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Long-term Exposure to Oxidant Gases and Mortality: Effect Modification by $PM_{2.5}$ Transition Metals and Oxidative Potential

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Background: Populations are simultaneously exposed to outdoor concentrations of oxidant gases (i.e., O_3 and NO_2) and fine particulate air pollution ($PM_{2.5}$). Since oxidative stress is thought to be an important mechanism explaining air pollution health effects, the adverse health impacts of oxidant gases may be greater in locations where $PM_{2.5}$ is more capable of causing oxidative stress.

Methods: We conducted a cohort study of 2 million adults in Canada between 2001 and 2016 living within 10 km of ground-level monitoring sites for outdoor $PM_{2.5}$ components and oxidative potential. O_x exposures (i.e., the redox-weighted average of O_3 and NO_2) were estimated using a combination of chemical transport models, land use regression models, and ground-level data. Cox proportional hazards models were used to estimate associations between 3-year moving average O_x and mortality outcomes across strata of transition metals and sulfur in $PM_{2.5}$ and three measures of $PM_{2.5}$ oxidative potential adjusting for possible confounding factors.

Results: Associations between O_x and mortality were consistently stronger in regions with elevated $PM_{2.5}$ transition metal/sulfur content and oxidative potential. For example, each interquartile increase (6.27 ppb) in O_x was associated with a 14.9% (95% CI = 13.0, 16.9) increased risk of nonaccidental mortality in locations with glutathione-related oxidative potential (OP^{GSH}) above the median whereas a 2.50% (95% CI = 0.600, 4.40) increase was observed in regions with OP^{GSH} levels below the median (interaction *P* value <0.001).

Conclusion: Spatial variations in $PM_{2.5}$ composition and oxidative potential may contribute to heterogeneity in the observed health impacts of long-term exposures to oxidant gases.

Keywords: Ox; Mortality; $PM_{2.5}$ components; Oxidative potential; Cohort study

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Outdoor fine particulate air pollution ($PM_{2.5}$) is a complex mixture of organic and inorganic components that contribute to the generation of reactive oxygen species and oxidative stress, which are thought to be important mechanisms underlying air pollution health effects.[1,2] Although $PM_{2.5}$ mass concentrations are recognized as an important contributor to the overall global burden of disease,[3] it is not clear how coexposure to specific $PM_{2.5}$ components may modify the long-term health effects of other common outdoor air pollutants such as oxidant gases (i.e., O_3 and NO_2). This is an important concept as populations are simultaneously exposed to *both* $PM_{2.5}$ and oxidant gases, and we must understand how these air pollutants interact to affect public health to adequately inform future regulatory interventions.

Specifically, it seems possible that associations between long-term exposure to oxidant gases and mortality risk may be stronger in regions where $PM_{2.5}$ composition has a greater capacity to cause oxidative stress (e.g., greater transition metal content and oxidative potential). This hypothesis is supported by existing evidence suggesting that outdoor $PM_{2.5}$ with higher oxidative potential is more strongly associated with adverse health outcomes.[1,2,4–8] However, this paradigm should not only apply to outdoor $PM_{2.5}$, but all air pollutants capable of contributing to oxidative damage in human systems. Therefore, if oxidant gases are harmful to human health

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primarily through their ability to cause oxidative stress, the health impacts of oxidant gases may be greater in populations coexposed to $PM_{2.5}$ that is more capable of causing oxidative stress because the *combined* oxidative impacts of $PM_{2.5}$ and oxidant gases may be greater in these locations. To our knowledge, no cohort studies to date have examined this question, but a recent systematic review highlighted substantial heterogeneity in existing studies of long-term exposure to oxidant gases and mortality.[9] One explanation for this heterogeneity may be that the long-term health impacts of oxidant gases depend in part on the composition/characteristics of outdoor $PM_{2.5}$ that is also present in the environment.

In this study, our aim was to determine if associations between long-term exposure to oxidant gases (O_x, expressed as a redox-weighted average of O_3 and NO_2) and nonaccidental, cardiovascular, and respiratory mortality are stronger in locations where $PM_{2.5}$ composition is more likely to contribute oxidative stress (i.e., with higher transition metal content and oxidative potential). To do this, we examined associations between long-term O_x exposures and mortality across strata defined by the content (i.e., mass proportions) of specific transition metals in outdoor $PM_{2.5}$ as well as three different measures of $PM_{2.5}$ oxidative potential using a database of ground-level measurements collected at 40 locations across Canada.

