ENDOTOXIN LEVELS AND THEIR ASSOCIATION WITH RESPIRATORY OUTCOME IN
TWO SASKATCHEWAN COMMUNITIES

A Thesis Submitted to the
College of Graduate and Postdoctoral Studies
In Partial Fulfillment of the Requirements
for the Degree of

Master of Science

In the Department of Community Health and Epidemiology
College of Medicine
University of Saskatchewan
Saskatoon, SK, Canada.

By

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ABSTRACT

There are a number of studies that investigate the association between endotoxin and respiratory outcomes, but few examine ambient endotoxin particularly in smaller industrial cities in Canada. In epidemiological studies of respiratory diseases, environmental factors are of major concern to health. The aims of this study are: (1) to assess ambient endotoxin levels in two communities in southern Saskatchewan (SK), Canada; and (2) to examine the association between ambient endotoxin levels and forced expiratory volume in one second (FEV$_1$) among older adult residents of the two communities. This work is part of the larger Air Quality and Lung Health Study conducted in the cities of Estevan and Swift Current, SK. Ambient environmental and personal respiratory health measures were collected in three periods (phases) of three month durations.

To assess ambient endotoxin levels in the two communities, samples were collected in spring 2013 (Phase 1), fall 2013 (Phase 2), and spring 2014 (Phase 3). The phase sampling strategy was employed to account for potential seasonal variations in endotoxin. Consecutive full-week (7 day accumulation) particle mass size fractions (PM$_{2.5}$ and PM$_{10}$) were gravimetrically collected for each of the communities for the 3-month duration of each phase (1-3) and analyzed for endotoxin load (EU/µg) and endotoxin concentration (EU/m$^3$). Geometric means were calculated for endotoxin load and concentration for each particle size fraction (PM$_{2.5}$ and PM$_{10}$), each community, and each phase. Differences were tested between particle size fractions, and between communities.

The highest levels of endotoxin (EU/µg) found in Estevan were 0.02 (Phase 1), 0.03 (Phase 2) and 0.01 (Phase 3), while endotoxin (EU/m$^3$) were 0.04 (Phase 1), 0.07 (Phase 2) and 0.02 (Phase 3). Similar trends were found in Swift Current with 0.04 (Phase 1), 0.10 (Phase 2), and 0.05 (Phase 3) in endotoxin (EU/µg), while 0.07 (Phase 1), 0.13 (Phase 2), and 0.08 (Phase 3) were found in endotoxin (EU/m$^3$). Estevan had no significant differences in endotoxin load (EU/µg) between size fractions for any of the phases. However, endotoxin load was significantly higher in the PM$_{2.5}$ size fraction as compared to the PM$_{10}$ for both Phases 2 and 3 for Swift Current. For both communities, in all phases, there was significantly greater endotoxin concentration (EU/m$^3$) in PM$_{2.5}$ as compared to PM$_{10}$. 


Comparing communities, Swift Current had significantly greater endotoxin load (EU/µg) in the PM$_{2.5}$ size fraction (in all phases) and the PM$_{10}$ size fractions in Phases 2 and 3 as compared to Estevan. Similar trends were observed for endotoxin concentration (EU/m$^3$) in PM$_{2.5}$ where mean concentrations were greater in Swift Current as compared to Estevan for all phases, but the difference between communities was significant only in Phase 3 (p<0.0001). For PM$_{10}$ only in Phase 3 were endotoxin concentrations significantly different between communities with Swift Current having higher mean levels (p<0.02).

Endotoxin load and concentration were evaluated against pulmonary function measures (FEV$_1$) in older adults in the two communities to test the association between ambient endotoxin levels and forced expiratory volume in one second (FEV$_1$) among older adult residents of the two communities. Twice daily (morning and evening) FEV$_1$ were collected from older adults (>50 years) from both communities over the duration of each of the three phases. Weekly (7 days) morning and evening mean, minimum and maximum FEV$_1$ were calculated to match endotoxin collection/analysis periods.

Despite the relatively low levels of endotoxin in these communities, personal respiratory health measures revealed that there were significant associations in Swift Current Phase 3 measures between mean and max FEV$_1$ and PM$_{2.5}$ endotoxin load and concentration, and these were the highest levels that were measured for either community or any phase.

Taken together these results indicate that endotoxin levels in ambient measures of particulate vary by size fraction with higher concentrations attributed to the smaller size fraction (PM$_{2.5}$). Endotoxin in ambient air particulate has the potential to influence respiratory outcomes. Even at what is considered low levels a continuous exposure to endotoxin can be associated with respiratory health effects as was seen in Swift Current during Phase 3.
CO-AUTHORSHIP

This thesis is made up of two manuscripts which were written by Awoyera Olasoji Olakunle in collaboration with the thesis supervisors, Drs. George Katselis and Shelley Kirychuk from the Canadian Centre for Health and Safety in Agriculture (CCHSA), University of Saskatchewan; committee members (J. Lawson) and members of the Airways Research Group (ARG) (J. Gordon, D. Rennie, D. Cockcroft) from the University of Saskatchewan.

“Assessment of endotoxin content in PM$_{2.5}$ and PM$_{10}$ particle mass of ambient aerosols in two Saskatchewan communities.”

Mr. Awoyera, under the supervision of Drs. Katselis and Kirychuk, developed research questions, carried out laboratory analysis of dust for endotoxin, interpreted results and developed this manuscript. Drs. Katselis and Kirychuk provided suggestions, guidance and constructive criticism in the development of this manuscript. Drs. Gordon, Cockcroft, Koehncke, Rennie, Lawson and the entire ARG team were involved in the initial idea, design, and data collection of the adult and environmental data in both Estevan and Swift Current. They also reviewed this manuscript.

“Association between ambient endotoxin and lung function among the adult residents in two Saskatchewan communities.”

Mr. Awoyera developed the research question and completed the data analysis, interpretation of results, and preparation of the manuscript under the tutelage of Drs. Katselis, Kirychuk and Lawson. They provided suggestions, advice on analysis, and rendered support in development of this manuscript. Drs. Gordon, Cockcroft, Koehncke, Rennie, Lawson and the entire ARG team were involved in the creation of the idea, design, and collection of adult and environmental data in both Estevan and Swift Current.
ACKNOWLEDGEMENTS

My gratitude to God for this unique opportunity. My appreciation goes to my supervisor, Dr. George Katselis, for his acceptance, guidance, support, and tolerance throughout the process of obtaining my Masters’ degree. Also, Dr. Shelley Kirychuk (Co-supervisor) is highly appreciated for her thoughtful insight, constructive criticism and valuable feedback throughout. I am grateful to other members of the committee: Dr. Josh Lawson and Dr. Bonnie Janzen, for their attentiveness and support during different cross-roads in the development of this thesis.

I would also like to thank the entire Airway Research Group (ARG) team for the opportunity provided for me to be part of their project. Thanks to the Canadian Centre for Health and Safety in Agriculture (CCHSA) at the University of Saskatchewan for the study space and facilities. In term of funding, I appreciate the Department of Community Health & Epidemiology, ARG and CCHSA for their monetary support via various scholarships. Thank you!

