for the 22 sites and used in the development of a LUR model for NO_2.

Land-use regression model: A regression model for NO_2 was derived using only predictors directly related to traffic volume or temporal/meteorological conditions. The restriction to this group of candidate predictors was undertaken to ensure specificity of exposures to locally derived traffic-related air pollution. The candidate predictor variables assessed included: weather variables (temperature, humidity, rainfall, hours of sunshine, evaporation, wind speed, atmospheric pressure), season, vehicle count per day, heavy vehicle count per day, light vehicle count per day, and volume of high, moderate and low vehicle roads within circular buffers at 50m increments up to 400m from the residence. Heavy and light vehicles were classified according to the AustRoads vehicle

classification system[24] and were expected to correlate with diesel and petrol vehicles, respectively. Model selection was based on adjusted R^2 and the square root of the mean square error (RMSE).

The final model selected for NO_2 incorporated the following three predictors - volume of high traffic road within 50m, the volume of moderate traffic road within 50m, and season - which in combination accounted for 86% of the total variation in NO_2 measurements. The partial R^2 for moderate and high traffic variables together was 0.32 and for season was 0.66. The amount of variation in NO_2 measurements was similar to that obtained from the cross-validation model and when actual traffic counts were used (Online Material).

Exposure assignment: The season effect from the LUR model was assigned to each day of pregnancy, and the mean of these values was calculated for each week of pregnancy. This ensured that the season effect for the week remained appropriate when weeks overlapped two seasons. These weekly season effects were then added to the traffic effects from the LUR model to obtain weekly estimates of NO_2. Although the seasonal pattern in NO_2 levels is consistent between years there may also have been longer-term variation. To account for longer-term temporal variation, the weekly estimates of NO_2 were multiplied by an annual adjustment factor. This factor was the ratio of the mean monitoring station measurement for the year of the week of exposure to the mean monitoring station measurement for the year that the passive samples were taken. Monitoring stations of NO_2 closest to the study area were used to make this adjustment. Means were then calculated over all completed weeks of each trimester and whole pregnancy.

Statistical methods and sub-group analyses

The study sample was restricted to women who gave birth to singletons greater than 30 weeks gestation because it is known that multi-fetal pregnancies have higher rates of adverse pregnancy outcomes and that early onset preeclampsia is frequently associated with growth restriction, and may have a differing underlying physiology[1]. Multiple variable logistic regression was applied to estimate odds ratios (OR) with 95% confidence intervals (CI) using SAS v9.1[25]. Adjustment was made for maternal age, both pre-gestational and gestational diabetes, Aboriginal status, parity, season of conception, maternal smoking during pregnancy, and the SEIFA score.

Analyses were conducted for the period of the pregnancy overall and for each trimester. Sub-group analyses (*a priori*) were also performed for women in each socioeconomic tertile (low, moderate, high), for women who self-reported as Aboriginal, for non-Aboriginal women, for women who did

not move house during pregnancy (according to electoral roll records), and for women who listed variants of 'home duties' as their occupation. Separate analyses were conducted for women where there was any indication of respiratory or circulatory disease: that is, (i) if asthma was stated on the midwife notification form; and/or (ii) if the mother was previously hospitalised and diagnosed with relevant respiratory and circulatory disease codes: J00-J99 (ICD10-AM), 460-519 (ICD9), I00-I99 (ICD10-AM), or 290-459 (ICD9). Separate analyses (*a posteriori*) were conducted for women aged ≤ 20 years or ≥40 years, and for women with pre-gestational or gestational diabetes as indicated on the midwife notification form.

A generalized additive model with a spline smoother was used to assess existence of nonlinear association between preeclampsia and mean traffic-related NO_2 over whole pregnancy. A quadratic term was consequently tested but found to be statistically non-significant. Therefore, NO_2 was examined as a linear term. Odds ratios were calculated using logistic regression (SAS 9.2) for the effect of an interquartile range (IQR) (75th percentile minus 25th percentile point) increase in exposure to traffic-related air pollutants on preeclampsia.

Ethics approval for this study was obtained from the University of Western Australia and the Department of Health Western Australia Human Research Ethics Committees.

