Within-city spatial variations of novel air pollution exposure metrics and their relationship with cardiovascular mortality and brain cancer incidence in the Canadian urban environment

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Abstract

Outdoor air pollution, including fine particulate matter ($PM_{2.5}$) air pollution, contributes to a range of adverse health outcomes and has a large population health impact. However, the standard method of measuring exposures to particulate air pollution as a mass concentration has limitations. Recently, emerging measures have been developed that account for the composition and toxicity of particles. The overall aim of this thesis was to describe within-city spatial variations in newly-developed measures of particle composition and toxicity (including multiple measures of particle oxidative potential as well as a measure of exposure to magnetite nanoparticles) across Canadian urban areas and to assess their effects on long-term health outcomes. To accomplish this aim, we completed three objectives that constitute the body of this manuscript-based thesis.

In Objective 1, we conducted monitoring campaigns at 124 sites in Montreal and 110 sites in Toronto, Canada to collect pollutant data, and developed land-use regression models to predict the spatial distributions of $PM_{2.5}$ oxidative potential, production of reactive oxygen species, and magnetite nanoparticles. We used Bayesian lasso regression models with land-use characteristics from Geographic Information Systems databases to predict pollutant measures at unobserved points in order to create high-resolution exposure surfaces. We observed high spatial variability of oxidative potential measures (coefficients of variation 42.0-66.0%) and magnetite (coefficients of variation 69.7-75.4%) within each city relative to $PM_{2.5}$ mass concentration (coefficients of variation 24.3-30.8%). Multivariable land-use regression models predicted elevated concentrations of oxidative potential, reactive oxygen species generation, and magnetite around highways, railways, and road intersections.

In Objective 2, we applied the estimates of exposure obtained in Objective 1 to determine how oxidative potential and the ability of particles to generate reactive oxygen species (ROS) may modify the relationship between long-term exposure to oxidant gases and cardiovascular mortality. We performed a retrospective cohort study of participants in the Canadian Census Health and Environment Cohort who lived in Toronto or Montreal. We used Cox proportional hazards models to estimate associations between outdoor concentrations of oxidant gases (O_x, a redox-weighted average of nitrogen dioxide and ozone concentrations) and cardiovascular deaths. Analyses were performed across strata of $PM_{2.5}$ oxidative potential and ROS concentrations. We observed that spatial variations in outdoor O_x were associated with an increased risk of cardiovascular mortality (HR per 5 ppb = 1.028, 95% CI: 1.001, 1.055). The effect of Ox on cardiovascular mortality was stronger above the median of each measure of $PM_{2.5}$ oxidative potential and ROS concentration (e.g., above the median of glutathione-based oxidative potential: HR = 1.045, 95% CI: 1.009, 1.081; below median: HR=1.000, 95% CI: 0.960, 1.043).

In Objective 3, we performed a retrospective cohort study in the Canadian Census Health and Environment Cohort to estimate associations between long-term exposure to magnetite nanoparticles in $PM_{2.5}$ and the incidence of brain cancer. Cox proportional hazards models were used to estimate the association between exposure to magnetite nanoparticles in outdoor $PM_{2.5}$ and incidence of brain cancer in Montreal and Toronto. We found no significant relationship between exposure to magnetite particles and incidence of malignant brain tumours (HR per IQR = 0.998, 95% CI: 0.988, 1.009). Moreover, we found no significant effect of $PM_{2.5}$ or NO_2 on brain cancer incidence.

Overall, these findings demonstrate the spatial variability of several emerging measures of particulate air pollution within the Canadian urban environment. As well, they highlight the potential for population health impacts of air pollution exposures within cities.

Résumé

La pollution de l'air extérieur, y compris la pollution atmosphérique par les particules fines ($PM_{2,5}$), contribue à une gamme d'effets néfastes sur la santé et a un impact important sur la santé de la population. Cependant, la méthode standard de mesure des expositions à la pollution atmosphérique particulaire en tant que concentration massique a des limites. Récemment, des mesures émergentes ont été développées qui tiennent compte de la composition et de la toxicité des particules. L'objectif général de cette thèse était de décrire les variations spatiales intra-urbaines des mesures nouvellement développées de la composition et de la toxicité des particules (y compris de multiples mesures du potentiel oxydatif des particules ainsi qu'une mesure de l'exposition aux nanoparticules de magnétite liées à la combustion) dans les zones urbaines canadiennes. et d'évaluer leurs effets sur les résultats de santé à long terme. Pour atteindre cet objectif, nous avons rempli trois objectifs qui constituent le corps de cette thèse manuscrite.

Dans l'objectif 1, nous avons mené des campagnes de surveillance sur 124 sites à Montréal et 110 sites à Toronto, au Canada, pour collecter des données sur les polluants et développer des modèles de régression de l'utilisation des terres pour prédire les distributions spatiales du potentiel oxydatif des $PM_{2,5}$, la production d'espèces réactives de l'oxygène et nanoparticules de magnétite. Nous avons utilisé des modèles bayésiens de régression lasso avec

des caractéristiques d'utilisation des terres provenant de bases de données de systèmes d'information géographique pour prédire les mesures de polluants à des points non observés afin de créer des surfaces d'exposition lissées. Nous avons observé une grande variabilité spatiale des mesures du potentiel oxydatif (coefficients de variation 42,0-66,0 %) et de la magnétite (coefficients de variation 69,7-75,4 %) dans chaque ville par rapport à la concentration massique de $PM_{2,5}$ (coefficients de variation 24,3-30,8 %). Des modèles de régression multivariés de l'utilisation des terres ont prédit des concentrations élevées de potentiel oxydatif, de génération d'espèces réactives de l'oxygène et de magnétite autour des autoroutes, des voies ferrées et des intersections routières.

Dans l'objectif 2, nous avons appliqué les estimations d'exposition obtenues dans l'objectif 1 pour déterminer comment le potentiel oxydatif et la capacité des particules à générer des espèces réactives de l'oxygène (ROS) peuvent modifier la relation entre l'exposition à long terme aux gaz oxydants et la mortalité cardiovasculaire. Nous avons réalisé une étude de cohorte rétrospective des participants de la cohorte santé et environnement du recensement canadien qui vivaient à Toronto ou à Montréal. Nous avons utilisé des modèles de risques proportionnels de Cox pour estimer les associations entre les concentrations extérieures de gaz oxydants (Ox, une moyenne pondérée redox de dioxyde d'azote et d'ozone) et les décès cardiovasculaires. Des analyses ont été effectuées sur les strates du potentiel oxydatif des $PM_{2,5}$ et des concentrations de ROS. Nous avons observé que les variations spatiales du Ox extérieur étaient associées à un risque accru de mortalité cardiovasculaire (HR pour 5 ppb = 1,028, IC à 95 % : 1,001, 1,055). L'effet d'O_x sur la mortalité cardiovasculaire était plus fort au-dessus de la médiane de chaque mesure du potentiel oxydatif des PM2,5 et de la concentration de ROS (par

exemple, au-dessus de la médiane du potentiel oxydatif à base de glutathion : HR = 1,045, IC à 95 % : 1,009, 1,081 ; en dessous médiane : HR = 1,000, IC à 95 % : 0,960, 1,043).

Dans l'objectif 3, nous avons réalisé une étude de cohorte rétrospective dans la cohorte santé et environnement du recensement canadien pour estimer les associations entre l'exposition à long terme aux nanoparticules de magnétite dans les $PM_{2,5}$ et l'incidence du cancer du cerveau. Des modèles à risques proportionnels de Cox ont été utilisés pour estimer l'association entre l'exposition aux nanoparticules de magnétite dans les $PM_{2,5}$ extérieures et l'incidence du cancer du cerveau à Montréal et à Toronto. Nous n'avons trouvé aucune relation significative entre l'exposition aux particules de magnétite et l'incidence des tumeurs cérébrales malignes (HR par IQR = 0,998, IC à 95 % : 0,988, 1,009). De plus, nous n'avons trouvé aucun effet significatif des $PM_{2,5}$ ou du NO_2 sur l'incidence du cancer du cerveau.

Dans l'ensemble, ces résultats démontrent la variabilité spatiale de plusieurs mesures émergentes de la pollution atmosphérique particulaire dans l'environnement urbain canadien. De plus, ils mettent en évidence le potentiel d'impacts sur la santé de la population des expositions à la pollution de l'air dans les villes.

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Contributions of Authors

Manuscript 1: Ripley S, Minet L, Zalzal J, Pollitt KJG, Gao D, Lakey PSJ, Shiraiwa M, Maher BA, Hatzopoulou M, Weichenthal S.

SR organized the field work, coordinated the study, performed statistical analyses and wrote the manuscript. LM and JZ extracted land use variables from Geographic Information Systems databases. KJGP and DG performed oxidative potential analyses. MS and PSJL provided estimates of reactive oxygen species generation from the mathematical KM-SUB-ELF model. BAM supervised magnetic analyses. MH assisted with design and coordination of field work. SW funded the study, oversaw all aspects of data collection and statistical analyses, and provided critical revisions of the manuscript. SR wrote the first draft of the manuscript and all authors participated in revising the manuscript.

Manuscript 2: Ripley S, Gao D, Pollit KJG, Lakey PSJ, Shiraiwa M, Hatzopoulou M, Weichenthal S.

SW conceptualized the study. SR refined the research question, prepared the data, performed all statistical analyses and wrote the manuscript. KJGP and DG led the oxidative potential analyses and advised on the oxidative potential data. MS and PSJL provided estimates of reactive oxygen species generation from the mathematical KM-SUB-ELF model. MH assisted with design and coordination of field work. SW funded the study, oversaw statistical analyses, and provided critical revisions of the manuscript. SR wrote the first draft of the manuscript and all authors reviewed and revised the final draft.

Manuscript 3: Ripley S, Maher BA, Hatzopoulou M, Weichenthal S.

SW conceptualized the study. SR refined the research question, prepared the data, performed all statistical analyses and wrote the manuscript. BAM supervised magnetic analyses and contributed to interpretation of the magnetite results. MH assisted with design and coordination of field work. SW funded the study, oversaw statistical analyses, and provided critical revisions of the manuscript. SR wrote the first draft of the manuscript and all authors reviewed and revised the final draft.

Contribution to Original Knowledge

Manuscript 1: This manuscript describes the spatial distribution of oxidative potential, reactive oxygen species generation, and anhysteretic remanent magnetization of fine particulate matter and identifies land-use predictors of these particle characteristics. These analyses use new data that I collected in exposure campaigns in 2018 and 2019. These exposures had not previously been measured in Montreal; although previous studies were conducted in Toronto, my manuscript contains data on a larger number of sites than previous studies. Further, the anhysteretic remanent magnetization of fine particulate matter had not previously been described in Canada.

Manuscript 2: This manuscript evaluates whether the association between exposures to long-term oxidant gases and cardiovascular mortality in Montreal and Toronto is modified by particle oxidative potential or reactive oxygen species generation. Some evidence supports the hypothesis that oxidative potential is an important effect modifier at the regional scale, but this question has not been evaluated within cities.

Manuscript 3: This manuscript assesses the association between anhysteretic remanent magnetization of fine particulate matter, a surrogate measure of magnetite nanoparticle concentrations, and incidence of brain cancer. This is the first study to assess the population health effects of exposures to magnetite nanoparticles.

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List of Abbreviations/Acronyms

AA: Ascorbate

ARM: Anhysteretic remanent magnetization

BMI: Body mass index

CanCHEC: Canadian Census Health and Environment Cohort

CAN-Marg: Canadian Marginalization Index

CANUE: Canadian Urban Environmental Health Research Consortium

CCHS: Canadian Community Health Survey

CI: Confidence interval

CIHR: Canadian Institutes of Health Research

COVID-19: 2019 novel coronavirus

Cu: Copper

CVD: Cardiovascular disease

DAG: Directed acyclic graph

DC: Direct current

DNA: Deoxyribonucleic acid

DTT: Dithiothreitol

ELF: Epithelial lining fluid

EPA: United States Environmental Protection Agency

ESR: Electron Spin Resonance

Fe: Iron

FSA: Forward Sortation Area

GAMM: Generalized additive mixed model

GSH: Reduced glutathione

HR: Hazard ratio

H_2O_2: Hydrogen peroxide

ICD-10: International Classification of Diseases, 10^{th} revision

IQR: Interquartile range

IRM: Isothermal remanent magnetization

IRR: Incidence rate ratio

K_{ARM}: Anhysteretic remanent magnetization normalized by volume

KM-SUB-ELF: Kinetic multilayer model for aerosol surface and bulk chemistry in epithelial lining fluid

Lasso: Least absolute shrinkage and selection operator

LT: Low temperature

LUR: Land-use regression

MDI: Material Deprivation Index

MOVES: Motor Vehicle Emission Simulator

NAPS: National Air Pollution Surveillance

NO: Nitric oxide

NO_X: Nitrogen oxides

NO_2: Nitrogen dioxide

NPRI: National Pollutant Release Inventory

OB: Oxidative burden

OH: Hydroxyl radical

OP: Oxidative potential

OP^{AA}: Oxidative potential measured by depletion of ascorbate

OP^{DTT}: Oxidative potential measured by depletion of dithiothreitol

OP^{ESR}: Oxidative potential measured by the electron spin resonance assay

OP^{GSH}: Oxidative potential measured by depletion of reduced glutathione

O_X: Redox-weighted combined effect of oxidant gases (i.e., ozone and nitrogen dioxide)

O_2: Oxygen

O_3: Ozone

PM: Particulate matter

$PM_{2.5:}$ Particulate matter with aerodynamic diameter less than 2.5 µm

$PM_{10:}$ Particulate matter with aerodynamic diameter less than 10 µm

PTFE: Polytetrafluoroethylene

QICSS: Quebec Interuniversity Centre for Social Statistics

Redox: Reduction-oxidation

ROS: Reactive oxygen species

RR: Risk ratio

RT: Room temperature

RTLF: Respiratory tract lining fluid

SD: Standard deviation

SES: Socioeconomic status

UFPs: Ultrafine particles (i.e., particles having diameter of 100 nm or less)

UK: United Kingdom

UPAS: Ultrasonic Personal Air Sampler

VOC: Volatile organic compounds

X_{ARM}: Anhysteretic remanent magnetization normalized by sampled particle mass

Chapter 1: Introduction

1.1 Background

Air pollution is a complex mixture of gases and suspended solids; the composition and concentration of these components vary over both time and space. Although technological advances have reduced the burden of outdoor air pollution in many parts of the world, air pollution remains an important public health hazard.[1] Air pollution is among the leading causes of morbidity and mortality worldwide[2] and is a ubiquitous exposure that affects entire populations. Notably, fine particulate air pollution ($PM_{2.5}$), which comprises solid suspended particles with an aerodynamic diameter of less than 2.5 µm, is known to contribute to a wide range of health outcomes.

$PM_{2.5}$ originates from a variety of combustion- and friction-related processes, both natural and anthropogenic, and is conventionally measured as a mass concentration (i.e., the total mass of particles having aerodynamic diameter less than 2.5 µm within a cubic meter of air, $\mu g/m^3$). This has been the basis for scientific measurement and regulation of $PM_{2.5}$ in Canada.[3] However, the mass-based measure of $PM_{2.5}$ has limitations since it treats all particles of a certain size fraction as equal in terms of their public health importance. In reality, $PM_{2.5}$ is not a single chemical entity, but rather a heterogeneous mixture of particles which derive from different sources and have different composition and toxicity.[4] Recently, research attention has turned to measures of particle composition and toxicity that move beyond $PM_{2.5}$ mass concentration alone with the goal of approaching a more biologically relevant exposure measure.[5-7]

Emerging measures of particulate air pollution exposure that account for the composition of particles have been an increasing focus of research.[6] These include *in vitro* laboratory measures of particle toxicity, such as oxidative potential;[8] measures of particle composition; estimates of the production of toxic species in the body using mathematical simulations;[9] and assessment of the magnetic properties of particles,[10, 11] among others. In recent years, several of these novel exposure measures have been described in terms of their spatial and temporal variability and have been linked to health outcomes in epidemiologic studies.[12, 13]

While there is a growing body of evidence on spatial and temporal variations in particle toxicity and composition at a regional scale, as well as the effects of these exposures on health outcomes, less is known about how these relatively novel measures vary at a smaller spatial scale, such as within cities. While $PM_{2.5}$ mass concentrations tend to vary on a regional scale, we expect that local-scale variations may be greater for these novel measures of particle toxicity and composition, given our understanding that local pollution sources affect the composition of particles (for example, metal-rich particles are produced by vehicular traffic and can be found in abundance near roadways and railways, with decreasing concentrations as distance from the source increases).[14] Given that the majority of the population of Canada lives in urban areas,[15] urban outdoor air pollution exposure has a large population health impact in Canada. Therefore, there is a need for more knowledge about how pollutants vary at a fine spatial scale in urban environments, and the implications for population health.

1.2 Research objectives and thesis structure

The overall goal of this thesis is to describe the spatial distributions of novel air pollution measures (fine particle oxidative potential, reactive oxygen species generation, and anhysteretic remanent magnetization susceptibility) across Canadian urban areas, and to apply these models in population-based cohort studies to identify particle characteristics that pose a risk to population health. The specific aims are the following:

> **Objective 1:** To develop land-use regression models for several emerging measures of particle toxicity and composition, namely $PM_{2.5}$ oxidative potential, reactive oxygen species generation, and magnetite nanoparticle composition, and to classify clusters of monitoring sites to identify possible sources associated with these particle characteristics.

To address objective 1, we conducted large-scale spatial monitoring campaigns in Montreal and Toronto, Canada's two largest cities, using a dense network of air pollution monitoring sites. We used these data to build land-use regression models and predict spatial variations at unobserved points across the study area. This objective is addressed in Chapter 3: Manuscript 1.

> **Objective 2:** To examine how within-city spatial variations in $PM_{2.5}$ oxidative potential and reactive oxygen species generation influence associations between long-term exposures to oxidant gases and cardiovascular mortality in Toronto and Montreal, Canada.

To address objective 2, we performed a retrospective cohort study of participants in four cycles of the Canadian Census Health and Environment Cohort. Our study population included

participants who lived in Toronto or Montreal for at least two years in the period 2002-2015. We estimated associations between outdoor concentrations of oxidant gases (specifically defined as a redox-weighted average of nitrogen dioxide and ozone) and mortality from cardiovascular causes. Analyses were performed across strata of three measures of $PM_{2.5}$ oxidative potential adjusting for relevant confounding factors such as individual-level and contextual socioeconomic status and demographic variables. This objective is addressed in Chapter 4: Manuscript 2.

> Objective 3: To estimate associations between exposure to fine particle anhysteretic remanent magnetization susceptibility (a measure of magnetite nanoparticle content) and brain cancer incidence.

To address objective 3, we performed a second retrospective cohort study of participants in four cycles of the Canadian Census Health and Environment Cohort. We followed participants from 2001 to 2016 in Toronto, and from 2001 to 2010 in Montreal. In this study, we estimated associations between exposure to anhysteretic remanent magnetization susceptibility of $PM_{2.5}$ samples (a surrogate measure of magnetite nanoparticle concentrations) and brain cancer incidence. As a secondary objective, we estimated the relationships of long-term $PM_{2.5}$ and NO_2 exposures with brain cancer incidence and examined if these relationships were modified by anhysteretic magnetic remanence susceptibility. This objective is addressed in Chapter 5: Manuscript 3.

Chapter 2: Literature Review

2.1 Components of air pollution exposure

Air pollution is among the top causes of premature death globally[16] and was associated with an estimated 6.67 million premature deaths in 2019.[16] Air pollution is a complex mixture of solid and gaseous components derived from a variety of different sources. This section of the literature review will provide a brief overview of several major types of ambient air pollution, namely fine particulate matter ($PM_{2.5}$) and oxidant gases (NO_2 and O_3).

2.1.1 Fine particulate matter ($PM_{2.5}$)

Particulate matter is the solid component of air pollution consisting of particles suspended in the air. Measuring and reducing exposures to particulate matter, most commonly fine particles, defined as particles with aerodynamic diameter less than 2.5 µm ($PM_{2.5}$), has been a focus of regulation in Canada as well as globally.[3, 17, 18] These particles are of notable health impact since their small size allows them to penetrate deep into the human respiratory tract.[19] Many epidemiologic studies of air pollution have examined relationships between exposures to particle mass concentrations (i.e., mass per volume of air) and various adverse health outcomes, in part because particle mass concentration (and especially $PM_{2.5}$) has been the most routinely measured pollutant for which data are available to be linked to health outcomes at the population level.[20] In Canada, concentrations of $PM_{2.5}$ are low relative to the global average, and have decreased over past decades (from approximately 10 µg/m^3 in 1990 to 7 µg/m^3 in 2019).[16] Nonetheless, much of Canada still exceeds[21] the $PM_{2.5}$ guideline of 5 µg/m^3 for health-based air quality set by the World Health Organization.[22]

2.1.2 Oxidant gases

In addition to particulate matter, the air pollution mixture also consists of gaseous components. Among these, two important gases are nitrogen dioxide (NO_2) and ground-level ozone (O_3). NO_2 in particular is among Canada's criteria air contaminants (i.e., air pollutants for which ambient air quality standards have been set).[23] NO_2 in urban areas is generated mainly from combustion processes, primarily vehicular exhaust, and is a marker of traffic-related air pollution.[24] NO_2 varies spatially and temporally in response to traffic patterns, local wind patterns, and land use.[25] Annual average outdoor NO_2 concentrations in Canada have decreased over time from approximately 20 ppb in 1988 to 11 ppb in 2013, largely due to regulations on vehicular emissions.[26] Long-term exposure to NO_2 has been linked to a number of adverse health outcomes including nonaccidental mortality and mortality from specific disease categories (including cardiovascular, lung cancer, cerebrovascular, diabetes, and respiratory causes).[27, 28] However, health effects are heterogeneous across different studies and geographic areas, and there remains debate regarding the role of NO_2 and the degree to which it is directly responsible for observed health effects, rather than being a marker for the effects of other toxic pollutants (e.g., fine particles) which are also emitted from the same sources.[29]

Ozone (O_3) at ground level is a secondary pollutant that is a product of complex photochemical reactions which occur between nitrogen oxides and volatile organic compounds in the presence of sunlight, particularly during the warm season.[30,31] These pollutants are emitted primarily by vehicular or industrial sources.[30] Exposure to ground-level ozone is associated with negative health effects including death from cardiovascular/respiratory disease, cerebrovascular disease, and ischemic heart disease.[32] Relative to NO_2, O_3 is a stronger oxidizing

agent.[33, 34] Because it is a strong oxidizing agent, O_3 in the human body plays a role in the oxidative stress pathway[35] which is a hypothesized mechanism by which some air pollutants induce adverse health outcomes (described in Section 2.2 below).

Since O_3 and NO_2 are correlated in space and time (as they have common sources and react together in complex chemical systems; the correlation tends to be inverse),[34] populations are exposed to both pollutants simultaneously and disentangling their effects is difficult. Further, both gases can induce oxidative stress,[36] which is a mechanism linking air pollution exposures to health effects. The combined effect of NO_2 and O_3 can be expressed as a redox-weighted average, O_X, which reflects the concentrations of both gases weighted by their ability to induce oxidative stress (through involvement in reduction-oxidation reactions) and thereby to adversely affect human health. Specifically, O_X is calculated as a weighted average of O_3 and NO_2 based on the following equation: $O_X = ((1.07 \times NO_2) + (2.075 \times O_3)) /3.14$.[33, 37] The weights in this equation represent reduction potential and reflect the greater ability of O_3 to act as an oxidizing agent relative to NO_2.

2.2 Oxidative stress

This section of the literature review will provide a brief description of oxidative stress, an important mechanism by which particulate air pollution and oxidant gases contribute to adverse health outcomes.

2.2.1 Oxidative stress

Oxidative stress is recognized as one of the mechanisms underlying the toxic effects of air pollution.[20, 38] Oxidative stress is characterized by an imbalance between the body's antioxidant

defenses and pro-oxidant free radicals (i.e., atoms with unpaired electrons) including reactive oxygen species (ROS). The body contains antioxidants which react with reactive oxygen species to form secondary products that are less toxic.[39, 40] In particular, the respiratory tract lining fluid, the thin layer of fluid that covers the epithelial surface of respiratory tract, contains high concentrations of antioxidants (such as glutathione, uric acid and ascorbic acid) which act as a first line of defense against inhaled pollutants.[41, 42] However, when the level of free radicals overwhelms the body's antioxidant defenses, surplus free radicals can provoke redox reactions that damage cells and tissues through processes including oxidation of lipids, protein, and genetic material (DNA).[43] Further, free radicals can provoke initiation of a proinflammatory cascade in which an influx of inflammatory cells to the injured site leads to a second wave of oxidative stress when inflammatory cells generate reactive species themselves as part of cellular signalling processes.[44] In short, oxidative stress is both induced by and induces inflammatory processes.[44] Oxidative stress is a plausible mechanism for the pulmonary inflammatory response observed following PM exposure, and may underlie other adverse health effects of air pollution exposure.[38, 45] Many of the pollutants that make up the ambient air pollution mixture can promote free radical reactions (e.g., particulates, O_3).[46]

2.2.2 Oxidative stress and $PM_{2.5}$

$PM_{2.5}$ exposure induces oxidative stress when inhaled particles react with antioxidants present in the respiratory tract lining fluid, depleting these defenses.[45] The ability of $PM_{2.5}$ to generate reactive oxygen species is driven not only by concentration of $PM_{2.5}$, but also by particle composition.[38] $PM_{2.5}$ is composed of particles of widely varying composition and toxicity.

For example, particles can contain transition metals such as iron and copper which are capable of acting as catalysts in the formation of reactive oxygen species through redox reactions.[38] The redox-active components in particles reach target sites in the lungs, vasculature, and heart to induce inflammation and oxidative stress.[47] Failure to overcome oxidative stress leads to the activation of additional cellular signalling cascades regulating the expression of cytokine and chemokine genes; the pro-inflammatory effects that result occur locally in target tissues directly exposed to PM as well as systemically, and can contribute to widespread pro-inflammatory effects remote from the site of damage.[48] Besides acting as a carrier for chemicals involved in redox reactions, each particle may also provide a reaction surface on which redox cycling chemistry can take place.[40] While focussing on individual chemical components (such as transition metals) can be useful to link specific sources of PM to health effects, investigating the individual health effects of specific components remains a challenge since components derived from the same sources tend to be correlated.[49, 50] In addition, particulate matter can contain organic components such as quinones and polycyclic aromatic hydrocarbons which also contribute to oxidative stress.[42] Quinones are highly redox-active molecules that directly reduce oxygen, resulting in the generation of reactive oxygen species, while polycyclic aromatic hydrocarbons indirectly contribute to the formation of reactive oxygen species after being transformed into quinones.[42] Finally, $PM_{2.5}$ also includes relatively non-reactive components that are unlikely to play a large role in the generation of oxidative stress, including dust, sand and sea salt.[51] These inert substances contribute to the mass of particles while having little biological effect.

2.2.3 Oxidative stress and oxidant gases

Unlike $PM_{2.5}$, which varies widely in composition and toxicity, O_3 and NO_2 are individual gaseous species and therefore their ability to generate oxidative stress is determined by their concentrations as their composition is homogenous. Ozone is a highly reactive gas that drives free radical reactions, contributing to the inflammatory cascade.[38, 52]When inhaled, ozone reacts with antioxidant substrates in the respiratory tract lining fluid and is consumed. However, when ozone exposures overwhelm antioxidant defenses, ozone can react with lipids and proteins, which leads to the generation of harmful secondary oxidation products that can initiate inflammatory processes in the lungs.[38] NO_2 is a free radical that reacts with substrates present in the lung lining fluid to produce oxidized species that initiate the secondary signalling cascade which brings an influx of inflammatory cells to the lung; NO_2 also initiates free radical generation, which results in protein oxidation, lipid peroxidation, and cell membrane damage .[38, 53, 54]

2.3 Alternative measures of particle toxicity and composition

This section of the literature review will provide a brief discussion of several different approaches to measuring exposures to $PM_{2.5}$, with a focus on moving beyond the traditional mass concentration measure (i.e., mass of $PM_{2.5}$ per volume of air). The complementary measures of $PM_{2.5}$ discussed in this section allow us to account for the composition and toxicity of particles rather than their mass concentration alone.

2.3.1 Oxidative potential

The ability of PM to generate oxidative stress can be measured through several assays using metric called oxidative potential (OP). OP represents an integrated measure that estimates the capacity of particles to oxidize target molecules by generating redox oxidizing species; a strength of OP is that it can reflect the combined presence and effect of several different correlated species.[5] OP is an important addition to the traditional particle mass measure of PM because $PM_{2.5}$ mass concentration treats all particles of a specific size fraction equally in terms of toxicity, but particles differ in chemical composition and consequently toxicity.[7] Therefore, measures of particle mass alone may not fully reflect the ability of particles to cause adverse health effects.[5] OP is increasingly used as an exposure measure in studies of air pollution health effects.[12]

Several assays exist to quantify OP including assays that measure the ability of PM to deplete antioxidants in a synthetic model of the respiratory tract lining fluid (RTLF).[8] The respiratory tract lining fluid is the first physical interface through which inhaled PM contacts the body. It contains high concentrations of antioxidants including ascorbate (AA)[55] and reduced glutathione (GSH)[56, 57] which constitute key defenses against oxidative stress. The RTLF assay is an acellular chemical model containing physiologically relevant concentrations of these antioxidants. Samples of PM with known mass are incubated for a defined time interval at human body temperature in a synthetic RTLF solution, following which antioxidant concentrations are measured. The extent to which antioxidants are depleted by exposure to PM over time is a direct measure of PM oxidative activity expressed as OP^{AA} (ascorbate-related oxidative potential) or OP^{GSH} (glutathione-related oxidative potential), respectively.[8, 57, 58] These complementary measures of OP respond to the presence of different chemical species in PM.

For example, OP^{AA} is more strongly associated with iron content of particles, while OP^{GSH} is related to aluminum;[59] however, both OP^{AA} and OP^{GSH} are sensitive to copper.[5] Studies frequently examine the effects of multiple measures of OP since there is no consensus on which measure is the most biologically or clinically relevant.[12] In addition to the RTLF assay, other OP assays include those based on the consumption of dithiothreitol (OP^{DTT})[60] or electron spin resonance related to the production of PM-induced hydroxyl radical species (OP^{ESR}).[61] Results from the epidemiologic literature show inconsistent effects when OP is used as an exposure measure in studies of health effects (Figure 2.1).