Finally, big thank to my Iyawo, Bolanle Hannah for her support, encouragement and constantly weighing my thoughts before giving me positive feedback. Your labour of love shall not be in vain. Families and friends, too numerous to mention in Canada and abroad, thank you for your roles in achieving my academic goal.
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LIST OF ABBREVIATIONS

AQHI: Air Quality Health Index
ARG: Airway Research Group
CD4: Cluster of Differentiation 4
EU: Endotoxin Units
FEV₁: Forced Expiratory Volume in 1 second
FVC: Forced Vital Capacity
GEE: Generalizing Estimating Equation
GM: Geometric Means
GSD: Geometric Standard Deviation
IL: Interleukin
KQCL: Kinetic Quantitative Chromogenic LAL
LAL: Limulus Amoebocytes Lysate
LPS: Lipopolysaccharide
PEF: Peak Expiratory Flow
PHAC: Public Health Agency of Canada
PM₂·₅: Particulate Matter with an aerodynamic diameter < 2.5 µm
PM₁₀: Particulate Matter with an aerodynamic diameter < 10 µm
TNF-α: Tumor Necrosis Factor Alpha
WHO: World Health Organization
CHAPTER 1 INTRODUCTION

1.1 Overview

Particulate pollution is comprised of small and large liquid and solid particles released from natural and human sources. Natural sources include dust, soil particulate, pollens, products of forest fires, and volcanic ash while human sources include fuel combustion from vehicles, power plant emissions, industrial processes, mining activities, cigarette smoking and wood stove burning [1]. The quantity, quality, and size of particulate matter (PM) in the ambient environment is influenced by the number and type of sources. PM$_{2.5}$ (fine inhalable particulate matter with a mean aerodynamic diameter of 2.5 µm or less) and PM$_{10}$ (inhalable particulate matter with mean aerodynamic diameter less than 10 µm) are important components of particulate pollution [1, 2] that have been associated with respiratory health outcomes [1, 3]. Less is known about the impact of microbial exposure, present in ambient particulate matter in different background environments, on respiratory health outcomes. Bacterial endotoxin represents such a microbial exposure.

Endotoxin, a molecule released into the air from the outer membrane of gram-negative bacteria [4], has been identified as an important biological component of particulate matter [5]. Endotoxin is a common component in organic dusts [5] such as agricultural environments, textile facilities, and waste processing areas [6, 7]. For instance, intensive animal facilities increase the levels of endotoxin in rural areas compared to urban areas [8, 9]. Studies suggest that endotoxin adsorbed in particulate matter is able to elicit respiratory health effects [10]. Inhalation of particulate matter embedded with endotoxin at different quantity and from various sources has been linked to respiratory outcomes including nose and throat irritation, chest tightness, cough, shortness of breath, acute airway flow restriction and inflammation in the lung [10]. However, respiratory health impairments with respect to endotoxin exposure from the ambient environment has not been widely studied although it has been well substantiated in occupational environmental studies [11]. Studies have revealed that endotoxin is an important microbial component in ambient particulate matter and an important modifier of toxicity [12, 13]. For instance, a study discovered that 20% of ambient airborne particulate matter might contain biological components and endotoxin concentration might reach levels as high as 30 EU/mg [9, 14].
The depth of penetration and response to endotoxin by the human respiratory system is strongly influenced by the size of particulate matter to which endotoxin is attached [1]. Studies suggest that the contribution of endotoxin to particulate matter toxicity depends on the size of inhaled particulate containing endotoxin [10]. Measuring the distribution of endotoxin in the particulate fractions of aerosols is therefore important in associations with respiratory health effects.

One of the fundamental measures of respiratory health is spirometry. Spirometry provides objective and highly reproducible measures of airflow limitation. The spirometry parameter, forced expiratory volume in 1 second (FEV₁) is a widely used assessment method in lung health studies to assess pulmonary function [15].

1.2 Rationale of the study

In 2004, a study in two rural communities in Saskatchewan found a higher prevalence of asthma in children in Estevan as compared to Swift Current [16]. The prevalence of ever asthma in Estevan was 21.4% compared to 16.2% in Swift Current. Furthermore, a higher proportion of girls (19.7%) in Estevan reported a history of asthma when compared to girls in Swift Current (12.5%) [16]. In addition to other factors, there are different primary industries in these two communities with Swift Current primarily involved in agricultural operations and Estevan with agricultural activities and industrial activities including coal mining operations and coal energy generation. These industries might contribute to the particulate air pollution, of which endotoxin can be associated, and potentially the respiratory health outcomes of residents [17]. There is limited knowledge on the composition of particulate matter in Saskatchewan especially with regard to the levels of endotoxin in the size fractions of ambient particulate matter and associations with respiratory outcomes.

Indoor and occupational endotoxin levels have been evaluated in various studies. However knowledge of the ambient composition of endotoxin is limited, particularly for small urban centers such as those common in Saskatchewan. Given differences in the prevalence of asthma rates in Estevan and Swift Current [16], and differences in pollution sources, we sought to evaluate the level of endotoxin and its association with respiratory outcomes in these communities. Therefore, this study focused on the assessment of ambient endotoxin distribution measured in inhalable
particulate fractions (PM$_{2.5}$ and PM$_{10}$). Furthermore, the study assessed the associations between endotoxin levels in PM$_{2.5}$ and PM$_{10}$ and FEV$_1$ as a respiratory health outcome measure.

1.3 Thesis organization

The approach used in this thesis was manuscript-style. This thesis was based around two separate, but related, manuscripts. The literature review addressed the knowledge around the field of study including the current knowledge around ambient particulate exposure, its composition and methods of assessment, and its relationship with respiratory health. Next, the overall methodology of the study was described. This was followed by two chapters, one for each paper in the thesis.

The first manuscript “Assessment of endotoxin content in PM$_{2.5}$ and PM$_{10}$ particle mass of ambient aerosols in two Saskatchewan communities” described the levels of ambient endotoxin in PM$_{2.5}$ and PM$_{10}$ collected from different phases of study in Estevan and Swift Current communities. This manuscript also compares: 1) ambient endotoxin levels found in PM$_{2.5}$ and PM$_{10}$ in each community and within each phase of the study; 2) ambient endotoxin levels found in PM$_{2.5}$ from Estevan and PM$_{2.5}$ from Swift Current, and likewise for PM$_{10}$, in each phase of the study.

The endotoxin levels from the first manuscript were used to investigate possible associations with the respiratory health outcome of Forced Expiratory Volume in 1 second (FEV$_1$) from a human study population in the second manuscript. The second manuscript “Association between ambient endotoxin and lung function among the adult residents in two Saskatchewan communities” furthered the significance of the findings in the first manuscript by examining the associations between the ambient endotoxin levels in the different particulate size fractions and lung function in terms of FEV$_1$ measured in the morning and evening from both Estevan and Swift Current communities, separately.

Finally, the thesis ends with the discussion chapter which brings together the two papers and how they fit into the current literature, discusses overall considerations of the study quality, limitations, and strengths, and ends with recommendations for future work.
1.4 References


particulate induces acute systemic inflammation in controlled human exposures.


CHAPTER 2 LITERATURE REVIEW

2.1 Scope of literature review

The purpose of this study was to assess the levels of ambient endotoxin by particle size fraction as well as associations between endotoxin levels and forced expiratory volume in 1 second (FEV$_1$) in two small urban communities in Saskatchewan. The thesis literature review aims to explore the knowledge, and previous research works about ambient endotoxin and ambient endotoxin effects on FEV$_1$. The review is organized into three sections to address the study purpose. The first section considers the entity of particulate pollution as an element of air pollution including endotoxin. This section aims to better understand endotoxin as a biological component in particulate pollution. The second section describes the respiratory health related to ambient air pollution and how it is assessed. The final section defines the current knowledge regarding ambient endotoxin and lung function.