METHODS

Study Population and Mortality Outcomes

Our cohort study population included members of multiple cycles (2001, 2006, 2011) of the Canadian Census Health and Environment Cohort (CanCHEC). Each CanCHEC is made up of adults (>25 years of age at enrollment) who completed the long-form census questionnaire capturing individual and household sociodemographic data, which were subsequently linked to vital statistics and tax records (for annual residential location).[10] O_x-mortality analyses were limited to cohort members residing within a 10-km radius of a monitoring site for $PM_{2.5}$ components/oxidative potential (described later). Individuals who were enumerated in more than one long-form census cycle were assigned to the cohort in which they first appeared. All participants were followed for mortality from census day (in either 2001, 2006, or 2011) until December 31, 2016. Our analyses focused on nonaccidental mortality (International Classification of Diseases, 10th revision codes (ICD-10): A00-R99) along with several specific causes of mortality including cardiovascular disease (ICD-10 codes: I10 to I69) and nonmalignant respiratory disease (ICD-10 codes: J00-J99). The CanCHEC database was created under the authority of the Statistics Act and approved by the Executive Management Board (reference number: 045-2015) at Statistics Canada. This is equivalent to standard research ethics board approval. Compared with the national CanCHEC cohorts, our analytical cohort had a slightly lower proportion (based on percentage of person-time) of younger subjects (14% of person-time among subjects 25–34 years of age compared with 18% in the full cohort) and a higher proportion of older subjects (26% of person-time among subjects 65–89 years of age compared with 19% in the full cohort) but was similar in terms of sex (52% female; 48% male), education (e.g., 18% with less than high school and 29% with a university degree), and employment status (63% employed).

Outdoor Oxidant Gas and $PM_{2.5}$ Concentrations

We did not examine models for O_3 or NO_2 separately as our focus was on the *combined* oxidant capacity of these gases (i.e., O_x) and how associations for O_x may be influenced by coexposure to outdoor $PM_{2.5}$ with varying abilities to cause oxidative stress. This is particularly important for NO_2 which often serves as a marker for a broad mix of combustion pollutants (e.g., traffic-related air pollution), which could have much different health impacts than NO_2 the molecule. Since we were interested in the combined oxidant capacity of the NO_2 and O_3 molecules themselves, we combined these exposures into a single redox-weighted average (i.e., a weighted average based on their redox potential) based on the following equation: $O_x = ((1.07 \times NO_2) + (2.075 \times O_3))/ 3.145$.[11,12]

Ozone data reflected the daily maximum of 8-hour average concentrations based on chemical transport modeling of surface observations in the warm season between 2002 and 2015 (i.e., the average of maximum values within the same 8-hour period each day during the warm season).[13] Hourly O_3 model output was fused with ground monitor data[14,15]; the spatial resolution of the O_3 model was 21 km^2 before 2009 and 10 km^2 in subsequent years of follow-up. NO_2 data were obtained from a national land use regression model for the year 2006, using 10 km^2 gridded remote sensing-derived NO_2 estimates and highly resolved land use data to produce a model with a resolution of 100 m^2.[16] We applied spatiotemporal adjustments to annual estimates of O_3 and NO_2 by first developing an annual time-series of both pollutants in Canada's 24 largest cities, based on ground monitoring data from 1981 to 2016. We then estimated yearly adjustment factors equal to the ratio of the observed concentration in the desired year to the average concentration in the reference year(s) (i.e., 2006 for NO_2 and the mean of 2002–2015 for O_3). We scaled the concentration estimates over the follow-up period using the annual adjustment factors based on the nearest city to that postal code location. Annual average outdoor NO_2 and O_3 concentrations were first assigned to the centroids of residential 6-digit postal codes for each cohort member and annual average O_x concentrations were then calculated using the formula above.

Annual average outdoor $PM_{2.5}$ mass concentrations ($\mu g/m^3$) were estimated using a model combining satellite retrievals of aerosol optical depth (AOD) at 1 km^2 resolution with simulations of the AOD-to-$PM_{2.5}$ relationship using GEOS-Chem (a chemical transport model).[17]

Ground-level Measurements of $PM_{2.5}$ Transition Metals and Oxidative Potential

Integrated $PM_{2.5}$ samples were collected on Teflon filters (using cascade impactors at a flow rate of 5 L/min) for 2 weeks

each month at 40 monitoring sites across Canada between 2016 and 2018.[7] Monthly samples were analyzed for OP and components and results were averaged over the 2-year monitoring period to estimate long-term average values for each site. The median number of monthly samples pooled within each site to estimate long-term average values for OP and transition metals was 27 (range: 15–41). Monitoring sites included the following locations across Canada (eFigure S1; http://links.lww.com/EDE/B958): Alberta: Anzac, Athabasca Valley, Calgary, Edmonton (3 sites), Fort Mackay, Fort McMurray, Red Deer, and St Albert; British Columbia: Courtenay, Duncan, Kamloops, Kelowna, Nanaimo, Prince George, Quesnel, Trail, and Victoria; Manitoba: Brandon, Flin Flon, and Winnipeg; New Brunswick: Fredericton and Saint John; Newfoundland and Labrador: Mt. Pearl and St. John's; Nova Scotia: Halifax; North West Territories: Yellowknife; Ontario: Hamilton, London, Ottawa (2 sites), and Windsor; Quebec: Montreal and Quebec City; Saskatchewan: Prince Albert, Regina, Saskatoon, and Swift Current; Yukon: Whitehorse. In most cities, monthly $PM_{2.5}$ samplers were located at provincial monitoring sites except for Ottawa and Montreal where monitors were located outside private residences.