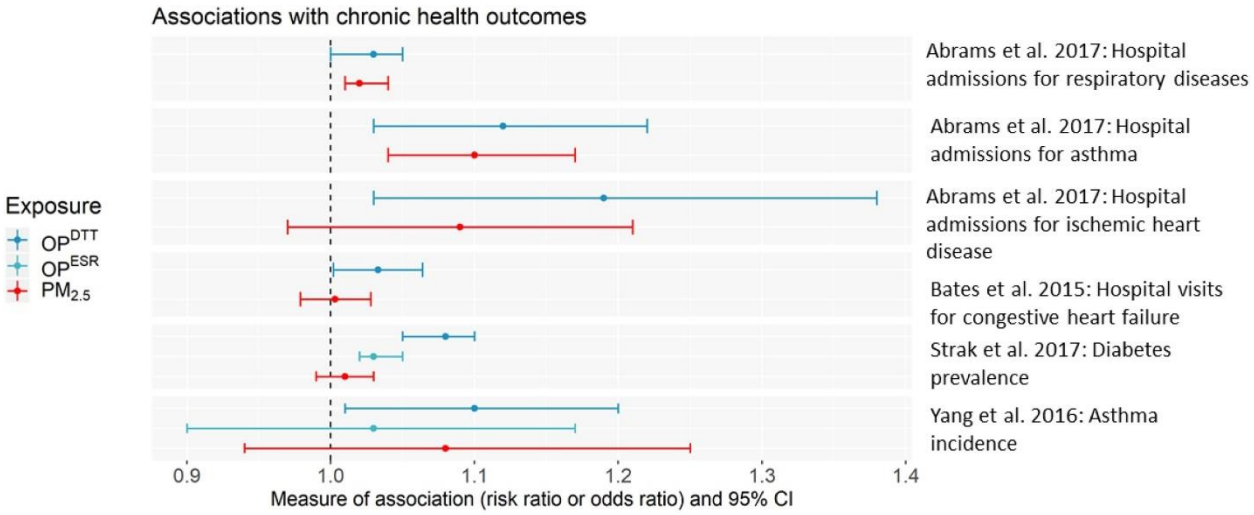

Figure 2.1. Associations of chronic health outcomes with a one-interquartile range increase of particle mass and oxidative potential exposures.[62-65] Figure adapted from Gao et al.[12]

As an alternative approach to the *in vitro* oxidative potential assays, the concentration of reactive oxygen species (ROS) in the epithelial lining fluid resulting from the ability of particles to generate ROS as well as the destruction of ROS by antioxidants can be estimated using a mathematical model based on the content of redox-active components including transition metals in particles.[9] Lakey et al. (2016) developed a mathematical model of the chemistry of the

human respiratory tract, KM-SUB-ELF, which estimates ROS concentrations generated in redox reactions in the epithelial lining in response to the iron and copper content of inhaled particles.[9] The KM-SUB-ELF model simulates mass transport and over 50 chemical reactions and estimates the concentration of ROS produced. This method reflects the combined importance of these two metals in their contribution to the generation of ROS in the lungs and offers an innovative way to estimate the impact of inhaled particles on oxidative stress in the human lung. Determining the individual contributions of specific metal components to health effects is a challenge due to strong correlations between the elements, but this method takes an alternative approach by examining the combined impact of the metals based on a shared mechanism of action. ROS generation estimated by the KM-SUB-ELF model is treated as complementary to the in vitro OP assays; Fang et al. demonstrated that correlations between different ROS species (e.g., H_2O_2, OH) and measures of OP varied substantially.[66] It is also important to note that neither OP assays nor ROS generation estimated from KM-SUB-ELF accounts for the endogenous biological generation of ROS by macrophages, which is a part of cellular signaling processes.[66, 67]

2.3.2 Characterization of particle composition

In addition to their ability to generate oxidative stress, PM samples can also be characterized by particle size and composition. One component of particular interest is the magnetite nanoparticle content of $PM_{2.5}$. Magnetite nanoparticles are small iron oxide particles that are produced during high-temperature combustion and friction processes including tailpipe emissions (e.g., from iron impurities found in fuel), vehicle brake-wear, railways, and industrial combustion processes.[10, 68-70] To assess magnetite content, the magnetic properties of PM

samples can be analyzed by a number of different methods.[71-75] Among these is anhysteretic remanent magnetization (ARM). Substances with remanent magnetization have the ability to remain in a state of magnetization in the absence of an active magnetic field.[14] ARM is obtained by applying an alternating magnetic field to a PM sample and measuring the response of the metals in the sample using a magnetometer.[75] ARM reflects both the concentration and size of magnetic particles.[14]

Magnetite nanoparticles are able to enter the brain directly through the olfactory nerve and have been observed in abundant quantities in human body tissues including the brain,[76] heart,[77] and placenta.[78] Toxicology studies suggest that magnetite nanoparticle exposures are relevant to disease development. In human lung cells *in vitro*, genotoxicity and increased production of ROS were observed in 24 hours after exposure to magnetite nanoparticles.[79] Conversely, some argue that magnetite in the brain does not play a role in the oxidative damage of brain neurons.[80] The population health impacts of exposure have not been assessed in epidemiologic studies. Much uncertainty remains regarding the spatial distribution and health effects of these little-studied particles.[81]

2.4 Within-city variations in $PM_{2.5}$ mass concentration, $PM_{2.5}$ characteristics, and oxidant gases

A growing area of research interest is the degree to which measures of air pollution exposures including particle toxicity and composition vary at small spatial scales, such as within cities. This section will describe the degree of spatial variation observed at the within-cities spatial scale for $PM_{2.5}$ mass, complementary $PM_{2.5}$ exposure measures, and oxidant gases.

2.4.1 Within-city variations in $PM_{2.5}$ mass concentration

$PM_{2.5}$ concentrations show relatively little spatial variation within cities relative to other pollutants.[82-85] $PM_{2.5}$ particles can remain suspended in the atmosphere for days to weeks and can be transported long distances from their original sources.[86] $PM_{2.5}$ is a mix of primary particles formed through combustion and friction processes, and secondary particles formed through atmospheric reactions between precursors.[87] About 80% of $PM_{2.5}$ in the Great Lakes region (including much of southern Ontario, Canada) is secondary, formed through condensation and chemical reactions among precursor species.[88] Consequently, $PM_{2.5}$ mass concentrations depend on regional background levels as well as on local sources.

2.4.2 Within-city variations in particle composition and oxidative potential

Small-scale spatial variations in particle composition are much greater than variations in overall $PM_{2.5}$ mass concentrations owing to greater contributions from local sources.[82, 83] Weichenthal et al. found considerable spatial variations in the concentration of iron and copper in $PM_{2.5}$ samples at the within-city scale in Toronto, Canada.[83] Consequently, the estimated production of reactive oxygen species in the epithelial lung lining fluid, which can be expressed as a function of copper and iron concentrations in particles, was also spatially variable.[83] Particle oxidative potential is also highly variable within cities.[82, 89-91] For example, in a study of PM collected at urban roadway and background locations, Boogard et al. found that the oxidative potential of PM_{10} (i.e., particles with aerodynamic diameter < 10 µm) sampled from major streets was 3.6 times higher than at urban background locations, which exceeded the spatial

contrasts observed for PM mass and all measured PM chemistry characteristics; even greater contrasts were observed between major streets and suburban background locations.[91] Similarly, in a study in Toronto, Ontario, Weichenthal et al. found substantial spatial variations in multiple measures of oxidative potential at the within-city scale.[89] Finally, although spatial distributions of exposures to magnetite nanoparticles are little studied, there is also evidence that they vary sharply in relation to local sources. For example, in a study in Poland, elevated magnetic susceptibility parameters were measured in soil within 3 m from the edge of minor roads and up to 15 m from major roads.[14]

2.4.3 Within-city variations in oxidant gases

Concentrations of NO_2 can be highly variable at small spatial scales. Differences in annual average ambient NO_2 concentrations of more than 50 mg/m^3 (27 ppb) have been observed between sampling locations less than 50 m apart in the United Kingdom.[92] Within Canadian cities, residential exposures to NO_2 vary substantially.[93] Indeed, NO_2 within cities is locally variable enough that NO_2 concentrations are correlated with neighborhood-level measures of socioeconomic status such as low income, low educational attainment, and low dwelling value.[93, 94] Land-use regression models also point to the importance of local sources: in land use regression models for NO_2 in Toronto, variables such as traffic and roadway length within a 300 m buffer, traffic counts, distance to roadways and in particular expressways, and industrial and residential land use were the strongest predictors of NO_2 levels.[94] Within-city variations in NO_2 exposures result from the interaction of a variety of factors, including local wind patterns, traffic patterns, and land use.[25, 95]

O_3 is formed as a secondary pollutant in complex relationships with nitrogen oxides (NO_X, including NO and NO_2) and volatile organic compounds (VOCs) in the presence of sunlight.[96] Although ozone is formed as a product of NO_2, it also combines with NO to form O_2 and is thus removed from the air. Therefore, reductions in NO_2 concentrations can result in increased O_3 concentrations.[97] Ground-level ozone concentrations depend on both local sources and distant sources that influence background levels.[97] When the combined effect of O_3 and NO_2 (i.e., O_X) is considered, the ozone concentrations are effectively a regional phenomenon contributing to the regional background O_X level, whereas the contribution of NO_X is a local phenomenon which correlates with the level of primary pollutants.[97] O_3 and consequently O_X are less spatially variable at fine spatial scales relative to NO_2.[98]

2.5 Long-term air pollution exposure and health outcomes

In this section, a brief overview of the literature linking selected outdoor air pollution exposures to cardiovascular health effects and brain cancer will be provided. This thesis focuses on these two different health outcomes that are both potentially related to long-term outdoor air pollution exposure. First, we will examine cardiovascular mortality, since the effect of air pollution on cardiovascular mortality is largely influenced by the oxidative stress pathway. Our examination of emerging measures of particle toxicity that account for the ability of particles to induce oxidative stress is relevant to this outcome. Second, we will examine brain cancer incidence. In this objective, we are specifically interested in the effects of magnetite nanoparticle exposure on the brain. There is evidence of long-term exposure to nanoparticles in outdoor air pollution causing incident brain cancer;[99] further, given that magnetite nanoparticles can

translocate to the brain and have been found in brain samples,[76] magnetite nanoparticle exposures are plausibly related to neurological dysfunction.

2.5.1 $PM_{2.5}$ and cardiovascular mortality

The association of outdoor particulate air pollution exposure with acute and chronic cardiovascular outcomes is well established.[44] As early as the 1990s, epidemiologic studies showed clear associations between acute and chronic exposures to particulate air pollution and cardiovascular mortality.[100, 101] Subsequent epidemiologic studies have demonstrated consistent associations of $PM_{2.5}$ with cardiovascular morbidity on a global scale.[43] Even at the relatively low exposure levels observed in Canada, $PM_{2.5}$ is robustly associated with a variety of acute and chronic health outcomes including cardiovascular mortality, with exposure effects modelled at concentrations as low as 2 $\mu g/m^3$.[102, 103] For example, a study of 2.6 million Canadians by Pinault et al. estimated that each 10 $\mu g/m^3$ increase in average $PM_{2.5}$ exposure at residential location was associated with a hazard ratio of 1.25 (95% CI: 1.19, 1.31) for cardiovascular mortality over 20 years of follow-up.[104] Similar results were summarized in a systematic review and meta-analysis of the existing evidence on the relationship between long-term $PM_{2.5}$ exposure and cardiovascular mortality which found a pooled risk ratio of 1.11 (95% CI: 1.09, 1.14) per 10 $\mu g/m^3$ increase in $PM_{2.5}$ mass concentrations across 21 studies.[105]

2.5.2 Oxidant gases and cardiovascular mortality

Oxidant gas exposures are also linked to cardiovascular outcomes. Long-term exposures to NO_2[106] and O_3[107] have been associated with deaths caused by cardiovascular disease at

exposure levels typical of those observed in Canada. The combined effect of NO_2 and O_3 (i.e., O_X) has also been identified as potentially modifying the effects of $PM_{2.5}$ exposures. For example, in a Canadian cohort study, O_X modified the effects of $PM_{2.5}$ exposures on cardiovascular mortality, as well as nonaccidental and respiratory mortality, with higher $PM_{2.5}$ effects observed in the highest tertile of O_X, a finding which was attributed to the combined oxidant effect of exposures to both $PM_{2.5}$ and O_X.[37] Similarly, Christidis et al. examined the association of $PM_{2.5}$ with mortality in both low- and high-O_x person-years and found a 24% higher risk in high-O_X person-years.[102] These findings suggest that the oxidative stress induced by particulate and gaseous pollutants may each be magnified in the presence of the other.

2.5.3 Oxidative potential and health outcomes: summary of epidemiologic evidence

Although epidemiologic evidence on the use of $PM_{2.5}$ OP as an exposure measure remains limited, patterns are beginning to emerge as the literature base grows; these were highlighted in our systematic review of the literature published in 2020.[12] Specifically, the OP^{DTT} and OP^{GSH} assays were most consistently associated with adverse health outcomes, including acute and chronic outcomes related to the cardiovascular and respiratory systems.[62-64, 108-111] The OP^{ESR} assay may have some utility but has only been assessed in relation to health outcomes in a small number of studies.[64, 65, 108] OP^{AA} has been linked with biomarkers of cardiovascular and systemic inflammation.[112, 113] However, to date associations remain inconsistent with respect to patterns across different measures of OP. Nonetheless, these studies provide some insight into the potential mechanisms behind the larger-scale clinical effects observed in studies of long-term exposure.

Similarly, some studies have directly assessed the concentrations of reactive oxygen species produced in response to particle exposures using the mathematical KM-SUB-ELF model and have examined relationships between the ROS estimates and health outcomes. Zhang et al. examined associations between long-term exposures to ROS concentrations and the incidence of asthma, chronic obstructive pulmonary disease (COPD), COPD mortality, pneumonia mortality, and respiratory mortality; positive associations were identified between ROS and all outcomes.[114] Likewise, Stieb et al. found a positive association between ROS generation at the neighbourhood level within Toronto and COVID-19 incidence (incidence rate ratio = 1.07; 95% confidence interval, 1.01–1.15 per interquartile range ROS).[115]

2.5.4 Modification of air pollution exposure effects by oxidative potential

Studies of health effects of $PM_{2.5}$ oxidative potential (OP) in Canada have primarily examined how the effects of pollutants may differ across levels of OP (i.e., how OP acts as an effect modifier). Several epidemiologic studies in Canada have evaluated the extent to which spatial differences in OP modify the health impacts of $PM_{2.5}$. I performed a systematic review and narrative synthesis of epidemiologic studies using OP as an exposure metric or effect modifier; results are summarized in brief here but can be accessed in full in the published manuscript.[12] Weichenthal et al. conducted a case-crossover study of the risk of emergency room visits for respiratory causes across 15 cities in Ontario, Canada.[116] Daily average $PM_{2.5}$ mass concentrations were collected from 19 fixed-site monitoring stations and OP^{AA} and OP^{GSH} were estimated from $PM_{2.5}$ samples collected from the same sites. Effect modification by OP^{GSH} was apparent at low $PM_{2.5}$ concentrations (≤10 μg/m^3), suggesting that short-term changes in

$PM_{2.5}$ mass concentrations increase the risk of acute respiratory issues most when OP^{GSH} is high, but this was not observed for OP^{AA}. Similarly, a case-crossover study in 16 cities across Ontario examined emergency department visits for acute myocardial infarction.[117] In this study, short term changes in $PM_{2.5}$ were more strongly associated with acute myocardial infarction in cities with higher OP^{GSH}. Specifically, each 5 $\mu g/m^3$ increase in $PM_{2.5}$ exposure was associated with a 7.9% (95% CI: 4.1, 12) increase in the risk of myocardial infarction in cities above the 90^{th} percentile of OP^{GSH}, whereas a 4.1% (95% CI: 0.26, 8.0) increase was observed in cities above the 75th percentile and no association was observed below the 50th percentile.[117] Evidence of effect modification was not observed for OP^{AA}.

Similar results were also observed in cohort studies. A cohort study in Ontario, Canada examined the impact of oxidative burden (i.e., the product of oxidative potential and $PM_{2.5}$ mass concentration) on the long-term health effects of exposure to ambient $PM_{2.5}$.[118] Oxidative burden was calculated by weighting $PM_{2.5}$ mass concentrations by OP metrics (e.g., glutathione-related oxidative burden = $PM_{2.5}$ mass x OP^{GSH}). Glutathione-related oxidative burden was associated with elevated risks of mortality from cardiometabolic causes (hazard ratio: 1.029, 95% CI: 1.002–1.057) as well as lung cancer and all non-accidental causes. These associations were consistently larger than those for $PM_{2.5}$ not weighted by OP^{GSH}. This effect was not observed for ascorbate-related oxidative burden. Taken together, these studies constitute a body of evidence supporting the idea that $PM_{2.5}$ effects on cardiovascular health may be stronger in areas where particles have greater ability to induce oxidative stress.

The effects of oxidant gases may also be modified by particle oxidative potential. In a case-crossover study across 34 Canadian cities, associations between short-term $PM_{2.5}$ and

oxidant gas exposures and respiratory hospitalizations in children were modified by OP^{GSH}.[49] Specifically, the effect of redox-weighted oxidant gas concentrations (O_X) on children's respiratory hospitalizations were higher when OP^{GSH} was above the median relative to when OP^{GSH} was below the median.[49] Conversely, associations were stronger when OP^{AA} was below the median, and no associations were observed above the median.[49] Further evidence is needed to clarify the health effects of long-term exposures to O_X and how they may be modified by particle characteristics.

At the within-city spatial scale, it remains unclear whether OP modifies the long-term cardiovascular health effects of exposures to oxidant gases or $PM_{2.5}$ mass concentrations. There have been few epidemiologic studies of health effects at this scale. Tonne et al. studied changes in carotid artery intima-media thickness, a measure of subclinical atherosclerosis, in a cohort of 2348 people in London, England; the association for PM_{10} mass weighted by OP^{GSH} (i.e., oxidative burden) was weaker than for PM_{10} mass alone.[111] However, OP estimates were based on 34 sites across greater London, UK, an area of approximately 1500 km^2, and it is possible the spatial variability of OP, which is strongly influenced by local sources, was not captured fully; this could obscure the effect of OP at fine spatial scales.[111]

Overall, these results suggest that interventions to reduce OP may be a way to reduce morbidity related to PM exposure even in areas where PM mass concentrations are already relatively low. A review of the literature suggests that studies using sufficiently spatially dense OP measurements could be useful to inform traditional mass-based interventions by identifying areas where regulation may be most efficient in reducing the public health impacts and related costs of $PM_{2.5}$ exposures.[12] While this approach represents a departure from the standard

method of implementing reductions across all locations, some evidence suggests that risk estimates for $PM_{2.5}$ may vary markedly across regional differences in oxidative potential; consequently, the expected benefits of reductions in $PM_{2.5}$ (and potentially other pollutants such as oxidant gases) may differ greatly between locations and particle oxidative potential may allow regulators to target mass-based interventions most efficiently. This may be especially true in Canada, where $PM_{2.5}$ mass concentrations are relatively low, and many areas share a similar mass concentration but may have meaningful differences in particle composition or biological activity. However, more evidence is needed to strengthen these findings, particularly at fine spatial scales.

2.5.5 $PM_{2.5}$ and brain cancer

While most epidemiologic studies of outdoor air pollution have focused on the respiratory and cardiovascular systems, recent interest has turned to other systems including the brain. For example, a cohort study in Toronto and Montreal found that concentrations of ultrafine particles (<100 nm in diameter) at residential locations were associated with an increased incidence of brain tumours, although the relationship was not observed for $PM_{2.5}$ mass concentrations.[99] A Danish study found positive associations between primary carbonaceous particles in $PM_{2.5}$ (components indicating a combustion source) and brain cancer incidence.[119] However, other studies have reported null or inconclusive results. A European cohort study found a positive association between $PM_{2.5}$ and malignant brain tumours (HR = 1.67, 95% CI: 0.89, 3.14) but the estimate was imprecise due to the small number of cases (n = 466).[120] Similarly, Jorgenson et al. studied the association of ambient air pollution (specifically $PM_{2.5}$, PM_{10}, total nitrogen oxides

[NO_X], and NO_2) with brain cancer incidence in the Danish Nurse Cohort, a cohort of 25,143 female nurses who were followed for a mean of 15.6 years.[121] Over the follow-up period, 121 incident brain cancer cases were recorded. A positive but imprecise and non-significant association with $PM_{2.5}$ was identified (HR: 1.06, 95% CI: 0.80, 1.40 per 3.37 $\mu g/m^3$ $PM_{2.5}$). A cohort study in the United States and Puerto Rico explored potential associations between particulate and gaseous air pollutants and brain cancer mortality in adults.[122] In an analysis of 1,284 deaths from brain cancer, particulate matter was not found to increase the risk of brain cancer mortality. Similarly, results from a large American registry of 127 898 incident brain tumours found no significant association with $PM_{2.5}$ exposures.[123]

2.5.6 Oxidant gases and brain cancer

Studies examining the association between NO_2 or total nitrogen oxides (NO_X, i.e., NO_2 and nitric oxide [NO] which quickly oxidizes to NO_2) exposures and brain cancer incidence have had mixed or inconclusive results. A Danish study found an association of NO_X exposures (IRR per 100 $\mu g/m^3$: 2.28, 95% CI: 1.25, 4.19) and roadway proximity of 50 m or less (IRR: 1.89, 95% CI: 1.07, 3.36) and brain tumour incidence.[124] Similarly, the Danish Nurse Cohort studied by Jorgenson et al. as described previously in Section 2.5.5 found a weak, non-statistically significant positive association between total brain tumours and NO_2 (HR per IQR: 1.09, 95% CI: 0.91, 1.29) and NO_x (HR per IQR: 1.02, 95% CI: 0.93, 1.12).[121] In the American cohort study of 1,284 deaths from brain cancer described above,[122] four gaseous pollutants (sulfur dioxide, nitrogen dioxide, carbon monoxide, and ozone) were not found to increase the risk of brain cancer mortality, and some exposures were unexpectedly protective. There is little evidence in the published literature

of an association between ground-level ozone exposure and brain cancer incidence; however, Valberg et al. investigated whether county-by-county brain cancer incidence rates and mortality rates were correlated with patterns of local ambient air pollution and identified a single positive but weak correlation with ozone (r = 0.15).[125] This was an ecological study with exposure estimated only at the country level, even though individual exposures likely varied.

Overall, substantial uncertainty remains regarding the association of particulate and gaseous air pollution exposures with brain cancer incidence. Brain cancer studies have often been limited by small sample size, since brain tumours are relatively rare, and by a lack of understanding about which species or components of the air pollution mixture are relevant, as well as over which time interval individuals are at risk of exposure effects.[126, 127] The availability of exposure data at the correct spatial resolution scale is an additional methodological issue. Nonetheless, identifying risk factors for brain tumour incidence is important; currently, prevention of brain cancer is challenging because there is little knowledge on modifiable risk factors and identifying such risk factors could reduce the burden of disease. Consequently, additional work is needed to further evaluate the relationship between outdoor air pollution and brain cancer.

2.6 Knowledge gaps

An increasing body of epidemiologic research describes exposures to $PM_{2.5}$ using methods that incorporate information on particle characteristics such as toxicity and composition, rather than assessing exposures to $PM_{2.5}$ mass concentration alone. However, there is little information available on how $PM_{2.5}$ characteristics vary on fine spatial scales, such as within cities, and

whether those small-scale variations are relevant to health outcomes. This thesis will explore the health effects of long-term air pollution exposures at a *within-city* level with a focus on specific components and characteristics of $PM_{2.5}$.

In Objective 1, I develop models to predict variations in outdoor $PM_{2.5}$ oxidative potential (OP) and reactive oxygen species concentrations (ROS) on a fine spatial scale within Toronto and Montreal, which is important since previous spatial monitoring campaigns for OP[89] and ROS[83] indicate that these measures are much more spatially variable than $PM_{2.5}$ mass concentrations. Oxidative potential of particulate air pollution is increasingly measured in exposure studies, but most existing studies measure OP at a small number of sites and thus do not capture complex *within-city* spatial variations in particle OP.[12] Additionally, I develop models to predict within-city spatial variations in outdoor magnetite nanoparticles which has not been done to date, but is important because these pollutants are potentially relevant to the development of chronic diseases.

In Objective 2, I apply estimated OP exposures to existing population-based cohort data to estimate the associations between oxidant gases and cardiovascular mortality in Toronto and Montreal across strata of OP. While the phenomenon of OP modifying exposures to $PM_{2.5}$ and oxidant gases has been observed previously within Canada, it has not been examined at the within-cities scale. Since nearly 3 out of every 4 Canadians live in large urban centres,[128] it is important to understand urban air pollution exposures and their health effects to maximize the health of the majority of the Canadian population.

Finally, Objective 3 is the first epidemiologic study to date to evaluate the health effects of exposure to magnetite nanoparticles which make up part of the air pollution mixture in urban

areas but remain largely uncharacterized in terms of their spatial distributions and health effects. Although these pollutants have been identified in human brain tissues and have been associated with negative health effects in toxicological studies, they have been virtually ignored in human epidemiologic studies owing to the absence of environmental exposure data.

Chapter 3: Manuscript 1

3.1 Preface

This chapter contains the first of three manuscripts in this dissertation. The goal of this chapter was to describe the spatial distributions of several measures of the potential toxicity and composition of $PM_{2.5}$ within Montreal and Toronto, Canada at a high spatial resolution. I conducted $PM_{2.5}$ monitoring campaigns across Montreal and Toronto and developed land-use regression models to predict spatial variations in multiple characteristics of $PM_{2.5}$. Specifically, we estimated the ability of $PM_{2.5}$ to generate reactive oxygen species in lung lining fluid (using a previously published mathematical model), measured $PM_{2.5}$ oxidative potential based on the depletion of the antioxidants ascorbate and glutathione in a synthetic respiratory tract lining fluid assay, and measured anhysteretic remanent magnetization susceptibility as an indicator of magnetite nanoparticles in $PM_{2.5}$ samples. This manuscript was peer-reviewed and published in *Environmental Science and Technology*.

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3.2 Predicting spatial variations in multiple measures of $PM_{2.5}$ oxidative potential and magnetite nanoparticles in Toronto and Montreal, Canada

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Abstract

There is growing interest to move beyond fine particle mass concentrations ($PM_{2.5}$) when evaluating the population health impacts of outdoor air pollution. However, few exposure models are currently available to support such analyses. In this study, we conducted large-scale monitoring campaigns across Montreal and Toronto, Canada during summer 2018 and winter 2019 and developed models to predict spatial variations in: 1) the ability of $PM_{2.5}$ to generate reactive oxygen species in the lung fluid (ROS), 2) $PM_{2.5}$ oxidative potential based on the depletion of ascorbate (OP^{AA}) and glutathione (OP^{GSH}) in a cell-free assay, and 3) anhysteretic magnetic remanence (X_{ARM}) as an indicator of magnetite nanoparticles. We also examined how exposure to PM oxidative capacity metrics (ROS/OP) varied by socioeconomic status within each city. In Montreal, areas with higher material deprivation, indicating lower area-level average household income and employment, were exposed to $PM_{2.5}$ characterized by higher ROS and OP. This relationship was not observed in Toronto. The developed models will be used in epidemiologic studies to assess the health effects of exposure to $PM_{2.5}$ and iron-rich magnetic nanoparticles in Toronto and Montreal.

Introduction

Exposure to outdoor air pollution is a major global health concern.[1] Particulate air pollution is of noted importance.[2] Fine particles, defined as particles with an aerodynamic diameter less than 2.5 µm ($PM_{2.5}$), are small enough to penetrate deep into the human respiratory tract and are typically measured as mass concentrations that only consider the bulk mass of airborne particles.

However, $PM_{2.5}$ is a mixture of compounds derived from different sources including combustion emissions from vehicles, industry, biomass burning, brake- tire-, and rail-wear from vehicles and trains, and resuspension of dust. These sources release particles of different chemical composition and consequently varying toxicity.[3] Therefore, measures of particle mass alone do not fully capture the ability of ambient PM to cause adverse health effects.[4]

Complementary measures of particulate air pollution have been developed that incorporate information on the effects that particles may have inside the body. Among these is particle oxidative potential (OP), which is a measure of the ability of PM to cause redox reactions in the human respiratory tract.[4, 5] Several assays exist to quantify OP, including assays that measure the ability of PM to deplete antioxidants in a synthetic model of the respiratory tract lining fluid.[6] An alternative approach to estimating air pollution impacts on oxidative stress considers the ability of particles to generate reactive oxygen species. Specifically, inhaled particles containing transition metals undergo redox reaction cycles in the respiratory tract lining fluid, generating reactive oxygen species (ROS).[7] Lakey et al. (2016) developed a mathematical model of the chemistry of the human respiratory tract that estimates ROS concentrations generated in response to the iron (Fe) and copper (Cu) content of inhaled particles.[7]

In addition to their ability to induce oxidative stress, PM can be characterized by particle size and composition. Specifically, magnetite, iron oxide, has been a component of recent interest due to its prevalence in urban air samples.[8] Magnetite nanoparticles are produced during high-temperature combustion processes such as those involved in tailpipe emissions (e.g., from iron impurities found in fuel) and friction processes such as vehicle brake-wear,[8, 9] as well as

industrial processes.[10,11] Magnetite nanoparticles have been observed in abundant quantities in human brains,[12] hearts,[13] and placental tissues.[14] The presence of magnetite nanoparticles in human brains, including in amyloid plaques characteristic of Alzheimer's disease,[15] suggests that exposures to these particles may be a potential risk factor for neurological diseases;[12] however, the spatial distributions of these particles within cities have yet to be systematically described, and hence the health consequences of exposure remain largely unknown.

In this study, we conducted spatial monitoring campaigns in Canada's two largest cities using a dense network of air pollution monitoring sites. The primary aim of the study was to develop land-use regression models for $PM_{2.5}$ ROS and OP as well as magnetite nanoparticles (using anhysteretic remanence magnetization) using these data as well as measurements of estimated ROS and OP from a previous study in Toronto conducted in 2016. The models developed can be used in epidemiologic studies to assess the health effects of exposure to $PM_{2.5}$ in Toronto and Montreal. Additionally, we identified clusters of monitoring sites to identify possible sources associated with these particle characteristics. We also examined how levels of OP and ROS generation levels vary by neighborhood socioeconomic status across each city.

Methods

Spatial monitoring studies

Outdoor $PM_{2.5}$ monitoring campaigns were conducted in Toronto and Montreal, Canada during summer 2018 and winter 2019. Monitoring sites were identified to capture the variability of ambient $PM_{2.5}$ mass concentrations in each city while maximizing spatial coverage of the study area (Figure S3.1). In total, 110 sites were monitored in Toronto (a geographic area of 630.2 km^2)

and 124 sites in Montreal (472.6 km^2) in the summer, with a subset of 67 sites monitored in Montreal in the winter. Daily mean temperatures for the summer sampling ranged from 14.4°C to 23.7°C in Montreal and 19.8°C to 26.6°C in Toronto. Winter daily average temperatures in Montreal ranged from -14.9°C to 4.5°C. Integrated 2-week $PM_{2.5}$ samples were collected at each site using Teflon filters with a mix of Ultrasonic Personal Air Sample (UPAS) monitors (Access Sensor Technologies, Fort Collins, CO) at a flow rate of 1 L/min and cascade impactors at a flow rate of 5 L/min. All samples were collected simultaneously in each city using preset timers.

