

Ambient air pollution and inflammatory effects in a Canadian pregnancy cohort

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Background: Epidemiologic studies have consistently reported associations between air pollution and pregnancy outcomes including preeclampsia and gestational diabetes. However, the biologic mechanisms underlying these relationships remain unclear as few studies have collected relevant biomarker data. We examined relationships between ambient PM_{2.5} and NO₂ with markers of inflammation during pregnancy in a prospective cohort of Canadian women.

Methods: We analyzed data from 1170 women enrolled in the Maternal-Infant Research on Environmental Chemicals study. Daily residential PM_{2.5} and NO₂ exposures during pregnancy were estimated using satellite-based and land-use regression models and used to create 14-day and 30-day exposure windows before blood-draw. Inflammatory markers C-reactive protein, interleukin-6, interleukin-8, and tumor necrosis factor- α were measured in third trimester plasma samples. Multivariable linear regression was used to estimate associations for an interquartile range (IQR) increase in PM_{2.5} and NO₂ and markers of inflammation, while adjusting for individual-level confounders.

Results: Fourteen-day (IQR: 6.85 $\mu\text{g}/\text{m}^3$) and 30-day (IQR: 6.15 $\mu\text{g}/\text{m}^3$) average PM_{2.5} exposures before blood-draw were positively associated with C-reactive protein after adjustment for covariates (24.6% [95% CI=9.4, 41.9] and 17.4% [95% CI=1.0, 35.0] increases, respectively). This association was found to be robust in several sensitivity analyses. Neither PM_{2.5} nor NO₂ exposures were associated with interleukin-6, interleukin-8, or tumor necrosis factor- α .

Conclusion: Exposure to ambient PM_{2.5} is positively associated with maternal inflammatory pathways in late pregnancy. This may contribute to positive associations between ambient PM_{2.5} and risk of adverse pregnancy outcomes.

Keywords: Air pollution; PM_{2.5}; NO₂; Inflammation biomarkers; Pregnancy; C-reactive protein

Ambient air pollution is associated with many pregnancy complications and birth outcomes, including gestational hypertension, preeclampsia, gestational diabetes, low birth weight, and cognitive development.^{1–8} Particulate matter with diameter of

2.5 μm or less (PM_{2.5}) and nitrogen dioxide (NO₂) are the most commonly measured air pollutants.^{6,9–13} PM_{2.5} is a complex mixture containing persistent organic pollutants, heavy metals, and endotoxins.^{14,15} NO₂ is thought to be a proxy measure of traffic emissions such as benzene, although some studies suggest an independent role of NO₂ on health outcomes after adjustment for other pollutants,^{16,17} as well as on inflammation directly.^{18–20}

Exposure to ambient air pollution elicits inflammatory responses,^{11,21–24} and several reviews support relationships between inflammation biomarkers and pregnancy and birth outcomes.^{25–29} Some markers of inflammation are reported to increase as a response to normal pregnancy, peaking in the third trimester.^{30,31} This, along with pregnancy-specific biologic processes, provides added complexity in disentangling the role of inflammatory markers as potential causal intermediates.^{32,33}

Despite the large body of literature on associations between air pollution and inflammation in general population cohorts, only two previous investigations have explored this association during pregnancy.^{34,35} These investigations assessed C-reactive protein (CRP) only and were unable to assess lower-dose exposures in their respective populations. In addition, one of the studies reported higher mean levels of PM_{2.5} (mean 16.4 $\mu\text{g}/\text{m}^3$)³⁴

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compared with exposures reported in Canadian pregnancy cohorts (mean 4.0–9.0 $\mu\text{g}/\text{m}^3$).^{8,36,37} The second study did not report $\text{PM}_{2.5}$ exposures but had higher mean levels of PM_{10} and NO_2 than would be expected in a Canadian setting (30.3 $\mu\text{g}/\text{m}^3$ $\text{PM}_{2.5}$, 39.9 ppb NO_2). As relationships observed at high exposure levels may be inconsistent with effects at lower exposures, it is of interest to explore and provide information regarding the dose-response relationship between air pollution and inflammation at lower exposures, such as those experienced by the Canadian population.