RESULTS

Characteristics of the study population are shown in Table 2. There were 23,452 women and singleton births of greater than 30 weeks duration between 2000 and 2006 in the study area. Of these women, 943 (4%) developed preeclampsia. There were 1,446 women (6.17%) who lived within 50m of either a moderate or high traffic road. Given that the middle of first trimester is separated by approximately 6 months from the middle of third trimester, the relationship between NO_2 levels between these two time points was assessed. A negative Pearson correlation between first and third trimester NO_2 levels was observed (r=-0.62). Pearson correlations between NO_2 levels for other pregnancy trimesters were less than 0.10. Whole pregnancy NO_2 levels were strongly correlated with levels in second trimester (r=0.72), but weakly correlated with levels in first trimester (r=0.29) and third trimester (r=0.20).

After adjustment for risk factors, the risk of preeclampsia increased by 30% (95% CI: 7%, 58%) for each IQR increase in third trimester NO_2, and by 12% (95% CI: 1%, 25%) for each IQR increase in NO_2 estimated over whole pregnancy (Table 3). In the subgroup analyses, effect sizes were generally larger for increases in third trimester exposure. Effect estimates for women who did not change residence and those who spent more time at home were generally negligible to low. There was no discernible difference between effects among socioeconomic groups. Effects of NO_2 exposure on risk of preeclampsia among women with circulatory or respiratory morbidity were comparable with

those of the whole population. For Aboriginal women, an IQR increase in whole pregnancy exposure to NO_2 was associated with a 35% (95% CI: 0%, 82%) increase in risk of preeclampsia after adjustment. Similar effect sizes were observed for women aged ≤ 20 years or ≥40 years, for whom this increase in exposure was associated with a 34% (95% CI: 5%, 72%) increase in adjusted odds of preeclampsia. Strongest effects were observed for women with pre-existing or gestational diabetes. Among this group, IQR increases in exposure during pregnancy was associated with a 53% (95% CI: 7%, 219%) increase in the adjusted odds of preeclampsia.

DISCUSSION

We observed moderate-sized effects of traffic-related air pollution (NO_2) exposure on the risk of preeclampsia. When elevated traffic-related air pollution exposures were assessed across the entire period of pregnancy, the likelihood of preeclampsia was significantly increased by 12% for each IQR increase in NO_2. This effect was even more pronounced when the third trimester exposures were examined, for which a 30% increase in risk was observed for each IQR increase. Taken together, these results suggest that a more elevated risk of preeclampsia was associated with increased exposure later in pregnancy, but also that risk may be more generally associated with increased traffic-related air pollution exposure throughout pregnancy. The results were not explainable by fixed cohort bias or the confounding effects of ambient temperature (Online Material).

The effect of air pollution on endothelial function is equivocal with studies showing significant alteration in endothelial function evident by changes in diameter and wall thickness of the brachial artery and blood velocity profiles among healthy subjects exposed to ambient air or gaseous pollutants, such as diluted diesel exhaust or concentrated ambient fine particles plus ozone[26, 27].

Although the underlying cause(s) of preeclampsia are not known, one of the leading mechanisms identified in relation to preeclampsia is endothelial dysfunction resulting in abnormal placentation and failed vascular remodelling followed by hyper-inflammation and endothelial activation which may trigger the maternal syndrome of preeclampsia[28]. We observed elevated odds of preeclampsia for increases in exposure late in pregnancy. Air pollution has been shown to promote oxidative stress and systemic inflammation, and has been shown to affect the levels of some of the biomarkers for cardiovascular disease[29]. It is plausible that anti-angiogenic mechanism could be a relevant mechanism as adverse associations with traffic-related air pollution have been observed previously[19].

Although negligible effects were observed for many of the sub-populations investigated, these findings were subject to a loss of statistical power due to smaller sample sizes for these sub-populations. An IQR increase in whole pregnancy exposure to traffic-related air pollution was associated with a 35% increase in risk of preeclampsia for among Aboriginal women. An IQR