In Toronto, ROS generation and OP were previously estimated from samples taken in a 2016 campaign at an additional 67 sites using similar methods and an integrated 2-week monitoring period.[16, 17] We used these data to supplement the 2018 data in order to maximize spatial coverage and ensure that our final model captured all available OP and ROS generation data in the study region.

Analysis of $PM_{2.5}$ samples: mass, metal composition, reactive oxygen species generation and oxidative potential

$PM_{2.5}$ mass concentration was determined gravimetrically and the concentrations of Fe and Cu in $PM_{2.5}$ samples were determined by X-ray fluorescence according to EPA Method IO-3.3 in Compendium of Methods for the Determination of Metals in Ambient Particulate Matter (EPA 625/R-96/010a). Reactive oxygen species generation was estimated using the KM-SUB-ELF model described in full detail by Lakey et al.[7] Briefly, this model simulates over 50 chemical reactions that occur in the epithelial lining fluid of the respiratory tract in response to Fe and Cu content of inhaled particles. This model provides estimates of exogenous ROS concentrations

generated in response to Fe and Cu in $PM_{2.5}$, but does not account for ROS generated through biological interactions or immune responses.[16] While OP is generally expressed as the capability of PM in catalyzing the oxidation of antioxidants or cellular reductants, and the subsequential generation of reactive oxygen species, in this study we distinguish between the two measures of OP in OP^{AA} and OP^{GSH} and the estimation of ROS generation in ELF using the mathematical model developed by Lakey et al.[7] for clarity as the process of estimating these quantities differs.

Oxidative potential of $PM_{2.5}$ samples was analyzed using two acellular in vitro assays: the ascorbate (AA) assay and the glutathione (GSH) assay, according to the procedures described in detail previously.[18, 19] Briefly, $PM_{2.5}$ samples were extracted, re-suspended and then incubated with a synthetic human respiratory tract lining fluid for 4 h at 37 °C. This fluid was a 200 µM composite solution of physiologically-relevant antioxidants including ascorbate (AA) and glutathione (GSH). $PM_{2.5}$ oxidative potential was measured by depletion of AA (% change in absorbance at 260 nm wavelength) and GSH (oxidized glutathione-reductase-5,5′-dithio-bis(2-nitrobenzoic acid) recycling assay. Urate was not included as previous evidence suggests that PM does not have an important impact on urate depletion. [6, 20, 21] Each measure of $PM_{2.5}$ oxidative potential was expressed per unit volume (% depletion/m^3).

Measurement of Magnetite Nanoparticles using Anhysteretic Remanent Magnetization

Magnetic remanence measurements were used to quantify the airborne concentrations of iron-rich magnetite particles in $PM_{2.5}$ samples. Specifically, anhysteretic remanent magnetization (ARM) was measured as this parameter is sensitive to the presence of magnetic nanoparticles with diameters between 30–50 nm.[22, 23] Samples were exposed to 4 different

direct current (DC) biasing fields of 0.06 mT, 0.08 mT, 1.0 mT and 1.2 mT, and subsequently a 2G RAPID cryogenic magnetometer was used to measure the magnetic response of the samples. X_{ARM} was then calculated as the slope of the ARM(DC field) linear function. ARM was normalized by sampled air volume. Henceforth the parameter X_{ARM} will be referred to as "concentration of magnetite nanoparticles" for clarity.

Derivation of land use and built environment predictors for model development

Land use and built environment attributes were derived for each monitoring site using ArcMap 10.8.1 (ESRI, Redlands, CA) (Table S3.1). Land use variables were calculated within circular buffers of 50, 100, 200, 300, 500, 750 and 1000 meters. Categories of land use variables included residential, commercial, governmental/institutional, resource/industrial, parks, open area, water, and building footprints (DMTI Spatial Inc. Database 2014). Additionally, roadway characteristics were captured by length of roads, bus routes, railways, and number of bus stops within each buffer. Data were derived from DMTI Spatial 2014, Montreal Open Data Portal, and Toronto Open Data Portal. Emme (INRO, Montreal, Quebec, Canada), and USEPA Motor Vehicle Emission Simulator (MOVES) were used to calculate average and total NO_X emissions for each buffer. Additionally, distance to industrial facilities registered to the National Pollutant Release Inventory of the Government of Canada (NPRI 2014) for NO_X or PM production (NPRI 2014) was estimated.

Statistical Analyses

Bayesian linear regression models were developed to predict spatial variations in ROS, OP, and magnetite nanoparticle concentrations. Specifically, we developed Bayesian lasso

models which perform variable selection simultaneously with coefficient estimation by shrinking the coefficients on less influential variables.[24] The Bayesian lasso provides estimates of credible intervals that can guide variable selection, enabling selection of a small number of relevant predictors. The lasso tends to perform well for prediction tasks because the regularization penalty reduces learning from the data and thereby reduces overfitting.[25, 26] Therefore, lasso is well suited to contexts in which the values of the coefficients themselves are less important than the accuracy of prediction. However, unlike the ordinary least squares regression estimator, the lasso approach does not generate unbiased coefficients,[24] and values of individual coefficients should be interpreted with caution. Variable selection was performed by fitting the model on all available variables, as well as quadratic transformations and logarithmic transformations where appropriate; variables for which the 80% credible interval excluded the null value of 0 were retained and the final model was fit using the selected variables. Weakly informative priors allowed the posterior predictive distribution to be informed primarily by the data. Regression coefficients were fit with a Laplace (double-exponential) prior, and the penalty component of the Lasso regression was assigned a Cauchy prior distribution. In order to investigate the spatial dependence of the model errors, models were fit with and without a spatially correlated error structure and the models with better predictive performance (as assessed by the expected log predictive density) were retained.[27]

For Montreal, annual mean concentrations at each monitored site were generated by averaging values across the summer and winter seasons. Since winter sites were a subset of summer sites, annual values were available for all winter sites and a subset of summer sites. For Toronto, data were available only for the summer season. For the Toronto models, in which we

included data from a 2016 campaign in which ROS generation and OP were monitored at a different set of sites,[16, 17] an indicator variable for campaign year was included in the model in order to generate predictions representing an average of posterior means across both years. Therefore, the data used to build land-use regression models included averages between two summer sampling campaigns (2016 and 2018) in Toronto, and between two seasons in a single year period (summer 2018 and winter 2019) in Montreal. Analyses were performed in R version 4.0.1[28] and RStan version 2.19.3.[29] Convergence of the Markov chains was checked by examination of trace plots, and models were evaluated using a leave-one-out cross-validation procedure, a process in which a single observation is left out and then predicted based on the model fit to the remaining data. This process is repeated iteratively for all observations and the predictive accuracy of the model is assessed using the sum of values over all observations.[30]

Mapping the predicted exposure surfaces

To generate maps of the exposure surfaces, each city was divided into grid cells of 100 × 100 meters and the previously developed land-use regression models were used to predict exposures at each mid-point to develop an exposure surface across the entire study area. Additionally, uncertainty surfaces were generated using the posterior standard deviation generated for each grid point (Supplementary Figure 3.2).

Cluster identification

We used k-means clustering on the 2018 sample data to identify clusters of sites that were most similar with respect to observed ROS generation, OP^{GSH}, OP^{AA}, and magnetite nanoparticles to better understand potential source characteristics. Briefly, this method

partitions sites into clusters using an iterative algorithm that minimizes the Euclidean distance between observations and cluster centers.[31] K-means cluster analysis was performed in R using the *cluster* package.[32] Each site was uniquely identified to a single cluster. The optimal number of clusters, *k*, was the value that produced the greatest decrease in within-cluster sum of squares for a range of values of *k*. In the K-means clustering analysis, only filters with complete measures for all four exposures were used (N=100 in Montreal and N=59 in Toronto).

Relationship with neighborhood-level socioeconomic status

To describe the relationship between neighborhood-level material deprivation and neighborhood-level exposure to ROS generation, OP^{AA} and OP^{GSH}, we aggregated data by Forward Sortation Area (FSA) (i.e., a geographical unit denoted by the first three digits in a Canadian postal code). We used the Material Deprivation Index (MDI), which includes socioeconomic characteristics of the population living in a small area (e.g., income, educational attainment, and employment), as a proxy for neighborhood-level socioeconomic status.[33, 34] We took the median value of MDI percentile within each FSA as well as the median predicted concentration of ROS, OP^{AA} and OP^{GSH} within each FSA. We then used Generalized Additive Mixed Models (GAMM)[35] to visualize the relationship between each $PM_{2.5}$ characteristic and MDI across FSAs.

Results

The distributions of ROS generation, OP and magnetite values estimated from $PM_{2.5}$ samples taken in each city and season are described in Figure 3.1. $PM_{2.5}$ ROS generation, OP^{AA}, and OP^{GSH} were slightly lower in Montreal than in Toronto, while median magnetite nanoparticle

concentrations were slightly lower in Toronto than in Montreal. In Montreal, winter values were slightly lower than summer. Within each city, estimates of ROS generation and measures of OP had greater spatial variation (coefficients of variation 42.0-66.0%) relative to $PM_{2.5}$ mass concentration (coefficients of variation 24.3-30.8%). The efficiency of the methanol extraction process for OP was 97.6% (SD=10.6%). For magnetite nanoparticles, differences between cities and seasons were less apparent, but within-city spatial variation was also high (coefficients of variation 69.7-75.4%). Since sampling and analysis techniques were the same for all filters, recovery is assumed to be constant for all sites, so it is likely there is no differential bias in recovery across the cities.

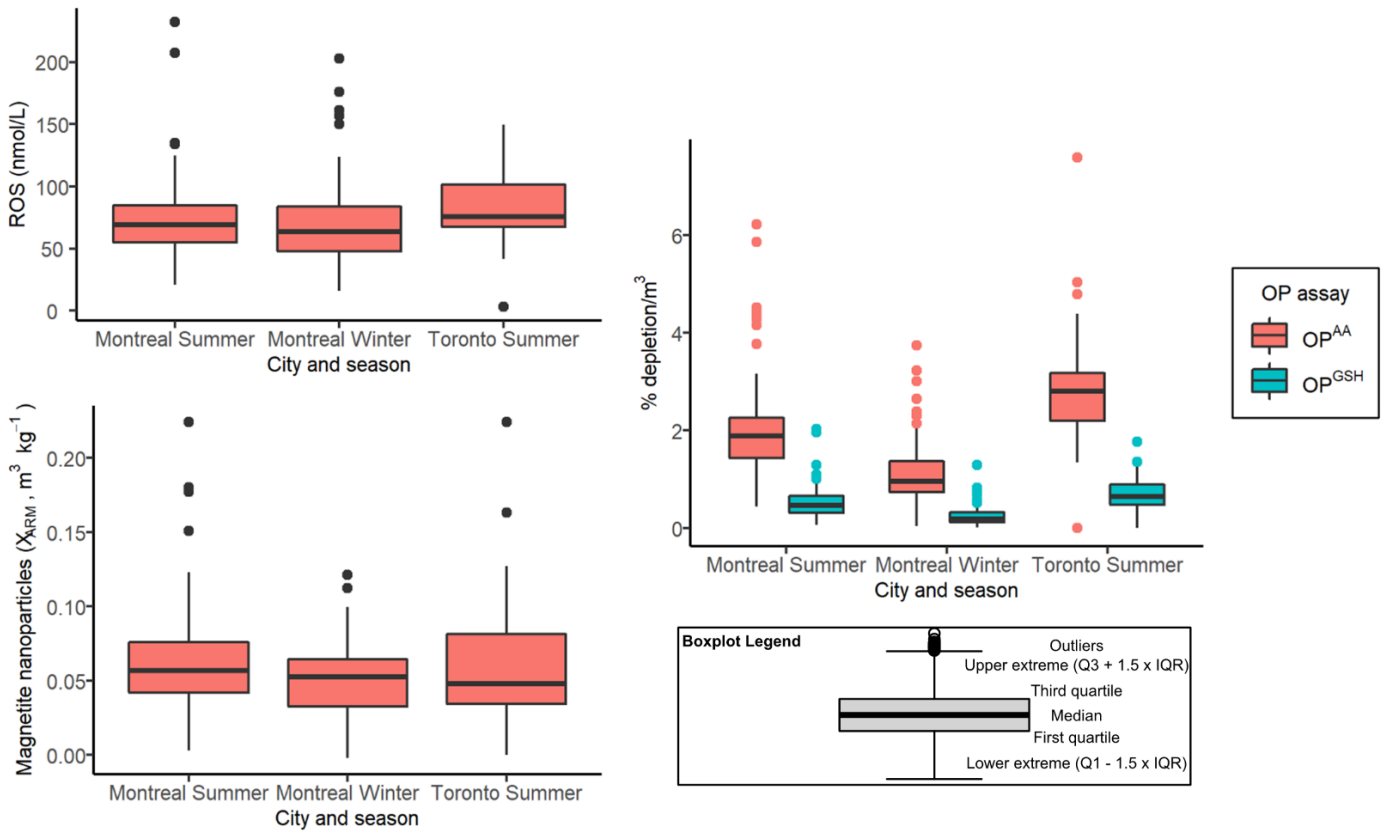


Figure 3.1. Distributions of $PM_{2.5}$ reactive oxygen species generation, magnetite nanoparticle concentrations, OP^{AA} and OP^{GSH} from 2018 monitoring campaigns in Toronto and Montreal, Canada.

Multivariable land-use regression models

Predicted spatial distributions of OP, ROS generation, and magnetite nanoparticles are shown in Figures 3.2 and 3.3. These surfaces highlight elevated concentrations of OP, ROS generation and magnetite around highways, rail lines, and road intersections. Several predictors of ROS entered into both Toronto and Montreal models, specifically length of rail-line within a buffer, as well as proximity to rail-lines, NPRI PM emitting sites, and highways (Figure 4). Distinct relationships with geographic coordinates (latitude and longitude) were observed in each city, potentially containing information on sources of PM that may not have been captured by the other variables in the models. Models for OP^{GSH} in both Toronto and Montreal contained variables related to commercial activity and NPRI PM emitting facilities. In OP^{AA} models, commercial activity and bus stops entered models in both cities; an inverse relationship with distance to rail also appeared in both cities, indicating higher predicted OP^{AA} with closer proximity to rail-lines. Final models did not use spatially correlated errors, as the inclusion of a spatially correlated error structure decreased predictive accuracy. Mean absolute errors of the land-use regression models are presented in Supplementary Table 3.2.

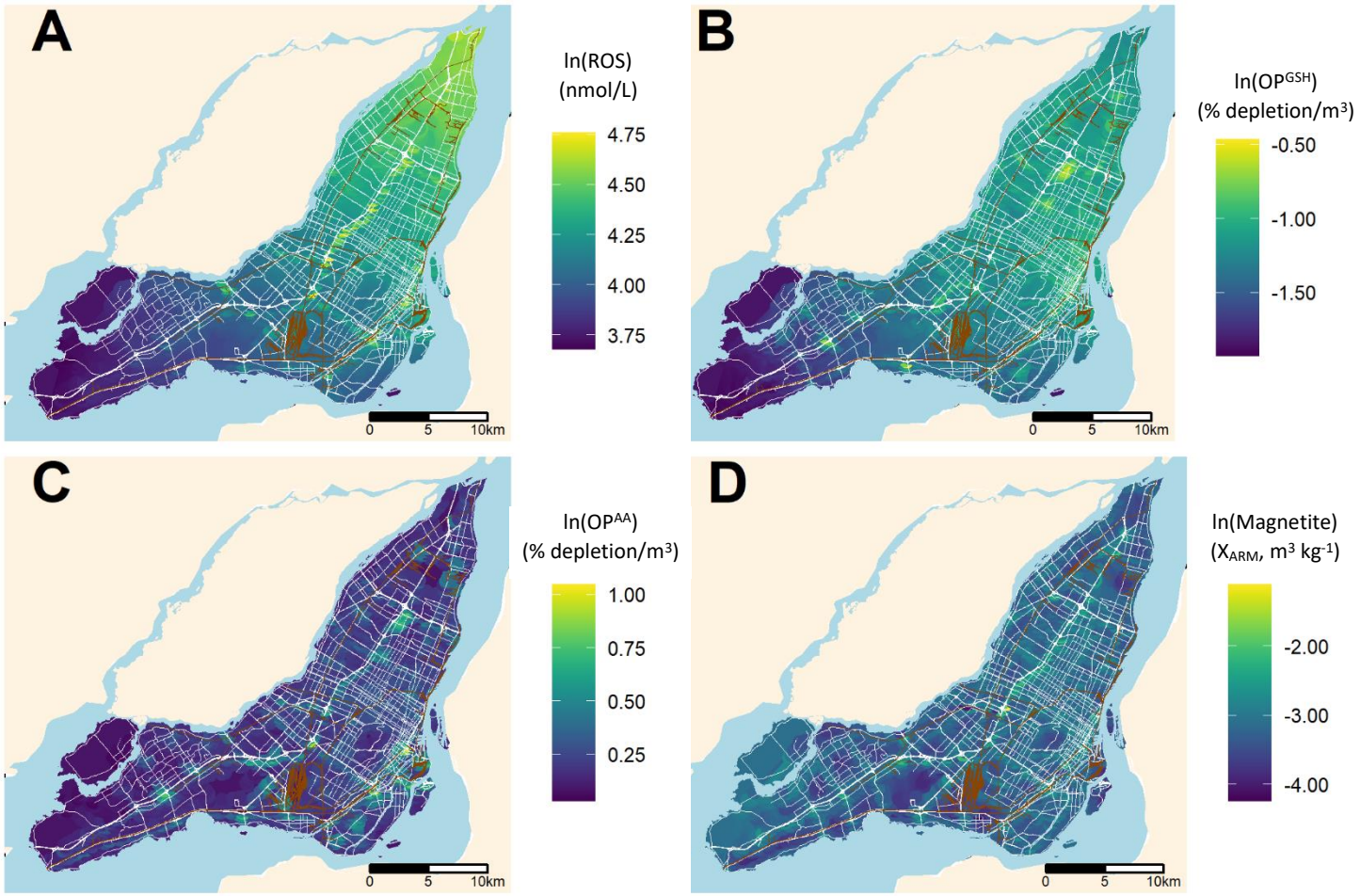


Figure 3.2. Predicted spatial distributions of $PM_{2.5}$ reactive oxygen species generation (A), OP^{GSH} (B), OP^{AA} (C), and magnetite nanoparticles (D) in Montreal, Canada.

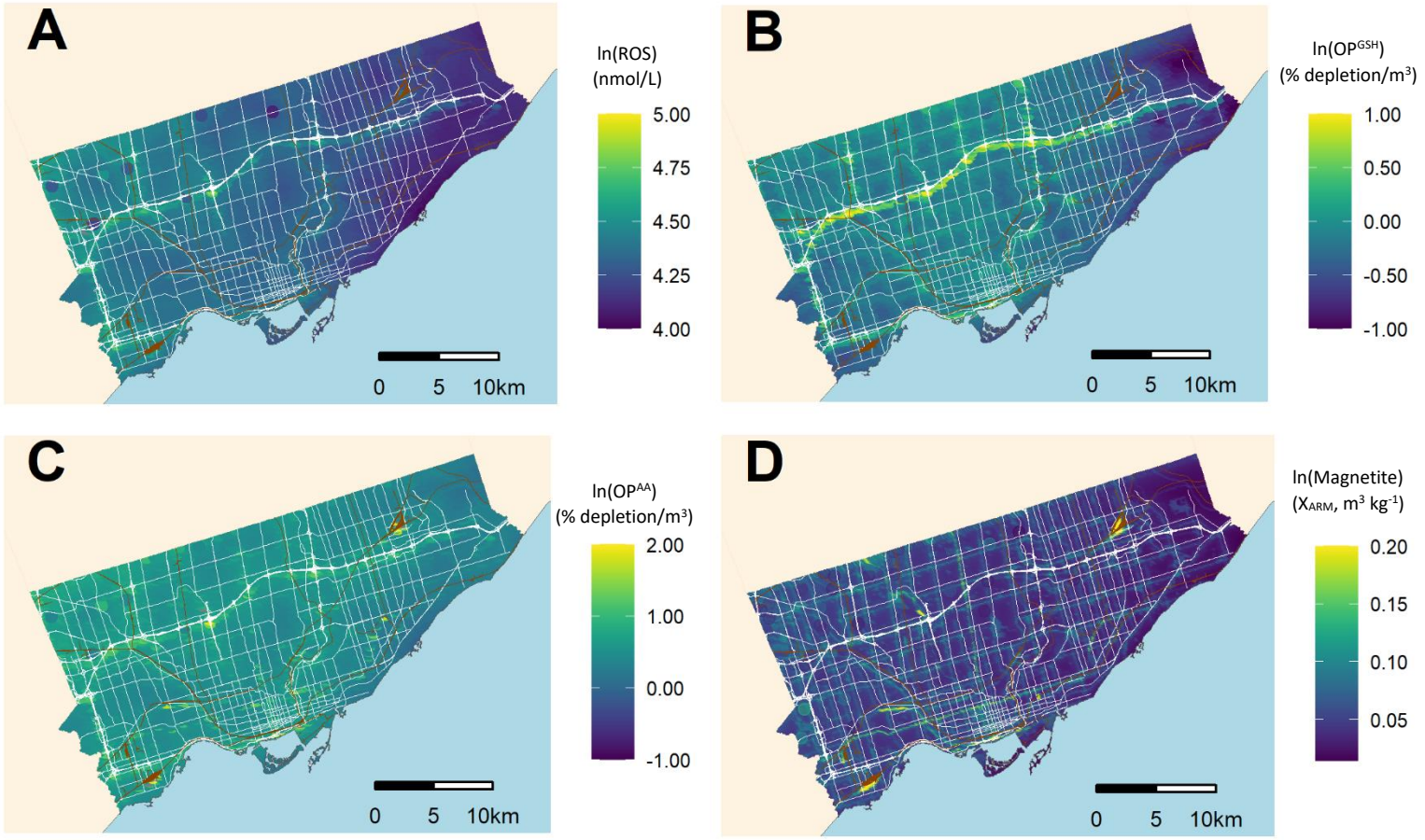


Figure 3.3. Predicted spatial distributions of $PM_{2.5}$ reactive oxygen species generation (A), OP^{GSH} (B), OP^{AA} (C), and magnetite nanoparticles (D) in Toronto, Canada.

In models for magnetite, traffic counts and proximity to major roads were associated with increased magnetite concentrations in both Montreal and Toronto. Other predictors that were associated with higher magnetite concentrations in Montreal included traffic counts, length of major roads, intersections, open space, and distance to the shore (Figure 3.4). In Toronto, length of railways, governmental land use, commercial land use, area of buildings, and north and east directions were associated with higher magnetite nanoparticle concentrations (Figure 3.4).

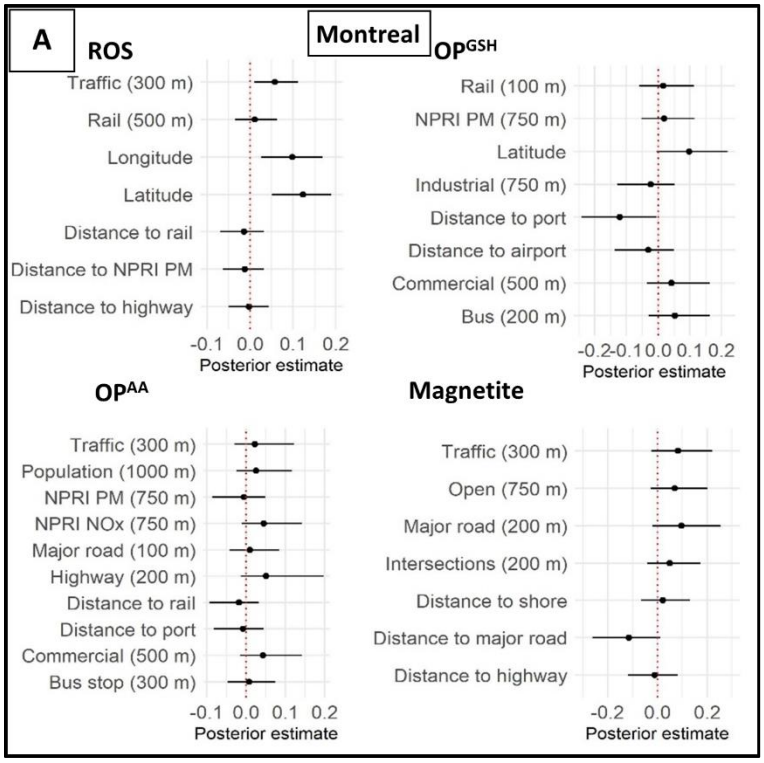
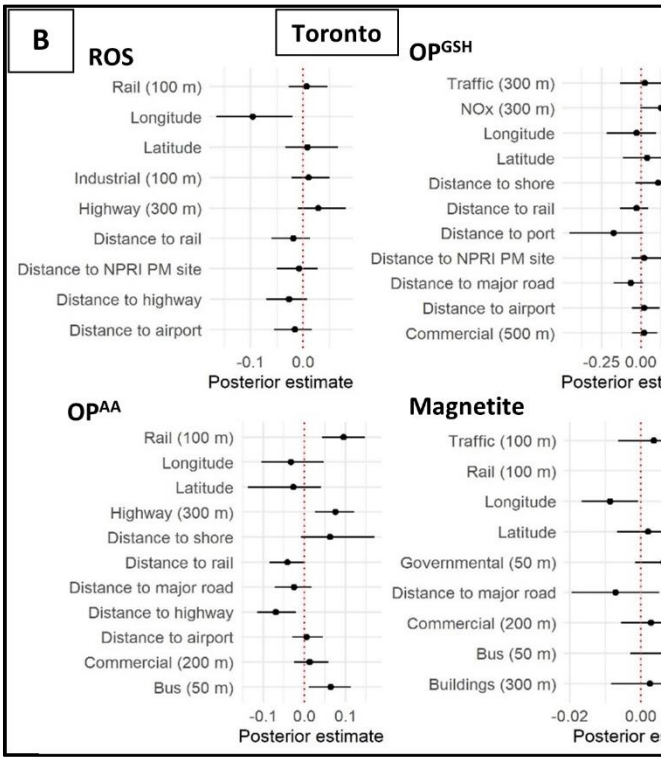


Figure 3.4. Coefficients from land use regression models for $PM_{2.5}$ characteristics in Montreal, Canada (A) and Toronto, Canada (B). Coefficients reflect change in the pollutant concentration for a one standard deviation increase in the predictor.



K-means clustering

Three groups were identified using the k means method (Figure 3.5). In both cities, the identified clusters had similar patterns with measured PM metrics. Specifically, Group 1 had the lowest values of ROS generation, OP^{AA}, OP^{GSH}, and magnetite. Group 2 had moderate values of ROS generation, OP^{AA} and OP^{GSH}, and had the highest magnetite nanoparticle concentrations. Group 3 was characterized by the highest values of ROS generation, OP^{AA} and OP^{GSH}, and moderate values of magnetite nanoparticle concentrations.

In Montreal, Group 1 (N= 66 sites, low OP/ROS generation/magnetite) consisted of sites that were mainly located in residential areas, although some of these sites were also located in proximity to vehicle emission sources (Figure 3.5). Sites in the Group 2 cluster (N = 27 sites, moderate OP and ROS generation, high magnetite) were located in Montreal's east end, where land use is dominated by industrial activity. Group 3 (N = 7 sites, high OP and ROS generation, moderate magnetite) sites in Montreal were mainly located in the downtown core and the southeast, where particle sources include vehicular traffic, construction and rail.

In Toronto, sites in Group 1 (N = 30) were generally located in residential neighborhoods farther from major traffic- or rail-related emission sources (Figure 3.5). However, some sites located close to high-traffic areas were also assigned to Group 1. Group 2 sites (N = 21) were in the east end of the city, with the exception being sites near the railyards in the west end. Group 3 (N = 8) corresponded to sites concentrated in the northwest corner of the city, where three highways intersect, and which borders on the city's major airport. Sites in Group 3 were also prevalent in the downtown core of the city as well as along the major highway that crosses the city in the east-west direction; the high exposures observed in Group 3 are consistent with these locations.

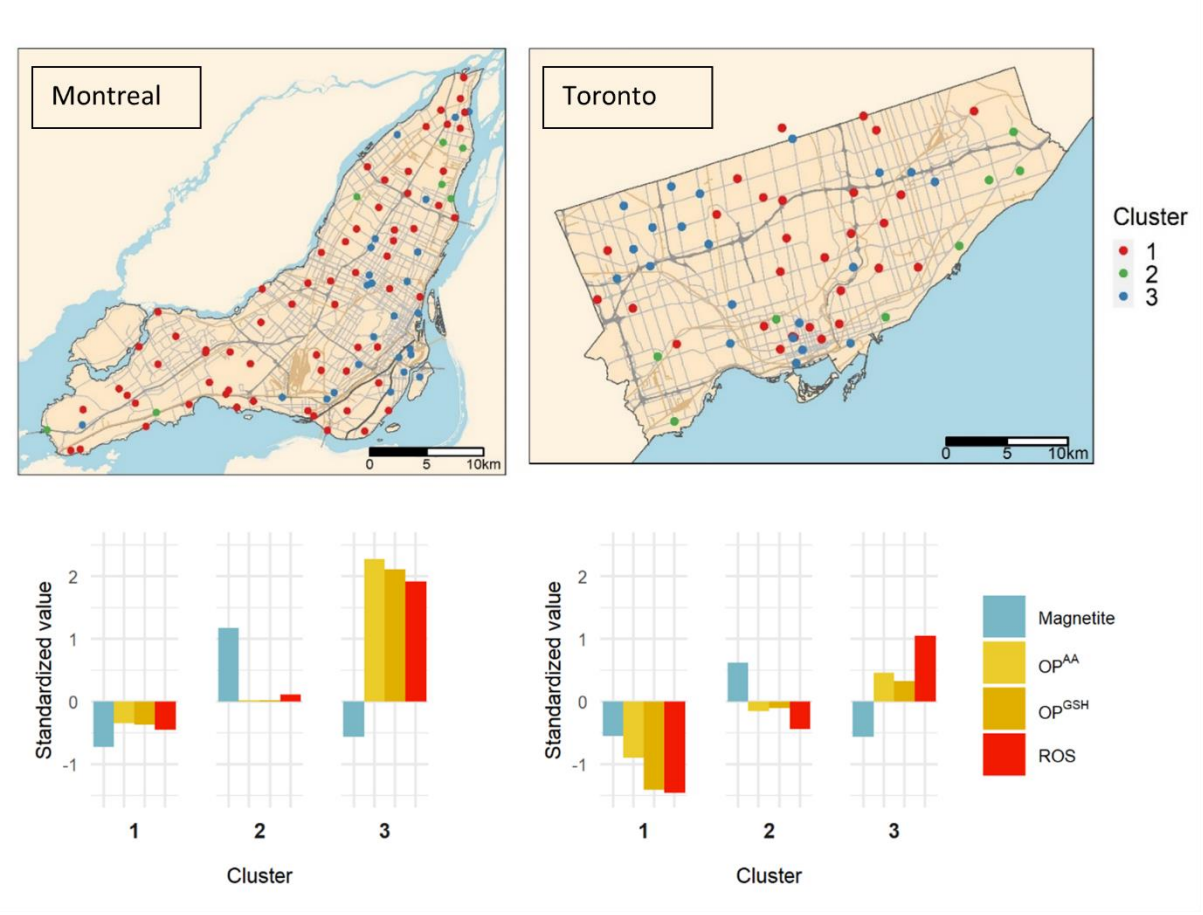


Figure 3.5. Clusters identified through K-means clustering of sites in Montreal and Toronto by ROS generation, OP^{AA}, OP^{GSH}, and magnetite nanoparticles. Upper panels show spatial locations of sites in each cluster. Lower panels show median values of ROS generation, OP^{AA}, OP^{GSH}, and magnetite nanoparticles in each cluster, standardized for uniform scale.