Our objective was to examine associations between $\text{PM}_{2.5}$ and NO_2 concentrations with markers of inflammation measured during the third trimester of pregnancy and characterize the dose-response relationship. We hypothesized that increased $\text{PM}_{2.5}$ and NO_2 exposure would be associated with elevated CRP, interleukin-6, interleukin-8, and tumor necrosis factor- α levels. We also examined the potential for effect modification by fetal sex. The biological sex of the fetus may impact inflammation pathways and metabolism during pregnancy, and previous studies report that mothers carrying male fetuses may have increased inflammation biomarker levels.^{38–41}

Methods

Study population and design

We used data from the Maternal-Infant Research on Environmental Chemicals (MIREC) Study.⁴² Briefly, between 2008 and 2011, 2001 pregnant women were recruited during their first trimester across ten cities in Canada and followed to delivery.⁴² Women were eligible for inclusion if they were 18 years of age or older, <14 weeks gestation, able to communicate in English or French, and planning to deliver at a local hospital.⁴² Biomarker data were available only for women who had a live-singleton birth and provided a blood sample during the third trimester. A complete case restriction was applied for the current study: only participants with complete exposure and outcome data, and residing in a Forward Sortation Area (first 3 digits of Canadian postal locator) less than 10×10 km ($n=1,170$) were included in the analysis. The original study was approved by the Health Canada and Public Health Agency of Canada Research Ethics Board (file no. REB 2016-017H), as well as the Research Ethics Committees of Sainte-Justine University Hospital in Montreal, Canada, and all study affiliated sites. Ethics approval for this nested analysis was obtained through Queen's University's Health Sciences Research Ethics Board (file no. 6030809). Informed consent was obtained from all participants.

Assessment of $\text{PM}_{2.5}$ and NO_2

$\text{PM}_{2.5}$ and NO_2 exposure estimates were developed using exposure surfaces.^{43,44} Surface-based $\text{PM}_{2.5}$ estimates were derived from a combination of satellite estimates, a chemical transport model, and geographically weighted regression. Details are described elsewhere.⁴³ The surface-based $\text{PM}_{2.5}$ estimates had a spatial resolution of 1×1 km. Ambient NO_2 concentrations for exposure estimates were derived from a national land use regression model, which included a large set of land-use characteristics, and satellite data to estimate ground-level NO_2 . The NO_2 surface had a resolution of <100 m.⁴⁴ These surfaces have been extensively used in Canadian epidemiologic investigations.^{8,37,45–49}

$\text{PM}_{2.5}$ and NO_2 exposures were assigned using the centroid of participants' self-reported Forward Sortation Area. The Forward Sortation Area corresponds to the first three digits of the Canadian residential postal code. Temporal resolution was added to the surface data for both $\text{PM}_{2.5}$ and NO_2 by using values from daily National Air Pollution Surveillance monitoring data located within a 30 km centroid of participants' Forward

Sortation Area to scale surface-based estimates.⁵⁰ Participants living further than 30 km from a monitoring station lacked temporal data on changes in pollutant exposures and were excluded from analyses. Residential information was collected during the first and third trimesters, and exposure estimates therefore accounted for residential mobility.

The relevant time-windows for the relationships between air pollution and changes in inflammatory pathways are not fully established. Studies assessing changes in inflammatory processes from air pollution using multiple exposure windows typically find the strongest associations between air pollution and inflammatory biomarkers approximately 1 week to 1 month before biomarker sample collection.¹¹ Daily mean concentrations of ambient air pollution were therefore averaged to derive exposure metrics that corresponded to 14 days and 30 days before the collection of the bio-specimen for inflammation biomarker measurement.

Measurement of biomarkers

Third trimester maternal whole blood was treated with ethylenediaminetetraacetic acid and phenylmethylsulfonyl fluoride, and was clarified to obtain plasma following a previously reported procedure.⁵¹ The average weeks gestation at blood draw for participants was 31.6. CRP, interleukin-6, interleukin-8, and tumor necrosis factor- α were measured in all women who had singleton live births and provided a blood sample during the third trimester. Biomarkers were analyzed using affinity-based multiplex protein array assays using Bio-Plex Pro Human panels (Bio-Rad, Canada) and Milliplex Map kits (Millipore, Canada).⁵² Overall, inter- and intraassay coefficients of variation for all biomarkers were below 12%.