2.2 Methods of the review

The literature search was completed using search engines such as PubMed, Google Scholar, the University of Saskatchewan’s Library literature and hardcopy journals in the library for current scientific research studies. Some articles were identified through the reference sections of relevant articles. The search words included ‘endotoxin’, ‘exposure’, ‘airborne’, ‘lung function’, ‘respiratory illnesses’, ‘respiratory diseases’, ‘particulate matter’ ‘ambient pollutant’, ‘spirometry’ ‘force expiratory volume in 1 second (FEV$_1$)’ and the combination of these words. Studies that used animals for the experiment and those not written in English Language were excluded from the review.

2.3 Ambient particulate air pollution and the role of endotoxin

2.3.1 Air pollution

Air pollution is a complex entity that varies in space and time and contains hundreds of compounds [1]. It has been widely recognized as a threat to human respiratory health [2]. Ambient air contains vapours and particulate matter with contaminants classified as human generated, natural, or secondary (from chemical reactions in the air) [3]. Particulate air pollution occurs when
substances, including particulates and biological molecules, are released or introduced into the atmosphere [4].

2.3.1.1 Major constituents of air pollution

The quality of air can be polluted due to the presence of one or more pollutants considered as main pollutants, including ground level ozone \([O_3]\), sulphur oxides \([SO_x]\), nitrogen oxides \([NO_x]\), and particulates [5]. The levels of the pollutants in the air vary and are influenced by several factors ranging from proximity to emission sources, weather condition, chemical reaction in the air and the transport over long distances by winds [5]. Pollutant measurements are influenced by wind speed and direction, temperature, relative humidity and air pressure [6].

Ozone is formed in the lower atmosphere when precursor gases such as nitrogen oxides and volatile organic compounds react in sunlight. Ground level ozone is harmful to human health. Ozone causes breathing problems and reduced lung function and aggravates asthma and other lung diseases. The World Health Organization (WHO) recommends an ozone limit of 100 µg/m\(^3\) (50 ppb) 8-hour concentration [5].

Sulphur dioxide \([SO_2]\) is emitted when fuel or raw material containing sulphur is burned or used in industrial processes [6]. The major source of \(SO_2\) in Canada is combustion of fuel for electricity generation and heating processes [6]. People with asthma have shown changes in pulmonary function and respiratory symptoms after \(SO_2\) exposure as short as 10 minutes. \(SO_2\) causes inflammation of the respiratory tract and coughing, mucus secretion, aggravation of asthma and susceptibility to infections of the respiratory tract. The WHO recommended limit is 500 µg/m\(^3\) (191 ppb) for 10 minutes duration [5].

Nitrogen dioxide \([NO_2]\) is another gas emitted into the atmosphere from high temperature combustion processes such as vehicle engines, power plants, and industrial processes. It is toxic and causes inflammation of airways. The current WHO guideline value is 200 µg/m\(^3\) (53 ppb) in 1 hour and 40 µg/m\(^3\) annual (21 ppb) [5].

Particulate matter, according to WHO, affects more people than any other pollutant [5]. Particulate pollution has health impacts even at very low concentrations [7]. The WHO recommended maximum limit of exposure is 25 µg/m\(^3\) in 24 hour mean for PM\(_{2.5}\), and 50 µg/m\(^3\) in 24 hour mean for PM\(_{10}\) [5].
2.3.1.2 *International Comparison of Air Quality*

Figure 2.1 is an international comparison of annual urban air quality indicators for fine particulate matter, sulphur dioxide, ozone and nitrogen dioxide. In 2014, China had the highest fine particulate matter at 30 μg/m³, about 3 folds higher than the levels found in urban areas in Canada (<10 μg/m³) [8]. The level of ozone pollution was ≤50 ppb in the USA, while the highest in Canada was <35 ppb. Sulphur dioxide was less than 1 ppb in Canada while it was 6 ppb in China. Nitrogen oxide was approximately 50% less in Canada, at ~15 ppb compared to >35 ppb in China [8].
Annual average concentrations of fine particulate matter for selected Canadian and international urban areas, selected years

Annual average concentrations of ozone for selected Canadian and international urban areas, selected years

Annual average concentrations of sulphur dioxide for selected Canadian and international urban areas, selected years

Annual average concentrations of nitrogen dioxide for selected Canadian and international urban areas, selected years

Figure 2-1. International comparison of urban air quality indicators. Taken from: Environment and Climate Change Canada (2016) Canadian Environmental Sustainability Indicators: International Comparison of Urban Air Quality; pp 6-9. Available at: www.ec.gc.ca/indicateurs-indicators/default.asp?lang=en&n=FDBB2779-1.
2.3.1.3 Canadian and Saskatchewan Air Quality

The annual concentration of major pollutants in Canada, nationally and regionally, for the year 2014 are briefly summarized in Table 2-1. In general, the air pollutants in Canada are below the World Health Organization Guidelines [5]. In 2014, pollutants in southern Ontario, which is comprised of Toronto and Ottawa, were consistently higher than the national pollutant levels. This might be due to population density in those cities. Pollutant levels in the Prairies and Northern Ontario region, which includes Saskatchewan, were less than the national values. The Prairies, which includes Saskatchewan, has air pollution levels below the national average.

Table 2-1. Annual concentrations of major pollutants in Canada (2014).

<table>
<thead>
<tr>
<th></th>
<th>O₃ (ppb)</th>
<th>SO₂ (ppb)</th>
<th>NO₂ (ppb)</th>
<th>PM₉.₅ (µgm⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>National levels</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32.9</td>
<td>1.3</td>
<td>8.9</td>
<td>7.7</td>
</tr>
<tr>
<td><strong>Regional levels</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atlantic Canada</td>
<td>32.5</td>
<td>1.1</td>
<td>4.4</td>
<td>6.0</td>
</tr>
<tr>
<td>Southern Quebec</td>
<td>33.2</td>
<td>1.7</td>
<td>8.4</td>
<td>8.1</td>
</tr>
<tr>
<td>Southern Ontario</td>
<td>36.6</td>
<td>2.4</td>
<td>9.8</td>
<td>8.3</td>
</tr>
<tr>
<td>Prairies &amp; Northern Ontario</td>
<td>32.7</td>
<td>0.8</td>
<td>7.9</td>
<td>7.4</td>
</tr>
<tr>
<td>British Columbia</td>
<td>27.7</td>
<td>1.3</td>
<td>10.5</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Source: Environment and Climate Change Canada (2016) Canadian Environmental Sustainability Indicators: Air Quality. Ozone, O₃ - annual average of the daily maximum 8 hours average concentration; sulphur dioxide, SO₂ - annual average of the hourly concentration; nitrogen dioxide, NO₂ - annual average of the hourly concentration; particulate matter size 2.5, PM₉.₅ - annual average of daily 24 hours average concentrations; ppb - parts per billion; µgm⁻³ - micrograms per cubic metre.

Figure 2-2 comprises the annual averages for major air pollutants in Saskatchewan for the years 2000-2016. In Saskatchewan, air pollutants show a decreasing trend over time, with fine particulate matter at lower levels as compared with coarse particulate [9].
2.3.1.4 Canadian Air Quality Standards

Ambient air quality monitoring through National Air Pollution Surveillance of the Canadian Air and Precipitation Monitoring Network (CAPMoN) provides the foundation for air quality management in Canada, including the evaluation of progress relative to the Canadian Ambient Air Quality Standards (CAAQS). The CAAQS are a national set of voluntary standards. These standards were established under the Canadian Environmental Protection Act, 1999 on May 25, 2013. The two pollutants that fall within the CAAQS are ozone and fine particulate matter (PM2.5). The CAAQS standards are shown Table 2-2.
Table 2-2. Canadian Ambient Air Quality Standards.