Relationship with Material Deprivation Index

In Montreal, predicted curves from Generalized Additive Mixed Models generally displayed increasing trends, indicating that areas that had higher values of the Material Deprivation Index (i.e., lower average household income, higher unemployment rate, and lower attainment of high school education) also had generally higher exposures to ROS generation and

OP^{GSH} (Figure 3.6). Specifically, areas with higher values of the Material Deprivation Index were prevalent in the eastern side of the city, where estimated exposures to ROS and OP^{GSH} were also higher. We observed a U-shaped relationship between MDI and OP^{AA}, as estimated OP^{AA} exposures were higher in areas with the highest and lowest median MDI, and lower in areas with moderate MDI. In Toronto, this pattern was not evident and minimal trend was observed (Supplementary Figure 3.3). This finding may reflect Toronto's geography, as traffic- and airport-related exposures are higher in the west side of the city and decline to the east, while some of the most deprived neighborhoods are in the east. In general, MDI was not a good predictor of any of the measures of oxidative stress (R^2 were all less than 5%, indicating less than 5% of variation in the oxidative stress measures was explained by MDI), with the exception of ROS generation in Montreal (R^2 = 0.24).

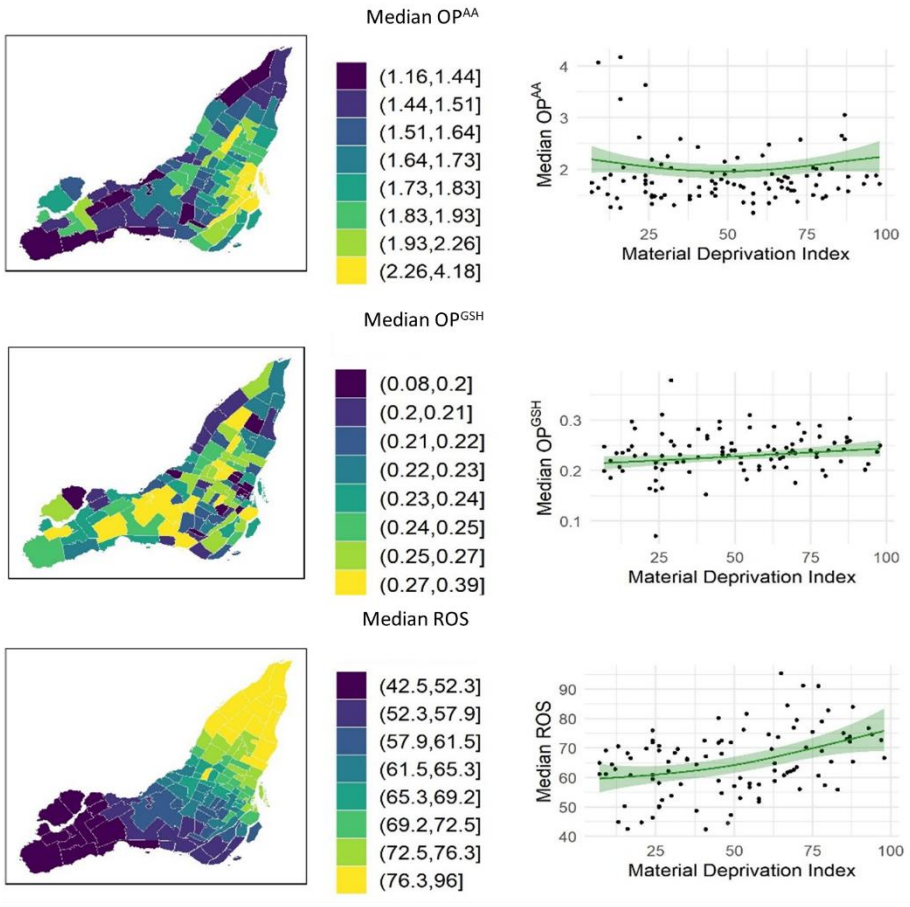


Figure 3.6. Montreal median predicted OP^{AA}, OP^{GSH} and ROS aggregated by Forward Sortation Area (left panels) and the relationship between each measure and median Material Deprivation Index, with local regression model fit (right panels). Higher MDI indicates a greater degree of deprivation.

Discussion

We developed models to predict the within-city spatial distribution of several biologically-relevant measures of $PM_{2.5}$ in Canada's two largest cities. We observed that estimated levels of PM-related ROS generation as well as measured concentrations of PM OP and magnetite vary on a fine spatial scale within Canadian cities. Further, our results suggest possible sources, primarily related to vehicular emissions. In predictive models, ROS generation, OP^{AA} and OP^{GSH} were primarily associated with vehicle-related land-use predictors (e.g., length of roads, number of road intersections, average daily traffic volume), as well as average NO_X traffic emissions, and rail-related land-use predictors (e.g., distance to railway, length of railways). Industrial land use was also a predictor of OP^{AA} in Montreal, but not in Toronto.

Our results suggest that OP^{AA} and OP^{GSH} are mainly driven by vehicle-related emissions, a finding that is consistent with the few land use regression models previously developed. For example, Yanosky et al.[36] modelled PM_{10} OP^{GSH} using 34 monitoring sites in London, U.K., and found brake- and tire-wear emissions to be positive predictors of OP^{GSH}. Similarly, Gulliver et al.[37] developed land use regression models for $PM_{2.5}$ OP^{AA} and OP^{GSH} in five cities in Europe. Both OP^{AA} and OP^{GSH} were predicted by traffic variables (e.g., traffic volume, road length) in addition to other variables such as natural land use and population distribution. However, it is important to note that findings from the present study are shaped by the use of measures of oxidative stress that are sensitive primarily to inorganic components of PM. All three measures of redox activity (OP^{AA}, OP^{GSH} and ROS generation) tend to reflect the impact of transition metals Cu and

Fe.[4, 7] Therefore, the measures of oxidative stress measured in this study reflect similar chemical components that may derive from primarily vehicular sources, which may account for the primarily vehicular sources identified as predictors of higher OP levels in the land-use regression models. Other measures could better reflect the impact of bulk sources of organic-abundant species, including those related to non-vehicular sources.

In our models for ROS generation, a measure that is a function of Fe and Cu concentrations, we found vehicular traffic-related variables such as traffic volume (in Montreal) and length of roadways (in Toronto) to be important predictors in models for ROS generation. A previous LUR model was developed for ROS generation in the Toronto area in 2016/2017; these data are incorporated into the models developed in the present study, and results of the LUR similarly show relationships between ROS generation and vehicular traffic-related variables.[16] Additionally, de Hoogh et al. developed LUR models for elemental Fe and Cu in 10 cities in Europe and found that the most important predictors were vehicle-related variables, such as traffic volume and road length. [38] Other studies assessed the direct generation of reactive oxygen species using the electron spin resonance assay, rather than the KM-SUB-ELF method of Lakey et al.[7] For example, in a study of 10 regional background, 12 urban background, and 18 street sites conducted in the Netherlands and Belgium, Yang at al. found high spatial correlations between OP^{ESR} and traffic-related PM components (such as Fe and Cu).[39] Conversely, Hellack et al. found combustion-related PM_{10} and semi-natural and forested areas to be the most important predictors of OP^{ESR} ;[40] however, this study included a larger number of sites in rural areas relative to the strictly urban sites in the present study.

To our knowledge this is the first characterization of within-city spatial variations in magnetite nanoparticle concentrations estimated from $PM_{2.5}$ samples in Canadian cities. A study in urban roadside and background sites in Lancaster and Birmingham, UK found brake-wear to be the strongest source of magnetite particles, and the concentration of magnetite in vehicle tailpipe emissions was much lower than in roadside PM collected from heavy-traffic areas,[8] which indicates that non-tailpipe vehicular emissions may be the most important contributor to magnetite emissions. In the present study, the final models for magnetite nanoparticles included vehicle- and rail-related land-use variables, which may reflect a combination of tailpipe- and non-tailpipe emissions, but cannot be differentiated in our models. Nonetheless, our models provide a description of the distribution of magnetite nanoparticles across the study area which can be applied in future studies of health outcomes, while future work can elucidate the sources of magnetite nanoparticles in $PM_{2.5}$ samples.

Using k-means clustering we were able to identify clusters of sites with similar combinations of ROS generation, OP^{AA}, OP^{GSH} and magnetite nanoparticle levels in each city, potentially reflecting different particle sources. This method has been used to identify sites with similar pollutant profiles at a regional or national scale: for example, to group sites by $PM_{2.5}$ composition across the United States,[41] and similarly to identify groups of cities in Portugal with similar profiles with respect to SO_2 and PM_{10} mass concentration.[42] Although unsupervised learning algorithms such as k-means clustering have been less used at the within-city spatial scale, the high spatial variability of OP and magnetite measures in our data allowed several

distinct clusters to be identified within Toronto and Montreal. Cluster results could be used in future health studies to examine the effect of combinations of exposures.

We identified distinct relationships of ROS generation and OP with socioeconomic status in Montreal and Toronto. Specifically, in Montreal there was a positive association between material deprivation and exposure to ROS/OP^{GSH}. In Canadian cities, there is some evidence that exposure to ambient air pollution is related to socioeconomic status: Pinault et al. found that in Montreal, Toronto, and Vancouver, certain measures of material and social deprivation were associated with higher outdoor NO_2 concentration, a traffic-related gaseous pollutant.[43] Our assessment of the relationship between material deprivation and ROS generation and OP was preliminary, but suggests a potential environmental justice concern. Areas with similar $PM_{2.5}$ exposures face greater health burdens from air pollution exposure when OP is high; if OP is higher in poorer areas, residents experience higher health risks, including respiratory illness[44] as well as myocardial infarction[45] and low birth weight[46] from PM exposure due to effect modification by OP. However, it is important to note that these patterns depend on locations and types of sources as well as the geography of each city. In Toronto, we did not observe the consistent pattern of increasing ROS generation and OP levels with increasing MDI observed in Montreal.

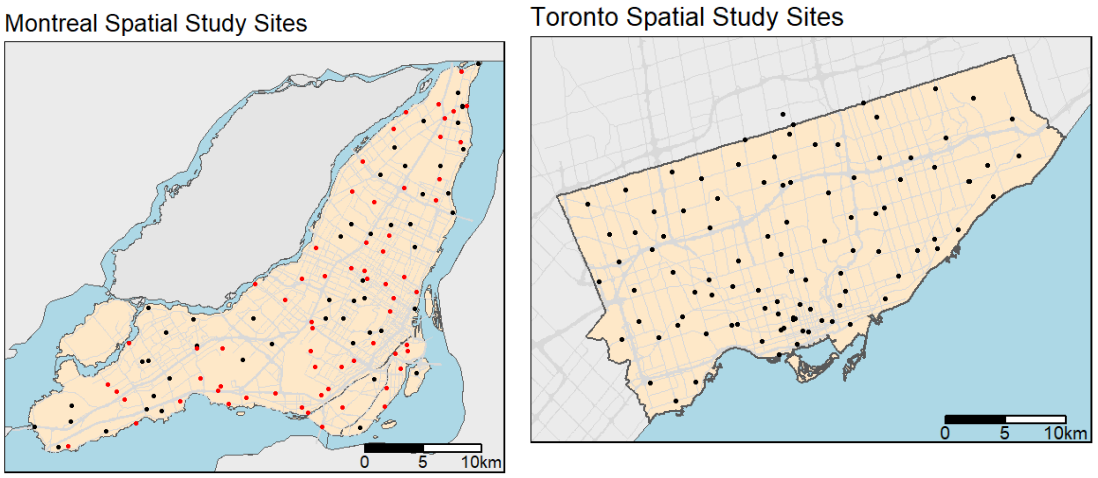
This study had several important strengths including the use of pre-set timers to allow simultaneous monitoring of all monitoring sites, eliminating the need for temporal adjustment, as well as a dense network of monitoring sites allowing us to describe variations in $PM_{2.5}$ ROS generation, OP and magnetite at a fine spatial scale. Since ROS generation, OP and magnetite are

more spatially variable than $PM_{2.5}$ mass concentrations, capturing this spatial variability is important in developing an accurate land use regression model.[47] Additionally, we incorporated earlier data from a 2016 monitoring campaign into the Toronto ROS generation and OP models, which may allow our predictions to better approximate long-term average exposures in comparison to a single monitoring period.

We acknowledge some limitations of the study. First, we were unable to obtain data for the winter season in Toronto, which also prevented us from calculating annual-average estimates of exposure in Toronto. Additionally, although the number of monitoring sites was relatively large, our sample sizes were not sufficiently large to investigate more complex non-linear associations between predictors and pollutant concentrations which might have allowed improved model performance.

In conclusion, we conducted large-scale spatial monitoring studies of multiple measures of $PM_{2.5}$ toxicity and composition in Toronto and Montreal, Canada. Our findings suggest that $PM_{2.5}$ ROS generating capacity, OP^{AA}, OP^{GSH} and magnetite nanoparticles vary on a fine spatial scale within cities. Moreover, we developed land-use regression models and found that land use variables related to vehicular traffic were most strongly predictive of these particle characteristics. Further work is needed to understand spatial and temporal variations in sources of ROS/OP and magnetite to identify possible regulatory interventions should these be warranted in the future.

3.3 Supplementary Material Manuscript 1



Supplementary Figure 3.1. Locations of sampling sites in Montreal and Toronto, Canada in the 2018 campaign. Red points indicate sites that were monitored in the summer and winter season; black sites are those that were monitored in summer only.

Supplementary Table 3.1. Predictors used in the development of land-use regression models in Toronto and Montreal

Description	Source	Buffer sizes
Building Area	DMTI 2013	50, 100, 200, 300, 500, 750, 1000m
Commercial Area	DMTI 2013	50, 100, 200, 300, 500, 750, 1000m
Government and Institutional Land Use Area	DMTI 2013	50, 100, 200, 300, 500, 750, 1000m
Open Area	DMTI 2013	50, 100, 200, 300, 500, 750, 1000m
Parks and recreational Area	DMTI 2013	50, 100, 200, 300, 500, 750, 1000m
Residential Area	DMTI 2013	50, 100, 200, 300, 500, 750, 1000m
Resource and Industrial Area	DMTI 2013	50, 100, 200, 300, 500, 750, 1000m
Waterbody Area	DMTI 2013	50*, 100, 200, 300, 500, 750,

		1000m
Length of Highways	DMTI 2013	50*, 100, 200, 300, 500, 750, 1000m
Length of Major Roads	DMTI 2013	50, 100, 200, 300, 500, 750, 1000m
Length of Major Roads, Highways and Local Roads	DMTI 2013	50, 100, 200, 300, 500, 750, 1000m
Distance to the closest highway	DMTI 2013	-
Distance to the closest major road	DMTI 2013	-
Length of Bus Routes	Toronto Transit Commission, Open Data, 2012 Montreal STM	50, 100, 200, 300, 500, 750, 1000m
Number of Bus Stops	City of Toronto, Open Data, 2018 City of Montreal Open Data Portal, 2018	50, 100*, 200*, 300, 500, 750, 1000m
Number of Intersections	City of Toronto, Open Data, 2018 City of Montreal Open Data Portal, 2018	50, 100, 200, 300, 500, 750, 1000m
Average Daily Traffic Volume	EMME	50, 100, 200, 300, 500, 750, 1000m
Total Daily Traffic Volume	EMME	50, 100, 200, 300, 500, 750, 1000m
Average NOx Traffic Emissions	EMME + MOVES	50, 100, 200, 300, 500, 750, 1000m
Total NOx Traffic Emissions	EMME + MOVES	50, 100, 200, 300, 500, 750, 1000m
Number of PM2.5 NPRI Chimneys	NPRI 2014	50, 100*, 200, 300, 500, 750, 1000m
Number of NOx NPRI Chimneys	NPRI 2014	50, 100*, 200, 300, 500, 750, 1000m
Distance to the closest NOx NPRI Chimney	NPRI 2014	-
Distance to the closest PM NPRI Chimney	NPRI 2014	-
Distance to the closest airport	DMTI 2013	-
Distance to the closest railline	DMTI 2013	-

Distance to the closest port	World Port Index, 2019 City of Montreal Open Data Portal, 2018	-
Distance to the shore	Statistics Canada 2011	-
Total population	Statistics Canada 2011	50, 100, 200, 300, 500, 750, 1000m
Length of rail road	DMTI 2013	50, 100, 200, 300, 500, 750, 1000m
Distance to commercial woodburning*	Montreal Direction de Santé Publique 2018	-
Number of commercial woodburning sites*	Montreal Direction de Santé Publique 2018	50, 100, 200, 300, 500, 750, 1000m

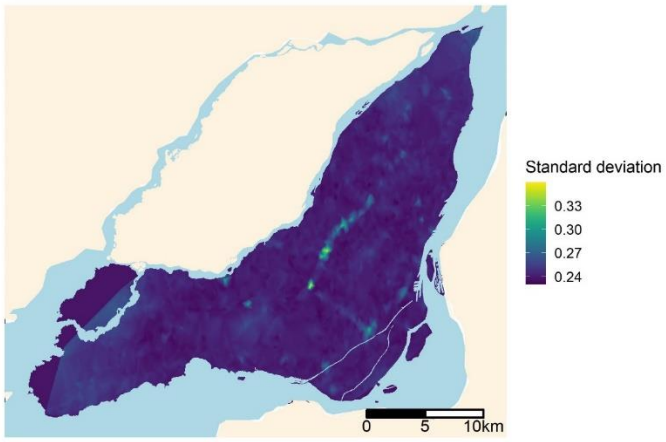
* Montreal only; † Toronto only

Supplementary Table 3.2. Performance of land use regression models for prediction of ROS /OP and magnetite nanoparticles.

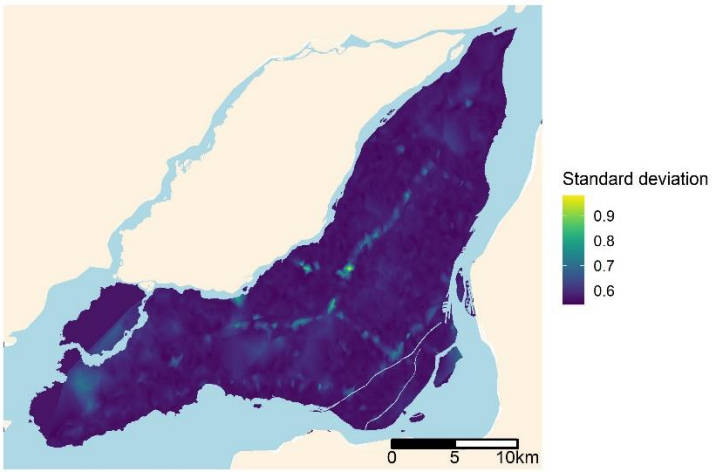
	Mean absolute error
ROS	
Montreal Annual	16.20
Toronto Summer	8.91
OP^{AA}	
Montreal Annual	0.24
Toronto Summer	0.30
OP^{GSH}	
Montreal Annual	0.13
Toronto Summer	0.16
Magnetite nanoparticles	
Montreal Annual	0.0167
Toronto Summer	0.0208

Supplementary Figure 3.2. Uncertainty surfaces for land use regression models.

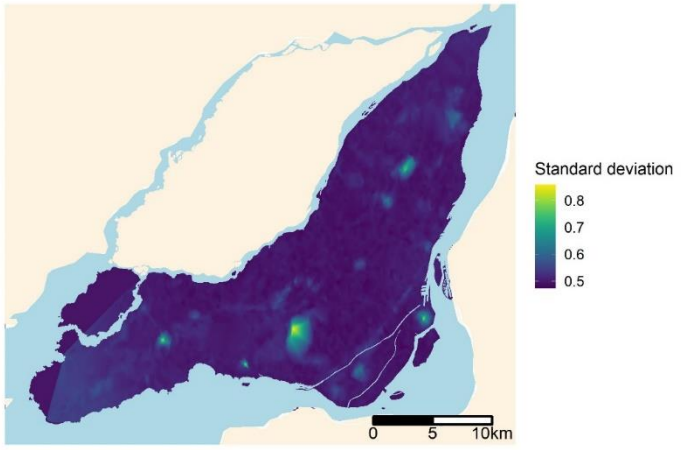
ROS



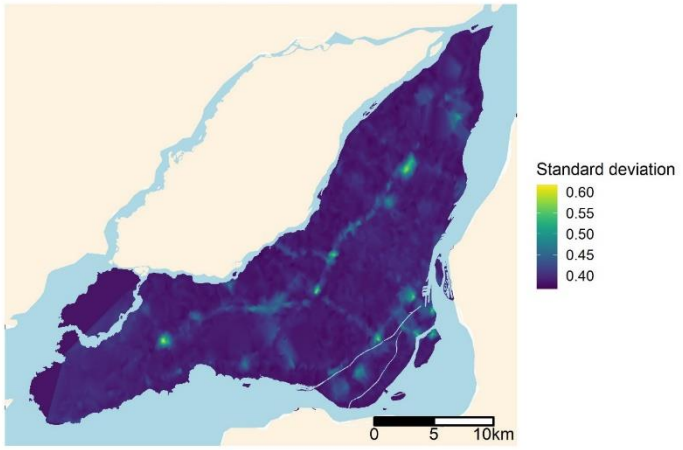
Magnetite



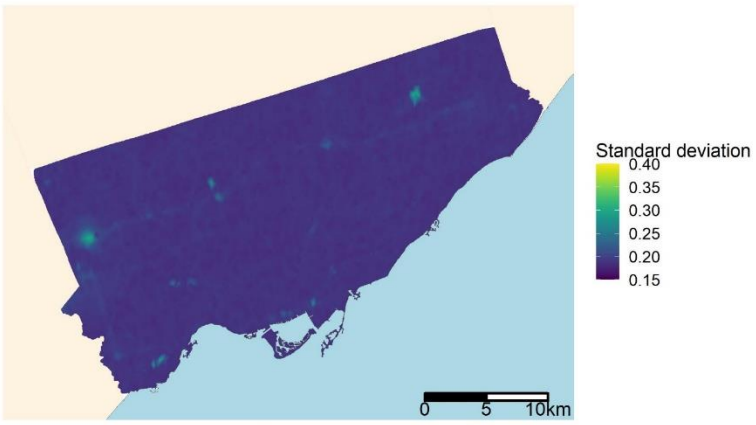
OP^{GSH}


Standard deviation
0.8
0.7
0.6
0.5
0
5
10km

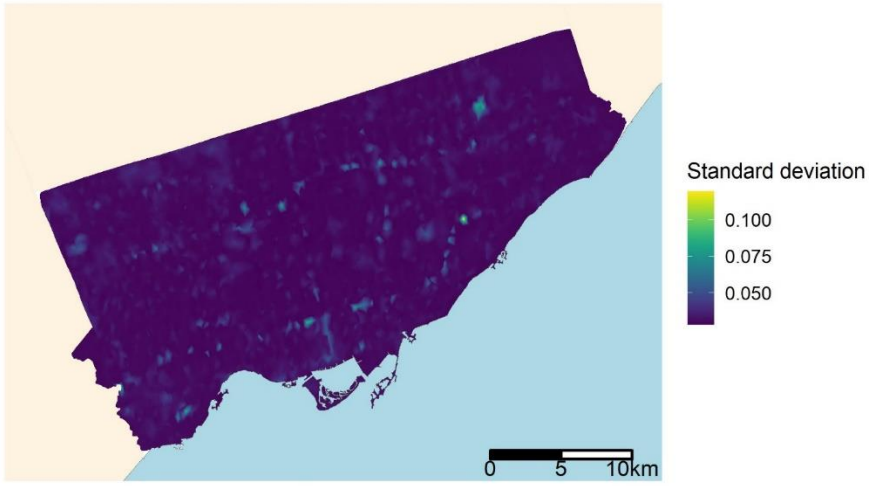
OP^{AA}


Standard deviation
0.60
0.55
0.50
0.45
0.40
0
5
10km

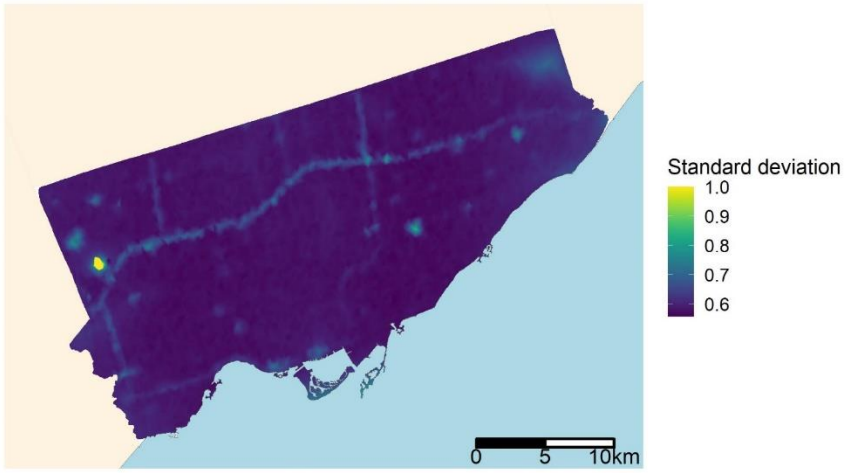
ROS



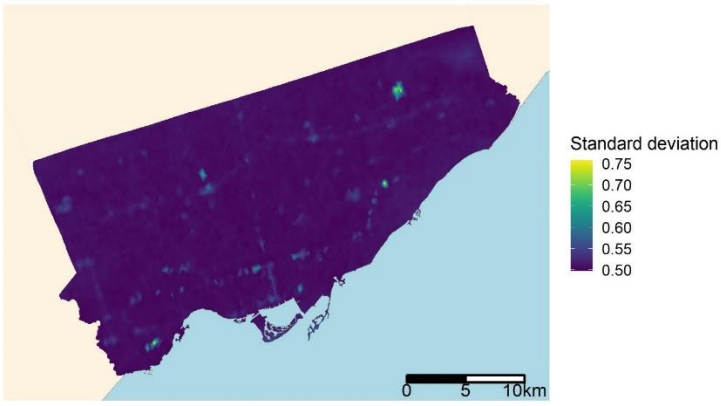
Magnetite



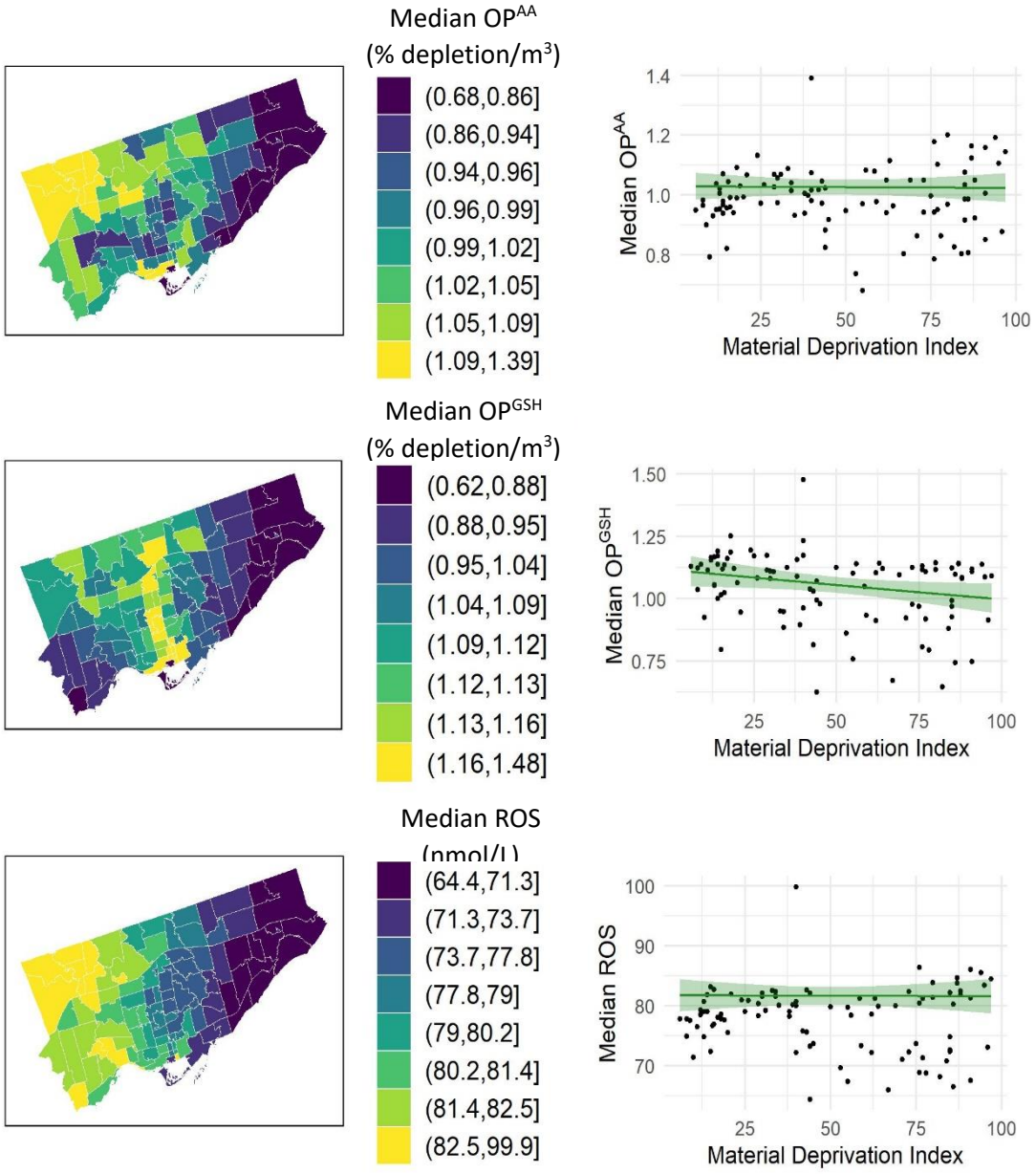
OP^{GSH}


Standard deviation
1.0
0.9
0.8
0.7
0.6
0
5
10km

OP^{AA}


Standard deviation
0.75
0.70
0.65
0.60
0.55
0.50
0
5
10km

Supplementary Figure 3.3. Toronto median predicted OP^{AA}, OP^{GSH} and ROS aggregated by Forward Sortation Area (left panels) and the relationship between each measure and median Material Deprivation Index, with local regression model fit (right panels). Higher MDI indicates a greater degree of deprivation.