Covariates

Lifestyle and demographic information was obtained from questionnaires collected throughout the pregnancy. Several covariates were considered as potential confounders in our analyses, based on their relationships as predictors of inflammation biomarkers of interest⁵³ or as potential confounders in previous studies.^{32,34,35} Relationships between covariates were also visualized using a directed acyclic graph (eFigure S1; <http://links.lww.com/EE/A151>). Covariates included: household income (below the median Canadian household income vs. above), education (high-school or less vs. any postsecondary), race (White vs. other), prepregnancy body mass index (underweight/normal, overweight, obese), season of blood draw, and age (years). We also considered self-reported information on housing characteristics (home type, attached garage, heating fuel, fireplace, type of cooking appliance, furnace in home, and second-hand smoke exposure in home). Covariate information on pregnancy-specific behaviors included alcohol consumption (none vs. any), smoking status (never, former, quit during this pregnancy), acetylsalicylic acid (aspirin) use (yes vs. no), prenatal multivitamin use, folic acid supplementation, other supplement use (any use vs. no use), activities (walking and biking hours per week), outdoor time (days spent outside for a minimum of 30 minutes), and gestational weight gain across pregnancy (kilograms). Recruitment site was related to both air pollution exposures and inflammation biomarkers and was included as a covariate in all models.

Statistical analysis

All analyses were performed using SAS Enterprise Guide 7.1 (SAS institute, Cary, NC). Multivariable linear regression was used to separately assess the relationship of an interquartile range (IQR) increase in 14-day and 30-day average $\text{PM}_{2.5}$ and NO_2 with each biomarker of interest. All biomarkers were

right-skewed and were log-transformed. For each biomarker of interest, a covariate-only model was built separately to select covariates into the model, employing backwards elimination ($P < 0.15$), which allowed for consistency and comparability among models for each outcome of interest with different air pollution indicators and exposure windows. Values below the limit of detection for interleukin-6 ($n = 10$) were assigned a value of half the limit of detection (0.2 pg/ml). No values were below the limit of detection for other biomarkers. All coefficient estimates (β) were transformed using the following formula to represent the % difference in biomarker levels per IQR: $100 \times (e^\beta - 1)$. All multivariable linear regression results described below represent a % change in biomarker levels per IQR increase in exposure.

The shapes of exposure-outcome relationships were explored using exposure categorization at equidistant cut-points and with the inclusion of quadratic terms in models, as well as through restricted cubic splines.⁵⁴

Sensitivity analysis

In two sensitivity analyses, we derived third trimester and total pregnancy averages for $\text{PM}_{2.5}$ and NO_2 , to ensure that our findings would be robust to our exposure window definition.

Conditions such as preeclampsia, impaired glucose tolerance, and gestational diabetes may result from increases in the biomarkers of interest; however, such complications may also lead to increases in inflammation.⁵⁵ We conducted a sensitivity analysis excluding women who had already developed preeclampsia, impaired glucose tolerance, or gestational diabetes before the time windows of interest in our investigation (maximum n after exclusion = 771).

Since CRP is a measure of both acute and chronic inflammation, we conducted a sensitivity analysis where we excluded participants with levels $> 100 \text{ mg/L}$, which may be indicative of an acute infection at the time of the bio-specimen collection (maximum n after exclusion = 1,028).³⁵ Lead is a potential component of $\text{PM}_{2.5}$ that has been independently linked to markers of inflammation.⁵⁶ We therefore conducted a sensitivity analysis controlling for third trimester blood lead in analyses with $\text{PM}_{2.5}$. Blood lead levels were measured in whole blood, and detailed methods are described elsewhere.⁵⁷

We also explored the potential for effect modification by fetal sex through the use of interaction terms in modeling, and presentation of stratified results.

Finally, quantitative bias analysis was used to better understand the potential for nondifferential error in exposure classification in our study. As exposure assessment was conducted at the ecologic level, but the true exposure of interest was personal exposure, there is potential for effect estimates to be biased towards the null. A reliability coefficient representing the correlation between ecologic and personal air pollution exposures can be used to better estimate the potential true association in the absence of nondifferential error. Therefore, we corrected the regression coefficients for statistically significant associations from the main analysis using a reliability coefficient of 0.3, as reported in a Canadian cohort of pregnant women comparing personal and ambient air pollution exposures,⁵⁸ where:

$$\text{corrected coefficient estimate} = \frac{\text{observed estimate}}{\text{reliability coefficient}} \quad .59$$

Results

Participant characteristics are presented in Table 1. For both $\text{PM}_{2.5}$ and NO_2 , 14-day and 30-day exposure estimates were similar (Table 2). Distributions of inflammation biomarkers are presented in Table 3.