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>2015 standard</th>
<th>2020 standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM$_{2.5}$ Annual</td>
<td>10 µg/m$^3$</td>
<td>8.8 µg/m$^3$</td>
</tr>
<tr>
<td>PM$_{2.5}$ for 24-hours</td>
<td>28 µg/m$^3$</td>
<td>27 µg/m$^3$</td>
</tr>
<tr>
<td>Ozone for 8-hours</td>
<td>63 ppb</td>
<td>62 ppb</td>
</tr>
</tbody>
</table>

ppb = parts per billion

2.3.2 Particulate matter as a pollutant

2.3.2.1 Particulate matter (PM)

Airborne particulate matter, a complex mixture of organic and inorganic substances, is an important component of air pollution [5]. Particulate matter exceeding 2.5 microns in aerodynamic diameter, but less than 10 microns (PM$_{10}$) is generally defined as coarse particles, while particles smaller than 2.5 microns (PM$_{2.5}$) are defined as fine particulate matter [10]. Fine particles have a greater total surface area than larger particles and typically possess a porous surface that can adsorb and retain toxic substances [2]. Particulate pollution is comprised of emissions from both natural and human activities. Naturally, particles emerge from wind-blown dust, volcanic ash, pollens, soil particles, the products of forest fire and oxidation of biogenic reactive gases. Human activities include fuel combustion from vehicles and power plants, industrial processes, construction work, quarrying and mining activities [11]. The conduct of particles in the air and within the respiratory system is largely determined by their physical properties which depend on size. Particulates of less than 2.5 micrometres infiltrate the alveoli and terminal bronchioles while the larger size of up to 10 micrometres deposits mostly on bronchi and the upper respiratory tract [11].

2.3.2.2 Constituents of particulate matter

The array of species localized on the surface of PM act as catalysts in the health effects associated with ambient PM [11]. The composition of PM varies greatly and depends on many factors, for instance, source, climate, season and humidity [2]. Coarse particulate matter is composed mainly of an insoluble crust-derived, mineral, and material of biologic origin such as
endotoxin. Fine particles are made up of carbonaceous aggregates with metals and organic species adsorbed on the surface cavities [2].

2.3.2.3 Factors affecting deposition of particulate matter

Particulate size and shape in conjunction with size and dimensional characteristics of the respiratory tract influence the deposition of particles [11]. Particle settlement on the respiratory tract is guided by three main mechanisms, the first of which is impaction which regulates the deposition by the use of speed and mass inertia of the particulate matters. Large particles have the greatest chance of impacting the large respiratory tract by colliding with the airways wall. The smallest particles with smallest mass are capable of following the main stream of airflow to branching airways [12]. The second mechanism is sedimentation which is the process by which the particles fall down due to gravitational effects in the airway tracts. The speed at which a particle drops is relative to its density and to the square of its diameter. At the lower tract and alveoli, Brownian diffusion (third mechanism), which is the motion due to the kinetic energy of a particle, becomes the primary mechanism contributing to particle deposition due to linear velocity and mass flow approaching zero [13].

When large particulate matter of 10 micrometres or greater is inhaled, it usually ends on the walls of the nose and pharynx [11]. These parts of the body are effective in clearing the inhaled air of the suspended particles and in turn reduce the volume of large particles at the lower airways. Most of the inhaled particulate matter in the diameter range of 2.5 to 10 micrometres are believed to deposit on the tracheobronchial surface while deposition at the alveoli level occurs with particles in the range of 0.1 to 2.5 micrometres [13].

2.3.3 Endotoxin as a component of particulate matter

Toxicity of particulate matter on the respiratory tract depends on the size and composition of the particulate. Size determines the degree of penetration into the tract and the composition of the particulate contributes to the PM-induced health effects [11,14]. The most significant macromolecule adsorbed on the surface of organic particulate matter is endotoxin [15], therefore it is imperative to evaluate levels of endotoxin in particulate matter. For example, the role of endotoxin becomes clearer by the results of a study conducted in three regions of Mexico City. The study showed that PM$_{10}$ in the presence of endotoxin, induced interleukin 1 beta (IL-1β), a
cytokine protein which is as an important mediator of inflammatory response [16]. Huang et al., 2002, found in their study that presence of endotoxin in PM$_{2.5}$ and PM$_{10}$ was responsible for 36% and 24% production of tumor necrosis factor alpha (TNF-$\alpha$), a cell signal protein which involved in systemic inflammation respectively [17]. Similarly, another study on endotoxin and PM showed a 30-fold macrophage inflammatory proteins production, which is crucial in the development of inflammation [18]. Given that Saskatchewan has low population density with agriculture as its predominant industry, ambient particulate pollution from organic particulate matter, and therefore endotoxin is an important consideration. Endotoxin is the most significant component of particulate matter in grain dust, and is associated with the development and aggravation of airway diseases in working populations [15]. In the absence of cigarette smoke, significant levels of endotoxin in domestic environments may relate to respiratory disease severity [19]. The levels of endotoxin can be thousands times higher in a high exposure environment, such as agricultural, compared to non-direct exposure environment, such as outdoor air, and based on distance to the source [20].

2.3.3.1 Endotoxin

Endotoxin is a complex chemical component of the outer membrane of Gram-negative bacteria, and plays an important role in cell integrity and interaction of the cell with the extracellular environment [21]. Organic matter Gram-negative bacteria are found in either solid (e.g. settled dust, household waste, plants) or liquid (e.g. waste-water, metal working fluids, waterlines) mediums [21]. Endotoxin is released into the air during cell growth and after occurrence of bacteria’s death [22]. It can stay in the air for long periods of time due to its small size [23].

2.3.3.2 Chemical structure of endotoxin

Endotoxin is an integral part of cell walls and is made of thermostable lipopolysaccharide, (LPS), proteins, and phospholipids [24]. Lipopolysaccharide is further made up of Lipid A, polysaccharide nucleus, and polysaccharide chains (Figure 2-3). Lipid A is similar in all species of bacteria and is responsible for the toxic effects of endotoxin [25]. Endotoxin derives its strength from the polysaccharide nucleus while polysaccharide chains are responsible for immunogenicity. The toxin is resistant to heat and heating at 180$^\circ$C over 4 hours is recommended for sterilization
Three forms of endotoxin are present in the air: a pure form consisting of small lipopolysaccharides molecules having molecular weights of 2000 to 20000 Dalton; a second form is pure lipopolysaccharides associated with cell wall components measuring less than 1 or 2 µm; a third form which is associated with biological and/or non-biological aerosol particles. The last two forms are generally considered to be more prevalent in the environment.

Figure 2-3. A general structure of lipopolysaccharides (LPS). [Taken from Radon (2006); reproduced after obtaining written permission].

The structural differences in the chemical nature of endotoxin influence its biological activity. A study showed that LPS from *Pseudomonas aeruginosa* is distinctly less toxic compared to *Enterobacterial* LPS due to the number of acyl-groups in the Lipid A, particularly the hexaacyl and pentacyl groups.

2.3.3.3 Measurement of endotoxin

Endotoxin is mostly measured in term of associated biological or non-biological aerosol in an environment. Endotoxin can be measured in two forms. The bioactive form of endotoxin is identified as being responsible for respiratory challenges while total endotoxins are comprised of those still embedded in the bacteria cell wall and the bioactive form. Bioactive endotoxin can be quantified by assays such as the Limulus Amoeocyte Lysate (LAL) assay.
crab, in the presence of endotoxin [26]. The assay measures the endotoxin potency based on the fatty acid content of Lipid A, the polysaccharide content, and the aggregation properties [30].