increase in third trimester exposure to traffic-related air was associated with a near doubling of the odds ratio (OR 1.91), but this was not statistically significant. Specific studies targeting this sub-population are required to assess whether the effects of traffic-related air pollution on risk of preeclampsia are disproportionally higher than those for the general population, possibly due to greater vulnerability as a result of increased risk of other co-morbidities. A study from South Australia has shown that Aboriginal women were more likely to have chronic hypertension in pregnancy and develop gestational hypertension and superimposed preeclampsia, however it is not known whether Aboriginal women might be more susceptible to the adverse effects of traffic-related air pollution on pregnancy outcomes[30], or whether these differences could relate to potential confounders and effect modifiers such as body mass index, pre-pregnancy blood pressure, or genetic differences[31, 32]. Traffic-related air pollution might be a promote preeclampsia among those already at elevated risk as effects were higher for women with diabetes and for younger/older women (≤20 years or ≥40 years). For women with diabetes, a strong risk factor for preeclampsia, an IQR increase in third trimester exposure to traffic-related air pollution was associated with more than a three-fold increase in odds (OR 3.26). Although some findings for sub-population analyses (Table 3) may have occurred by chance owing to multiple comparisons, consistency in the direction (i.e., increased risk of preeclampsia) of observed associations for the vast majority of the results tends to suggest a true association.

In this study, NO_2 was investigated as a marker for the complex mixture of chemical compounds that constitute exhaust from motor vehicle traffic. Three previous studies have directly investigated associations between preeclampsia and ambient air pollution[9, 13, 21]. Adverse associations were observed between preeclampsia and elevated levels of CO among a small pregnancy cohort of 3,509 women in Washington, but the authors of this study reported that further adjustment for year of conception nullified the association[21]. We expect that our LUR-modelled exposure was less prone to such confounding because it captured a high degree of spatial variation in traffic-related air pollution levels due to the larger number of monitoring sites. Adverse findings were observed among two cohorts in California, but the authors of these studies did not report higher risks for elevated levels of exposure at different stages of pregnancy[9, 13]. Therefore, further studies are required to confirm the effects we observed for elevated exposure in third trimester.

An advantage of our study was that we conducted a targeted air sampling campaign to take field measurements of a marker for traffic-related air pollution (NO_2). The LUR model was constructed using only traffic predictors, which meant that it was specific to locally generated traffic-related air pollution. We also simultaneously measured actual traffic counts while taking passive NO_2 samples, which enhanced confidence in the appropriateness of the selected LUR model. At a subset of sites we took simultaneous indoor and outdoor passive NO_2 samples. In summary, we were able to assess

and confirm some of the inherent assumptions in this type of study: (i) that annual traffic counts or road types are sufficient proxies for actual traffic counts as predictors in LUR models, and (ii) that LUR estimates of ambient NO_2 are also predictive of indoor levels.

As preeclampsia was ascertained by records at delivery, a limitation of this study was that the exposure estimates did not exclude exposures after first diagnosis. However, resulting misclassification is expected to be minimal since more than 90% of cases of preeclampsia are diagnosed after week 34 of pregnancy[33], and the remaining period of pregnancy is short relative to the seasonal periods for which NO_2 levels varied in this study. A further limitation, not previously identified by other studies, was that LUR performed reasonably well in predicting outdoor measurements but did not perform well at predicting indoor levels (Online Material). Therefore, the LUR model cannot be considered a reliable indicator of the absolute level of indoor exposure at the residence, but instead must be interpreted as a correlated marker for the portion of traffic-related air pollution that penetrates indoors.

CONCLUSION

Elevated exposure to traffic-related air pollution in pregnancy, particularly late in pregnancy, was associated with increased risk of preeclampsia. There was little evidence to suggest that effects were greater among lower socioeconomic groups or women with circulatory or respiratory disease. The magnitude of the estimated effects appeared greater for women aged under 20 or over 40 years, Aboriginal women, and women with pre-existing and gestational diabetes than those for the whole population.

Competing Interest

Competing Interest: None to declare.

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Contributorship Statement

GP was involved in the conceptual design, conducting analysis, interpretation of the results and writing of the paper. FG, AS, AC, CB and NN were involved in the conceptual design, revisions of the paper and interpretation of results.