In multivariable linear regression, 14-day and 30-day average $\text{PM}_{2.5}$ exposures before blood draw were associated with higher CRP levels in the third trimester. Each IQR change in 14-day average $\text{PM}_{2.5}$ was associated with 24.6% higher CRP levels

(95% CI = 9.4, 41.9), and results were comparable for 30-day average (17.4%; 95% CI = 1.0, 35.0) (Table 4). No statistically significant associations were observed between NO_2 and CRP, or for other exposure-biomarker relationships (Table 4).

In separate models, quadratic exposure terms were not statistically significant (data not shown). Use of equidistant cut-points for $\text{PM}_{2.5}$ exposure resulted in increases in CRP consistent with a monotonic dose-response relationship for both 14-day and 30-day average exposures (eTable S1; <http://links.lww.com/EE/A151>). We found that compared with $\text{PM}_{2.5}$ exposures at less than $5 \mu\text{g}/\text{m}^3$, exposures greater than $15 \mu\text{g}/\text{m}^3$ were associated with 50.7% higher CRP levels (95% CI = 8.3, 107.5). Although a similar, but attenuated dose-response relationship was found for 30-day average $\text{PM}_{2.5}$, the results were not statistically significant. Categorization of $\text{PM}_{2.5}$ and NO_2 did not suggest additional linear or nonlinear relationships with other biomarkers (eTable S1; <http://links.lww.com/EE/A151>). Visualization of relationships between 14-day and 30-day average $\text{PM}_{2.5}$ and CRP via restricted cubic splines were also consistent with a linear trend (eFigures S2 and S3; <http://links.lww.com/EE/A151>).

In sensitivity analyses, the association between third trimester $\text{PM}_{2.5}$ exposure and CRP was comparable to results in the main analysis (eTable S2; <http://links.lww.com/EE/A151>), however, $\text{PM}_{2.5}$ exposure across the entire pregnancy was not associated with CRP.

For 14-day and 30-day average $\text{PM}_{2.5}$ exposure and CRP levels, effect estimates were moderately attenuated after excluding women with preeclampsia, impaired glucose tolerance, and gestational diabetes (eTable S3; <http://links.lww.com/EE/A151>), and only the result for 14-day average $\text{PM}_{2.5}$ exposure was statistically significant (19.7%; 95% CI = 0.1, 39.1). Similarly, in the sensitivity analyses excluding individuals with CRP levels greater than 100 mg/L , effect estimates were attenuated for both 14-day and 30-day average $\text{PM}_{2.5}$ exposures, and were statistically significant only for 14-day average exposures (15.0%; 95% CI = 1.0, 29.7) (eTable S4; <http://links.lww.com/EE/A151>). Effect estimates for other relationships were small and not statistically significant.

When third-trimester blood-lead concentrations were included in the analysis, the effect estimates were slightly stronger and were statistically significant for both 14-day (24.6%; 95% CI = 9.4, 43.3) and 30-day (18.5%; 95% CI = 2.0, 36.3) average $\text{PM}_{2.5}$ exposures and CRP levels, respectively (eTable S5; <http://links.lww.com/EE/A151>). Effect estimates for other associations remained small and nonsignificant.

For all exposure-biomarker relationships, interactions by fetal sex were not statistically significant (eTable S6; <http://links.lww.com/EE/A151>).

Using a quantitative bias analysis, we estimate that an unbiased percent increase in CRP after accounting for nondifferential ecologic exposure measurement error could be as high as 108% and 70% per observed IQR change in 14-day and 30-day $\text{PM}_{2.5}$, respectively.⁵⁸

Discussion

We investigated potential relationships between $\text{PM}_{2.5}$ and NO_2 with biomarkers of inflammation, including CRP, interleukin-6, interleukin-8, and tumor necrosis factor- α during the third trimester of pregnancy. Our results suggest a positive linear association between $\text{PM}_{2.5}$ and CRP levels in third trimester pregnancy, despite the relatively low exposures to ambient air pollution experienced in this population. This was most consistent using a 14-day average (before blood draw), although similar findings were observed using 30-day and third-trimester averages. This finding was generally robust to several sensitivity analyses. $\text{PM}_{2.5}$ was not associated with interleukin-6, interleukin-8, or tumor necrosis factor- α in any analyses. Similarly, NO_2 was not associated with any of the selected markers of inflammation.

Table 1.
Characteristics of MIREC participants and air pollution exposures (n = 1,170).