The LAL is sensitive for measuring low endotoxin levels less than 10 EU/m³, such as that found in slightly contaminated exposed environment such as outside air [31]. Variation in endotoxin determination can be impacted by a number of factors including the solution used in the extraction, the extraction methods, and the interference of beta-1,3-glucan [32, 33], and inter-laboratory variation [34, 35]. Other methods, including a chemical analytical method utilizing gas chromatography-mass spectrometry, have been utilized to detect both biologically active and inactive endotoxins, but have been found to be less sensitive [36].

2.3.3.4 Limulus amebocyte lysate assay

The most common endotoxin measurement, and the one used in this thesis study, is LAL assay. In this assay a sample is mixed with LAL reagent in an incubating plate and read over time, using a plate reader (spectrophotometer), for the appearance of a yellow colour. The assay is described in detail in Chapter 4. The reaction is composed of three serine protease zymogens and one clottable protein (coagulogen). As shown in Figure 2-4, in the presence of endotoxin, a glycoprotein of 123 kDa, called Factor C is autocatalytically activated to active Factor C. Factor B, the second serine protease zymogen, is activated by active Factor C by limited proteolysis. Active Factor B activates Proclotting enzyme to clotting enzyme, which catalyzes the transformation of coagulogen to insoluble coagulum gels [37]. Using the initial absorbance reading of each well as its own blank, the time required for the absorbance to increase to 0.2 absorbance units is considered the reaction time. The reaction time is inversely proportional to the amount of endotoxin present. The more the quantity of endotoxin the shorter the reaction time and vice versa. The amount of endotoxin in unknown samples is referenced to a standard curve which is prepared on the same plate as the known samples. Logarithmic (log/log) linear correlations are used to compute endotoxin levels in unknowns. The assay is typically optimized to be linear in the range of 0.005 EU/mL to 50.0 EU/mL [37].
Endotoxin (LPS)

Factor C ➔ Activated factor C

Factor B ➔ Activated Factor B

Proclotting enzyme ➔ Clotting Enzyme


(Artificial substrate)

Figure 2-4. Summary of Limulus protease activation pathway. Taken from: 1,3-Beta-D-Glucan Detection Reagent Kit Manual, Cambrex, March 2007.

2.3.3.5 Endotoxin limit

Despite the observed occupational exposure effects on the health of workers, an exposure limit for endotoxin is not yet established. The indoor, outdoor or workplace safe range of endotoxin exposure is still inconclusive. In an occupational exposure study done on a poultry farming, Donham et al. recommended an exposure limit of 14 EU/m$^3$ for total endotoxin and 7.15 EU/m$^3$ for respirable endotoxin [38]. Rylander also calculated the limit of exposure to be 33 ng/m$^3$ [39]. Different methods of sample collection, extraction methods, inter-laboratory procedures, sample storage time before analysis or transport [28, 32-33], and lack of correlation between data makes it impossible to establish a clear dose-effect relationship or an exposure limit for humans [40]. Studies commonly reference the Dutch Expert Committee on Occupational Standard’s recommendation of an exposure limit of 50 EU/m$^3$ in a working environment for a period of 8 hours [41-44].

2.3.3.6 Distribution of endotoxin on particulate matter

Endotoxins have been measured in ambient particulate size matter PM$_{2.5}$ and PM$_{10}$ [45-47]. The size distribution of aerodynamic particles of airborne endotoxin plays a vital role in determining endotoxin toxicity and its health effects [48]. A summary of ambient endotoxin assessment in varying locations worldwide can be found in Table 2-2, spanning a period of 13
years (2001-2014). A majority of the studies were performed in Europe, Asia and in North America.
Table 2-3. Summary of ambient endotoxin measured in different studies.

<table>
<thead>
<tr>
<th>Authors (Year published)</th>
<th>Location and country</th>
<th>Total number of samples</th>
<th>Seasons/year of study</th>
<th>Levels of ambient endotoxin</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long et al., (2001) [49]</td>
<td>Massachusetts, USA</td>
<td>13</td>
<td>Spring-summer 1998</td>
<td>PM$_{2.5}$ 2.0 EU/mg</td>
<td>Seasonal variation was not considered</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fall-winter 1998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heinrich et al., (2003) [47]</td>
<td>Sachsen-Anhalt, Germany</td>
<td>21</td>
<td>Jan- June 2002</td>
<td>1st City: PM$<em>{2.5}$ 0.8 EU/mg, 0.006 EU/m$^3$; PM$</em>{10}$ 11.7 EU/mg, EU/m$^3$ 0.063 2nd City: PM$<em>{2.5}$ 0.7 EU/mg, 0.008 EU/m$^3$; PM$</em>{10}$ 10.7 EU/mg, EU/m$^3$ 0.071</td>
<td>Probably the similarities of weather in first and second city drive the correlation between both areas</td>
</tr>
<tr>
<td>Mueller-Anneling et al., (2004) [31]</td>
<td>California, USA</td>
<td>104</td>
<td>1-year sampling</td>
<td>PM$_{10}$ 0.44 EU/m$^3$</td>
<td>Other pollutants did not affect the level. High in summer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PM$_{10}$ 13.6 EU/mg</td>
<td></td>
</tr>
<tr>
<td>Morgenstern et al., (2005) [50]</td>
<td>Munich, Germany</td>
<td>206</td>
<td>March 1999 to July 2000</td>
<td>PM$<em>{2.5}$ 1.30 EU/mg, 0.019 EU/m$^3$; PM$</em>{10}$ 3.91 EU/mg, EU/m$^3$ 0.081</td>
<td>No clear spatial pattern of endotoxin due to site selection</td>
</tr>
<tr>
<td>Menetrez et al., (2007) [51]</td>
<td>North Carolina, USA</td>
<td>342</td>
<td>August 2003 to January 2004</td>
<td>PM$<em>{2.5}$ 0.15 EU/m$^3$; 0.016 EU/µg PM$</em>{10}$ 0.16 EU/m$^3$; 0.045 EU/µg</td>
<td>No seasonal variation</td>
</tr>
<tr>
<td>Traversi et al., (2010) [52]</td>
<td>Piedmont (Turin) Italy</td>
<td>116</td>
<td>January 2007 to December 2007</td>
<td>In PM$_{10}$: Winter 5.02 EU/mg Spring 13.30 EU/mg Summer 16.41 EU/mg Autumn 7.49 EU/mg</td>
<td>Levels associated with temperature and wind while inversely correlated with humidity</td>
</tr>
<tr>
<td>Study</td>
<td>Location</td>
<td>City/Region</td>
<td>Dates</td>
<td>Concentrations</td>
<td>Notes</td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
<td>-------------</td>
<td>-------</td>
<td>----------------</td>
<td>-------</td>
</tr>
<tr>
<td>Allen <em>et al.</em>, (2011) [46]</td>
<td>British Columbia, Canada</td>
<td>City A: 226  City B: 234</td>
<td>October 2005 to September 2006.</td>
<td>City A: PM$<em>{2.5}$ 0.15 EU/m$^3$; PM$</em>{10}$ 0.40 EU/m$^3$  City B: PM$<em>{2.5}$ 0.16 EU/m$^3$; PM$</em>{10}$ 0.67 EU/m$^3$</td>
<td>High temperature and moderate relative humidity. Wind direction has no effect. Highest level of endotoxin in summer and fall while the lowest in winter and spring.</td>
</tr>
<tr>
<td>Nilsson <em>et al.</em>, (2011) [45]</td>
<td>Stockholm, Sweden</td>
<td>35</td>
<td>May to September 2009</td>
<td>PM$<em>{2.5}$ 0.015 EU/m$^3$; 2.2 EU/mg  PM$</em>{10}$ 0.05 EU/m$^3$; 3.7 EU/mg</td>
<td>No clear effects of traffic or meteorological factors.</td>
</tr>
<tr>
<td>Wheeler <em>et al.</em>, (2011) [82]</td>
<td>Regina, Canada</td>
<td>179</td>
<td>January–March, and June–August, 2007</td>
<td>PM$_{10}$  Summer 1.57 EU/m$^3$; 0.18 EU/µg  Winter 0.12 EU/m$^3$; 0.05 EU/µg</td>
<td>Use of air conditioning and fan might influence the source of endotoxin.</td>
</tr>
<tr>
<td>Cheng <em>et al.</em>, (2012) [36]</td>
<td>Pearl River Delta Region, China</td>
<td>City 1: 68  City 2: 34  City 3: 32</td>
<td>February 2008 to March 2009</td>
<td>City 1: PM$<em>{2.5}$ 0.085 EU/m$^3$  City 2: PM$</em>{2.5}$ 0.148 EU/m$^3$  City 3: PM$<em>{2.5}$ 0.099 EU/m$^3$  City 1: PM$</em>{10}$ 0.345 EU/m$^3$  City 2: PM$<em>{10}$ 0.386 EU/m$^3$  City 3: PM$</em>{10}$ 0.350 EU/m$^3$</td>
<td>City 2 is an urban city with different manufacturing industries and sampling site was situated on the 23-storey building.</td>
</tr>
<tr>
<td>Guan <em>et al.</em>, (2014) [53]</td>
<td>Beijing, China</td>
<td>321</td>
<td>March 2012 to February 2013</td>
<td>In PM$_{2.5}$: 0.65 EU/m$^3$; 10.25 EU/mg</td>
<td>Levels vary with meteorological influence e.g., temperature showed positive correlation in winter but negative correlation in spring, summer and autumn.</td>
</tr>
</tbody>
</table>
The studies presented in Table 2-2 were conducted prior to 2013 in large, urban metropolitan areas, as well as interior mainland (BC Canadian study), rural areas, and villages around the world. Results are reported in endotoxin units of mg or µg of particulate and/or cubic metre of sampled air. Outdoor sampling sites varied from backyards of residential buildings to tops of multi-storey buildings (5-23), and sampling season varied. Nilsson et al., [45] studied urban endotoxin air in Sweden and impact of traffic with other meteorological factors. The study was comprised of five study sites. The study showed that endotoxin ranged from 0.015 EU/m³ to 0.1 EU/m³ in particulate size 2.5 and 10, while traffic and meteorological factors had no observable effects on the endotoxin level. In each of the sampling units, the endotoxin level found in PM₁₀ was higher than in PM₂.₅. A study from two communities in British Columbia, Canada, found a higher concentration of endotoxin in PM₁₀ than in PM₂.₅ in both communities [46]. The reason for this is not clearly understood. These outdoor levels of endotoxin are generally less than 50 EU/m³, below the occupational level recommended by the Dutch Committee for occupational exposures. The depth of penetration and behaviour of endotoxin in the human respiratory system is strongly influenced by their size of PM [54, 55]. Measuring the distribution of endotoxin in aerodynamic fractions of particulate is important step in determining toxicity and health effects.