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Table 1: Number of two-week passive samples of NO_2 by season and traffic volume at the sampling site

	Summer	Autumn	Winter	Spring	Total
Number of two-week passive NO_2 samples	29*	10	18	10	67
Number of sites	14	10	13	10	22**

*Both one and two-week passive samples were taken at five sites to assess whether results were consistent over both averaging periods

**Total number of unique sites

Table 2: Maternal, pregnancy and infant characteristics of singleton births in metropolitan Perth, 2000-2006

Maternal and infant characteristics (N=23,452)	N	%
Maternal characteristics		
Maternal age (years)		
Less than 20	829	4
20-24	2,962	13
25-29	5,669	24
30-34	8,272	35
35 or over	5,720	24
Birth order		
First birth	10,823	46
Second birth	7,938	34
Third birth	3,046	13
Fourth birth or more	1,645	7
Smoked during pregnancy	3,337	14
Pre-existing or gestational diabetes	1,049	5
Preeclampsia	943	4
Sub-population of women		
Women at home	3,254	14
Women who did not move house	13,437	57
Women with respiratory morbidity*	2,434	10
Women with pre-existing or gestational diabetes	1,049	4
Women aged ≤ 20 years or ≥40 years	2,360	10
Aboriginal	887	4
Low socioeconomic status**	7,556	32
Mid socioeconomic status**	7,939	34
High socioeconomic status**	7,941	34
Infant characteristics		
Infant sex		
Male	12,117	52
Female	11,335	48
Maternal traffic-related air pollution exposure (NO_2)	Mean	SD
Trimester 1	23.29	10.19
Trimester 2	23.27	10.29
Trimester 3	22.54	9.87
Pregnancy	23.04	4.14

*Women with asthma or previous circulatory or respiratory-related hospitalization

** Defined according to the tertile of the socioeconomic index for areas (SEIFA)

Table 3: Odds ratios for risk of preeclampsia for an IQR increase in traffic-related air pollution (NO_2) exposure by pregnancy period of exposure and sub-population

	Preeclampsia		Odds Ratios (95% CI)	
	N (%)	IQR (ppb)	Unadjusted OR	Adjusted OR
All women (N=23,452)	943 (4)			
Trimester 1		15.99	1.03 (0.93, 1.14)	1.07 (0.91, 1.27)
Trimester 2		16.97	1.04 (0.93, 1.15)	1.04 (0.86, 1.26)
Trimester 3		16.64	1.11 (0.99, 1.24)	**1.30 (1.07, 1.58)**
Pregnancy		5.63	**1.10 (1.01, 1.2)**	**1.12 (1.01, 1.25)**
Women who spent more time at home (N=3,254)	103 (3)			
Trimester 1		15.99	0.96 (0.7, 1.32)	1.05 (0.64, 1.73)
Trimester 2		16.97	0.84 (0.61, 1.16)	1.08 (0.57, 2.04)
Trimester 3		16.64	1.14 (0.82, 1.58)	0.99 (0.53, 1.85)
Pregnancy		5.63	0.88 (0.67, 1.17)	0.95 (0.63, 1.42)
Women who did not move house (N=13,437)	531 (4)			
Trimester 1		15.99	0.98 (0.85, 1.12)	1.04 (0.83, 1.3)
Trimester 2		16.97	0.98 (0.85, 1.13)	1.00 (0.77, 1.29)
Trimester 3		16.64	1.08 (0.94, 1.25)	1.17 (0.89, 1.52)
Pregnancy		5.63	1.00 (0.89, 1.13)	1.04 (0.89, 1.21)
Women with circulatory or respiratory morbidity (N=2,434)	140 (6)			
Trimester 1		15.99	0.92 (0.70, 1.2)	0.73 (0.46, 1.15)
Trimester 2		16.97	1.14 (0.85, 1.51)	1.39 (0.83, 2.32)
Trimester 3		16.64	1.11 (0.83, 1.47)	1.33 (0.79, 2.23)
Pregnancy		5.63	1.10 (0.87, 1.38)	1.08 (0.81, 1.45)
Women with pre-existing or gestational diabetes (N=1,049)	67 (6)			
Trimester 1		15.99	1.14 (0.77, 1.67)	1.53 (0.77, 3.04)
Trimester 2		16.97	1.29 (0.85, 1.95)	1.08 (0.52, 2.23)
Trimester 3		16.64	1.42 (0.94, 2.14)	**3.26 (1.48, 7.16)**
Pregnancy		5.63	**1.49 (1.11, 2.00)**	**1.53 (1.07, 2.19)**
Women aged ≤ 20 years or ≥40 years (N=2,360)	143 (6)			
Trimester 1		15.99	1.21 (0.93, 1.59)	1.46 (0.93, 2.28)
Trimester 2		16.97	1.14 (0.86, 1.50)	1.10 (0.68, 1.80)
Trimester 3		16.64	1.07 (0.81, 1.42)	1.55 (0.94, 2.55)
Pregnancy		5.63	**1.27 (1.03, 1.56)**	**1.34 (1.05, 1.72)**
Aboriginal women (N=887)	55 (6)			
Trimester 1		15.99	1.15 (0.75, 1.75)	1.47 (0.78, 2.77)
Trimester 2		16.97	1.34 (0.86, 2.09)	1.43 (0.69, 2.95)
Trimester 3		16.64	1.24 (0.80, 1.90)	1.91 (0.96, 3.83)
Pregnancy		5.63	**1.33 (1.01, 1.75)**	**1.35 (1.00, 1.82)**
Non-aboriginal women (N=22,565)	888 (4)			
Trimester 1		15.99	1.02 (0.92, 1.13)	1.05 (0.88, 1.25)
Trimester 2		16.97	1.02 (0.91, 1.14)	1.01 (0.83, 1.24)
Trimester 3		16.64	1.10 (0.98, 1.23)	**1.26 (1.03, 1.55)**
Pregnancy		5.63	1.08 (0.99, 1.18)	1.09 (0.98, 1.23)
Women in lowest SEIFA tertile (N=7,556)	333 (4)			
Trimester 1		15.99	1.01 (0.85, 1.2)	1.17 (0.88, 1.56)
Trimester 2		16.97	1.00 (0.83, 1.2)	1.17 (0.83, 1.64)
Trimester 3		16.64	1.10 (0.91, 1.33)	1.14 (0.81, 1.61)
Pregnancy		5.63	1.06 (0.91, 1.23)	1.16 (0.96, 1.41)
Women in middle SEIFA tertile (N=7,939)	319 (4)			
Trimester 1		15.99	1.07 (0.90, 1.27)	0.97 (0.73, 1.29)
Trimester 2		16.97	1.11 (0.92, 1.33)	1.09 (0.79, 1.52)
Trimester 3		16.64	1.07 (0.89, 1.29)	1.38 (0.99, 1.91)
Pregnancy		5.63	**1.18 (1.02, 1.36)**	1.15 (0.96, 1.38)
Women in highest SEIFA tertile (N=7,941)	291 (4)			
Trimester 1		15.99	1.01 (0.84, 1.21)	1.09 (0.81, 1.46)
Trimester 2		16.97	1.00 (0.83, 1.22)	0.87 (0.62, 1.22)
Trimester 3		16.64	1.16 (0.95, 1.41)	1.37 (0.97, 1.93)
Pregnancy		5.63	1.08 (0.93, 1.25)	1.05 (0.87, 1.27)