Characteristic	n (%)	PM _{2.5} (14-day average)	NO ₂ (14-day average)
		Mean ± SD	Mean ± SD
Baseline			
Income (\$CDN) ^a			
Household < \$80,000	414 (35.4)	10.5 ± 5.3	17.2 ± 11.5
Household ≥ \$80,000	693 (59.2)	8.9 ± 4.7	17.2 ± 11.5
Do not know/refuse to answer	49 (4.2)	8.9 ± 4.04	18.3 ± 11.8
Missing	14 (1.2)	12.4 ± 5.7	20.8 ± 8.2
Education			
High-school or less	78 (6.7)	10.5 ± 4.9	15.7 ± 10.2
College or University	1,089 (93.0)	9.4 ± 5.0	17.2 ± 11.2
Missing	3 (0.3)	6.5 ± 1.8	21.3 ± 12.1
Race			
White	971 (82.9)	9.5 ± 5.0	16.0 ± 10.8
Other	199 (17.0)	9.7 ± 4.9	22.6 ± 11.2
Prepregnancy BMI			
Under/normal weight	705 (60.2)	9.6 ± 4.9	18.2 ± 11.3
Overweight	227 (19.4)	9.4 ± 5.1	15.3 ± 10.6
Obese	151 (12.9)	9.2 ± 5.9	13.9 ± 10.7
Missing	87 (7.4)	9.8 ± 5.6	18.3 ± 10.6
Current home type			
Single detached	566 (48.3)	8.6 ± 4.5	13.8 ± 10.3
Duplex or townhouse	339 (28.9)	10.5 ± 5.6	18.8 ± 10.6
100% residential building	246 (21.0)	10.0 ± 4.6	21.7 ± 11.1
Mix residential and commercial	18 (1.5)	11.2 ± 7.0	27.6 ± 12.4
Missing	1 (0.1)	4.5 -	12.4 -
Attached garage			
Yes	430 (36.8)	9.0 ± 4.6	15.0 ± 10.6
No	740 (63.3)	9.8 ± 5.2	18.4 ± 11.2
Main heating fuel			
Electric	375 (32.1)	11.5 ± 6.0	18.0 ± 10.2
Natural gas	643 (54.9)	8.9 ± 3.9	17.5 ± 11.7
Fuel oil	92 (7.8)	6.3 ± 4.7	9.7 ± 7.7
Other	14 (1.2)	7.1 ± 3.6	15.8 ± 8.9
Missing	46 (3.9)	9.8 ± 4.3	21.3 ± 11.3
Fireplace			
No fireplace	715 (61.2)	10.3 ± 5.1	18.0 ± 10.9
Natural gas	214 (18.3)	7.9 ± 3.9	16.5 ± 11.4
Propane	15 (1.3)	7.4 ± 6.2	7.1 ± 3.3
Wood/wood pellets	162 (13.8)	8.4 ± 5.0	15.8 ± 11.4
Other	61 (5.1)	9.5 ± 5.1	15.7 ± 11.6
Missing	3 (0.3)	6.8 ± 4.1	9.3 ± 5.1
Type of cooking appliances used			
Electric stove only	388 (33.2)	10.4 ± 4.9	19.9 ± 10.7
Electric and other (gas stove, wood stove, charcoal BBQ, propane/gas BBQ)	781 (66.8)	9.1 ± 4.9	15.7 ± 11.1
Missing	1 (0.1)	9.3 -	26.3 -
Furnace in home			
No	440 (37.6)	11.2 ± 5.8	20.0 ± 10.5
Yes	689 (59.0)	8.5 ± 4.1	15.3 ± 11.1
Missing	41 (3.5)	8.4 ± 4.3	16.6 ± 12.2
Second-hand smoke exposure			
No	1,122 (95.9)	9.5 ± 4.9	17.3 ± 11.1
Yes	46 (3.9)	10.5 ± 6.0	12.7 ± 10.9
Missing	2 (0.2)	4.3 ± 2.5	9.8 ± 2.7
During pregnancy			
Alcohol consumption			
No consumption	951 (81.3)	9.4 ± 4.9	16.4 ± 10.8
Any consumption	218 (18.6)	10.1 ± 5.2	20.4 ± 12.1
Missing	1 (0.1)	8.1 -	19.9 -
Smoking status			
Never	730 (62.4)	9.3 ± 4.9	16.9 ± 11.1
Former	305 (26.0)	9.6 ± 4.9	18.1 ± 11.2
Quit during this pregnancy	87 (7.4)	10.6 ± 5.3	16.8 ± 11.8
Current	48 (4.0)	10.6 ± 5.5	15.5 ± 11.1
ASA use			
None	1,138 (97.3)	9.6 ± 5.0	17.1 ± 11.2
Occasional	32 (2.7)	7.6 ± 4.6	17.4 ± 9.0
Prenatal multivitamin use			
No	145 (12.4)	10.8 ± 5.2	16.3 ± 10.2
Yes	1,025 (87.6)	9.3 ± 4.9	17.2 ± 11.3