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Chapter 4: Manuscript 2

4.1 Preface

This chapter contains the second of three manuscripts in this thesis. Our goal was to examine how $PM_{2.5}$ oxidative potential modifies the effects of gaseous air pollutants on cardiovascular mortality at the within-city scale. Previous evidence demonstrated that increases in particle oxidative potential may lead to stronger effects of exposures to $PM_{2.5}$ mass concentrations or oxidant gases, but this has not been examined on the within-city scale. In this chapter, we conducted a retrospective cohort study of participants in the Canadian Census Health and Environment Cohort in Montreal and Toronto. We estimated associations between outdoor concentrations of oxidant gases (specifically defined as O_X, a redox-weighted average of nitrogen dioxide and ozone) and mortality from cardiovascular causes. Analyses were performed across strata of several measures of $PM_{2.5}$ oxidative potential adjusting for relevant confounding factors such as individual-level and contextual socioeconomic status and demographic variables.

At the time of thesis submission this manuscript is under review at *Environmental Epidemiology*.

Citation: Ripley S, Gao D, Pollitt KJG., Lakey PSJ, Shiraiwa M, Hatzopoulou M, Weichenthal S. Within-city spatial variations in long-term average outdoor oxidant gas concentrations and cardiovascular mortality: Effect modification by oxidative potential in the Canadian Census Health and Environment Cohort (CanCHEC).

4.2 Within-city spatial variations in long-term average outdoor oxidant gas concentrations and cardiovascular mortality: Effect modification by in oxidative potential in the Canadian Census Health and Environment Cohort (CanCHEC)

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Abstract

Background: Health effects of oxidant gases may be enhanced by components of particulate air pollution that contribute to oxidative stress. Our aim was to examine if *within-city* spatial variations in the oxidative potential of outdoor fine particulate air pollution ($PM_{2.5}$) modify relationships between oxidant gases and cardiovascular mortality.

Methods: We conducted a retrospective cohort study of participants in the Canadian Census Health and Environment Cohort who lived in Toronto or Montreal, Canada, from 2002-2015. Cox proportional hazards models were used to estimate associations between outdoor concentrations of oxidant gases (O_x, a redox-weighted average of nitrogen dioxide and ozone) and cardiovascular deaths. Analyses were performed across strata of two measures of $PM_{2.5}$ oxidative potential and reactive oxygen species concentrations (ROS) adjusting for relevant confounding factors.

Results: $PM_{2.5}$ mass concentration showed little within-city variability, but $PM_{2.5}$ oxidative potential and ROS were more variable. Spatial variations in outdoor O_x were associated with an increased risk of cardiovascular mortality (HR per 5 ppb = 1.028, 95% CI: 1.001, 1.055). The effect of O_x on cardiovascular mortality was stronger above the median of each measure of $PM_{2.5}$ oxidative potential and ROS (e.g., above the median of glutathione-based oxidative potential: HR = 1.045, 95% CI: 1.009, 1.081; below median: HR=1.000, 95% CI: 0.960, 1.043).

Conclusion: Within-city spatial variations in $PM_{2.5}$ oxidative potential may modify long-term cardiovascular health impacts of O_x. Regions with elevated O_x and $PM_{2.5}$ oxidative potential may be priority areas for interventions to decrease the population health impacts of outdoor air pollution.

What this study adds: The effects of gaseous air pollutants may be stronger in areas where particulate matter has greater toxicity, for example, as measured by particle oxidative potential or concentrations of ROS generated in the epithelial lining fluid (ROS). This effect has not been assessed at small spatial scales. We used a population-based cohort in Montreal and Toronto, Canada to assess the association of long-term exposure to oxidant gases with cardiovascular mortality, and whether that relationship varied by particle oxidative potential and ROS. We observed stronger effects of oxidant gas exposures in areas above the median of oxidative potential and ROS.

Introduction

Exposure to fine particulate air pollution ($PM_{2.5}$) is a known risk factor for cardiovascular morbidity and mortality.[1, 2] An important mechanism by which $PM_{2.5}$ induces cardiovascular dysfunction is oxidative stress, which occurs when levels of reactive oxygen species exceed normal levels and overcome the body's antioxidant defenses.[1] $PM_{2.5}$ is only one component in the complex mixture of chemicals that comprise air pollution. Oxidant gases (expressed as O_x, which represents a weighted average of the gases nitrogen dioxide, NO_2, and ozone, O_3) also contribute to outdoor air pollution. These gases can induce oxidative stress and are associated with cardiovascular mortality.[3, 4]

$PM_{2.5}$ concentrations depend on regional background levels (i.e., particles transported from distant sources) as well as local sources (e.g., local vehicular traffic or industrial activity)[5] but show relatively little spatial variability within cities.[6-9] However, O_X concentrations vary considerably within cities (this is driven primarily by local production of NO_2).[10,11] As well, small-scale spatial variations in $PM_{2.5}$ components are much greater than variations in total $PM_{2.5}$ mass concentrations owing to greater differences in composition from local sources.[6, 7] Moreover, PM components are not equally toxic, and many $PM_{2.5}$ components (e.g., transition metals) are associated with increased production of free radicals.[12] Particle oxidative potential (OP) presents an integrated approach to estimating the ability of particles to induce oxidative stress, and existing evidence suggests that OP is highly variable within cities.[6, 13] OP can be determined by a number of different acellular assays,[14] frequently by measuring depletion of antioxidants (most commonly ascorbate [AA] and glutathione [GSH]) using a cell-free assay based on a synthetic

respiratory tract lining fluid exposed to $PM_{2.5}$ sample extracts.[15] Alternatively, the concentration of reactive oxygen species (ROS) resulting from the ability of particles to generate ROS and the destruction of ROS by antioxidants can be estimated using a mathematical model based on the content of redox-active components including transition metals in particles.[16]

Recent evidence suggests that regional variation (i.e., between cities) in $PM_{2.5}$ OP can modify the acute and chronic health effects of O_X.[17, 18] Specifically, in a time-stratified case-crossover study across 34 Canadian cities, associations between short-term O_X exposures and respiratory hospitalizations in children were stronger when monthly average glutathione-based OP (OP^{GSH}) was higher.[18] Similarly, long-term effects were assessed in a cohort study of Canadian adults living within 10 km of one of 40 monitoring sites across the country, and associations between O_X and mortality (nonaccidental, cardiovascular, and respiratory mortality) were consistently stronger in regions with elevated $PM_{2.5}$ transition metal/sulfur content and oxidative potential (OP^{AA}, OP^{GSH} and OP estimated from a dithiothreitol-based assay).[17] However, neither of these previous studies examined how $PM_{2.5}$ OP and ROS concentrations may modify the health impacts of O_X *within* cities.

In this study, we examined how *within-city* spatial variations in $PM_{2.5}$ OP and ROS concentrations influenced associations between long-term exposures to O_X and cardiovascular mortality in Toronto and Montreal, Canada. Our cohort analysis included more than 1 million members of the Canadian Census Health and Environment Cohort (CanCHEC) and our estimates of $PM_{2.5}$ OP and ROS were based on dense spatial monitoring campaigns conducted across each city.

Methods

Cohort description and mortality outcomes

The Canadian Census Health and Environment Cohort (CanCHEC) has been described previously.[19, 20] Briefly, this is a population-based cohort established in 2008, when the 1991 Canadian Census was linked to 10 years of death records, and includes non-institutionalized Canadians (aged 25 and older) who were among the approximately 20% of households selected for enumeration by the long-form Census questionnaire. The cohort now includes multiple cycles of follow-up.[21] These datasets were linked to postal code histories for annual place of residence using Historical Tax Summary Files. CanCHEC includes information from Census questionnaires on socioeconomic indicators, ethnicity, and place of residence, as well as neighborhood-level characteristics including environmental conditions.[19] Cause-specific mortality data were linked from the Canadian Vital Statistics Death Database using deterministic and probabilistic methods. The CanCHEC dataset was created under the authority of the Statistics Act and approved by the Executive Management Board at Statistics Canada (reference: 045-2015). This is equivalent to standard research ethics board approval. Informed consent was waived because the database used in this study contains only deidentified individual records.

The present study population was limited to individuals in the 1991, 1996, 2001 or 2006 CanCHEC cycles who were between the ages of 25 and 90 years and who lived in Toronto or Montreal for at least 2 years during follow-up. Individuals who were enumerated in more than one long-form census cycle were assigned to the earliest cohort in which they appeared. All

participants were followed for cardiovascular mortality (ICD-10 codes I10-I69) from the date they entered the study area (on or after census day in 2001 for the 1991, 1996, and 2001 cohorts, or on or after census day in 2006 for the 2006 cohort) to December 31, 2015. This restricted follow-up period was implemented to reduce potential error caused by extrapolation of OP and ROS exposures far into the past (i.e., for the 1991 cohort).

Exposure assignment

Outdoor concentrations of O_X, $PM_{2.5}$ mass concentrations, and $PM_{2.5}$ OP and ROS were assigned to the residential postal codes across each city (6-digit postal codes, about the size of one city block face). Residential postal code histories from annual income tax filings were used to estimate time-varying exposures for O_x (and $PM_{2.5}$) over the duration of the follow-up period to account for residential mobility within and between cities (i.e., between Montreal and Toronto). Specifically, exposures were assigned as 3-year moving averages with a 1-year lag (e.g., an individual's exposure for 2008 was equal to the mean of their exposures for 2005, 2006, and 2007), as in Pinault et al. 2017.[22] This exposure assignment procedure ensured that the exposure always preceded the event. Although $PM_{2.5}$ OP and ROS exposures were measured or estimated based on 2018 data, they were updated annually to account for residential mobility. Person-time was considered at risk of exposure effects if the individual resided in the study area during at least two of the preceding three years.

Outdoor oxidant gas and $PM_{2.5}$ concentrations

Outdoor O_X concentrations were calculated as a redox-weighted average of ozone (O_3) and nitrogen dioxide (NO_2) based on the following equation: $O_X = ((1.07 \times NO_2) + (2.075 \times O_3))$

/3.14.[3, 23] O_3 data were estimated using chemical transport models of surface observations incorporating ground monitor data.[24, 25] O_3 concentrations reflected the daily maximum of eight-hour average concentrations[26] and were assigned as annual averages to postal codes. The O_3 models had a spatial resolution of 21 km^2 before 2009 and 10 km^2 from 2009-2015. Annual average outdoor NO_2 concentrations were estimated from a land-use regression model[27] developed from 2006 data, combining NO_2 estimates derived from remote sensing and National Air Pollution Surveillance monitoring data. The NO_2 model had a spatial resolution of 100 m^2. NO_2 and O_3 data indexed to DMTI Spatial Inc. postal codes were provided by CANUE (Canadian Urban Environmental Health Research Consortium). The resulting calculated O_X exposures combined NO_2 exposures estimated at a 100 m^2 spatial scale with lower-resolution O_3 exposures estimated at a larger spatial scale of 10 km^2 (21 km^2). Finally, annual average outdoor $PM_{2.5}$ mass concentrations were estimated using previously developed models.[28] Briefly, $PM_{2.5}$ concentrations were estimated at a resolution of 1 × 1 km using a combination of aerosol optical depth, a chemical transport model, and land-use data.[28, 29]

Spatial monitoring studies and laboratory analyses for $PM_{2.5}$ oxidative potential and modeling reactive oxygen species concentrations

Outdoor $PM_{2.5}$ monitoring campaigns were conducted in 2018 in Toronto and Montreal, Canada. Monitoring sites were identified to capture the variability of ambient $PM_{2.5}$ in each city with maximal spatial coverage.[22] In total, 110 sites were monitored in Toronto (a geographic area of 630.2 km^2) and 124 sites in Montreal (472.6 km^2) in the summer season; daily mean

temperatures ranged from 14.4°C to 23.7°C in Montreal and 19.8°C to 26.6°C in Toronto. Integrated 2-week $PM_{2.5}$ samples were collected at each site using Teflon filters with a mix of Ultrasonic Personal Air Sample (UPAS) monitors (Access Sensor Technologies, Fort Collins, CO) at a flow rate of 1 L/min and cascade impactors at a flow rate of 5 L/min. Samples were collected simultaneously in each city using preset timers, eliminating the need for temporal adjustment of estimates.

The oxidative potential of $PM_{2.5}$ samples was analyzed using two acellular *in vitro* assays, namely the ascorbate (AA) assay and the glutathione (GSH) assay, according to procedures described previously.[15, 30] Briefly, $PM_{2.5}$ samples were extracted, re-suspended and then incubated with a synthetic human respiratory tract lining fluid for 4 h at 37 °C. This fluid was a composite solution of physiologically-relevant antioxidants including equimolar concentrations (200 µM) of ascorbate (AA), glutathione (GSH), and urate. $PM_{2.5}$ oxidative potential was measured by depletion of AA (% change in absorbance at 260 nm wavelength) and GSH (% change in absorbance at 405 nm wavelength). Measures of AA-related and GSH-related $PM_{2.5}$ oxidative potential were expressed per unit mass (% depletion/µg).

In addition to the OP assays, the concentration of reactive oxygen species (ROS) in the epithelial lining fluid due to generation from redox reactions of transition metals and destruction by reactions with antioxidants was estimated using the KM-SUB-ELF model described by Lakey et al.[16] This mathematical model simulates chemical reactions that occur in the respiratory tract's epithelial lining fluid following inhalation of particles as determined by the particles' Fe and Cu content. Concentrations of Cu and Fe in $PM_{2.5}$ samples were determined by X-ray fluorescence

according to EPA Method IO-3.3 in Compendium of Methods for the Determination of Metals in Ambient Particulate Matter (EPA 625/R-96/010a).

Spatial variations in outdoor $PM_{2.5}$ OP and ROS were assigned based on the measurements collected across each city (i.e., the closest monitoring site to a given residential postal code centroid).[22] In sensitivity analyses, OP values were also estimated from a previously developed land-use regression model;[6] however, the primary analysis used measured values rather than modelled estimates since the measured sites were densely concentrated across the study areas. We were not able to extrapolate OP and ROS estimates into the past due to the absence of historical data. However, we assume relative stability of the spatial distributions of OP and ROS over time since these measures are driven largely by vehicular traffic and the locations of major roadways/highways have not changed significantly over the time period of follow-up.

Statistical analyses

We used stratified Cox proportional hazards models to estimate hazard ratios describing relationships between within-city spatial variations in O_x concentrations and cardiovascular mortality, overall and within strata of OP/ROS of $PM_{2.5}$. Models were stratified by age (10-year age groups), sex (male/female), Census cohort year (1991, 1996, 2001, and 2006), city of residence (Toronto/Montreal), and immigrant status (Canadian-born/immigrant). Covariates were chosen with the aid of a Directed Acyclic Graph (DAG) (see Supplemental Figure 4.1). Additionally, models were adjusted for several indicators of socioeconomic status including

visible minority status, occupational level, educational attainment, labour force status, marital status, and income quintile, as well as an additional variable indicating relative age within the 10-year age group (to address possible residual confounding by age), and $PM_{2.5}$ mass concentration. In addition, we included neighbourhood-level variables for four dimensions of the Canadian Marginalization Index (CAN-Marg) which describes inequalities in terms of material deprivation, residential instability, dependency, and ethnic concentration.[31]

Follow-up time started with census day 2001 for the 1991, 1996 and 2001 cohorts, and census day 2006 for the 2006 cohort. Subjects were censored if they moved outside the cities of Montreal or Toronto, if they were lost to follow-up, at the end of the study period, or at time of death from a non-cardiovascular cause. Data were accessed and analyzed in the secure facilities of the McGill-Concordia Research Data Centre located at McGill University. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary NC). Hazard ratios were expressed per 5 ppb increase in O_X.

The CanCHEC datasets lack information on potential individual-level confounders such as smoking and body mass index (BMI). Although these are not likely to be strong confounders of the relationship between outdoor concentrations of O_x and cardiovascular mortality (i.e., because individual level smoking is not a cause of long-term average outdoor O_X concentrations), we evaluated correlations between O_X and smoking and BMI in order to assess the potential for confounding by chance correlations with O_X. To do this, we used the Canadian Community Health Survey (CCHS) cohort population, an ancillary population-based cohort which has individual-level data on these lifestyle variables. Within each city, we observed weak inverse

correlations between outdoor O_X concentrations and smoking (r = -0.032 (Toronto); r = -0.056 (Montreal)) and BMI (r = -0.013 (Toronto); r = -0.0055 (Montreal)). Thus, residual confounding by these factors would tend to lead to negative confounding[32] and an underestimate of the magnitude of association between O_X exposures and cardiovascular mortality.

Results

Cohort characteristics

In total, approximately 36 800 deaths from cardiovascular causes were included in the analyses, occurring over 10 987 500 person-years (rounded to the nearest 100 to comply with Statistics Canada confidentiality requirements) in approximately 1.1 million individuals (Table 4.1).

Table 4.1. Descriptive statistics at baseline for the study cohort of people living in Toronto or Montreal (1991, 1996, 2001, and 2006 CanCHEC cohorts)

Characteristic	Person-Years	Participants	Cardiovascular deaths
Total	10 987 500	1 121 000	36 800
Sex			
Male	4 994 700	520 400	19 400
Female	5 992 800	600 700	17 400
Immigrant status			
Non-immigrant	5 811 100	606 400	20 500
Immigrant	5 176 400	514 600	16 300
City of residence			
Toronto	6 012 200	616 400	19 500
Montreal	4 975 300	504 600	17 400
Age group			
25-34	917 100	108 300	NA
35-44	2 662 100	259 800	700
45-54	2 855 300	271 800	2 000

55-64	2 050 600	197 400	4 100
65-74	1 495 400	151 100	9 600
75-84	781 800	99 500	13 800
85-89	225 300	33 200	6 500
Occupational class			
Management	927 100	92 400	1 200
Professional	1 748 300	169 800	1 500
Skilled, technical & supervisory	2 035 100	202 300	2 700
Semiskilled	2 441 900	242 900	3 300
Unskilled	800 800	79 200	1 500
No occupation	3 034 500	334 300	26 700
Labour force status			
Employed	7 078 100	697 500	8 100
Unemployed	618 500	62 700	1000
Not in labour force	3 290 900	360 800	27 700
Income quintile			
Lowest	2 194 000	224 500	6 100
Second lowest	2 193 700	236 200	13 900
Middle	2 202 800	226 400	7 400
Second highest	2 195 700	220 700	5 000
Highest	2 201 300	213 200	4 400
Educational attainment			
Less than high school graduation	2 936 300	305 010	18 880
High school graduation with/without trades certificate	3 242 700	330 300	10 300
Some postsecondary or college diploma	1 986 800	206 100	3 800
University degree	2 821 700	279 600	3 900
Marital status			
Divorced/separated/widowed	1 561 200	173 500	10 700
Married (including common law)	7 265 400	720 900	21 100
Single	2 160 810	226 600	5 000
Visible minority status			
Not defined as visible minority	8 672 300	881 100	33 100

Visible minority	2 315 100	239 900	3 800
Marginalization index			
CAN-Marg: Residential instability			
Lowest	2 212 300	206 700	5 700
Second lowest	2 185 600	214 300	6 700
Middle	2 189 000	226 100	6 900
Second highest	2 202 300	234 600	7 600
Highest	2 198 400	239 500	10 000
CAN-Marg: Material deprivation			
Lowest	2 214 100	214 000	7 400
Second lowest	2 178 200	213 200	7 100
Middle	2 192 600	223 800	7 200
Second highest	2 207 700	231 700	7 300
Highest	2 194 700	238 200	7 800
CAN-Marg: Dependency			
Lowest	2 186 700	241 200	5 400
Second lowest	2 233 900	232 900	6 000
Middle	2 171 600	219 100	6 300
Second highest	2 204 100	218 100	7 500
Highest	2 191 200	209 800	11 800
CAN-Marg: Ethnic concentration			
Lowest	2 181 000	209 200	8 000
Second lowest	2 214 700	220 500	7 900
Middle	2 202 700	221 100	7 100
Second highest	2 185 400	224 700	7 300
Highest	2 203 700	245 500	6 600

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.

Table 4.2. Descriptive statistics for ambient pollutant concentrations across all person-years.

Pollutant	Mean (SD)	Median	IQR	Percentile	
				1st	99th
$PM_{2.5}$ (μg/m^3)	9.5 (1.3)	9.5	1.6	7.0	13.0
OP^{AA} (% depletion/μg)	0.092 (0.036)	0.085	0.034	0.016	1.036
OP^{GSH} (% depletion/μg)	0.337 (0.070)	0.332	0.068	0.182	1.442
ROS (nmol/L)	71.474 (10.807)	73.399	14.311	44.525	95.110
NO_2 (ppb)	20.027 (5.475)	19.80	7.60	8.64	35.00
O_3 (ppb)	38.697 (5.081)	37.75	6.73	29.41	51.88
O_x (ppb)	32.345 (3.804)	31.88	5.01	25.56	42.66

IQR: interquartile range; SD: standard deviation.

Characteristics of pollutant exposures

O_X and $PM_{2.5}$ exposures across all person-years, as well as $PM_{2.5}$ OP and ROS, are summarized in Table 4.2. Ox and $PM_{2.5}$ distributions were similar within strata of $PM_{2.5}$ OP and ROS (see Supplementary Tables 4.1-4.2). In Montreal, the median distance from postal code centroids to the nearest monitored site was 916.5 m, with a maximum of 5550.2 m. In Toronto, the median distance was 1338.3 m and maximum 4863.1 m. O_X exposures were weakly/moderately correlated with measures of $PM_{2.5}$ oxidative potential (Pearson correlation coefficients as follows: OP^{AA}, 0.33; OP^{GSH}: 0.51; ROS, 0.29). The three OP and ROS measures were positively correlated, with the highest correlation observed between OP^{AA} and OP^{GSH} (Pearson correlation = 0.54), and weaker correlations between ROS and OP^{GSH} (Pearson correlation = 0.49) and ROS and OP^{AA} (Pearson correlation = 0.17) .

Relationship between O_X and cardiovascular mortality within strata of particle OP/ROS

Figure 4.1 shows the hazard ratios for associations between O_x and cardiovascular mortality. Overall, each 5 ppb increase in O_x (IQR = 5.01) was associated with an increased risk of cardiovascular mortality (HR: 1.028, 95% CI: 1.001, 1.055). The relationship was stronger above the median of OP^{AA}, OP^{GSH} and ROS concentrations (for numeric values see Supplementary Table 4.3). The overall effect of O_x was stronger in women than in men (HR for women: 1.039, 95% CI: 1.000, 1.080; HR for men: 1.019, 95% CI: 0.983, 1.055), but differences in HRs for O_X across strata of OP were larger in men (see Supplementary Table 4.4). Little variation in outdoor $PM_{2.5}$ mass concentration exposures was observed across the study area (IQR = 1.60 $\mu g/m^3$) and was not associated with the risk of cardiovascular mortality (HR per 1 $\mu g/m^3$: 0.988, 95% CI: 0.960, 1.017). Although our primary focus was on the combined weighted redox capacity of NO_2 and O_3, we also observed higher effects above the median of OP^{AA}, OP^{GSH}, and ROS for NO_2 (see Supplementary Table 4.5) and O_3 (see Supplementary Table 4.6). For example, overall O_3 exposures were associated with an increased risk of cardiovascular mortality (HR per IQR (6.73 ppb): 1.035, 95% CI: 1.010, 1.062), and a higher risk above the median OP^{GSH} (HR: 1.021, 95% CI: 1.008, 1.079) than below the median OP^{GSH} (HR: 1.014, 95% CI: 0.973, 1.022).

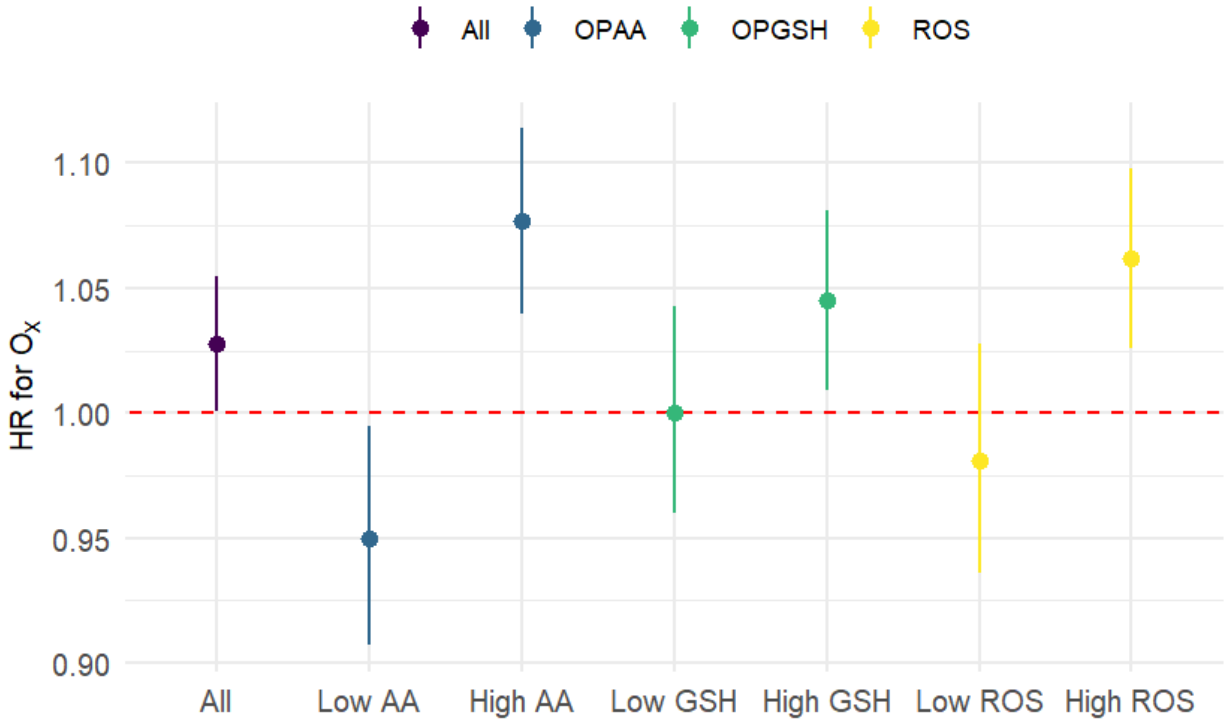


Figure 4.1. Hazard ratios (95% CI) of O_X exposures (per 5 ppb) on cardiovascular mortality overall and across strata (low = below median, high = above median) of $PM_{2.5}$ oxidative potential (ascorbate (AA), glutathione (GSH)) and reactive oxygen species concentrations (ROS).

Spatial distributions of co-occurring high levels of O_X and $PM_{2.5}$ OP

In Montreal, areas with co-occurring high levels of O_X and OP and ROS (i.e., above the median) tended to be near major highways, in the downtown core, and in the east end of the city where industrial activity is prevalent (Figure 4.2). Similarly, in Toronto, these areas occurred in the north-west quadrant of the city where two major highways intersect near an international airport, as well as in the eastern area of the city where there is a major north-south highway (Figure 4.3).

In Montreal, areas with higher marginalization appeared to have a more harmful mixture of pollutants in terms of combined levels of O_X and OP/ROS (Figure 4.3). Specifically, co-exposure to both O_X above the median and OP above the median (for all three OP measures) was weakly to moderately correlated with material deprivation (a measure of access to and attainment of basic material needs, which includes factors such as percent unemployment and percent without a high school degree) in Montreal (Spearman's rank correlations of 0.377-0.421) (see Supplementary Table 4.7). Similarly, the CAN-Marg dimension of residential instability (which includes indicators that measure types and density of residential accommodations as well as family structure characteristics) was correlated with co-exposure to both O_X above the median and OP above the median (Spearman's rank correlations of 0.233-0.436) in Montreal (see Supplementary Table 4.7). However, the CAN-Marg dimensions of ethnic concentration and dependency were not consistently correlated with combined OP/O_X exposures. Significant correlations between marginalization variables and combined OP/O_X were not observed in Toronto.

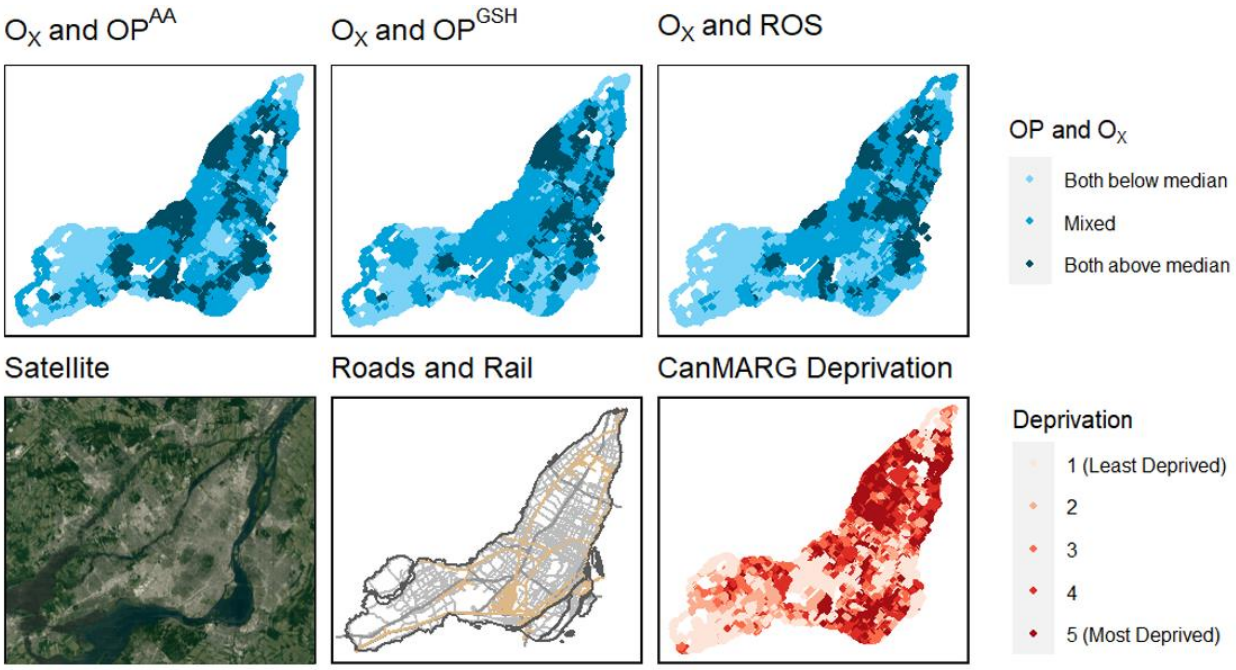


Figure 4.2. Spatial distributions of co-occurring oxidative potential measures and O_X concentrations at postal codes in comparison to land-use patterns, traffic infrastructure and quintiles of socioeconomic deprivation in Montreal, Canada.