Monthly $PM_{2.5}$ filters were analyzed for sulfur and transition metal content using x-ray fluorescence (United States Environmental Protection Agency Method IO-3.3) and oxidative potential as described below. The following transition metals were selected *a priori* for inclusion in our analyses based on previous evidence suggesting an association with particle OP: Cu, Fe, Zn, Ni, Mn.[1,2,18,19] Sulfur was included in our $PM_{2.5}$ component analyses as we recently reported that the acute cardiovascular health impacts of $PM_{2.5}$ mass concentrations were stronger in men when *both* S and transition metals were elevated (S makes the particles more acidic and is thought to make the metals more biologically available).[8,20] Therefore, we wanted to examine how *combinations* of these components may modify the health impacts of O_x.

For oxidative potential analyses, $PM_{2.5}$ samples were first extracted into HPLC grade methanol by vortexing at 1,800 revolutions per minute for 20 minutes and sonicating for 10 minutes. Decanted methanol was evaporated under a gentle flow of nitrogen. PM samples were resuspended in ultrapure water containing 5% HPLC methanol to a final storage concentration of 200 μg PM/mL. Resuspended $PM_{2.5}$ samples were analyzed in triplicate using three OP metrics: the ascorbate (AA), glutathione (GSH), and dithiothreitol (DTT) assays. Ascorbate and glutathione oxidative potential (OP^{AA} and OP^{GSH}) were assessed using the acellular respiratory tract lining fluid (RTLF) OP assay.[21] Briefly, $PM_{2.5}$ samples were incubated at a concentration of 75 μg/mL in a 96 well plate for 4-hours at 37°C with synthetic respiratory tract lining fluid (RTLF) containing 200 μM of each AA, GSH, and uric acid in an ultraviolet—visible plate reader (Molecular Devices, Spectra Max 190) alongside positive controls (0.5 μM $Cu(NO_3)_2$, 0.02% H_2O_2) and blanks. Ascorbate depletion was calculated over the 4-hour incubation period, and GSH depletion was measured using the glutathione-reductase enzyme recycling assay.[22–25]

Dithiothreitol oxidative potential (OP^{DTT}) was assessed using an adapted version of the DDT assay. Briefly, resuspended $PM_{2.5}$ samples were incubated with 100 μM DTT in a 96 well plate alongside positive controls (0.5 μM $Cu(NO_3)_2$), blanks, and DTT standards (containing 0−100 μM DTT) for 35 minutes at 37°C, with constant shaking. After 5, 15, 25, and 35 minutes, the remaining DTT was measured by adding 1.0 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to each well and measuring absorbance at 412 nm. Samples were initially analyzed at a concentration of 50 μg/mL; however, if DTT depletion exceeded 25% after 35 minutes, the sample was reanalyzed at a lower concentration. All OP values are expressed in units of pmol/min/μg; values below detection were replaced with half the limit of detection.

Statistical Analysis

Cox proportional hazard models were used to estimate associations between outdoor O_x concentrations and mortality outcomes across strata defined by ground-level measurements of oxidative potential (OP^{GSH}, OP^{AA}, and OP^{DTT}) (pmol/min/μg) and mass proportions of transition metals (i.e., Cu, Fe, Zn, Ni, Mn) and sulfur (S) in $PM_{2.5}$. Strata for OP, metals, and sulfur were based on median values observed across the 40 monitoring sites (eFigure S1; http://links.lww.com/EDE/B958). Median values were used to define strata of OP/components for three reasons. First, a smaller number of strata were needed to ensure sufficient power to detect possible effect modification across categories of OP/components. Second, since OP/component data were collected at the end of the follow-up period, we thought that error in classifying locations as above/below median values would be less than for classifications across 3 or more categories. Finally, random error across levels of a dichotomous effect modifier will tend to diminish the observed effect modification[26]; thus, we felt this decision was conservative in that we would not overestimate possible differences across strata (assuming misclassification was independent of O_x). Models were first examined across strata of each component/characteristic separately, followed by models examining strata with *both* high sulfur and high metals (i.e., both above the median) or *both* low sulfur and low metals (i.e., both below the median). Intermediate categories (e.g., high S/low metals) were not examined as far fewer cases (~80% fewer cases) were available for these strata.

All Cox proportional hazards models were stratified by age (5-year groups), sex, immigrant status, and census cycle, and included covariates for 3-year moving average outdoor $PM_{2.5}$ mass concentration with a 1-year lag (i.e., the same as for O_x), individual-level income, educational attainment, marital status, Indigenous identity, employment status, occupational class, and visible minority status. In addition, we included neighborhood-level variables for four dimension

of the Canadian Marginalization Index (CAN-Marg) which describes inequalities in terms of material deprivation, residential instability, dependency, and ethnic concentration.[27] Individual-level data on smoking and body mass index (BMI) are not available in CanCHEC; however, we did evaluate correlations between outdoor O_x concentrations and smoking (6 levels: never, former-occasional, former-daily, occasional, occasional-former daily, daily) and BMI (continuous) in the Canadian Community Health Survey (CCHS) cohort population over the same time-period (CCHS is an ancillary population-based cohort with individual-level data on smoking and BMI). In the CCHS cohort, weak inverse correlations were observed between outdoor O_x concentrations and both smoking ($r = -0.045$) and BMI ($r = -0.038$); thus, suggesting that any residual confounding by these factors in the CanCHEC cohort would tend to underestimate the magnitude of associations for O_x.