2.4 Respiratory outcomes and ambient air pollution

2.4.1 Respiratory health measurements

2.4.1.1 Questionnaires

When measuring respiratory health in adults and children, certain measures have been commonly used in various studies, this includes questionnaires and diary to assess symptoms and objective testing measures such as spirometry to assess airflow or airflow obstruction [56]. Questionnaires are widely used in respiratory health epidemiological studies. The presence and absence of symptoms or diseases can be assessed and defined depending on the information collected. This method is cheap, safe, non-invasive, and requires little time of the participant [57]. The main challenge of using questionnaires is accurate recall of the participant which might be influenced by psychosocial and social factors [57].
2.4.1.2 Spirometry

Spirometry is one objective measure of lung function. Lung function is an excellent operative marker of the effects of air pollution in the general population [58]. It is objective, quantitative, and an early predictor of cardiorespiratory morbidity and mortality [59, 60]. Lung function testing has the capacity to measure volume and airflow and can be performed using a spirometer [61]. The measurement records the volume of air exhaled and how quickly air is exhaled over a period of time [61]. Spirometry can be used to evaluate and diagnose a wide range of lung diseases, to measure the severity of these conditions, and to evaluate efficacy of treatment of lung disease [62]. Some of the maneuvers with spirometry measure passive regular breathing, while others require forced exhalation after a deep breath. Training on appropriate maneuver techniques improves the quality of the measures and minimizes error due to technique.

Lung function testing through spirometry is performed in a standardized fashion. There are specific guidelines that allow for this standardization and reproducibility of results and to reduce variability from the use of different techniques or equipment. The American Thoracic Society and the European Respiratory Society have developed such guidelines [61]. There is a standard to ensure appropriate equipment assessment and maintenance, spirometry calibration, and number of spirometry maneuvers performed [63]. A maximum of eight spirometry maneuvers should be performed and at least three minimum attempts. It is important to be able to identify if adequate spirometry is performed in order to be able to utilize the ensuing data appropriately. Recognizing the limitations and quality of lung function testing allows for proper understanding and use of the spirometry results [39].

Several studies have ascertained that spirometry parameters are good indicators to know the state of lung function. Maximum forced vital capacity (FVC) and maximum forced expiratory volume in 1 second (FEV₁) is commonly used parameters in research studies as the early pointer of chronic respiratory and systemic inflammation [64, 65]. Important factors in lung function include: height, sex, age, ethnic background and smoking history [66].

2.4.1.3 Factors affecting spirometric measurement

Height is a crucial factor that affects the expected lung volume [67]. The taller the individual, the higher the lung volume. The disparity found with lung volume increases with
Lung volume rises as the lungs develop from birth to 20 years of age in females, and 20 to 24 years of age in males [67, 68]. Subsequently, lung capacity drops with age because the lungs lose elastic recoil with reduced expiratory flows [67, 68]. Adult males have higher lung volumes than adult females of the same age and height. African Americans have lung volumes approximately 10% lower than Caucasians while Asians have lung volumes 2% to 8% lower than Caucasians [69].

2.4.1.4 Spirometric parameters

Important measurements of lung function can be obtained using spirometry through the forced expiratory measurements [62]. These include forced vital capacity (FVC), forced expiratory volume (FEV1), peak expiratory flow (PEF), and forced expiratory flow between the 25th and 75th percentile of FVC (FEF25-75%). FVC measures the volume of air one can exhale forcefully after deep inhalation. PEF measures how quickly the breath is exhaled. FEV measures the volume of air one can forcefully exhale in one breath over a measured time, typically in the first second (FEV1) [56,66]. The ratio of FEV1 and FVC (FEV1/FVC) can provide a useful measure for evaluating obstructive and restrictive lung disease. FEV1 is regarded as the typical measurement to detect airflow caliber. This index shows the quality of both large and small airways [55]. A decrease in ratio resulting from a decreased FEV1 is seen with obstructive lung disease, which would have a reduced (FEV1/FVC) ratio. In restrictive lung disease, both FEV1 and FVC are reduced, so the ratio may be normal or even increased [70].