Adjustment was made for maternal age, diabetes, Aboriginal status, parity, season of conception, maternal smoking during pregnancy, and the socioeconomic index for areas (SEIFA) score except where sub-populations were stratified by these variables.

METHODS

Data sources

Description of data sources: Midwives' notifications are completed for every birth from 20 weeks gestation or 400g birth weight attended by a midwife or medical practitioner. This source contains information on maternal characteristics and pregnancy conditions, labour, delivery and infant outcomes. Birth registration forms are completed by parents for all births from 20 weeks gestation and also contain parental demographic information such as Aboriginal status, country of birth and occupation. Death registrations contain information about timing and cause of death. Hospital morbidity records are completed for all hospital admissions, both public and private. These records list the principal diagnosis and additional diagnoses recorded for every admission and coded according to the International Classification of Diseases (ICD). Records from each of these databases are linked using probabilistic linkage with over 98.5% matching[1].

Exposure assessment

Validation of the use of roads categorised by traffic volume in the LUR: The selected LUR model for NO_2 used the total length of moderate and high traffic roads within a 50m radius as predictors. The state road authority (MRWA) classifies roads by traffic volume using long-term averages of traffic counts, typically an annual average. As it was unclear as to the influence of short-term variability in traffic levels, we compared the R^2 of the selected LUR model to a model in which actual vehicle counts were obtained at the same time as the passive sampling. The R^2 of 86% for the selected LUR model for NO_2 was approximately the same as a model that used the actual roadside count of light vehicles per day, count of heavy vehicles per day, and season as predictors (R^2=85%).

