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$_3$ 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$_3$ 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$_3$ 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$_3$ 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 = 0.60, 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: O$_3$; Mortality; PM$_{2.5}$ components; Oxidative potential; Cohort study

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Outdoors 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. Although PM$_{2.5}$ mass concentrations are recognized as an important contributor to the overall burden of disease, 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. 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...
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. 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$_3$ 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). 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 I52) 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:

$$O_x = \left(\frac{1.07 \times NO_2 + 2.075 \times O_3}{3.145}\right)$$

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). Hourly O$_3$ model output was fused with ground monitor data; 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$^2$. 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 (µg/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). 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. 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: Anzac, Athabasca Valley, Calgary, Edmonton, 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. 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). Therefore, we wanted to examine how combinations of these components may modify the health impacts of O$_3$.

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 (OPAA and OPGSH) were assessed using the acellular respiratory tract lining fluid (RTLF) OP assay. Briefly, 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$_3$ concentrations and mortality outcomes across strata defined by ground-level measurements of oxidative potential (OPGSH, OPAA, and OPDTT) (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; thus, we felt this decision was conservative in that we would not overestimate possible differences across strata (assuming misclassification was independent of O$_3$). 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$_3$), 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. Individual-level data on smoking and body mass index (BMI) are not available in CanCHEC; however, we did evaluate correlations between outdoor $O_3$ concentrations and smoking (6 levels: never, former-occasional, former-daily, 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 $O_3$ 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 $O_3$.

All hazard ratios for outdoor $O_3$ are expressed per inter-quartile change in outdoor concentration (6.27 ppb); interaction $p$-values were estimated by including an interaction term between $O_3$ and indicator variables for strata. $O_3$ 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 µg/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 $O_3$ concentration was 28.75 ppb (5th = 20.41 ppb; 95th = 36.58 ppb) and $O_3$ 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 $O_3$ concentrations were moderately correlated with outdoor PM$_{2.5}$ mass concentrations ($r = 0.62$). Correlations between outdoor $O_3$ 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 $O_3$ 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 µg/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 $O_3$ 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 $O_3$ 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 $O_3$ 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 $O_3$ 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).

Hazard ratios for $O_3$ 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

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

(Continued)
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 \(\text{O}_x\) 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 \(\text{O}_x\) 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 \(\text{O}_x\) and mortality and we noted several interesting results.

First, our findings demonstrated a clear and consistent pattern of stronger associations between outdoor \(\text{O}_x\) and mortality.
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 1.** Hazard ratios (95% CI) for Ox 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 Ox concentrations (per 6.27 ppb) and nonaccidental, cardiovascular, and respiratory mortality across strata of 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 co-benefits to further reductions in transition metals and sulfur in PM$_{2.5}$ as these reductions could reduce the health impacts of outdoor PM$_{2.5}$ 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$_{2.5}$ 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 non-tail 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

**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.
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). 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 CCCHS 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 CCCHS 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.

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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