Lung function measures can be interpreted through absolute and percent predicted values. Absolute values of lung function are the recorded measurements from spirometry for a patient or individual. Predicted values of lung function are calculated based on age, sex, and height of a patient, which is typically, based on large population studies of healthy none smoking patients [62]. Lung function is expressed as a percentage of the predictive value by comparing the absolute value to the predicted value. This allows classification of the severity of impairment in lung function relative to a ‘normal’ population [62,68].

Repeated spirometry testing is useful to assess patterns or changes including progression in disease status, medication change requirements, or changes as a result of exposures such as occupational or environmental.
Portable spirometry meters, such as PIKO-1 are commonly used when repeated measures over time for the same subject are required, particularly in the home setting. The value of this functional, affordable, high quality, portable measuring device has been fully recognized [71]. This small device measures peak expiratory flow (PEF) and FEV$_1$. The device is capable of date and time stamping measures, and able to store up to 96 measurements with an FEV$_1$ range from 0.15 to 9.99 and an accuracy of ±3.5% (0.05 L) [72].

2.4.2 Global burden of air pollution

The impact of exposure to very high levels of particulate pollution is well documented. However, there is a significant discourse on the impact of low level particulate pollution exposures. Globally, air pollution is a major health risk, including increased risk of heart disease, stroke and lung cancer. Air pollution is attributed to 5.5 million premature mortalities worldwide, or one in 10 deaths, the fourth leading mortality risk in the world.[73] A recent report on the health impacts of air pollution in Canada estimates 14,400 annual mortalities attributed to air pollution [74].

Epidemiological studies have shown associations between airborne particles and respiratory health problems [59,75-76]. Cohen et al., [77] studied the global burden of disease due to outdoor air pollution in 2005. The analyses of the study were based on fine and coarse particulates which showed that these exposures accounted for 3% of adult cardiopulmonary disease mortality and 5% of respiratory tract cancer [77].

The health response to particulate pollution varies between individuals and subgroups of the population. Individual vulnerability affects the level at which health effects are observed or the rate of increase in symptoms as the pollution increases. Factors that increase vulnerability include underlying chronic respiratory or cardiovascular condition [78] and metabolic diseases [79]. Children and the elderly are highly susceptible to air pollutants. Children are susceptible due to higher metabolic rates, spending more hours outside the house, and a developing immune system. The elderly population is susceptible to adverse effects of air pollutants because of a less efficient immune system, decrease in chest wall compliance, and functional decline of organ systems [80-84]. Epidemiological studies have demonstrated a strong association between exposure to air pollution and respiratory health [59]. Low to moderate levels of ambient air pollutants can greatly increase respiratory problems in older adults [14]. For instance, Ye and co-workers (2001) studied adult residents in Tokyo, Japan for 15 years in relation to particulate matter
pollution. They found that increasing airborne outdoor PM$_{10}$ concentrations were associated with significantly higher rates of asthma and bronchitis with p-value less than 0.0001 [85].

2.4.2.1 Burden of respiratory outcomes in Canada and Saskatchewan

Respiratory disease is a vast worldwide health problem. Respiratory symptoms and disease are common and the prevalence increases with age [86]. In general, and including tuberculosis, pulmonary hypertension, and other occupational lung diseases, more than 1 billion people are suffering from chronic respiratory conditions [87]. Asthma is the most common chronic disease locally and globally. It is estimated that approximately 250 million people worldwide suffer from asthma [89], more than 200 million people suffer from COPD [88], and as much as 6% of the adult population experience sleep-disordered breathing [88].

According to Public Health Agency of Canada (PHAC), tobacco smoking and air quality (indoor and outdoor) played the foremost role in the development and exacerbation of respiratory illnesses in Canada [90]. According to the Canadian Lung Association, one in every five Canadians, both adults and children, has a respiratory problem. A disease associated with the lung is the third leading cause of death among Canadians [91]. In comparison with other developed countries, prevalence of asthma and asthmatic-like symptoms in Canada (14.1%) is somewhat similar to United States (10.9%) and very similar to United Kingdom (>15%), New Zealand (15.1%), Australia (14.7%) and the Republic of Ireland (14.6%) [92]. Overall, in the North American population one-tenth has asthma [92]. According to Canadian Community Health Survey (CCHS) of 2005 adults over the age of 34 surveyed, 4.4% reported that they had been diagnosed by a health professional with COPD (includes self-report of COPD, chronic bronchitis or emphysema) [93]. Lung cancer is the leading cause of death due to cancer in Canada. It causes approximately 29% of cancer deaths among men and 22% among women. In 2007, an estimated 23,300 Canadians were expected to develop lung cancer [94]. In 2005, 1,616 cases of new and relapsed active cases of tuberculosis were reported in Canada (5.0 per 100,000). The incidence rates were highest among the 65-74 and 75 and over age groups [95]. In general, 3 million Canadians are dealing with one of the five serious respiratory diseases – asthma, chronic obstructive pulmonary disease (COPD), lung cancer, tuberculosis (TB), and cystic fibrosis. These and other respiratory diseases affect individuals of all ages, cultures and backgrounds from children to parents to grandparents [96].
In Saskatchewan, in 2005, an estimated 8.8% of the adult population had been diagnosed with asthma [97], while COPD was estimated to be 4.3% [98], lung cancer incidence 53.7 per 100,000 population [99] during the same year. According to Saskatchewan provincial tuberculosis working group in 2013, the rates of tuberculosis in Saskatchewan are higher than national rates, with 7.5 cases per 100,000 people in Saskatchewan compared to the Canadian average of 4.7 per 100,000 [100].

The problem of respiratory illness is substantial in Canada [91]. Asthma and related respiratory health problems put remarkable limitations on all aspects of life including physical, emotional, social, economical and professional characteristics of an individual especially in the absence of proper treatment [101]. According to the Conference Board of Canada, in 2010 the burden of lung diseases was about $3.4 billion in term of direct health care costs while $8.6 billion was spent on indirect expenses [102]. A panel survey between 2002 and 2007 in the United States attributed a total cost of $56 billion to asthma [103]. The Lung Association of Canada projected annual economic liability to increase two-fold by 2030 if no strategy was devised to cope with the respiratory disease [104]. A substantial portion of this burden could be alleviated through improvement in ambient air quality [105].

2.4.3 Respiratory outcomes and the role of endotoxin

2.4.3.1 Respiratory outcomes

Respiratory disease is a pathological process with characteristic sets of signs and symptoms. Several factors impact respiratory outcomes and disease including personal (age, sex, genetics and smoking) and environmental factors (damp housing and environmental exposures) [83,106]. Respiratory disease is common in both adults and children and accounts for a great deal of personal and health care burden. The most common respiratory diseases include asthma, chronic obstructive pulmonary disease, lung cancer, influenza and tuberculosis [107]. Respiratory symptoms are subjective evidence and phenomenon that is experienced by an individual affected either as a precursor to disease or as a component of the disease state [86]. Common respiratory symptoms include wheezing, chest tightness, cough, phlegm, and shortness of breath [86].

Respiratory symptoms are mostly characterized as acute or chronic. Acute is when a condition is rapid in onset and subsiding after a relatively short period. Chronic respiratory
conditions persist for a longer time period (typically categorized as greater than 3 months) and exhibit slow progression [106,107]. The symptoms can present as partially reversible airway obstruction, airway inflammation, and/or airway hyperresponsiveness [106]. Allergic respiratory conditions are caused by hypersensitivity of the immune system to something in the environment (allergen). The conditions include hay fever, allergic asthma and sinusitis while the allergic respiratory symptoms include sneezing, coughing, phlegm, wheezing, shortness of breath [107]. Respiratory and associated sputum production symptoms are important markers of developing acute or chronic respiratory diseases in adults [86].