All hazard ratios for outdoor O_x are expressed per interquartile change in outdoor concentration (6.27 ppb); interaction p-values were estimated by including an interaction term between O_x and indicator variables for strata. O_x exposures were assigned as 3-year moving average with a 1-year lag (to ensure that exposure preceded the outcome); exposures were updated for residential mobility and subjects were censored if they moved to a location more than 10-km from one of our monitoring sites. We did not examine $PM_{2.5}$-mortality associations across strata of components/OP as long-term average outdoor $PM_{2.5}$ mass concentrations did not vary meaningfully across our study sites (IQR: 6.37–9.77 $\mu g/m^3$).

RESULTS

In total, our study population included 2 million adults with 153,800 nonaccidental deaths, 44,200 cardiovascular disease deaths, and 13,700 respiratory disease deaths occurring during the follow-up period (Table 1). The median O_x concentration was 28.75 ppb (5th = 20.41 ppb; 95th = 36.58 ppb) and O_x distributions were similar within strata of $PM_{2.5}$ OP and components (eTable S1; http://links.lww.com/EDE/B958). Long-term estimates of mass proportions of transition metals and sulfur in $PM_{2.5}$ varied substantially across study locations (eTable S2; http://links.lww.com/EDE/B958). Spearman correlations between $PM_{2.5}$ OP and $PM_{2.5}$ components are shown in eFigure S2; http://links.lww.com/EDE/B958. OP^{DTT} was weakly correlated with transition metals in $PM_{2.5}$ ($-0.15 < r < 0.10$) as well as OP^{AA} and OP^{GSH} ($-0.02 < r < 0.17$). OP^{GSH} and OP^{AA} were moderately correlated with each other ($r = 0.67$) and with Cu ($0.68 < r < 0.72$). Outdoor O_x concentrations were moderately correlated with outdoor $PM_{2.5}$ mass concentrations ($r = 0.62$). Correlations between outdoor O_x concentrations and long-term estimates of $PM_{2.5}$ OP and mass proportions of $PM_{2.5}$ components across study sites were as follows: OP^{GSH} ($r = 0.28$), OP^{AA} ($r = 0.43$), OP^{DTT} ($r = 0.17$), Cu ($r = 0.26$), S ($r = 0.60$), Fe ($r = 0.35$), Zn ($r = 0.13$), Mn ($r = 0.24$), Ni($r = -0.13$).

Overall, each IQR increase in O_x was associated with an increased risk of nonaccidental (HR = 1.092, 95% CI = 1.079, 1.104), cardiovascular (HR = 1.153, 95% CI = 1.129, 1.177), and respiratory mortality (HR = 1.084, 95% CI = 1.045, 1.126) (eTable S3; http://links.lww.com/EDE/B958). In general, these association tended to be stronger among women: nonaccidental mortality (women: HR = 1.114, 95% CI = 1.097, 1.131; men: HR = 1.064, 95% CI = 1.047, 1.082); cardiovascular mortality: (women: HR = 1.179, 95% CI = 1.147, 1.212; men: HR = 1.117, 95% CI = 1.082, 1.152); respiratory mortality (women: HR = 1.113, 95% CI = 1.058, 1.170; men: HR = 1.049, 95% CI = 0.993, 1.109). Outdoor $PM_{2.5}$ mass concentrations (per IQR of 3.4 $\mu g/m^3$) were not associated with an increased risk of mortality across the limited concentration range captured by our study sites: nonaccidental mortality (HR = 0.996, 95% CI = 0.989, 1.003); cardiovascular mortality (HR = 0.971, 95% CI = 0.958, 0.984); respiratory mortality (HR = 0.982, 95% CI = 0.959, 1.006).

Hazard ratios (and 95% CIs) for outdoor O_x concentrations across strata of long-term estimates of outdoor $PM_{2.5}$ OP are shown in Figure 1 (eTables S3; http://links.lww.com/EDE/B958). The magnitudes of associations between O_x and nonaccidental, cardiovascular, and respiratory mortality were consistently greater when $PM_{2.5}$ OP was above the median. This pattern was observed for all three metrics of OP (with larger differences observed across strata for OP^{GSH} and OP^{AA}) and was most apparent for nonaccidental and cardiovascular mortality as hazard ratios for respiratory mortality were less precise owing to a smaller number of events (interaction *P* values across strata of $PM_{2.5}$ OP are shown in eTable S3; http://links.lww.com/EDE/B958). The results of O_x analyses conducted across strata of long-term estimates of mass proportions of transition metals and sulfur in outdoor $PM_{2.5}$ are shown in Figure 2 (eTable S4; http://links.lww.com/EDE/B958). Consistently stronger associations were observed between O_x and nonaccidental, cardiovascular, and respiratory mortality when the transition metal and sulfur content of outdoor $PM_{2.5}$ was elevated (interaction *P* values across strata of $PM_{2.5}$ components are shown in eTable S4; http://links.lww.com/EDE/B958). Again, this pattern was most consistent for nonaccidental and cardiovascular mortality and across strata for mass proportions of S and Ni in $PM_{2.5}$. Similar patterns were observed across strata of $PM_{2.5}$ OP and $PM_{2.5}$ components when analyses were conducted separately by sex, with stronger associations observed among women (Tables S5–S10; http://links.lww.com/EDE/B958).