(Continued)

Table 1.
(Continued)

Characteristic	n (%)	PM _{2.5} (14-day average)	NO ₂ (14-day average)
		Mean ± SD	Mean ± SD
Folic acid use			
No	816 (78.3)	9.7 ± 4.9	17.2 ± 11.4
Yes	344 (29.4)	9.1 ± 5.2	16.8 ± 10.4
Missing	10 (0.9)	9.7 ± 7.0	24.7 ± 14.1
Other supplement use			
No	807 (68.9)	9.9 ± 5.1	16.9 ± 11.1
Yes	354 (30.3)	8.6 ± 4.6	17.8 ± 11.2
Missing	9 (0.7)	9.7 ± 7.0	24.7 ± 14.1
Season of blood-draw			
Winter	263 (22.5)	9.3 ± 5.0	19.8 ± 13.2
Spring	334 (28.6)	7.5 ± 3.8	16.1 ± 11.2
Summer	284 (24.3)	11.7 ± 5.6	15.3 ± 8.7
Fall	289 (24.7)	9.9 ± 4.6	17.8 ± 10.7
Continuous characteristics		Mean ± SD	Missing (%)
Age (years)		32.3 ± 5.0	0
Gestational weight gain (kg)		15.4 ± 5.9	37 (3.0)
Activity (hours per week)		17.8 ± 15.8	1 (0.1)
Outside time (days spent outside for ≥ 30 minutes between 9 AM and 4 PM in past month)		13.7 ± 10.3	4 (0.3)

^aIncome cutoff chosen based on income threshold for two-parent Canadian household.

ASA indicates acetylsalicylic acid.

Table 2.**Descriptive statistics for air pollution exposures in MIREC participants (n = 1,170)**

Variable	Concentration					Pearson correlation ^a		
	Mean (SD)	25th Percentile	50th Percentile	75th Percentile	IQR	PM _{2.5} (30-day average)	NO ₂ (14-day average)	NO ₂ (30-day average)
PM _{2.5} (14-day average) µg/m ³	9.5 (5.0)	5.6	8.7	12.5	6.9	0.92	0.33	0.30
PM _{2.5} (30-day average) µg/m ³	9.5 (4.5)	6.0	9.0	12.1	6.2	–	0.32	0.33
NO ₂ (14-day average) ppb	17.1 (11.2)	7.0	16.3	25.0	18.0	–	–	0.98
NO ₂ (30-day average) ppb	17.3 (11.1)	7.0	17.0	25.3	18.3	–	–	–

^aAll Pearson correlation coefficient $p < 0.0001$.

TABLE 3.**Descriptive statistics for inflammation biomarkers in MIREC participants (n = 1,170)**

	Geometric mean	Minimum	25th Percentile	Median	75th Percentile	Maximum	Spearman correlation		
							IL-6	IL-8	TNFα
CRP (mg/L)	16.9	0.1	8.1	16.9	35.9	1,602.6	0.19, $p < 0.001$	0.04, $p = 0.15$	0.15, $p < 0.001$
IL-6 (pg/ml)	1.7	0.1	0.9	1.6	2.8	136.2	–	0.42, $p < 0.001$	0.31, $p < 0.001$
IL-8 (pg/ml)	2.0	0.2	1.4	1.9	2.6	40.4	–	–	0.43, $p < 0.001$
TNFα (pg/ml)	4.3	0.2	3.2	4.3	5.7	28.7	–	–	–

IL-6 indicates interleukin-6; IL-8, interleukin-8; TNFα, tumor necrosis factor-alpha.