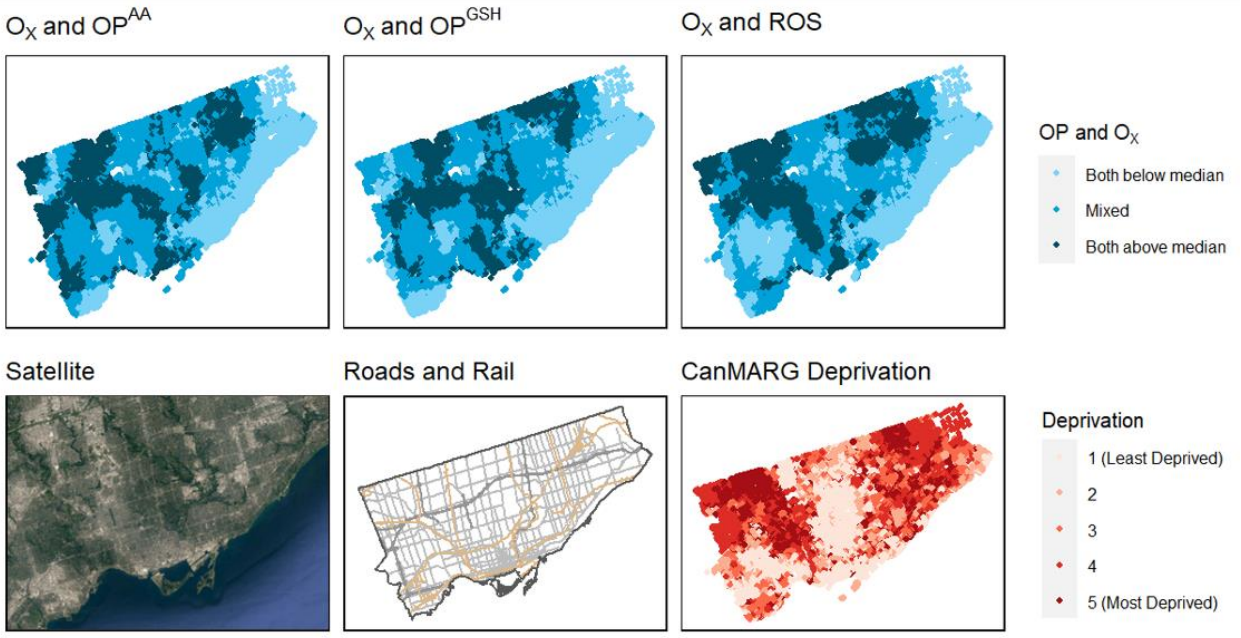


Figure 4.3. Spatial distributions of co-occurring oxidative potential measures and O_X concentrations at postal codes in comparison to land-use patterns, traffic infrastructure and quintiles of socioeconomic deprivation in Toronto, Canada.

Discussion

In this study we investigated how *within-city* spatial variations in outdoor $PM_{2.5}$ OP and ROS concentrations may modify associations between long-term exposure to O_x and cardiovascular mortality. Estimates of spatial variations in OP/ROS were based on monitoring campaigns of 110 sites in Toronto and 124 sites in Montreal to estimate the intraurban spatial variability of OP/ROS exposures.

Our results suggest that exposures to O_X are associated with higher risks of cardiovascular mortality in areas where the ability of $PM_{2.5}$ to induce oxidative stress is elevated. Recent work has demonstrated effect modification of the effects of O_X by OP at the regional scale. For example, between cities, associations between O_X and mortality (nonaccidental, cardiovascular, and respiratory mortality) were consistently stronger in regions where $PM_{2.5}$ oxidative potential was higher.[17] Similarly, another study found that the association of O_X with respiratory hospitalizations in children was higher when monthly average glutathione-based OP (OP^{GSH}) was higher.[18] However, each of these studies was conducted across a wide spatial area using a single site monitor or a small number of monitors in each city/region, so complex small-scale spatial variations in OP were not captured. Our study is the first to present evidence that $PM_{2.5}$ OP can influence the effect of O_X *within* cities.

Although the number of studies showing the influence of OP on O_X exposures is small, a substantial body of evidence demonstrates that OP modifies the effect of $PM_{2.5}$ mass concentration exposures on health outcomes. For example, in a cohort study in Ontario, OP^{GSH}-related oxidative burden (i.e., $PM_{2.5}$ mass weighted by OP^{GSH}) was more strongly associated with elevated risks of mortality than $PM_{2.5}$ mass concentration alone, but this was not observed for OP^{AA}-related oxidative burden.[33] Further, in a case-crossover study conducted in 16 studies across Ontario, the strongest associations between $PM_{2.5}$ and emergency room visits for myocardial infarction occurred in areas where both O_X and OP^{GSH} were high.[34] Given that both Ox and $PM_{2.5}$ mass concentration exposures induce oxidative stress, which is thought to be an important mechanism contributing to the observed adverse health effects, it is plausible that

areas where $PM_{2.5}$ exposures have a greater ability to induce oxidative stress will see greater health effects from both $PM_{2.5}$ mass concentrations and O_X. In our study, spatial variations in $PM_{2.5}$ exposures were minimal at the within-city scale and were not associated with an increased risk of cardiovascular mortality; however, since O_X varies substantially within cities, it was possible to identify an effect of spatial variations in O_X exposures on cardiovascular mortality and to examine how OP enhances that effect.

Areas in Montreal and Toronto with high co-occurring levels of O_X and OP or ROS concentrations appeared to be spatially distributed near sources of traffic emissions. This is consistent with previous work that showed traffic-related variables to be the strongest predictors of OP^{AA}, OP^{GSH}, and ROS in this study area.[6] Transition metals including Cu and Fe are drivers of OP derived from vehicular non-tailpipe emissions; however, OP also responds to other, non-metal components of $PM_{2.5}$ (e.g., organic compounds).[14] Importantly, our findings suggest that neighborhoods in Montreal with higher material deprivation and residential instability (i.e., lower socioeconomic status) may also face a more dangerous mix of air pollution in terms of combined exposure Ox and OP/ROS. We previously described a relationship between material deprivation and measured OP which was evident in Montreal but not Toronto;[6] given our present findings that combined OP/O_X exposures tend to be elevated in areas of Montreal with greater deprivation, it is likely that more-deprived neighborhoods are exposed to a more toxic air pollution mixture relative to less-deprived neighborhoods. Existing evidence suggests that a disproportionate burden of traffic-related air pollution falls upon marginalized populations in Canada's largest cities.[11, 35-38] Moreover, recent evidence suggests that targeted, location-

specific reductions in emissions can efficiently reduce national inequalities in $PM_{2.5}$ exposures[39] and exposures to metals in $PM_{2.5}$.[40] In the same manner, identifying areas in cities where both O_X and $PM_{2.5}$ OP are elevated may be an efficient approach to targeting local interventions aimed at reducing the population health impacts of air pollution.

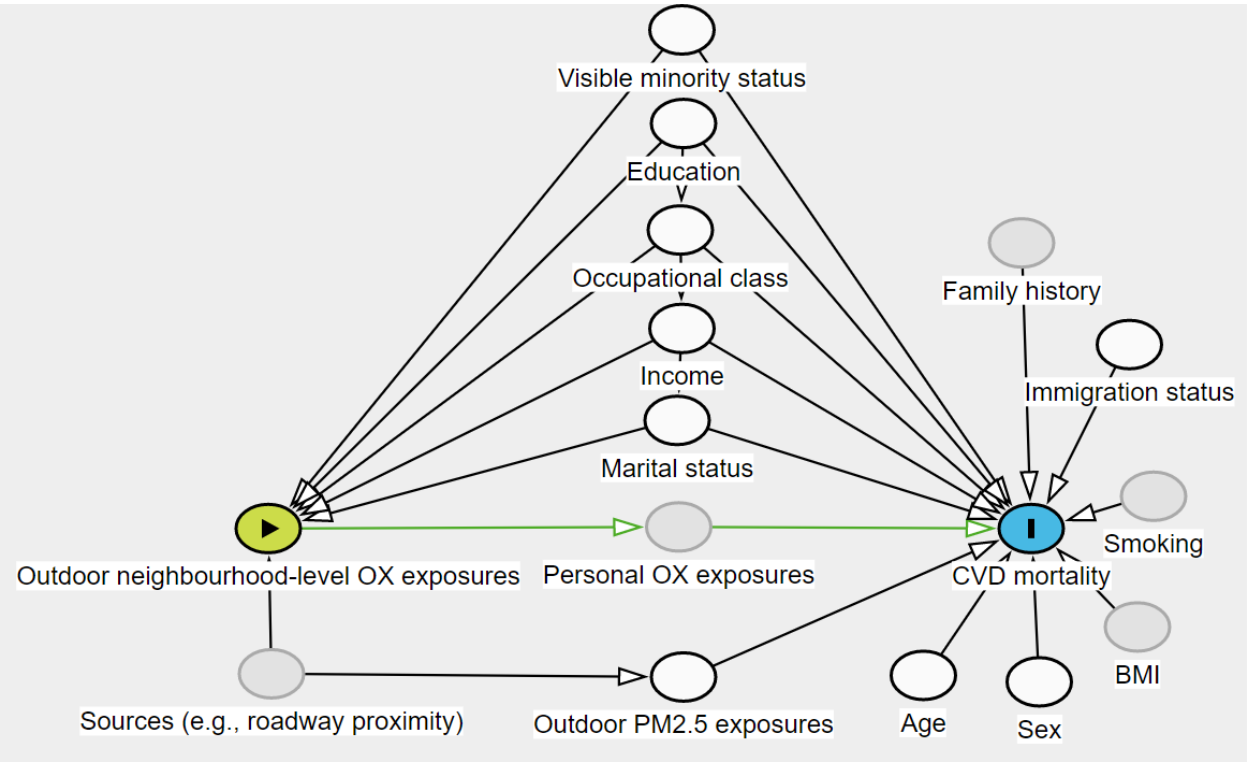
The study has several notable strengths including the availability of annual updated exposure information (for NO_2, O_3 and $PM_{2.5}$), a large study population, and detailed individual-level information on socioeconomic factors. Additionally, we have exposure data from a dense network of monitors allowing measurement of $PM_{2.5}$ oxidative potential/ROS at a high spatial resolution. Nonetheless, we acknowledge some limitations of the study. First, we assume that the use of 2-week monitoring periods represents a sufficient approximation to long-term average spatial variations in $PM_{2.5}$ OP. This assumption is supported by previous studies that have suggested that the spatial pattern of pollutant concentrations derived from short-term monitoring campaigns remains relatively stable over time.[41, 42] Although the OP measurements were collected after the end of follow-up, spatial contrasts are assumed to be representative of earlier spatial contrasts within each city during the follow-up period. Since spatial contrasts were classified as above/below the median of OP or ROS, this error would only change the observed results if it resulted in a location moving across categories (i.e., above/below the median). While we cannot rule out this possibility entirely, the locations of major roadways/highways and industrial areas have not changed over the duration of follow-up and thus we do not expect considerable differences in the spatial distribution of OP/ROS over the follow-up period. Finally, there is potential for exposure measurement error in the assignment of OP values from sites

directly to monitors, as some people live closer to a monitor than others. However, in comparison to previous studies which assigned exposures to individuals residing within a 10 km radius of a measured site,[17] our median distance-to-monitor of approximately 1 km yields a more accurate exposure assignment.

In conclusion, our findings suggest that within-city variations in $PM_{2.5}$ oxidative potential may modify associations between long-term exposure to O_x on mortality from cardiovascular diseases. Additional studies are needed to confirm these results since these patterns may differ in other cities. Nonetheless, our findings suggest that the effect modification previously observed at a regional scale is relevant even at a much smaller spatial scale. Moreover, our results suggest that areas with greater material deprivation and residential instability in Montreal are more likely to be exposed to both O_X and OP above the median, which is a potential environmental justice issue given the increased risk of cardiovascular mortality in these areas.

4.3 Supplementary Material Manuscript 2

Supplementary Figure 4.1. Directed Acyclic Graph for estimating the direct effect of exposure to outdoor oxidant gases (O_X) concentrations on cardiovascular (CVD) mortality. Parameters in grey are unobserved. The green line indicates the estimated association.



Supplementary Table 4.1. Distribution of 3-year average outdoor O_X (ppb) across strata of $PM_{2.5}$ oxidative potential (depletion of glutathione (OP^{GSH}) and ascorbate (OP^{AA})) and reactive oxygen species concentration (ROS).

		O_x	
Characteristic		**Median (25^{th} – 75^{th})**	**5^{th} – 95^{th}**
Overall		31.88 (29.72 – 34.73)	26.78 – 39.28
OP^{GSH}			
	< 50^{th}	30.14 (28.09 – 32.53)	26.24 – 36.27
	≥ 50^{th}	33.60 (31.51 – 36.37)	29.64 – 41.06
OP^{AA}			
	< 50^{th}	30.32 (28.22 - 32.68)	26.24 – 36.24
	≥ 50^{th}	33.45 (31.41 – 36.40)	28.69 – 41.04
ROS			
	< 50^{th}	30.95 (28.73 – 33.40)	26.64 – 37.02
	≥ 50^{th}	32.85 (30.78 – 36.02)	27.13 – 41.01

Supplementary Table 4.2. Distribution of 3-year average outdoor $PM_{2.5}$ mass concentrations ($\mu g/m^3$) across strata of $PM_{2.5}$ oxidative potential (depletion of glutathione (OP^{GSH}) and ascorbate (OP^{AA})) and reactive oxygen species concentration (ROS).

Characteristic		$PM_{2.5}$	
		Median (25^{th} – 75^{th})	5^{th} – 95^{th}
Overall		9.5 (8.7 – 10.3)	26.78 – 39.28
OP^{GSH}			
	< 50^{th}	10.0 (9.3 – 10.8)	8.4 – 12.5
	≥ 50^{th}	9.0 (8.2 – 9.6)	7.3 – 11.0
OP^{AA}			
	< 50^{th}	9.9 (9.1 – 10.6)	7.9 – 12.2
	≥ 50^{th}	9.2 (8.4 – 9.8)	7.4 – 11.1
ROS			
	< 50^{th}	9.6 (8.9 – 10.5)	7.4 – 12.1
	≥ 50^{th}	9.4 (8.6 – 10.1)	7.5 – 11.3

Supplementary Table 4.3. Hazard ratios (95% CI) for the effect of O_x (per 5 ppb) on incidence of cardiovascular mortality across strata of $PM_{2.5}$ oxidative potential (glutathione-based, OP^{GSH}, and ascorbate-based, OP^{AA}) and reactive oxygen species concentration (ROS)

$PM_{2.5}$ Components	Cardiovascular mortality	
	Deaths	HR (95% CI)*
Overall	36 800	1.028 (1.001, 1.055)
OP^{GSH}		
$\leq 50^{th}$	18 800	1.000 (0.960 - 1.043)
$> 50^{th}$	18 100	1.045 (1.009 - 1.081)
Interaction p-value	0.0149	
OP^{AA}		
$\leq 50^{th}$	18 000	0.950 (0.907 - 0.995)
$> 50^{th}$	18 800	1.077 (1.040 - 1.114)
Interaction p-value	0.3712	
ROS		
$\leq 50^{th}$	18 300	0.981 (0.936 - 1.028)
$> 50^{th}$	18 500	1.062 (1.026 - 1.098)
Interaction p-value	0.001	

All Cox proportional hazards models were stratified by age (10-year groups), sex, immigrant status, census cycle, and city, and included covariates for relative age within age group, $PM_{2.5}$ mass concentrations, individual-level income, educational attainment, marital status, employment status, occupational class, and visible minority status. In addition, we included neighbourhood-level variables for four dimensions of the Canadian Marginalization Index (CAN-Marg) which describes inequalities in terms of material deprivation, residential instability, dependency, and ethnic concentration.

Supplementary Table 4.4. Hazard ratios (95% CI) for O_x (per 5.00 ppb) and cardiovascular mortality across strata of $PM_{2.5}$ oxidative potential and ROS concentration stratified by sex.

$PM_{2.5}$ Components	Cardiovascular Mortality			
	Males		Females	
	Deaths	HR (95% CI)	Deaths	HR (95% CI)
Overall	19 400	1.019 (0.983 – 1.055)	17 400	1.039 (1.00 – 1.080)
OP^{GSH}				
$\leq 50^{th}$	9 600	0.967 (0.912 – 1.025)	9 200	1.032 (0.974 – 1.094)
$> 50^{th}$	9 900	1.047 (1.001 – 1.096)	8 200	1.042 (0.988 – 1.098)
OP^{AA}				
$\leq 50^{th}$	9 400	0.927 (0.869 – 0.989)	8 700	0.974 (0.911, 1.040)
$> 50^{th}$	10 100	1.075 (1.027 – 1.126)	8 700	1.075 (1.027, 1.137)
ROS				
$\leq 50^{th}$	9 300	0.950 (0.889 – 1.015)	9 000	1.007 (0.942 – 1.076)
$> 50^{th}$	10 100	1.043 (0.996 – 1.091)	8 400	1.089 (1.034 – 1.146)

Supplementary Table 4.5. Hazard ratios (95% CI) for NO_2 (per interquartile range = 7.60 ppb) and cardiovascular mortality across strata of $PM_{2.5}$ oxidative potential and ROS concentration

$PM_{2.5}$ Components		Cardiovascular mortality	
		Deaths	HR (95% CI)*
Overall		36 800	0.992 (0.971 – 1.013)
OP^{GSH}			
	$\leq 50^{th}$	18 800	0.986 (0.957 – 1.015)
	$> 50^{th}$	18 100	1.017 (0.979 – 1.057)
OP^{AA}			
	$\leq 50^{th}$	18 000	0.927 (0.895 – 0.961)
	$> 50^{th}$	18 800	1.049 (1.014 – 1.086)
ROS			
	$\leq 50^{th}$	18 300	0.933 (0.905 – 0.962)
	$> 50^{th}$	18 500	1.061 (1.028 – 1.095)

Supplementary Table 4.6. Hazard ratios (95% CI) for O_3 (per interquartile range = 6.73 ppb) and cardiovascular mortality across strata of $PM_{2.5}$ oxidative potential and ROS concentration

$PM_{2.5}$ Components		Cardiovascular mortality	
		Deaths	HR (95% CI)
Overall		36 800	1.035 (1.010 – 1.062)
OP^{GSH}			
	$\leq 50^{th}$	18 800	1.014 (0.973 – 1.022)
	$> 50^{th}$	18 100	1.042 (1.007 – 1.079)
OP^{AA}			
	$\leq 50^{th}$	18 000	0.979 (0.938 – 1.022)
	$> 50^{th}$	18 800	1.062 (1.026 – 1.10)
ROS			
	$\leq 50^{th}$	18 300	1.057 (1.015 – 1.101)
	$> 50^{th}$	18 500	1.028 (0.993 – 1.064)

Supplementary Table 4.7. Spearman rank correlations for the relationship between four dimensions of the Canadian Marginalization Index and co-occurring high/low (above/below the median) levels of O_X and OP (OP^{AA}, OP^{GSH} and ROS concentration)

	O_X/OP^{GSH} Spearman rank correlation[1]	P-value[2]	O_X/OP^{AA} Spearman rank correlation	P-value	O_X/ROS Spearman rank correlation	P-value
Montreal						
Residential instability	0.41	<0.01	0.38	<0.01	0.42	<0.01
Material deprivation	0.29	<0.01	0.32	<0.01	0.23	0.02
Ethnic concentration	0.13	0.19	-0.06	0.58	0.04	0.68
Dependency	0.17	0.09	0.17	0.86	0.02	0.06
Toronto						
Residential instability	0.21	0.10	0.21	0.09	0.23	0.07
Material deprivation	-0.06	0.64	-0.02	0.88	0.18	0.16
Ethnic concentration	-0.10	0.42	0.03	0.78	-0.08	0.49
Dependency	0.23	0.06	0.21	0.10	0.27	0.03

1. Correlation between 3-level variable for co-occurring OX and OP (i.e., both below the median; mixed; or both above the median) and 5-level variable for quintiles of the Canadian Marginalization Index.
2. P-value from Spearman's rank sum test.

Supplementary Table 4.8. Regression results for hazard ratios of O_X exposures (per 5 ppb) on cardiovascular mortality overall and across strata of $PM_{2.5}$ oxidative potential (OP) and reactive oxygen species concentrations (ROS) using OP and ROS estimated from land use regression models

Measure/strata	Hazard ratio (95% confidence interval)
OP^{GSH} below median	0.9586 (0.9202, 0.9983)
OP^{GSH} above median	1.1502 (1.1154, 1.186)
OP^{AA} below median	0.9812 (0.9398, 1.024)
OP^{AA} above median	1.1709 (1.135, 1.208)
ROS below median	0.9587 (0.9162, 1.0079)
ROS above median	1.1506 (1.1154, 1.1868)

OP^{AA}: ascorbate-related oxidative potential; OP^{GSH}: Glutathione-related oxidative potential; ROS: estimated ability of $PM_{2.5}$ to directly generate reactive oxygen species.

References

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Chapter 5: Manuscript 3

5.1 Preface

In addition to their ability to induce oxidative stress (examined in Objective 2), fine particles can be characterized by other measures of composition and toxicity. Recently, attention has turned toward exposures to magnetite nanoparticles, which are extremely small iron oxide particles that make up part of the pollution mixture in the urban environment. These particles are noteworthy due to their transition metal composition, magnetic properties, and small size which allows them to translocate to the brain.[76] Since existing evidence suggests that ultrafine particles are associated with brain cancer incidence,[99] the magnetite nanoparticle content of $PM_{2.5}$ represents a plausible health risk. However, epidemiologic studies have yet to evaluate the potential effects of this exposure. In this study, we estimated associations between outdoor magnetite nanoparticle concentrations and brain cancer incidence at the within-city scale.

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5.2 Within-city spatial variations in $PM_{2.5}$ magnetic properties and brain cancer incidence in Toronto and Montreal, Canada

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Abstract

Introduction: Magnetite nanoparticles are small, strongly magnetic iron oxide particles which are produced during high-temperature combustion and friction processes and form part of the outdoor air pollution mixture. These particles can translocate to the brain and have been found in human brain tissue. However, the health impacts of exposure to magnetite nanoparticles in air pollution have yet to be assessed in epidemiologic studies. In this study, we estimated associations between within-city spatial variations in the concentration of magnetite nanoparticles in outdoor fine particulate matter ($PM_{2.5}$) and brain cancer incidence. We also estimated associations between long-term exposures to outdoor $PM_{2.5}$ and nitrogen dioxide (NO_2) (as a marker of traffic-related air pollution) and brain cancer incidence and examined if these relationships were modified by the magnetite nanoparticle content of $PM_{2.5}$.

Methods: We performed a cohort study using four cycles of the Canadian Census Health and Environment Cohort in Montreal and Toronto, Canada. We followed 1.29 million participants for incident malignant brain tumours from 2001 to 2010 in Montreal and from 2001 to 2016 in Toronto. As a proxy for magnetite nanoparticle content, the susceptibility of anhysteretic remanent magnetization (χ_{ARM}) was measured in $PM_{2.5}$ sampled across both cities (N = 124 in Montreal, N = 110 in Toronto), and values were assigned to residential locations, updated annually to account for residential mobility within and between cities. Stratified Cox proportional hazards models were used to estimate hazard ratios (per IQR change in volume-normalized χ_{ARM} $PM_{2.5}$, a unitless quantity) adjusting for socioeconomic and demographic factors as well as exposures to $PM_{2.5}$ mass concentrations and NO_2.

Results: We identified 1,300 incident brain tumours during the follow-up period. Within-city spatial variations in volume-normalized χ_{ARM} were not associated with brain tumour incidence (HR = 0.998, 95% CI: 0.988, 1.009) after adjusting for $PM_{2.5}$, NO_2, and sociodemographic factors, and applying an indirect adjustment for unmeasured potential confounders (cigarette smoking and body mass index). Outdoor $PM_{2.5}$ mass concentrations and NO_2 were also not associated with increased brain tumour incidences, and further stratification by mass-normalized χ_{ARM} (above/below the median) did not alter these findings.

Conclusion: We found no evidence of an important relationship between within-city spatial variations in the content of airborne magnetite nanoparticles and brain tumour incidence. Further research is needed to evaluate this understudied exposure as magnetite nanoparticles present in the external environment are known to reach the human brain.

Introduction

Outdoor air pollution is among the leading causes of death and disease worldwide and has many harmful effects on human health, including associations with numerous cancers.[1] Evidence suggests positive associations between brain cancer and some components of the air pollution mixture, including carbonaceous particles (components indicating a combustion source) in fine particle air pollution ($PM_{2.5}$)[2] as well as nitrogen oxides (NO_X, a marker of traffic-related air pollution)[3] and ultrafine particles;[4] conversely, a number of studies have also reported null associations for $PM_{2.5}$[4-6] and nitrogen dioxide (NO_2)[7] concentrations with brain cancer incidence. Since there are few known modifiable risk factors for brain cancer, identifying the effects of modifiable environmental exposures may be an important way to reduce brain cancer incidence.

While existing studies of air pollution and brain cancer generally focus on the most commonly measured air pollutants such as fine particle mass concentrations ($PM_{2.5}$), there is increased interest in novel air pollution exposure measures that account for composition, toxicity, and/or size of particles. Specifically, measures that account for particle composition, toxicity, and size may vary spatially at finer scales compared to $PM_{2.5}$ mass concentrations, which could make them more useful in epidemiologic studies of exposure variations within cities. One measure of interest is the magnetite nanoparticle content of outdoor $PM_{2.5}$. Magnetite nanoparticles are small (<<100 nm in diameter), strongly magnetic iron oxide particles that are produced during high-temperature combustion and friction processes including both vehicle tailpipe emissions and brake-wear as well as industrial activity.[8-10] Moreover, existing evidence suggests that outdoor concentrations of magnetite nanoparticles measured in $PM_{2.5}$ samples vary substantially within cities, much more so than traditional $PM_{2.5}$ mass concentrations.[11]

The relationship between magnetite nanoparticles and brain cancer is of particular interest as these pollutants can enter the brain directly through the olfactory nerve, the neuroenteric system and via

the circulation, and have been identified in human brains.[12-14] Once in the brain, magnetite may pose a risk to brain health by provoking redox activity that leads to oxidative stress.[12] In a study in Mexico City, neuroinflammatory markers were correlated with presence of metals in the frontal lobe in children and young adults.[12, 15] The magnetite nanoparticles observed in human brains are co-associated with a range of other, potentially toxic, exogenous metal-bearing particles, including aluminium, titanium, nickel, platinum.[12, 13] Although populations are known to be exposed to magnetite nanoparticles, and it is biologically plausible that such exposures could contribute to adverse health outcomes, to date there have been no epidemiologic studies of the health effects of exposure to magnetite nanoparticles in ambient air pollution. The aim of this study was to estimate the association between within-city spatial variations in the concentration of magnetite nanoparticles, as represented by laboratory measurement of the anhysteretic remanent magnetization susceptibility (χ_{ARM}) of outdoor $PM_{2.5}$ (as described below) and incidence of brain cancer in Montreal and Toronto, Canada. As a secondary aim, we investigated whether associations between brain tumours and outdoor concentrations of nitrogen dioxide (a marker of the broader traffic-related air pollution mixture) and $PM_{2.5}$ mass concentrations were modified by mass-normalized ARM susceptibility of $PM_{2.5}$.

Methods

Cohort description

The Canadian Census Health and Environment Cohort (CanCHEC) is a population-based cohort that has been described previously.[16, 17] The cohort includes multiple cycles of follow-up of Canadian Census records and includes non-institutionalized Canadians (aged 25 and older) who were among the approximately 20% of households selected for enumeration by the long-form Census questionnaire in one of the eligible census years.[18] These datasets were linked to postal code histories to obtain annual

place of residence from Historical Tax Summary Files. CanCHEC includes information from Census questionnaires on individual-level and contextual variables including socioeconomic indicators, ethnicity, and place of residence, as well as environmental conditions.[16] Mortality data were linked from the Canadian Vital Statistics Death Database and cancer incidence data were linked from the Canadian Cancer Registry. The CanCHEC dataset was created under the authority of the Statistics Act and approved by the Executive Management Board at Statistics Canada (reference: 045-2015). This is equivalent to standard research ethics board approval. Informed consent was waived because the database used in this study contains only deidentified individual records.

Our study population includes individuals in the 1991, 1996, 2001 or 2006 CanCHEC cohorts aged 25 to 90 years at baseline who lived in Toronto or Montreal for at least 2 years during follow-up. Since approximately 20% of households were randomly assigned to complete the long-form census in each cohort cycle, some individuals were enumerated on more than one long-form census. These individuals were assigned to the earliest cohort in which they appeared.

Ascertainment of cancer diagnosis

Cancer diagnoses in CanCHEC were identified using data linked to the CanCHEC cohorts from the Canadian Cancer Registry, a database that records incident primary cancers diagnosed for each person since 1992.[19, 20] Participants were followed for first incidence of primary malignant brain tumour (defined by International Classification of Diseases, 10th Revision (ICD-10) codes C71.0 - C71.9). Follow-up time started with Census day 2001 for the 1991, 1996 and 2001 cohorts, and Census day 2006 for the 2006 cohort. This restricted follow-up period was implemented to reduce potential error caused by extrapolation of estimated ARM susceptibility of $PM_{2.5}$ into the past. For members of the study population living in Montreal, cases were only

identified in the period 2001-2010 as cancer diagnosis data were not available in the province of Quebec for diagnosis years from 2011 onward. Participants were excluded if they had any cancer diagnosis in the three years prior to the start of follow-up to reduce the possibility of confounding by potential exposure to ionizing radiation in cancer treatment. For a small number of individuals, exact date of diagnosis was unavailable. In cases where month and year of diagnosis was available but day of diagnosis was missing, diagnosis was assigned to the 15th day of the month. If only year of diagnosis was available, the individual was excluded from the dataset (N = 36).