Hazards ratios for O_x analyses conducted across strata of mass proportions of *both* transition metals and sulfur are shown in Figure 3 (Table S11; http://links.lww.com/EDE/B958) and demonstrate a consistent pattern of stronger associations when both transition metals and sulfur are elevated (i.e., above the median) compared with when both are below the median. As above, this pattern was most apparent for nonaccidental and cardiovascular mortality (interaction *P* values

TABLE 1. Descriptive Cohort Data (2001, 2006, 2011 CanCHEC cohorts) for Participants Living Within 10-km of a Ground-level Monitoring Location for $PM_{2.5}$ Oxidative Potential and Components

			Mortality Outcomes		
Characteristic	**Person-years**	**Participants**	**Nonaccidental**	**Cardiovascular**	**Respiratory**
All	15,807,300	2,001,600	153,800	44,200	13,700
Sex					
Male	8,382,400	1,047,500	71,300	19,200	6,300
Female	7,424,900	954,200	82,500	25,000	7,400
Age					
25–29	504,500	196,000	100	NA	NA
30–39	2,614,700	585,300	1,200	200	NA
40–49	3,511,300	734,700	4,700	1,100	200
50–59	3,608,000	730,800	14,000	3,200	700
60–69	2,677,100	556,300	26,100	6,000	1,700
70–79	1,822,000	380,500	44,400	12,000	4,200
80–89	1,069,700	235,600	63,400	21,600	6,800
Education					
Not completed high school	2,955,000	365,600	64,300	19,400	6,600
High school with/without trades certificate	4,538,600	555,300	45,300	12,900	3,800
Postsecondary nonuniversity	3,947,600	506,500	25,900	7,000	2,100
University degree	4,366,100	574,200	18,300	4,800	1,100
Employment status					
Employed	10,263,500	1,271,700	31,500	7,500	1,600
Unemployed	587,300	79,000	2,700	700	200
Not in labor force	4,956,500	651,000	119,600	36,000	11,900
Income deciles					
1st decile—highest	1,574,900	442,900	15,800	4,500	1,600
2nd decile	1,550,500	526,700	25,700	7,700	2,700
3rd decile	1,472,700	570,300	18,400	5,400	1,800
4th decile	1,430,100	588,300	14,800	4,200	1,400
5th decile	1,387,600	593,300	12,000	3,400	1,100
6th decile	1,348,800	583,500	9,700	2,700	700
7th decile	1,318,500	561,200	7,800	2,100	600
8th decile	1,307,100	523,100	6,400	1,700	400
9th decile	1,311,400	461,400	5,300	1,400	300
10th decile—lowest	1,442,300	353,700	5,300	1,300	300
Not applicable	1,663,500	128,300	32,600	9,800	2,800
Occupational level					
Management	1,281,900	156,800	4,100	1,000	200
Professional	2,541,100	306,200	6,300	1,400	300
Skilled, technical, and supervisory	3,332,900	422,300	11,200	2,700	600
Semiskilled	3,119,700	387,100	11,600	2,800	600
Unskilled	1,134,100	148,100	5,200	1,400	400
No occupation	4,397,500	581,200	115,500	35,000	11,600
Visible minority					
Not defined as visible minority	13,369,200	1,678,300	142,400	40,900	12,800
Visible minority	2,438,100	323,400	11,400	3,200	900
Immigration status					
Nonimmigrant	12,035,100	1,531,700	119,000	33,600	11,000
Immigrant	3,772,200	470,000	34,800	10,600	2,600
Marital status					
Single, never married	2,716,700	364,900	16,400	4,800	1,300
Common-law	1,647,400	223,200	6,700	1,600	500
Married	8,965,400	1,080,100	82,200	22,700	6,800
Separated	451,500	59,100	4,200	1,100	400
Divorced	1,200,700	154,200	14,700	4,100	1,400
Widowed	825,600	120,100	29,600	9,700	3,200

(*Continued*)

TABLE 1. (*Continued*)