C-reactive protein is an acute-phase protein that is synthesized in the liver.⁶⁰ It is a component of the nonspecific acute-phase response to inflammation, and considered a classic marker of inflammation.⁶⁰ It is unclear why significant associations were observed only for CRP, and not other inflammation biomarkers. One potential explanation could be that CRP is a more sensitive marker of inflammation compared with other selected biomarkers. The literature on CRP as a biomarker of pregnancy complications is more substantive than for other biomarkers of inflammation complications.^{25,29,61,62} It is possible that other inflammation biomarkers are not as relevant when studying the impact of environmental exposures on inflammation, and may not represent intermediates between exposures and health outcomes of interest.^{30,31,63}

To our knowledge, two previous studies have assessed relationships between air pollution and markers of inflammation during pregnancy. Van Den Hooven et al., studied the association of PM₁₀ and NO₂ with maternal first trimester CRP

levels (>8 mg/L, and ≤8 mg/L) in the Generation R Study in the Netherlands (n = 5,067).³⁵ Air pollutant exposure windows of 1, 2, and 4 weeks before bio-specimen collection, and total pregnancy were assessed. Participants in the 4th quartile (vs. 1st quartile) of PM₁₀ exposure had higher odds of elevated CRP levels 1 week before bio-specimen collection, with no association with CRP levels for those in the 2nd or 3rd exposure quartiles. Lee et al. studied the associations of several air pollution indicators, including PM_{2.5} and NO₂, on CRP (>8 mg/L, and ≤8 mg/L) during early pregnancy in a prospective cohort of 1696 pregnant women in Pennsylvania.³⁴ Several different exposure windows were assessed: 8-day, 22-day, 29-day, and same-day. Researchers found that longer PM_{2.5} exposure windows (22- and 29-day averages) led to higher odds of having elevated CRP levels.³⁴ Neither study demonstrated consistently positive or statistically significant associations with NO₂, which is consistent with our results and may be explained by a weak correlation between NO₂ exposure levels and the pollutants it is used as a proxy for,

Table 4.
Multivariable linear regression analysis for the relationship between short-term PM_{2.5} and NO₂ exposure and biomarkers of inflammation in MIREC participants

Exposure	Biomarker and percent difference ^a	P
CRP ^b (n = 1,081)		
PM _{2.5} (per IQR increase)		
14-day average	24.6% (9.4, 41.9)	0.001
30-day average	17.4% (1.0, 35.0)	0.03
NO ₂ (per IQR increase)		
14-day average	9.4% (-8.6, 31.0)	0.32
30-day average	8.3% (-10.4, 31.1)	0.39
IL-6 ^c (n = 1,003)		
PM _{2.5} (per IQR increase)		
14-day average	-1.9% (-12.2, 9.4)	0.74
30-day average	0.1% (-11.3, 12.7)	0.99
NO ₂ (per IQR increase)		
14-day average	0.0% (-13.9, 16.2)	0.99
30-day average	6.2% (-8.6, 24.6)	0.45
IL-8 ^d (n = 1,072)		
PM _{2.5} (per IQR increase)		
14-day average	3.1% (-3.9, 10.5)	0.39
30-day average	0.1% (-6.8, 8.3)	0.97
NO ₂ (per IQR increase)		
14-day average	0.3% (-8.6, 9.4)	0.99
30-day average	0.4% (-8.6, 11.6)	0.94
TNFα ^e (n = 1,083)		
PM _{2.5} (per IQR increase)		
14-day average	2.0% (-3.9, 7.3)	0.61
30-day average	2.0% (-3.9, 9.4)	0.47
NO ₂ (per IQR increase)		
14-day average	-1.9% (-8.6, 6.2)	0.63
30-day average	-1.0% (-8.6, 7.3)	0.81

Differing final counts in models due to differing availability of covariates selected for each biomarker.

^aPercent difference represents the percentage increase in biomarkers per IQR difference in pollutant.

^bModel for CRP controlled for recruitment center, alcohol, income, activity, body mass index.

^cModel for IL-6 controlled for recruitment center, maternal age, outside time, BMI, folic acid, main heating, furnace.

^dModel for IL-8 controlled for recruitment center, maternal age, alcohol, income, activity, body mass index, folic acid.

^eModel for TNFα controlled for recruitment center, maternal age, body mass index.

IL-6 indicates interleukin-6; IL-8, interleukin-8; TNFα, tumor necrosis factor-alpha.

such as benzene, volatile organic pollutants, and other tailpipe emissions.⁶⁴ Other explanations for differences in relationships between PM_{2.5} and NO₂ with inflammation markers could be due to the methods used to estimate exposure (PM_{2.5} relying primarily on satellite data and NO₂ relying primarily on land use regression), as well as differences in the spatial resolution of the pollutants (NO₂ being more heterogeneous).⁶⁵