Spatial monitoring studies and estimation of magnetite nanoparticle exposures using anhysteretic remanent magnetization

$PM_{2.5}$ samples were collected in outdoor monitoring campaigns conducted in 2018 in Montreal and Toronto, Canada. Sites where monitors were placed were selected to capture important sources of ambient $PM_{2.5}$ in each city while maximizing spatial coverage of the study area.[22] A total of 124 sites in Montreal and 110 sites in Toronto were monitored. Mean daily temperatures over the sampled period ranged from 14.4°C to 23.7°C in Montreal and 19.8°C to 26.6°C in Toronto. Integrated 2-week $PM_{2.5}$ samples were collected using Teflon filters and preset timers with a mix of Ultrasonic Personal Air Sample (UPAS) monitors (Access Sensor Technologies, Fort Collins, CO) at a flow rate of 1 L/min and cascade impactors at a flow rate of 5 L/min.

In order to quantify the content of magnetite particles in $PM_{2.5}$ samples, anhysteretic remanent magnetization (ARM) was measured. ARM is roughly proportional to the concentration of ferrimagnetic minerals within a sample[21] and specifically responds to the presence of magnetic nanoparticles with

diameters between 30–50 nm.[22, 23] First, $PM_{2.5}$ samples (on PTFE filters) were exposed to four different direct current (DC) biasing fields of 0.06 mT, 0.08 mT, 1.0 mT and 1.2 mT. Subsequently, a 2G RAPID cryogenic magnetometer (2G Enterprises, Mountain View, CA) was used to measure the magnetic response of the samples. ARM measurements were also made of 20 blank PTFE filters and the mean of the ARM measurements taken on these blanks was subtracted from sampled filter values. ARM was expressed as a susceptibility of ARM normalized by the direct current (DC) field, calculated as the slope of the ARM(DC field) linear function. ARM susceptibility values were then normalized by air sampled volume (expressed as K_{ARM}, a dimensionless quantity) for the primary analysis and by particulate mass (expressed as χ_{ARM}, in units of m^3/kg) in secondary analyses.

Outdoor $PM_{2.5}$ and nitrogen dioxide concentrations

To evaluate the effects of spatial variations in other co-occurring pollutants, we assigned long-term estimates of outdoor $PM_{2.5}$ and nitrogen dioxide (NO_2) concentrations at residential address to cohort members in the same manner. Annual average outdoor $PM_{2.5}$ mass concentrations were estimated using models described in detail previously.[24] Briefly, $PM_{2.5}$ concentrations were estimated at a 1 × 1 km resolution using aerosol optical depth, a chemical transport model, and land-use data.[24, 25] Annual average outdoor concentrations of NO_2 were estimated using a land-use regression model[26] in which estimates were derived from remote sensing and National Air Pollution Surveillance monitoring data; this model was developed from 2006 data and had a spatial resolution of 100 m^2. $PM_{2.5}$ and NO_2 data indexed to DMTI Spatial Inc. postal codes were provided by CANUE (Canadian Urban Environmental Health Research Consortium).

Exposure assignment

$PM_{2.5}$ ARM susceptibility values, as well as $PM_{2.5}$ and NO_2 concentrations, were assigned directly to residential 6-digit postal codes (an area equivalent to approximately one city block face in urban areas) from the value at the closest measured site. Postal codes were linked to monitored points using latitude and longitude from the master postal code list (CanMap Postal Suite, version v2015.3, DMTI Spatial Inc., Markham). In cases where a single postal code was represented by multiple points of latitude and longitude, an average estimate for the postal code was created by equally weighting the multiple pollutant values across points. Time-varying exposures were estimated using residential postal code histories from annual income tax filings, allowing for movement within and between cities. Exposures were assigned to cohort members at their residential address as 3-year moving averages with a 1-year lag (e.g., an individual's exposure for 2008 was the mean of their exposures for 2005, 2006, and 2007). This is consistent with the standard exposure assignment used in many studies using the CanCHEC cohort since ambient $PM_{2.5}$ is regulated in Canada based on a three-year time window.[27]

Statistical analyses

Stratified Cox proportional hazards models were used to estimate hazard ratios describing the relationship between $PM_{2.5}$ ARM susceptibility (volume-normalized, i.e., K_{ARM}) and incidence of brain tumours. Follow-up time started with time of entry into the CanCHEC cohort (e.g., Census day 2001 for the 2001 cohort). Subjects were censored if they moved outside the cities of Montreal or Toronto, if they were lost to follow-up, at the end of study period, or at time of death, whichever came first. Data were accessed and analyzed in the secure facilities of the McGill-Concordia Research Data Centre located at McGill University. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) and R 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

Variables used for regression adjustment were chosen based on a Directed Acyclic Graph (DAG) (Supplementary Figure 5.1). There are few well-established risk factors for primary brain tumours except for exposure to ionizing radiation and family history.[28] Nonetheless, we adjusted for a number of demographic and socioeconomic status variables which could confound the relationship through chance associations with the outcome. Specifically, we adjusted for age (5-year age groups as a strata variable), sex (male/female strata), immigration status (immigrant/nonimmigrant strata), Census cohort year (four categories as strata: 1991, 1996, 2001, and 2006), visible minority status, occupational level, educational attainment, marital status, and income quintile. Additionally, models were adjusted for $PM_{2.5}$ mass concentrations and NO_2 to evaluate the sensitivity of effect estimates to spatial variations in long-term exposures to these pollutants.

As an additional analysis, we examined the effects of $PM_{2.5}$ and NO_2 stratified by mass-normalized ARM susceptibility (χ_{ARM}). When stratifying by ARM susceptibility, our goal in the estimation of NO_2 effects on brain cancer incidence was to investigate whether the mixture of traffic-related air pollutants (of which NO_2 is a marker) is more harmful in areas with greater ARM susceptibility; in the $PM_{2.5}$ analysis, our goal was to evaluate whether the effect of exposure to fine particles was greater in areas where $PM_{2.5}$ ARM susceptibility is higher. We performed Cox proportional hazards regression as described above and below the median of χ_{ARM} values.

Sensitivity analyses

Some individual-level risk factor variables, notably cigarette smoking and body mass index (BMI), are not available in the CanCHEC database. Although the evidence linking smoking to brain cancer incidence is inconsistent, and some studies suggest no relationship,[29] cigarette smoking is an important cause of human cancer and meta-analysis suggests a possible association with brain cancer incidence.[30] Similarly, evidence suggests a possible assocation of obesity with some types of brain cancer.[31] Smoking and BMI are not causes of outdoor air pollutant concentrations, so they are not confounders in the standard definition.[32] Nonetheless, chance associations could confound the relationship between outdoor $PM_{2.5}$, NO_2 or ARM susceptibility and brain tumour incidence, and the indirect adjustment method was applied to address this possibility. The indirect adjustment method has been described in detail previously;[33] briefly, this method uses data on the correlation between measured covariates and unmeasured risk factors from a secondary data source, as well as estimates of the relationship between the missing risk factors and incidence of the outcome. We used data from multiple cycles of the Canadian Community Health Survey, a biannual national health survey that has the same target population as the Canadian census (i.e., the Canadian population) and collects data on health and lifestyle characteristics including smoking and BMI. The relationships between smoking and brain cancer, as well as BMI and brain cancer, were estimated from the literature based on systematic reviews and meta-analyses of the existing evidence.[30, 31]

Results

Cohort characteristics are presented in Table 5.1. In total, we identified approximately 1,300 eligible cases of malignant primary brain tumours over 13.6 million person-years of follow-

up in 1.29 million individuals (all numbers rounded to the nearest 100 to satisfy institutional confidentiality requirements). Incident brain tumours were identified at a higher rate in people of increased age, in men relative to women, and in people who identified as white relative to those who identified as visible minorities (Table 5.1).

Table 5.1. Descriptive statistics at baseline for the study cohort of people living in Toronto or Montreal (1991, 1996, 2001, and 2006 CanCHEC cohorts)

Characteristic	Person-Years	Participants	Incident brain tumours
Total	13,636,200	1,291,900	1300
Sex			
Male	6,258,300	608,300	700
Female	7,377,900	683,600	600
Immigrant status			
Non-immigrant	7,032,800	710,400	700
Immigrant	6,603,400	581,500	600
City of residence			
Montreal	5,079,400	564,600	500
Toronto	8,556,800	727,300	800
Age group			
25-34	2,014,600	193,700	200
35-44	3,071,500	288,300	300
45-54	3,057,900	287,900	300
55-64	2,175,500	205,700	200
65-74	1,457,900	137,400	200
75-84	1,284,700	123,300	200
85-89	574,100	55,500	100
Occupational class			
Management	1,151,500	107,300	100
Professional	2,132,000	193,500	200
Skilled, technical & supervisory	2,513,700	233,600	200
Semiskilled	3,032,200	279,200	300

Unskilled	993,800	89,800	100
No occupation/not in labour force	3,812,900	388,500	500
Income quintile			
Lowest	2,727,300	260,900	200
Second lowest	2,727,200	274,300	300
Middle	2,722,800	261,000	300
Second highest	2,731,500	254,200	300
Highest	2,727,400	241,400	300
Educational attainment			
Less than high school graduation	3,725,400	351,700	400
High school graduation with/without trades certificate	4,053,500	382,700	400
Some postsecondary or college diploma	2,405,900	238,000	200
University degree	3,451,400	319,500	300
Cohort			
1991	3,778,500	337,000	400
1996	5,225,900	465,700	600
2001	3,269,000	283,300	200
2006	1,362,800	205,900	100
Marital status			
Single	2,695,600	263,600	200
Common-law	929,600	101,000	100
Married	8,038,100	723,400	900
Separated	401,000	38,900	NA
Divorced	849,000	84,300	100
Widowed	722,900	80,600	100
Visible minority status			
Not defined as visible minority	3,704,000	962,300	1,100
Visible minority	9,932,200	329,600	200

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

The mean volume-normalized ARM susceptibility (K_{ARM}) across all eligible person-years was 5.8 × 10^{-14} (SD = 3.4 × 10^{-14}) and mean mass-normalized ARM susceptibility (χ_{ARM}) was 9.3 × 10^{-6} m^3/kg (SD = 6.7 × 10^{-6} m^3/kg). Spatial variations in ARM susceptibility were much greater than spatial variations in $PM_{2.5}$ mass concentrations. The mean $PM_{2.5}$ concentration was 9.4 μg/m^3 (SD = 1.3 μg/m^3) and the mean NO_2 concentration was 21.2 ppb (SD = 5.5 ppb) (Table 5.2). Spatial patterns of K_{ARM} and χ_{ARM} by postal code are mapped in Figure 5.1. ARM susceptibility parameters showed little correlation with other ambient pollutants. Spatial variations in K_{ARM} (volume-normalized) were very weakly correlated with $PM_{2.5}$ mass concentration (r = -0.0007) and NO_2 (r = 0.0496), and similarly spatial variations in χ_{ARM} (mass-normalized) were very weakly correlated with $PM_{2.5}$ (r = 0.023) and NO_2 (r = -0.028).

Table 5.2. Descriptive statistics for ambient pollutant concentrations and $PM_{2.5}$ ARM characteristics across all person-years.

Characteristic	Mean (SD)	Median	IQR	Percentile			
				1st	25th	75th	99th
Pollutant concentrations							
$PM_{2.5}$ (μg/m^3)	9.4 (1.3)	9.5	1.6	6.9	8.5	10.1	13
NO_2 (ppb)	21.2 (5.5)	21.0	7.5	10.4	17.1	24.6	36.4
ARM susceptibility parameters							
K_{ARM}, volume specific (× 10^{-14}, unitless)	5.8 (3.4)	4.9	3.1	0.1	4.0	7.1	18.0
χ_{ARM}, mass specific (× 10^{-6} m^3/kg)	9.3 (6.7)	7.0	4.0	2.0	6.0	10.0	39.0

ARM: anhysteretic remanent magnetization; IQR: interquartile range; NO_2: nitrogen dioxide; $PM_{2.5}$: fine particulate matter; SD: standard deviation.

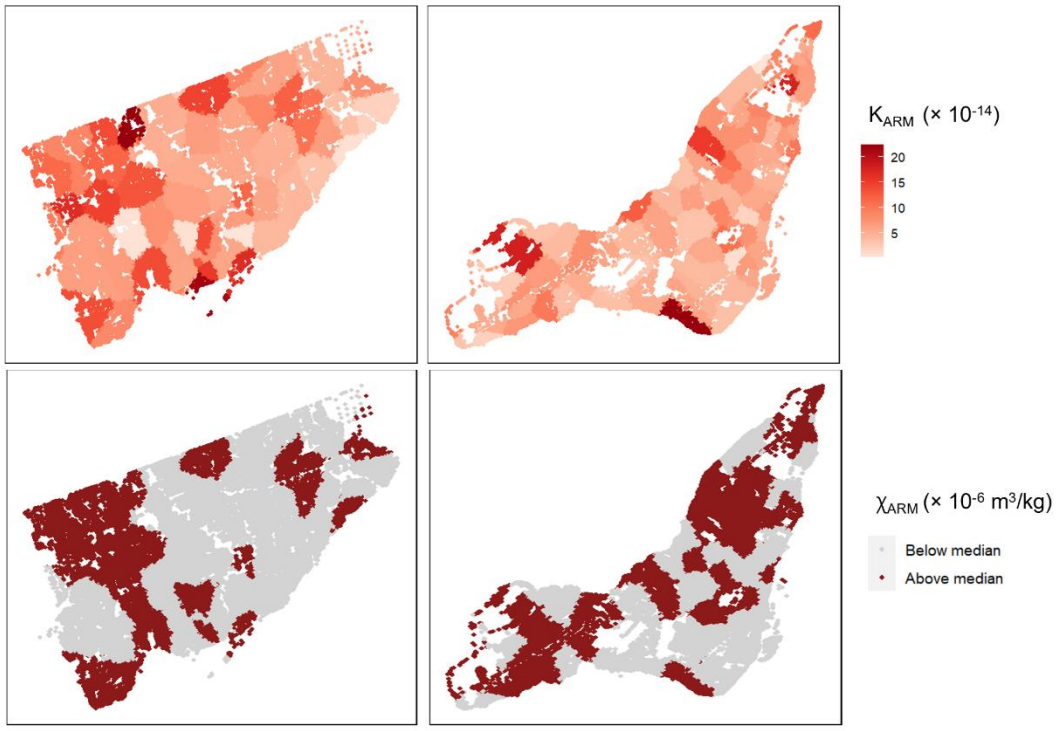

Figure 5.1. Spatial variations in volume-normalized ARM (K_{ARM}) and areas above/below the median mass-normalized ARM (χ_{ARM}) assigned to postal codes from measured sites in Toronto and Montreal, Canada.

Cox regression model results are presented in Table 5.3. Models showed no association between volume-normalized ARM susceptibility (K_{ARM}) and brain cancer incidence (HR per 3.0 × 10^{-14}: 0.998, 95% CI: 0.988, 1.009). Long-term exposures to $PM_{2.5}$ were inversely associated with brain cancer incidence (HR per 3 μg/m^3: 0.833, 95% CI: 0.681, 1.021). When stratified by mass-normalized ARM susceptibility (χ_{ARM}), the effect of $PM_{2.5}$ was closer to the null (i.e., indicating a less strong protective effect) above the median X_{ARM} (HR: 0.899, 95% CI: 0.774, 1.043) relative to below the median (HR: 0.711, 95% CI: 0.374, 1.350), but estimates were imprecise. Similarly, long-term average exposures to NO_2 were not associated with brain cancer incidence (HR per 10

ppb: 0.963, 95% CI: 0.876, 1.058). In stratified analyses, the point estimate of the effect of NO_2 above the median of χ_{ARM} (HR: 0.945, 95% CI: 0.848, 1.053) was protective whereas below the median of χ_{ARM} the effect was deleterious (HR: 1.020, 95% CI: 0.877, 1.195); however, confidence intervals were wide and overlapping. For all pollutants, indirect adjustment for smoking and body mass index had little effect on hazard ratios (Table 5.3).

Table 5.3. Crude and indirectly adjusted hazard ratios for incident primary malignant brain tumours.

Pollutant	Crude HR (95% CI)	Indirectly adjusted HR (95% CI)
$PM_{2.5}$ (per 3 μg/m^3)		
Overall	0.845 (0.692, 1.031)	0.833 (0.681, 1.021)
Below median ARM susceptibility (χ_{ARM})	0.736 (0.387, 0.898)	0.711 (0.374, 1.350)
Above median ARM susceptibility (χ_{ARM})	0.895 (0.771, 1.092)	0.899 (0.774, 1.043)
NO_2 (per 10 ppb)		
Overall	0.965 (0.881, 1.057)	0.963 (0.877, 1.058)
Below median ARM susceptibility (χ_{ARM})	1.037 (0.891, 1.206)	1.020 (0.877, 1.195)
Above median ARM susceptibility (χ_{ARM})	0.933 (0.841, 1.036)	0.945 (0.848, 1.053)
ARM susceptibility normalized by volume (K_{ARM}) (per 3.0 × 10^{-14})	1.000 (0.894, 1.118)	0.998 (0.988, 1.009)

K_{ARM}: volume-normalized anhysteretic remanent magnetization susceptibility of $PM_{2.5}$; X_{ARM}: mass-normalized anhysteretic remanent magnetization susceptibility of $PM_{2.5}$.

Discussion

We conducted a population-based cohort study examining the relationship between within-city spatial variations in fine particle ($PM_{2.5}$) ARM susceptibility on malignant brain tumour incidence in two Canadian cities. We found no relationship between exposures to volume-normalized ARM susceptibility of $PM_{2.5}$ and brain tumour incidence; moreover, we found no

evidence that the effects of other long-term outdoor pollutant exposures (i.e., $PM_{2.5}$ mass concentration and NO_2 as a marker for traffic-related air pollution) were modified by the mass-normalized ARM susceptibility of fine particles. In general, our findings do not support a relationship between spatial variations in ARM susceptibility of $PM_{2.5}$, a measure which corresponds to the presence of magnetite nanoparticles, and incidence of brain cancer. We identified a non-significant inverse effect of $PM_{2.5}$ on brain cancer incidence which we cannot explain. However, this result is similar to protective effects previously observed for the effect of $PM_{2.5}$ on brain cancer incidence, such as the effect by Jorgenson et al. (HR per 3 $\mu g/m^3$: 0.985, 95% CI: 0.635, 1.54),[34] by Harbo Poulsen et al. (OR per 3 $\mu g/m^3$: 0.992, 95% CI: 0.946, 1.039),[2] or by Weichenthal et al. (HR per 3 $\mu g/m^3$: 0.907, 95% CI = 0.762, 1.079).[4]

Although we found no effect of $PM_{2.5}$ ARM susceptibility on brain cancer incidence, nonetheless the health effects of exposures to magnetite nanoparticles merit further study, as laboratory analyses suggest that exposure to magnetite nanoparticles may be deleterious to human cells in vitro. Exposure to magnetite particles in human cells in vitro can induce reactive oxygen species generation,[35] which contributes to the oxidative stress pathway that may be responsible for many of the observed adverse health effects of PM exposure. Other in vitro studies suggest that exposure to magnetite may play a role in the development of neurodegenerative diseases such as Alzheimer's disease.[36] Magnetite nanoparticles observed in the human brain are co-associated with other exogenous metal-bearing nanoparticles, including titanium, aluminium, platinum, nickel, and cobalt.[12, 13] Finally, toxicological studies have suggested that metal-rich ultrafine particles (UFPs) are able to access all major organs,[37-40]

suggesting their relevance to health outcomes including those affecting the brain.[41] Given the toxicological evidence, there remains a need for future *epidemiologic* studies assessing the effects on health of magnetite nanoparticle exposures.

We observed spatial patterns of variation in ARM that suggest relationships between ARM and land use characteristics. In both cities, the spatial patterns of ARM exposures suggest primarily local sources that are likely related to vehicular traffic.[11] Previous work showed that traffic counts, proximity to major roads and proximity to road intersections were predictors of ARM, as well as proximity to railways (in Montreal but not in Toronto).[11] Additionally, our results show similar spatial patterns to previous studies of UFP, suggesting that ARM and UFP measures reflect similar sources and are likely correlated in space. Specifically, previous studies that assessed the spatial distributions of UFP in Toronto identified elevated concentrations of UFP in the northwest of the city, where two major highways intersect near an airport. [42, 43] We also identified elevated ARM in this northwest quadrant, as well as in the downtown core. Similarly, the areas with lowest ARM in Toronto were the residential neighbourhoods in the city centre. The major highway that runs across the city in the east-west direction appeared to be surrounded by higher ARM areas, which is also consistent with previous UFP studies. In Montreal, UFP concentrations were elevated in the industrial east end of the city, around highways, and in the downtown core.[43] We also observed slightly elevated ARM in the industrial east end, which suggests potential for some non-vehicular sources of magnetite nanoparticles. However, a previous study of UFP and cancer incidence did find a relationship, whereas we

found no relationship between ARM and brain tumour incidence; this suggests that ARM may be of limited use in characterizing the UFP fraction.

Future studies may benefit from exploring different measures of magnetite nanoparticles. We used the room temperature ARM susceptibility of $PM_{2.5}$ as a surrogate measure of magnetic nanoparticle content as it reflects the concentration of particles of approximately 30-50 nm in diameter.[22, 23] However, it is possible that the ARM susceptibility in $PM_{2.5}$ samples may not be a good proxy for sampling the actual nanoparticle size range. We are most interested in the smallest particles (e.g., UFPs, which have diameter less than 0.1 µm) as there is evidence that they may be relevant to the development of brain cancer: specifically, a previous study of brain cancer incidence in Montreal and Toronto, Canada found that residential UFP concentrations were positively associated with brain cancer incidence, but $PM_{2.5}$ mass concentrations were not.[4] Given the potential relevance of UFPs to brain health, a possible direction for future research could be the measurement of magnetic parameters on the ultrafine fraction of particulate matter: for example, Gonet et al. analyzed isothermal remanent magnetization (IRM, a measure of magnetic remanence which results from short-term exposure to strong magnetizing fields) on size-fractionated particles sampled from brake-wear emissions (using 14 size fractions ranging from 0.016 µm – 10 µm). Although an increasing body of literature exists describing magnetic parameters of particles collected from air samples[44, 45] or from tree leaf surfaces near roadways,[8, 46-48] it remains to be determined which measures of magnetic activity and which particle size fractions are most relevant in health studies; future studies may consider size-resolved evaluation of magnetic characteristics of particles as a step

towards assessment of their potential health impact. Additionally, a focus on low-temperature (LT) magnetic measurements may better characterize the UFP fraction. Muxworthy[44] LT magnetite measurements and found significantly higher concentrations of magnetite nanoparticles than previously estimated using room-temperature (RT) measures. In addition, Sheikh et al.[49] collected air samples from the London Underground and analyzed them using ARM as well as both RT-SIRM and LT-SIRM. Given evidence that the predominant size range of magnetite nanoparticles identified in the brain is 5-20 nm,[50] and emerging research suggests that magnetite nanoparticles in this size range are more accurately quantified using LT methods,[44] future studies of PM may be better served by LT magnetic measurements rather than the RT measures that we performed.

This study had several notable strengths, including high-resolution estimates of spatial variations in the ARM susceptibility of PM, the availability of updated exposure information for subjects moving within and between cities, and time-varying estimates of NO_2 and $PM_{2.5}$ exposures, as well as detailed individual-level data on potential confounders. A further advantage is the availability of data on incident, rather than prevalent brain tumour diagnoses. However, our study also had a number of limitations. First, the of $PM_{2.5}$ ARM susceptibility were based on measurements of air filters collected during 2-week monitoring periods in 2018 (i.e., after the end of the follow-up period), and due to the absence of historical measurements it was not possible for us to extrapolate ARM susceptibility estimates backward in time. This is a source of possible exposure measurement error; however, a systematic difference in the degree of exposure error between brain cancer cases and non-cases is not expected and therefore bias

would tend toward the null. Additionally, since major changes in spatial patterns of roadway infrastructure have not occurred during the study period, we did not expect major changes in the spatial patterns of tailpipe and brakewear emissions. Next, measurements of $PM_{2.5}$ ARM susceptibility were made at room temperature, rather than at low temperature (e.g., liquid nitrogen, 77 K, or helium, 4.2 K, temperatures). Recent, low temperature-based studies show that both the total magnetite content and the numbers of ultrafine particles <10 nm in size (magnetically 'invisible' at room temperature) are being routinely under-estimated in magnetic characterisation of particulate air pollution.[49] Further, we assume that the use of 2-week monitoring periods represents a sufficient approximation to long-term average spatial variations in $PM_{2.5}$ ARM susceptibility. We based this assumption on existing evidence that suggests that the spatial pattern of pollutant concentrations derived from short-term monitoring campaigns remains relatively stable over time.[51, 52] Although the ARM susceptibility measurements were collected after the end of follow-up, spatial contrasts are assumed to be representative of earlier spatial contrasts within each city during the follow-up period.

A second limitation was the absence of individual-level data on potential confounders such as smoking and body mass index. However, as described in the conceptual directed acyclic graph (Supplementary Figure 5.1), these individual-level variables are not likely causes of long-term air pollution exposures including $PM_{2.5}$ ARM susceptibility, so they are not strictly confounders. Nonetheless, we performed an indirect adjustment method to account for confounding that could occur by chance associations between $PM_{2.5}$ ARM susceptibility and individual-level variables. Similarly, we lacked individual-level data on other potential causes of

brain cancer (e.g., family history of brain cancer or exposures to ionizing radiation). Since these factors were not present in the ancillary database we used to perform the indirect adjustment, we were unable to adjust for them. It is possible that potential confounding by these or other unmeasured confounders remains, if there exists a systematic relationship between the potential confounders and spatial variations in outdoor $PM_{2.5}$, NO_2 or ARM susceptibility.

In conclusion, we performed the first cohort study of spatial variations in ARM susceptibility of outdoor $PM_{2.5}$ and incident brain tumours. We did not find an association between ARM susceptibility, a measure which is proportional to the concentration of magnetite nanoparticles, and brain cancer incidence. Further, we found non-significant protective effects of NO_2 and PM_{25} exposires and brain cancer incidence, and did not identify modification of these effects by mass-normalized ARM susceptibility. Nonetheless, future studies should further explore the association of exposures to magnetite nanoparticles, especially the finest (< 10 nm) nanoparticles, with health outcomes due to their high exposure prevalence in urban areas.

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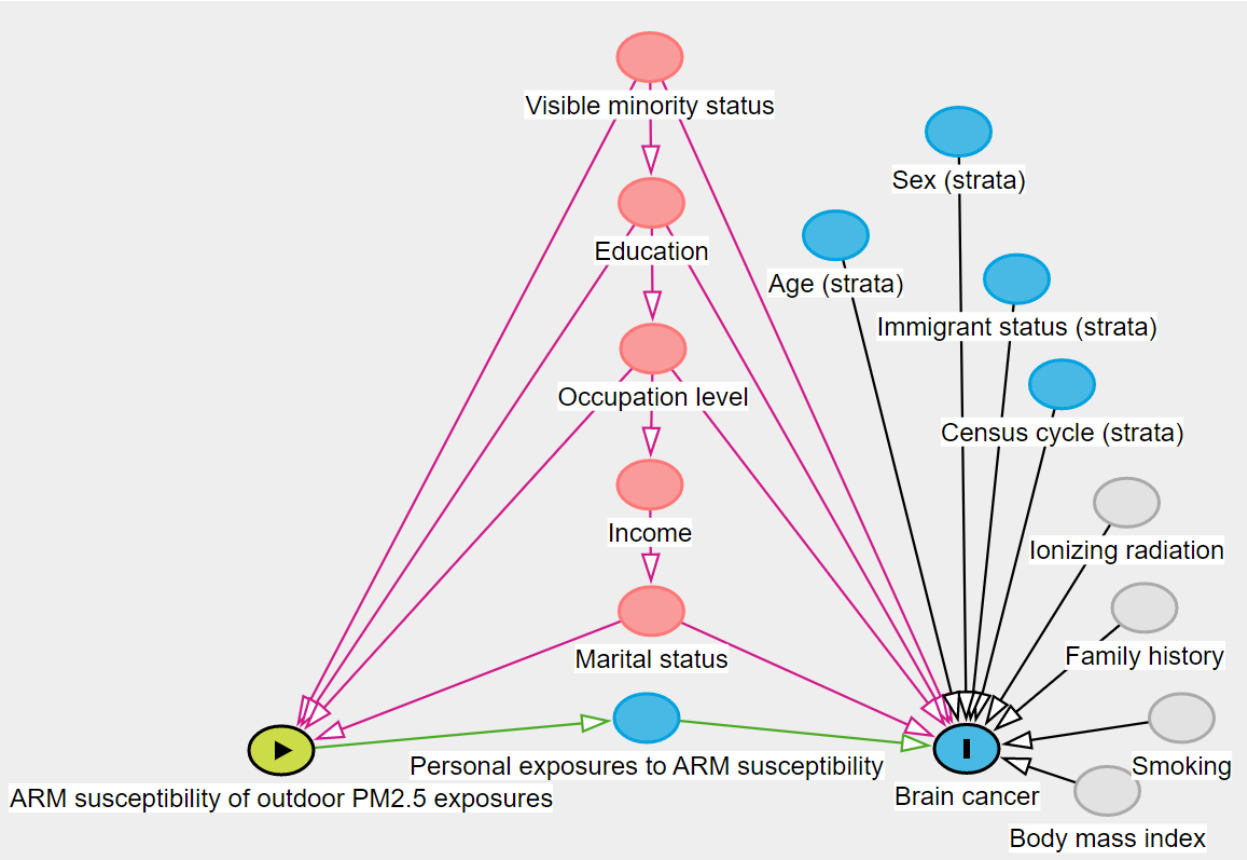
5.3 Supplementary Material Manuscript 3

Supplementary Figure 5.1. Directed acyclic graph for anhysteretic remanent magnetization (ARM) susceptibility of outdoor $PM_{2.5}$ concentrations (a surrogate measure of magnetite nanoparticle concentrations) and brain tumour incidence. Parameters in grey are unmeasured variables, parameters in red are potential confounding factors, and parameters in blue are included as strata variables in the Cox proportional hazards regression models.