Characteristic	Person-years	Participants	Mortality Outcomes		
			Nonaccidental	Cardiovascular	Respiratory
Can-Marg: Residential instability					
Q1—lowest	1,487,900	231,200	9,200	2,600	800
Q2	2,596,800	415,800	18,900	5,300	1,600
Q3	3,230,400	522,100	29,900	8,600	2,500
Q4	3,835,700	621,500	41,100	11,900	3,700
Q5—highest	4,641,200	756,300	54,700	15,700	5,000
Can-Marg: Dependency					
Q1—lowest	3,641,500	596,100	24,200	6,800	2,100
Q2	2,909,900	503,400	24,800	7,100	2,200
Q3	2,134,700	399,300	20,800	6,000	1,900
Q4	3,199,800	550,000	33,600	9,600	3,000
Q5—highest	3,906,000	625,300	50,300	14,600	4,400
Can-Marg: Material deprivation					
Q1—lowest	3,702,900	569,000	26,400	7,400	2,200
Q2	2,515,400	442,300	23,500	6,900	2,000
Q3	2,757,200	485,200	28,600	8,300	2,500
Q4	3,029,300	532,200	32,400	9,500	3,000
Q5—highest	3,787,100	599,400	42,800	12,100	4,000
Can-Marg: Ethnic concentration					
Q1—lowest	2,141,900	338,100	21,500	6,000	1,900
Q2	3,332,300	555,600	33,800	9,800	2,900
Q3	4,174,600	701,700	41,900	12,000	3,700
Q4	4,261,200	708,600	38,400	11,100	3,400
Q5—highest	1,882,000	331,300	18,000	5,200	1,700
Urban form					
Active urban core	3,312,300	601,700	37,500	11,100	3,400
Transit-reliant suburb	2,464,200	505,500	26,400	7,500	2,400
Car-reliant suburb	8,149,200	1,204,100	72,200	20,500	6,300
Exurban	271,700	52,800	2,100	600	200
Non-CMA/CA	1,610,000	228,500	15,700	4,500	1,400
Airshed					
Western	1,663,400	225,400	18,000	5,300	1,600
Prairie	4,163,900	530,100	34,000	10,400	3,200
West Central	1,273,100	157,500	14,300	4,400	1,200
Southern Atlantic	1,105,700	139,400	11,700	3,400	1,000
East Central	7,529,500	974,200	75,300	20,600	6,500
Northern	57,900	7,900	300	NA	NA

All numbers are rounded to the nearest 100 for confidentiality and may not add up to the total; NA denotes counts below 100 and are suppressed for confidentiality.

across strata of both metals and sulfur are shown in eTable S11; http://links.lww.com/EDE/B958). For respiratory mortality, the 95% confidence intervals for O_x hazards ratios only excluded the null when both metals and sulfur were above the median (Figure 3). eFigure S1 (Part B); http://links.lww.com/EDE/B958 shows the spatial distribution of locations across Canada where Ni and S content in outdoor $PM_{2.5}$ are both above/below median values.

DISCUSSION

In this study, we examined how spatial variations in long-term estimates of outdoor $PM_{2.5}$ oxidative potential and mass proportions of specific $PM_{2.5}$ components may modify the association between O_x and nonaccidental, cardiovascular, and respiratory mortality. Long-term estimates of spatial variations in $PM_{2.5}$ oxidative potential and $PM_{2.5}$ composition were based on prospective ground-level measurements collected at 40 locations across Canada between 2016 and 2018. To our knowledge, this is the first epidemiologic study to examine how $PM_{2.5}$ oxidative potential and $PM_{2.5}$ components may modify association between long-term exposure to O_x and mortality and we noted several interesting results.

First, our findings demonstrated a clear and consistent pattern of stronger associations between outdoor O_x

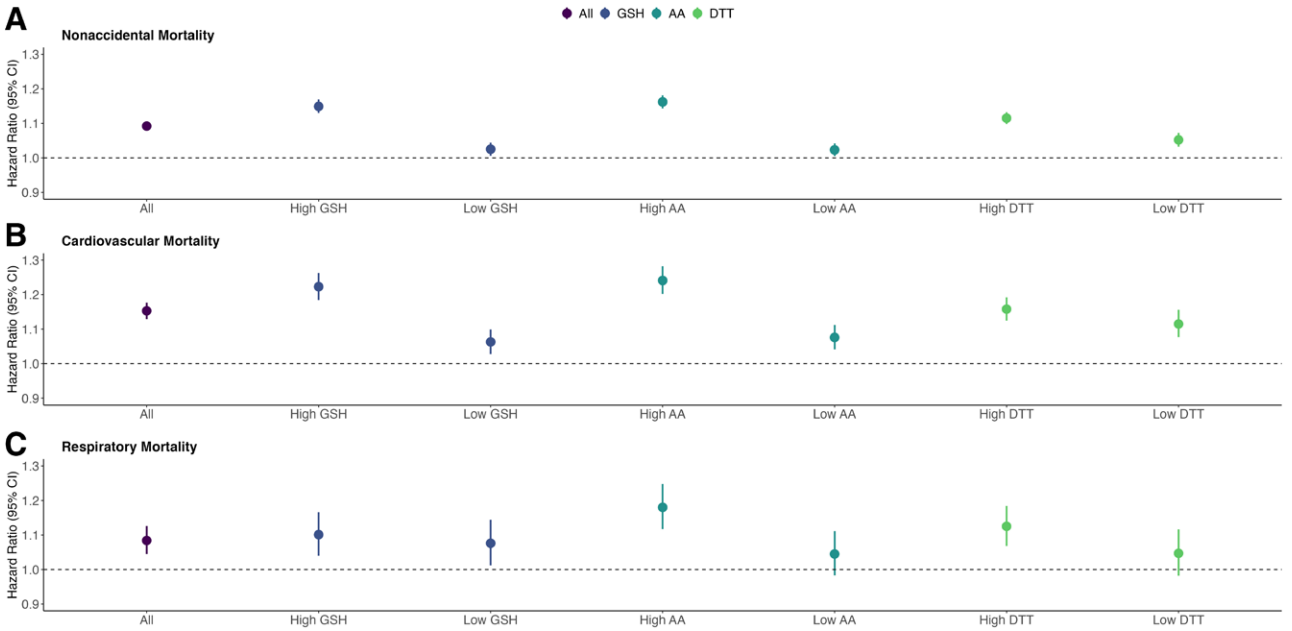

FIGURE 1. Hazard ratios (95% CI) for O_x concentrations (per 6.27 ppb) and nonaccidental, cardiovascular, and respiratory mortality across strata of $PM_{2.5}$ oxidative potential (glutathione (GSH), ascorbate (AA), and dithiothreitol (DTT)).