Internal cross-validation of the LUR model: Leave-out-one cross validation was applied to the LUR model for outdoor NO_2. This was conducted by sequentially removing data for each site and then estimating the NO_2 level using the remaining sites. This is the most common method for internal validation of LUR models[2]. There was only a slight decrease in the validation R^2 from the 86% obtained for the original model to 82%. The validation RMSE was 6ppb, which is the same as that obtained for the original model and less than half of the standard deviation in the observed NO_2 values (SD=15ppb). The intra-class correlation coefficient (ICC) between the original and validation study's prediction was 0.99.

Validation of the LUR model against indoor measurements: At 18 additional locations, we co-located an outdoor passive NO_2 sampler with a passive sampler placed inside each of two neighbouring houses. At two locations, one indoor sample could not be obtained due to lack of willing participants, and one indoor passive NO_2 sample was lost by a participant. Therefore, a final total of 33 indoor passive NO_2 samples matched to 18 outdoor passive NO_2 samples were available to ascertain the degree to which the LUR model predicted indoor levels. This sampling campaign was restricted to summer to minimise the influence of indoor heating. At each location, houses were selected so that they were on the same side of the road to minimise the influence of wind direction. Indoor samples were also taken during the summer sampling session for purposes of validation. Observed outdoor concentrations explained 51% of the total variation in observed indoor concentrations of NO_2. However, for every 1 ppb increase in predicted outdoor NO_2 using LUR, the observed indoor levels increased by only 0.13 ppb (95% CI: -0.01, 0.27).

DISCUSSION

Posthoc sensitivity analyses: The effects of colder weather, which is correlated with higher NO_2 levels, might provide an alternative explanation for the effects observed in this study[3-5]. In a Norwegian study, odds ratios for preeclampsia were highest for the winter months[5]. In an Indian study, the incidence of eclampsia (but not preeclampsia) was greater in the cooler monsoon season than the dry season[4]. The authors of those studies suggest that low ambient temperatures might be a precipitating cause by way of a "vasospasm that is part of the pathogenesis of preeclampsia"[5], or reduced plasma volume also observed in winter months compared to summer months[4]. Season of delivery was not associated with preeclampsia in a recent study conducted in Thailand[6]. Moreover, in that study, higher risk of preeclampsia was observed for women who conceived in the (warmer) dry season than the monsoonal season. We conducted a posthoc analyses to assess the sensitivity of our results to the effects of ambient (mean maximum) temperature. After inclusion of temperature in the fully adjusted models, the odds ratios of preeclampsia for IQR increases in third trimester and whole pregnancy NO_2 were 1.39 (95% CI: 1.14, 1.70) and 1.18 (95% CI: 1.06, 1.31) respectively. Therefore, the effects of traffic-related air pollution on preeclampsia reported in this study appear to be independent of ambient temperature.

Most of the variation in assigned exposure was due to timing of pregnancy (relative to season) rather than the location of residence (relative to proximity to major roads). Despite adjustment for season of conception, and adjustment for temperature in sensitivity analyses, we cannot completely exclude the possibility of confounding by an unmeasured time-varying factor. We repeated the analysis after matching women on the month-year of conception to remove temporal contrasts in exposure. The odds ratio of preeclampsia for an IQR increase in whole pregnancy NO_2 was 1.02 (95% CI: 0.87, 1.18), and 0.99 (95% CI: 0.85, 1.16) after adjustment. After restricting to the 1,446 women who lived within 50m of a moderate or high traffic road, the estimated odds of preeclampsia increased to 1.14 (95% CI: 0.87, 1.48), and 1.07 (95% CI: 0.81, 1.42) after adjustment. Therefore, there was insufficient evidence for an association after excluding temporal variation in exposure. However, lack of detection of an effect might have been due to insufficient statistical power due to the low level of spatial contrast in exposure.

The results of this study might also have been influenced by *fixed cohort bias,* a truncation bias caused by the different gestation lengths at the start and end of the study period. We conducted a further sensitivity analysis to quantify the magnitude of this bias using the method recently proposed by Strand, Barnett and Tong (2011)[7]. After adjustment for the fixed cohort bias, the odds ratios of preeclampsia for IQR increases in third trimester and whole pregnancy NO_2 were 1.31 (95% CI: 1.07, 1.59) and 1.12 (95% CI: 1.00, 1.25) respectively. Therefore, there was negligible influence of fixed cohort bias on the effects of traffic-related air pollution on preeclampsia reported in this study, possibly due to the long duration of the study (7-year period).

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