Additional information on construction and choice of covariates used in regression models

Covariates reported at baseline:

Categorical variables reported at baseline included marital status (single, common-law, married, separated, divorced, or widowed); income adequacy quintile; highest level of education attained (less than high school graduation, high school graduate with or without trade certificate, postsecondary non-university degree, university degree); occupational class (management, professional, skilled technical and supervisory, semi-skilled, unskilled, not applicable); visible minority status (visible minority, white/indigenous). Indigenous Canadians were included in the white/non-visible minority group for several reasons: because they represent a small fraction of the urban population, for consistency with previous studies,[1] and because white/indigenous Canadians both have elevated rates of brain cancer and other cancers relative to the visible minority group.[2] In addition, models were adjusted for a continuous variable representing the deviation, in years, from the median of each 5-year age category in order to reduce possible residual confounding by age.

Time-varying covariates updated annually:

Co-pollutant variables (i.e., annual average $PM_{2.5}$ and NO_2 concentrations) were assigned as neighbourhood-level variables using residential 6-digit postal codes. Annual average estimates of outdoor $PM_{2.5}$ concentrations were estimated as described previously using a using a combination of aerosol optical depth, a chemical transport model, and land-use modelling[3] and were assigned to residential postal codes with a spatial resolution of approximately 1 km^2. NO_2

exposures were estimated with a spatial resolution of 100 m x 100 m from a model developed from 2006 data, combining NO_2 estimates derived from remote sensing and National Air Pollution Surveillance monitoring data. $PM_{2.5}$ and NO_2 data indexed to DMTI Spatial Inc. postal codes were provided by CANUE (Canadian Urban Environmental Health Research Consortium. $PM_{2.5}$ and NO_2 exposures were assigned using a 3-year moving average with a 1-year lag (updated annually for residential mobility) for consistency with previous CanCHEC studies.[1]

Indirect adjustment methods

The indirect adjustment method described here was developed in further detail previously.[4] From the stacked CCHS cohorts (cycles 1.1-3.1), we selected a population having the same inclusion criteria as our selected CanCHEC population (i.e., aged between 25 and 89 years, living in Toronto and Montreal). We assigned $PM_{2.5}$, NO_2, and $PM_{2.5}$ ARM susceptibility values to these people in the CCHS cohort based on the postal code and year of survey.

We defined smoking as a three-level variable (never [reference category], former, current) and BMI as five categories (<25 kg/m^2 [reference category], 25-30 kg/m^2, 30-35 kg/m^2, 35-40 kg/m^2, >40 kg/m^2).

Next, in the selected CCHS cohort, we estimated the multivariate linear relationship between NO_2, $PM_{2.5}$, magnetite and both cigarette smoking habits (i.e., never, former, current) and BMI and smoking after controlling for all the variables included in the survival model (i.e., sex (strata), immigrant status (strata), cohort (strata), city, 5-year age group (strata), occupational class, income quintile, educational attainment, visible minority status).

The relationship between the adjustment variables and brain cancer incidence was obtained from the literature. The associations and their 95% confidence intervals for the six adjustment variables (two for smoking and four for BMI) are given in Supplementary Table 5.1. BMI was further adjusted by accounting for the median BMI in the selected CCHS cohort within each BMI category.

Supplementary Table 5.1. Associations between indirectly adjusted variables and brain cancer incidence taken from the literature.

Indirect adjustment variable	Risk ratio (95% CI)	Source
Smoking status (Reference = never smoker)		Li et al. 2016[5]
Former smoker	0.97 (0.88, 1.07)	
Current smoker	1.07 (0.98, 1.16)	
Body mass index (Reference = BMI < 25 kg/m^2)		Niedermaier et al. 2015[6]
25-30 kg/m^2	1.21 (1.01, 1.43)	
30-35 kg/m^2	1.54 (1.32, 1.79)	
35-40 kg/m^2	1.54 (1.32, 1.79)	
>40 kg/m^2	1.54 (1.32, 1.79)	

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Chapter 6: Overall Discussion

6.1 Summary of Findings

The overall goal of this thesis was to describe within-city spatial distributions of novel measures of fine particle ($PM_{2.5}$) air pollution (specifically multiple measures of $PM_{2.5}$ oxidative potential, reactive oxygen species generation, and magnetic susceptibility) and to evaluate the health impacts of these exposures.

In Chapter 3 (Manuscript 1), I conducted spatial monitoring campaigns in Canada's two largest cities, Montreal (sampled in summer and winter seasons) and Toronto (sampled in the summer season only), using a dense network of $PM_{2.5}$ sampling sites. I developed land-use regression models for the following $PM_{2.5}$ characteristics: $PM_{2.5}$ oxidative potential based on the depletion of ascorbate (OP^{AA}) and glutathione (OP^{GSH}) in a simulated respiratory tract lining fluid assay; the ability of $PM_{2.5}$ to generate reactive oxygen species in the lung fluid (ROS), as estimated by the mathematical KM-SUB-ELF model; and $PM_{2.5}$ anhysteretic magnetic remanence (ARM) susceptibility as a surrogate measure of magnetite nanoparticles. Additionally, I identified clusters of monitoring sites with similar profiles of these $PM_{2.5}$ characteristics. I also examined how levels of OP and ROS generation vary by neighborhood socioeconomic status in each city. We observed substantial spatial variations in each $PM_{2.5}$ characteristic within cities; importantly, these variations were considerably greater than variations in outdoor $PM_{2.5}$ mass concentration. Predictors of OP and ROS included primarily roadway variables indicating traffic-related tailpipe and brake-wear emissions as well as proximity to railways. ARM susceptibility also appeared to be related primarily to roadways. In Montreal, areas with higher material deprivation (a

neighbourhood-level measure of socioeconomic status) tended to be exposed to $PM_{2.5}$ characterized by higher ROS and OP (i.e., particles with greater ability to induce oxidative stress), but this relationship was not observed in Toronto.

In Chapter 4 (Manuscript 2), I examined how within-city spatial variations in the oxidative potential and reactive oxygen species generating capacity of $PM_{2.5}$ may modify the relationship between long-term exposures to oxidant gases and cardiovascular mortality. I accomplished these aims by performing a cohort study of participants in the Canadian Census Health and Environment Cohort who lived in Toronto or Montreal from 2002-2015. I used Cox proportional hazards models to estimate associations between exposures to oxidant gases (expressed as O_x, which is a redox-weighted average of nitrogen dioxide and ozone concentrations) and cardiovascular deaths. Analyses were performed across strata of two measures of $PM_{2.5}$ OP as well as $PM_{2.5}$ ROS and models were adjusted for relevant socioeconomic and demographic confounders. $PM_{2.5}$ mass concentrations had low spatial variability within cities, but $PM_{2.5}$ oxidative potential and ROS were more highly spatially variable, as was O_X (primarily due to the higher spatial variability of NO_2 within the O_X average). Spatial variations in outdoor O_x at residential addresses were associated with an increase in the risk of cardiovascular mortality (HR per 5 ppb = 1.028, 95% CI: 1.001, 1.055). We observed a consistent pattern in which the effect of Ox on cardiovascular mortality was stronger where $PM_{2.5}$ oxidative potential or ROS was higher.

In Chapter 5 (Manuscript 3), I examined associations between within-city spatial variations in the susceptibility of outdoor $PM_{2.5}$ to anhysteretic remanent magnetization (ARM) (a surrogate measure of magnetite nanoparticle content) and incidence of brain cancer. In addition, I examined how the effects of $PM_{2.5}$ and NO_2 exposures on brain cancer incidence were

modified by $PM_{2.5}$ ARM susceptibility. To accomplish this aim, I conducted a second cohort study using the Canadian Census Health and Environment Cohort in Montreal and Toronto. ARM susceptibility values were measured in $PM_{2.5}$ sampled across both cities and exposures were assigned to residential locations and updated annually to account for residential mobility. Stratified Cox proportional hazards models were used to estimate hazard ratios for the association of $PM_{2.5}$ ARM susceptibility with brain cancer. We identified 1,300 incident brain tumour cases during the follow-up period and found that within-city spatial variations in volume-normalized ARM susceptibility were not associated with brain tumour incidence (HR = 0.998, 95% CI: 0.988, 1.009) after adjusting for $PM_{2.5}$, NO_2, and sociodemographic factors.

6.2 Strengths and Limitations

Overall, this thesis makes several novel contributions to the field of air pollution epidemiology. Objective 1 provides a detailed description of the spatial variability of characteristics of $PM_{2.5}$ related to toxicity and composition (specifically two measures of $PM_{2.5}$ oxidative potential, reactive oxygen species generation, and ARM susceptibility) at the within-cities scale using an unprecedented density of sampling sites. Additionally, we assessed seasonal differences in these measures (in Montreal only) which has not previously been accomplished. Objective 2 demonstrates modification of the effects of oxidant gases on cardiovascular mortality by $PM_{2.5}$ oxidative potential and reactive oxygen species generation; this phenomenon had not previously been studied at the within-cities scale. Lastly, Objective 3 is the first epidemiologic study to evaluate the population health effects of exposures to magnetite nanoparticle air pollution (assessed by ARM susceptibility of $PM_{2.5}$). Together, the work

presented in this thesis represents a step forward in our knowledge of the role that particle toxicity and composition may play in the population health effects of $PM_{2.5}$ exposures.

In addition to these substantive contributions, this thesis has notable methodological strengths. Chiefly, relative to previous studies, exposure measurement error (specifically Berkson error) in the assignment of novel $PM_{2.5}$ exposure measures is likely reduced due to the abundance of monitors used in our sampling campaigns, which allowed us to describe fine-scale spatial variations in exposure measures. The high spatial resolution of monitors allowed us to assign measurements of $PM_{2.5}$ OP, ROS, and ARM susceptibility directly to participants, which eliminated modelling error that can occur when values predicted from land-use regression models are assigned (this is a complex form of measurement error that has a Berkson-like component and a classical-type component).[129, 130] Additionally, we consider the degree of confounding control in Objectives 2 and 3 to be fairly good due to the availability of both individual-level and area-level data on many potential demographic and socioeconomic status confounders in the CanCHEC database. Further, the use of CanCHEC to study health effects of environmental exposures has been extensively demonstrated in previous studies.[27, 99, 104, 131-133] Confounding by personal lifestyle behaviours was also reduced because we estimated associations for outdoor concentrations and not personal measures of outdoor air pollution, with the trade-off of reduced precision.[134] The availability of annually updated time-varying exposures for $PM_{2.5}$ mass concentrations and NO_2 is an additional strength, allowing us to account for observed time trends in exposures to these pollutants which have decreased over the time periods studied in Objectives 2 and 3.[26, 135]

Nonetheless, the work has methodological limitations. Firstly, we were unable to accomplish all the goals of our field campaigns (which produced the exposure estimates used in all three objectives). We had originally planned to perform a winter sampling campaign in Toronto, as well as Montreal, to better characterize seasonal variability in both cities. However, when conducting the winter sampling in Montreal, an extreme cold weather event caused premature shutdown of monitors. As a result, we had to implement a second winter monitoring campaign in Montreal to prolong the exposure monitoring period; due to funding limitations, we were then unable to perform the winter sampling to assess the seasonal variability of $PM_{2.5}$ OP/ROS/ARM susceptibility in Toronto. This remains to be characterized in future studies. As a result, the exposures we assigned in Objectives 2 and 3 were from summer campaigns only and do not reflect the full seasonal variability of OP/ROS/ARM susceptibility.

We would like to make note of instances where the work carried out deviated from the planned thesis work. Notably, although we initially had planned to use OP, ROS and magnetite exposure estimates generated from the land-use regression models in Objective 1 as the exposures assigned to individuals in Objectives 2 and 3, this was not done. Rather, we directly assigned observed values to the closest postal code. We were able to accomplish this due to our dense network of observed sites. This decision was made during discussions among the manuscript coauthors, with the rationale that the density of sampled sites was sufficient and that we could avoid introducing the error inherent in the land-use regression modelling process. There is evidence that using exposures estimated from land-use regression models can induce x and y error which is rarely accounted for in analyses of health outcomes;[129, 130, 136] therefore, having the data, we opted to use the directly measured estimates instead.

As in many epidemiologic studies of air pollution exposures, exposure measurement error also remains a concern in our studies. We were unable to back-cast estimates of $PM_{2.5}$ OP, ROS, and ARM susceptibility due to a lack of historical data. Therefore, the estimates of OP/ROS/ARM susceptibility that were assigned to individuals were in many cases measured after follow-up had ended due to participants being censored or experiencing the health events of interest (i.e., cardiovascular mortality in Objective 2; brain tumour incidence in Objective 3). We assumed that spatial variations in the exposures we measured reflected past spatial variations (i.e., because the locations of important sources like major roads did not vary over the study period) during the etiologic time window in which participants were at risk of exposure effects; however, we were unable to test this assumption. In addition, we assumed, as in previous studies,[83, 89, 137] that the two-week integrated $PM_{2.5}$ samples capture a sufficient time period to extrapolate observed spatial variations to long-term exposure contrasts, but it is possible that this assumption also contributed to exposure measurement error. Finally, as exposures were assigned at the postal code level rather than the individual level, this likely contributed to Berkson-type exposure error which would tend to decrease the precision of estimated associations though it would not induce bias in the magnitude or direction of effects.[138] Lastly, the residential postal codes assigned to individuals in the CanCHEC cohort rely on data from income tax records; for a small proportion of individuals, their residential tax filing address may not be associated with the primary place of residence (e.g., business address, tax preparer's address, failure to update address with the Canada Revenue Agency, etc.).[139]

Additionally, the possibility of residual confounding remains. Although we adjusted for numerous potential confounding variables in Objectives 2 and 3, some of these were available

only at baseline and were not updated in a time-varying fashion (e.g., income quintile, occupational class, marital status, etc.). It is possible that these variables would change over 10 years or more of follow-up, which means that our confounding control was imperfect and that some residual confounding remains after adjustment. Additionally, in Objective 3, it is possible that unmeasured confounders exist; the risk factors for brain cancer incidence are poorly understood, and there may exist in nature unknown confounders that were not included in our conceptual DAG. However, these unknown risk factors for brain cancer would also have to be associated with magnetite concentrations to confound our results.

Potential for selection bias exists in Objectives 2 and 3. There are two major possible sources of selection bias: specifically, the study inclusion/exclusion criteria, and loss to follow-up. First, the cohort inclusion and exclusion criteria may be sources of selection bias. Although the CanCHEC cohort is based on the Canadian census, which has as its target population the Canadian population, nonetheless not all Canadians are represented; specifically, the institutionalized population is not sampled. Since CanCHEC represents a sample of the census respondents, it also excludes institutional residents. This is a potential source of selection bias if institutional residents differ from the non-institutionalized population in both their exposures and their outcomes. Loss to follow-up could also be a source of selection bias if the loss to follow-up is a consequence of *both* exposure and outcome.[140] In the CanCHEC data, individuals are lost to follow-up if they stop filing tax returns, at which point their residential location is no longer known. Individuals are also lost to follow-up if they move outside the Montreal/Toronto area, since we are unable to assign their exposures to $PM_{2.5}$ OP/ROS/ARM susceptibility if they exit this study area as our exposure surfaces only exist for Montreal and Toronto. Some people

may be more likely to be lost to follow-up if they fail to file tax returns, which would make them more likely to be lost to follow-up relative to the rest of the population (e.g., people who are unhoused or unemployed may be in this category). If any of these factors that increases the likelihood of loss to follow-up is a consequence of both exposure (i.e., exposures to oxidant gases or magnetite nanoparticles) and study outcomes (i.e., cardiovascular mortality in objective 2, or brain cancer in objective 3), selection bias could result.

6.3 Public Health Significance

Outdoor air pollution is a major contributor to loss of life and loss of quality of life globally;[141] in Canada, although concentrations of traditional pollutants like $PM2_{.5}$ mass concentrations are relatively low, we still observe adverse health effects of outdoor air pollution.[102-104, 142] This study of air pollution epidemiology moved beyond assessing exposures to traditional measures of $PM_{2.5}$ mass concentrations. Specifically, my work contributes to our understanding of the spatial distribution of novel $PM_{2.5}$ exposure measures that incorporate information on particle characteristics such as particle toxicity and composition. In addition, my work contributes to the assessment of the implications of these spatial variations in novel $PM_{2.5}$ exposure measures to the health of Canadians.

Objective 1 is the most spatially dense description of spatial variability of $PM_{2.5}$ oxidative potential and reactive oxygen species generation in Canadian cities, as well as the first ever description of $PM_{2.5}$ ARM susceptibility. The surfaces we generated can be used in future epidemiologic studies or combined with new data to generate improved exposure estimates. Additionally, our data establishes a baseline which could be used to compare long-term temporal

trends in these measures as the existing fleet dominated by vehicles with internal combustion engines begins to transition into electric vehicles which may have a different emissions profile.[143, 144] The fine-scale spatial variation we observed in these characteristics of $PM_{2.5}$ toxicity and composition suggests that regulations that target specifically local sources may be an efficient means of reducing the health and economic impacts of pollutants.

In Objective 2, we observed stronger associations between outdoor oxidant gas concentrations and cardiovascular mortality in areas within cities where particle oxidative potential was higher. Cardiovascular mortality is the leading cause of death worldwide as well as in Canada, and air pollution is an important modifiable risk factor.[141, 145] OP measurements collected across a sufficient number of locations could be used to inform concentration-based interventions by highlighting regions where regulatory actions may be most efficient in reducing the public health impacts of $PM_{2.5}$, O_X and associated health care costs.[12] Additionally, previous analysis demonstrated that focusing on $PM_{2.5}$ mass concentration reductions in areas with higher particle oxidative potential may allow regulators to target mass-based interventions to achieve equivalent population health benefits at a lower cost (Figure 6.1).[12] Our results suggest, similarly, that regulation that targets $PM_{2.5}$ oxidative potential may reduce the health effects of oxidant gases; we observed stronger associations between oxidant gas concentrations and cardiovascular mortality in areas with higher oxidative potential. This finding is valuable since ozone in particular is difficult to regulate since it is not directly emitted but rather is formed as a secondary pollutant.[31] As an example, reductions in NO_X emissions during local lockdowns in response to the COVID-19 pandemic in Toronto in 2020 did not result in corresponding ozone reductions.[146] Our results suggest that interventions that maximize reductions in particle

oxidative potential could reduce the public health impacts of exposures to oxidant gases even if concentrations of oxidant gases remain unchanged. The findings of Objective 2 contribute to emerging evidence that the strength of associations between oxidant gas concentrations and health outcomes depend on the toxicity of co-exposures to $PM_{2.5}$,[49] which may contribute to the observed spatial heterogeneity in health effects of NO_2.[147]

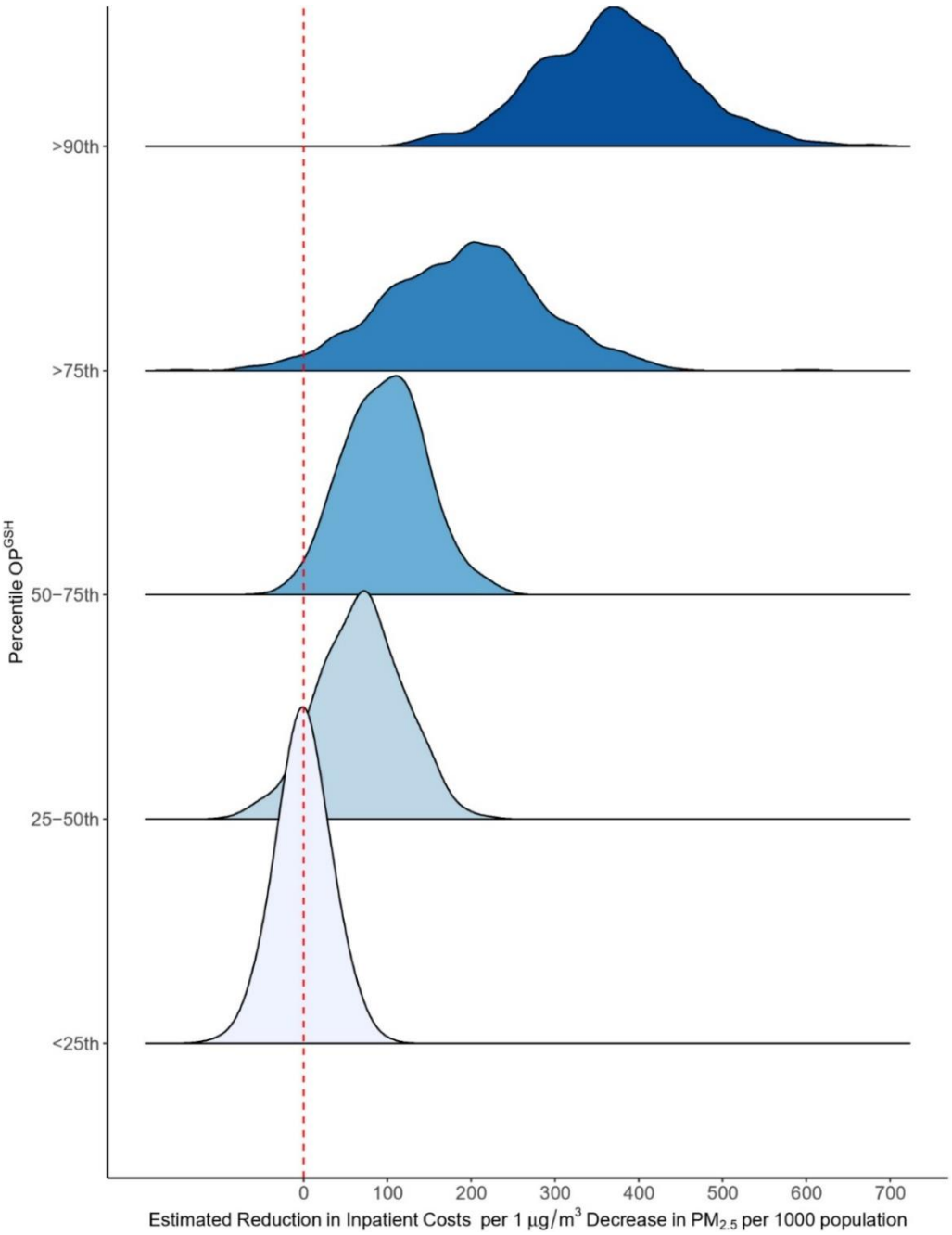

Figure 6.1. Population-standardized (per 1000 population) estimates of annual reductions in acute care inpatient costs for acute myocardial infarction across levels of $PM_{2.5}$ oxidative potential (OP^{GSH}) in 16 cities in Ontario, Canada. Figure adapted from Gao et al.[12]

Finally, Objective 3 investigated effects of $PM_{2.5}$ ARM susceptibility, a surrogate measure of magnetite nanoparticle concentrations, on malignant primary brain tumour incidence. Malignant brain tumours are an important cause of morbidity and mortality due to their ability to cause severe disability[148] or rapid mortality[149] and the burden they place on healthcare systems.[150] Incidence of primary malignant brain tumours appears to be increasing in Canada.[151] Since few modifiable risk factors for brain cancer have been identified, prevention remains difficult. Given the existing evidence that suggests an association between residential exposures to ultrafine particle concentrations and malignant brain tumour incidence,[99] we had a plausible rationale for studying the effects of spatial variations in ARM susceptibility on brain tumour incidence. Although we did not identify a relationship, further investigation into the health effects of exposures to magnetite nanoparticles (e.g., a possible link with neurodegenerative disease[76, 152]) is merited based on existing toxicological evidence.[79, 153]

6.4 Opportunities for Future Research

There are several opportunities for future research related to the subjects explored in this thesis.

Regarding Objective 1, the results of my $PM_{2.5}$ measurement campaigns pertain to a short time window; future studies could build upon our results by confirming whether the spatial distributions in $PM_{2.5}$ ROS, OP and ARM susceptibility we observed, as well as the relationships with land-use predictors, are consistent over time. Additionally, different modelling approaches could be used that do not require the availability of land-use predictors; for example, an increasing body of studies have used machine learning with images as inputs to predict pollutant

distributions with good results.[154, 155] These approaches require a larger body of training data, but the data I collected could be integrated with future data to develop more advanced exposure models. Additionally, although we conducted a preliminary assessment of the seasonal variation of ROS/OP in Montreal, seasonal differences in spatial distributions and sources remain to be characterized in Toronto as well as in other cities.

Regarding Objective 2, additional studies should confirm the results we observed in different locations, since patterns may be city-specific rather than representing a broadly generalizable phenomenon. Identifying specific components or sources that contribute to higher OP of outdoor $PM_{2.5}$ is also important when setting future policy goals and defining regulatory interventions. Additionally, results could be confirmed using different data sources; for example, the Canadian Community Health Survey (CCHS) cohort has now been linked to detailed information on mortality from the Canadian Vital Statistics Deaths Database. The Canadian Community Health Survey is a cross-sectional, survey that collects data on health status and determinants of health for the Canadian population and is designed to provide data at the health region level every 2 years. Like CanCHEC, the CCHS has a large sample of respondents; unlike CanCHEC, it has the advantage of including individual-level data on a wider range of potential confounders related to personal lifestyle behaviours (i.e., body mass index, smoking, nutrition, and more.[156] Although these lifestyle factors are unlikely to be strong confounders, as described above, results of my analyses in Objective 2 could be confirmed using this cohort to reduce the potential for residual confounding.

Regarding future research directions pertaining to Objective 3, we treated all malignant brain tumours as a single outcome, but it is plausible that specific tumour subtypes may be more

or less strongly associated with exposures to magnetite nanoparticles. The hazard ratios we estimated may underestimate the effect of magnetite nanoparticle exposures on some tumour subtypes, while overestimating the risk in others. However, we did not have sufficient power to discriminate by tumour type. Additionally, other measures of the magnetic properties of particles may be more relevant to health, and could be explored in future studies. As we discussed briefly in Objective 3, it may be more pertinent to focus future studies on magnetic parameters assessed in ultrafine particles, since these smaller particles have been shown to be associated with brain cancer incidence.[99]

Future research focused on the environmental justice implications of the work presented in this thesis may be interesting. The work presented in this thesis involved a brief examination of the relevance of our findings to environmental justice. In Objective 1, we found that areas in Montreal with higher material deprivation (measured by the Material Deprivation Index) tended to have slightly higher concentrations of ROS and OP^{GSH}, but not OP^{AA}, but this relationship was weak and was not observed in Toronto. In Objective 2, we examined spatial distributions of co-occurring high levels of redox-weighted oxidant gases and OP and noted that areas with higher levels of the CAN-Marg material deprivation and residential instability dimensions (but not the ethnic concentration and dependency dimensions) appeared to have a more harmful mixture of pollutants in terms of combined levels of O_X and OP/ROS in Montreal, but not in Toronto; correlations were weakly or moderately positive. However, these findings are preliminary and a full analysis of the implications to environmental justice from our work was beyond the scope of this doctoral thesis.

Lastly, our findings, particularly the stronger relationship we observed between oxidant gases and cardiovascular mortality in areas with higher oxidative potential, are interesting from the perspective of public health interventions. Although there is increasing evidence of the relative toxicity of different PM components/characteristics,[157] as well as their associations with different health outcomes, there is not yet a consistent body of literature that can tell us which components/characteristics should be targeted with interventions.[158] Although the work presented in this thesis contributes to the literature, more work is needed to identify clearly the sources and characteristics of $PM_{2.5}$ that are of greatest public health importance. Nonetheless, we can discuss several potential interventions that could reduce the toxicity of particles or their associated public health impacts.

First, e recommend development of a monitoring program that accounts for particle characteristics and toxicity such as oxidative potential would allow for better research studies of the effects of OP and other particle characteristics on health, as well as better understanding of how these particle characteristics and their sources vary over time. This richer data set could also allow source analysis using methods such as positive matrix factorization or principal components analysis to identify which sources may contribute most to particle toxicity. In addition, more extensive data on OP may strengthen the argument of where to target general reductions in $PM_{2.5}$ mass concentration.

In some cases, the expected health effects of $PM_{2.5}$ exposures (and therefore the expected benefits of reductions in $PM_{2.5}$ mass concentration) could vary between locations and particle oxidative potential may allow regulators to target mass-based interventions to achieve the greatest benefits to the health of the population for the lowest cost.[12] Several potential interventions exist to reduce general $PM_{2.5}$ mass concentrations. For example, the infiltration of outdoor particulate matter into indoor environments can be modified by changes to air exchange and building design; this pathway to reducing exposures to PM could be especially helpful in areas near major sources of particles of elevated toxicity (i.e., possible major roadways and intersections as well as industrial areas).[159] Land-use decisions can also be informed by known particulate sources: for example, avoiding the siting of residences, schools, and hospitals near major traffic arteries.[159] Another possible intervention on a local scale is the introduction of

low-emissions or ultra low-emissions zones, which have been piloted in several cities to reduce the emissions from vehicular traffic.[160] Analysis of the effectiveness of regulations and air quality management actions to reduce air pollution and its associated health impacts is an active area of research[161] and a full discussion of these interventions may be important in future research.

Nonetheless, the brief examination of environmental justice performed in this thesis may inform promising directions for future research. Our findings in Objective 1 suggest that the measures of $PM_{2.5}$ toxicity we studied vary highly spatially within cities, likely in response to local sources. A useful direction for future research would be to conduct studies that identify emission sources associated with elevated levels of these characteristics. This would allow identification of potential interventions. Additionally, studies that link emission sources, rather than simply ambient concentrations, to outcomes, as in Thakrar et al., are an important step toward identifying promising strategies for improving public health by reducing air pollution.[162] Our findings in Objective 2 suggest that the effects of oxidant gases on health may be stronger in areas where $PM_{2.5}$ toxicity is higher. Existing evidence suggests that local-scale variation in the quality of the air to which populations are exposed could contribute to disparities in health. For example, non-white people in North America are more exposed to disproportionately high $PM_{2.5}$ mass concentrations relative to whites overall[163] and in urban areas.[164] Further, research indicates that such racial-ethnic disparities in $PM_{2.5}$ exposure are more effectively addressed by targeting local sources in contrast to standard regulatory emission-reduction approaches.[165, 166] The focus on local exposure variations in this thesis is potentially relevant to environmental

justice concerns. This is important because inequalities in air pollution exposures with respect to ethnicity[167] and socioeconomic status[94, 167, 168] have been identified in Montreal and Toronto.

6.5 Conclusion

In conclusion, this dissertation described within-city spatial variations in several emerging measures of $PM_{2.5}$ toxicity and composition (Objective 1), demonstrated an apparent modification of the effect of oxidant gases on cardiovascular mortality by particle oxidative potential (Objective 2), and found no effect of $PM_{2.5}$ ARM susceptibility (a measure of magnetite nanoparticles) on brain cancer incidence (Objective 3). Through focusing on within-city spatial variations in $PM_{2.5}$ toxicity and composition, this dissertation aimed to provide an improved understanding of particulate air pollution in the Canadian urban environment and provides useful directions for future work that may clarify future environmental policy.

Chapter 7: References

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