FIGURE 2. Hazard ratios (95% CI) for O_x concentrations (per 6.27 ppb) and nonaccidental, cardiovascular, and respiratory mortality across strata of $PM_{2.5}$ transition metals and sulfur.

concentrations and mortality when $PM_{2.5}$ oxidative potential and mass proportions of $PM_{2.5}$ transition metals/sulfur were elevated. This pattern was most apparent for nonaccidental and cardiovascular mortality; fewer cases were available for respiratory mortality and hazard ratios for this outcome were less precise. The consistency of this observation is likely explained by the fact that all of the metals included in the analysis were selected *a priori* based on previous associations with OP[1,2,18,19] and the fact that they were all moderately to highly correlated with each other. Moreover, differences in associations across levels of OP were greatest for OP^{GSH} and OP^{AA} (which were more strongly correlated with metals in $PM_{2.5}$ than OP^{DTT}), again suggesting an important role for redox-active metals in the observed pattern of effect modification. For the specific components examined, differences were greatest across strata of mass proportions of Ni and S in

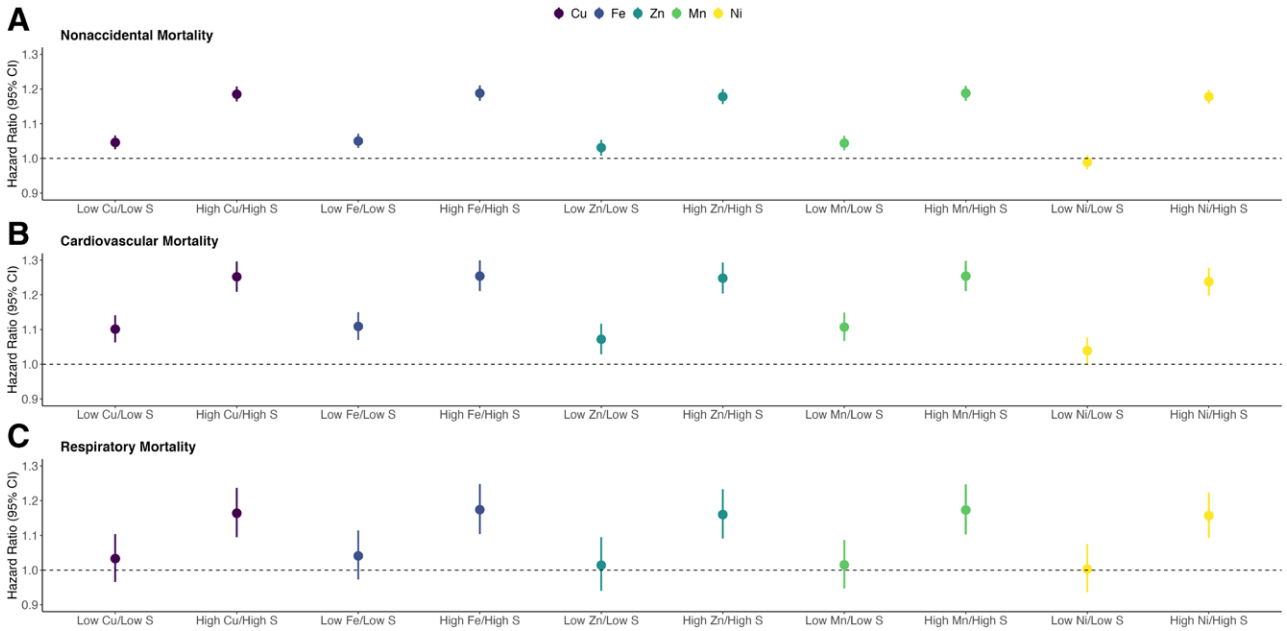

FIGURE 3. Hazard ratios (95% CI) for O_x concentrations (per 6.27 ppb) and nonaccidental (A), cardiovascular (B), and respiratory mortality (C) across strata of both $PM_{2.5}$ transition metals and sulfur.

$PM_{2.5}$. A similar pattern was detected when we examined O_x-mortality association across strata of *both* metals and sulfur, with stronger associations observed when mass proportions of both metals and sulfur were elevated. In this analysis, O_x was only positively associated with respiratory mortality (i.e., 95% CIs excluded the null) when both S and metals were above median values. Collectively, our findings suggest that associations between long-term exposure to O_x and mortality may be modified by coexposure to outdoor $PM_{2.5}$ OP and mass proportions of transition metals and sulfur in $PM_{2.5}$.