Biomarker levels in our study were comparable to other pregnancy cohorts for interleukin-6,^{66,67} interleukin-8,^{66,68} and tumor necrosis factor-α,^{67,68} but higher for CRP.³⁵ We analyzed blood samples from the third trimester, when CRP levels are thought to be highest, although previous studies evaluated biomarkers earlier in pregnancy.^{34,35} Median CRP levels in our study are 4-times higher than in the study by Van Den Hooven et al.,³⁵ and the two aforementioned previous studies categorized CRP levels at 8 mg/L, which would correspond to the 25th percentile in our sample. In addition, there are differences in exposure distributions between our study and these previous investigations. We reported a 14-day mean PM_{2.5} exposure of 9.5 μg/m³ for our study participants compared with a 7-day mean of 16.4 μg/m³ reported by Lee et al.³⁴ Nonetheless, we report comparable associations between PM_{2.5} and CRP in our study and we further demonstrate evidence of linear and monotonic associations between continuous exposure and outcome variables that were

not observed in other studies. Collectively, these results suggest that short-term exposure to PM_{2.5} during pregnancy may result in higher levels of CRP.

When applying the percent increase in CRP levels observed between the referent category of categorized 14-day PM_{2.5} exposure and the highest level of exposure, the change corresponds to a ~20 mg/L increase in CRP levels. For context, a meta-analysis of studies assessing changes in CRP and their relationship with preeclampsia found a weighted mean difference (after adjustment for body mass index) of 2.6 mg/L between preeclampsia cases and controls.⁶⁹ An Australian prospective study found a mean difference of 32.2 mg/L in CRP levels in the third trimester between women diagnosed with gestational diabetes compared with controls.⁷⁰ Research on the potential for elevated CRP levels in predicting pregnancy complications, such as preeclampsia or gestational diabetes, is ongoing. However, based on the current literature, the differences in CRP observed in the higher versus lower exposure levels in our cohort correspond to changes that may be considered clinically meaningful.

This study has some important limitations. First, we relied on an ecologic measure of ambient air pollution which may have led to nondifferential error in our estimates. We restricted all analyses to areas less than 10 × 10 km to minimize this exposure assessment limitation and employed a quantitative bias analysis to estimate the potential true effect size in the absence of nondifferential exposure misclassification. Although the reliability coefficient used for the quantitative bias analysis was not estimated from the MIREC cohort, it was based on a Canadian pregnancy cohort,⁵⁸ and allows us to observe the potential degree of misclassification occurring in our study. This reliability coefficient is not itself without error, and may be influenced by behaviors, indoor sources of exposure, home locations, and season. Nonetheless, we expect that this revised estimate likely reduces some nondifferential error, while informing on the degree of misclassification occurring in the analysis, and the impact of this on the observed estimate. Another potential concern was the limited understanding of the most appropriate time windows of exposure. We studied four different exposure windows to better understand relationships of interest, and found consistent associations for PM_{2.5} and CRP for 14-day, 30-day, and third-trimester average exposures.

Finally, we relied on a single measure of inflammatory markers in the third trimester. The timing of CRP measures in pregnancy is heterogeneous in studies of CRP and pregnancy complications.⁶⁹ Our finding that exposures around the third trimester were more strongly associated with CRP than exposures during other time points could reflect that these measures are simply more proximal to the timing of the outcome measure. It was not possible to evaluate the effect of different time windows of exposure in our study with only a single outcome measure. However, our findings are consistent with a recent meta-analysis on PM_{2.5} exposure and preeclampsia risk, which found that pregnant women may be most susceptible to PM_{2.5} exposures in the third trimester. This matches the time-window of exposure investigated in our study.⁷¹

Our analysis has several strengths. The MIREC Study is a prospective, multisite pregnancy cohort study, and is well positioned to study the impact of ambient air pollution on maternal health. To our knowledge, this is the first investigation of PM_{2.5} and NO₂ exposures and biomarkers of inflammation to study dose-response relationships during pregnancy. The use of objective exposure and outcome measures helps to mitigate both recall and selection bias in our analysis. Finally, we conducted several sensitivity analyses to more thoroughly understand the observed relationships. To our knowledge, this is also the first study of air pollution exposures during pregnancy and biomarkers of inflammation to employ a quantitative bias analysis.

We showed that short-term (14-day and 30-day) PM_{2.5} exposure was associated with higher third trimester CRP concentrations. This may represent one mechanism through which PM_{2.5} is associated with pregnancy complications. Future studies

should investigate longitudinal changes in CRP levels from air pollution exposure, especially at low-levels, such as those typically experienced by the Canadian population. This work adds to the growing literature underscoring the potential health impacts of air pollution in specific vulnerable populations, such as pregnant women.

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