We did not identify other cohort studies examining how outdoor $PM_{2.5}$ components/characteristics may modify the long-term health impacts of O_x. Anderson et al[28] examined how specific $PM_{2.5}$ components (i.e., sulfate, nitrate, silicon, elemental carbon, organic carbon, sodium, and ammonium) may confound ozone-mortality associations but did not evaluate how these components *modify* ozone-mortality relationships. Nevertheless, considerable heterogeneity exists in the current literature examining the relationship between oxidant gases and mortality,[9] and our findings suggest that spatial variations in outdoor $PM_{2.5}$ components/characteristics may be a contributing factor to differences observed across studies.

However, it is important to note that there were weak to moderate correlations between O_x and $PM_{2.5}$ OP/components ($-0.13 < r < 0.60$) and thus we cannot rule out that at a small portion of the observed patterns of associations for O_x were due to correlations with potentially harmful $PM_{2.5}$ components. Nevertheless, as shown in the maps displayed in eFigure S1; http://links.lww.com/EDE/B958, study locations with high/low OP and high/low transition metals were distributed across Canada (e.g., the distance between Whitehorse (Yukon) and St John's (Newfoundland) is approximately 5,000 km and both were classified as having high OP^{GSH}; the distance between Fredericton (New Brunswick) and St. John (New Brunswick) is approximately 100 km and they had different classifications for all three measures of OP), and it seems unlikely that our results are explained by O_x simply being a marker for a specific type of localized air pollution mixture.

If the observed health impacts of O_x are attributable the combined oxidative nature of the gases themselves, our results have important implications for future regulatory interventions aimed at reducing emissions of individual $PM_{2.5}$ components/sources. Specifically, our results suggest that there could be substantial cobenefits to further reductions in transition metals and sulfur in $PM_{2.5}$ as these reductions could reduce the health impacts of outdoor $PM_{2.5}$[8] as well as the long-term health effects of O_x. Of the specific components examined, differences across strata were greatest for Ni and S. Fuel oil combustion is an important source of Ni in $PM_{2.5}$[29] and both transportation and industrial sources contribute to sulfur (i.e., sulfate) in $PM_{2.5}$ (although sulfur emissions have decreased over time).[30] Other transition metals like Cu and Fe are emitted from nontail pipe sources like brake wear,[31] which will be an increasingly important contributor to outdoor $PM_{2.5}$ as the vehicle fleet transitions to electricity.[32] These sources are logical targets for future regulatory interventions related to transition metals in outdoor $PM_{2.5}$.

Although this study had many strengths including a large population-based cohort and prospective ground-level measurements of outdoor $PM_{2.5}$ oxidative potential and components it is important to recognize several limitations. First, ground-level measurements of $PM_{2.5}$ components and OP

were collected at the end of the follow-up period (2016–2018), and we assumed a stable spatial distribution of these factors over the duration of the follow-up period (2001–2016). As a result, if there were dramatic changes in sources of outdoor $PM_{2.5}$ components/OP in a given region over time, it is possible that some locations were misclassified in terms of being above/below median values for long-term estimates of $PM_{2.5}$ components/OP. If this error was independent of O_x concentrations, nondifferential error would tend to diminish observed effect modification across strata of $PM_{2.5}$ components/OP (i.e., because some locations that were actually "high" would be classified as "low" and some locations that were actually "low" would be classified as "high", in random fashion).[26] In this case, it is possible that differences observed across strata are in fact larger than reported in our analyses. Alternatively, if this error was differential (e.g., places with high O_x tended to be misclassified more often than places with low O_x or *vice versa,* although O_x levels were similar across strata) it could bias the pattern of effect modification in either direction. For example, if all locations above the median $PM_{2.5}$ metals were classified correctly but a large portion of locations classified as low in 2016–2018 were in fact high for most of follow-up (e.g., because of emission reductions over time) this too would tend to minimize differences across strata. A related issue concerns correlations between outdoor O_x concentrations and long-term estimates of $PM_{2.5}$ OP and mass proportions of $PM_{2.5}$ metals which were weak to moderate ($-0.13 < r < 0.60$); thus, we cannot rule out the possibility that a small portion of the observed pattern of association for O_x is attributable to correlations with potentially harmful $PM_{2.5}$ components. Importantly, we did update O_x exposures over time to incorporate changes in outdoor concentrations with residential mobility. Finally, as noted above, we did not have individual-level data for smoking or obesity in the CanCHEC cohort but ancillary data from the CCHS cohort suggests that residual confounding by these factors is not a likely explanation for our results as weak inverse correlations exist between outdoor O_x concentrations and these variables in the CCHS data (which is also a population-based Canadian cohort). More generally, as our primary exposure was an area-level measure of outdoor O_x concentrations, unmeasured individual-level factors are not a likely source of confounding bias in our analysis.[33]

In summary, we observed consistently stronger associations between outdoor O_x concentrations and mortality in Canada in regions with elevated $PM_{2.5}$ oxidative potential and $PM_{2.5}$ transition metal/sulfur content. Additional studies are needed to confirm these results in other locations, but our findings suggest that the characteristics/components of outdoor $PM_{2.5}$ may modify the long-term health impacts of O_x. This possibility should be explored in future population-based studies as there may be substantial co-benefits (i.e., reduced health impacts attributable to O_x) to directly targeting specific components/characteristics of outdoor $PM_{2.5}$ in future regulatory interventions.

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