The presence of symptoms can reflect irritation, inflammatory conditions, and/or respiratory disease. The respiratory tract develops signs when stimuli trigger a reaction on the smooth surface of the respiratory tract [106]. According to World Health Organisation (WHO) 2008, respiratory diseases are commonly characterized by swelling of bronchial tubes, causing narrowing of airways and breathlessness in a severe condition [89]. Several mechanisms have been suggested to explain the adverse respiratory effects of air pollutants. The common path of action is that when an air pollutant (such as particulate matter, ozone, and nitrogen oxides) rests on the respiratory epithelium, high concentrations of oxidants and pro-oxidants cause the formation of oxygen and nitrogen free radicals which induce oxidative stress. Non-neutralized free radicals by antioxidant defences start an inflammatory response and mediators that result in subclinical inflammation [5, 104].

2.4.3.2 Endotoxin and respiratory outcomes

Endotoxin enters the respiratory system through inhalation, which causes respiratory and systemic inflammatory responses including dry cough and decrease in lung function [21,32] Several studies have revealed that inhalation of bioactive endotoxin can trigger an immunological response and cause inflammation [108]. The lipid A part of bioactive endotoxin, after deposition on the respiratory tract, encounters alveolar macrophages carrying CD14 gene, which serves as receptor for LPS. Through tissue receptors, the macrophages are activated leading to release of pro-inflammatory cytokines, chemokines, and other mediators [109,110].

Mediators released as a result of endotoxin include TNF-α, IL-1β, IL-6 and IL-8 [109]. These cytokines trigger the assembly of neutrophils that result in local and systemic inflammation [109,110]. Individual genetic make-up is known to be accountable for how individuals respond to
endotoxins [111]. These immunological changes are minimal in a normal individual but more obvious in asthmatic subjects, which mean inhaled endotoxin may worsen respiratory health effects in individuals with underlying respiratory symptoms [112].

In some previous studies, endotoxin exposure has been considered to have protective effects, particularly in children. These studies suggested that early life exposure to endotoxin causes secretion of inflammatory cytokines which is responsible for maturation of immune system in the direction of T-helper cell 1, subduing the risk for atopic sensitization [27]. In a study that involved 61 infants at high risk for sensitization, it was found that the risk for atopic sensitization was inversely associated with the concentration of endotoxin in the house dust [113]. Another study in the USA by Perzanowski et al., 2006, found that exposure to endotoxin may reduce risk of developing childhood atopy [114]. In an association with wheeze and asthma among the children dwelling in the rural area, the exposure to endotoxin might be protective [115].

Studies of associations between endotoxin and respiratory health effects (1998 - 2015) are summarized in Table 2-3. In this summary, only the spirometry values were tabulated as the findings. Most of the studies were performed in Europe, Asia, and in North America and the majority examined exposed working populations rather than the public. As shown, endotoxin was found to be associated with respiratory effects (lung function; FEV1, PEF, FVC).

The studies were mostly conducted in workplaces, schools, and among workers and students. Endotoxin sampling included personal aerosol sampling and indoor area sampling. The duration of exposure in the articles reviewed ranged from 1 month to 25 years. At the time of developing this thesis, the author could not found a research article that considers association between ambient endotoxin and respiratory outcomes of FEV1. In general, exposure to endotoxin in a closed environment affects FEV1 at different concentrations and this may be attributed to the duration of exposure.
Table 2-4. Overview of studies of associations between endotoxin and lung function health effects (1998-2015).

<table>
<thead>
<tr>
<th>Authors (year published)</th>
<th>Country</th>
<th>Study population</th>
<th>Outcomes variables</th>
<th>Findings</th>
<th>Other comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorn &amp; Rylander, (1998) [112]</td>
<td>Sweden</td>
<td>Healthy subjects</td>
<td>Questionnaire for symptoms, spirometric testing and blood sampling</td>
<td>FEV₁ and FVC values were significantly lower 24 hours after inhalation of LPS.</td>
<td>Non-smokers, no current cold, no asthma</td>
</tr>
<tr>
<td>Vogelzang et al.,(1998) [116]</td>
<td>Netherlands</td>
<td>Pig farmers</td>
<td>Spirometric parameters: FEV₁ and FVC</td>
<td>After adjusting for age, baseline parameters and smoking, decline in FEV₁ &amp; FVC was strongly associated with endotoxin exposure at annual mean of 105 ng/m³ of endotoxin.</td>
<td></td>
</tr>
<tr>
<td>Kirychuk et al., (1998) [117]</td>
<td>Canada</td>
<td>Swine workers</td>
<td>FEV₁ and FVC</td>
<td>No statistical significant association was found between annual FEV₁ or FVC decrease and airborne endotoxin.</td>
<td>The observation might be due to sample size</td>
</tr>
<tr>
<td>Wang et al., (2003) [118]</td>
<td>China</td>
<td>Young female cotton workers</td>
<td>FEV₁ and FVC</td>
<td>Decline by 2% in FEV₁ &amp; FVC after 3 months, 5% in FVC after 12 &amp; 18 months, 2.8 % and 1.3% after 12 months and 18 months respectively</td>
<td>Newly hired, the parameters were compared to their first day at work</td>
</tr>
<tr>
<td>Sigsgaard et al., (2004) [119]</td>
<td>Denmark</td>
<td>Male paper mill workers</td>
<td>FEV₁ and FVC</td>
<td>Changes were observed in FEV₁ and FVC every year</td>
<td></td>
</tr>
<tr>
<td>Rabinovitch et al., (2005) [120]</td>
<td>USA</td>
<td>Asthmatic school-aged children</td>
<td>FEV₁ and asthma symptoms</td>
<td>Increases in personal endotoxin exposures were associated with decreased FEV₁ values and increased symptoms</td>
<td>Personal monitoring.</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Occupation</td>
<td>Outcome Measure</td>
<td>Findings</td>
<td>Additional Information</td>
</tr>
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<td>---------------------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Mehta et al., (2010) [141]</td>
<td>China</td>
<td>Cotton textile workers</td>
<td>FEV$_1$ and respiratory symptoms</td>
<td>Among all the workers, there were strong association with reduced FEV$_1$ especially with workers hired less than 5 years</td>
<td>Likewise, the association was found chronic cough</td>
</tr>
<tr>
<td>Lawson et al., (2011) [122]</td>
<td>Canada</td>
<td>School-aged children</td>
<td>Questionnaire for symptoms and spirometry: FEV$_1$ and FVC</td>
<td>High concentration of endotoxin on the mattress was associated with decline FEV$_1$ ($\beta = -0.25$, SE=0.07 ($p&lt;0.01$) among the female case group, while similar associated was found play area with FVC $\beta= -0.17$, SE=0.09 ($p&lt;0.10$)</td>
<td>Sex and tobacco smoking influence the association</td>
</tr>
<tr>
<td>Cyprowski et al., (2015) [123]</td>
<td>Poland</td>
<td>Sewage treatment plant workers</td>
<td>FEV$_1$ and FVC</td>
<td>Despite the low endotoxin levels, significant impact was observed on across-shift FEV$_1$</td>
<td>The result was independent of dust concentrations and smoking habits</td>
</tr>
</tbody>
</table>
2.4.4 The Air Quality Health Index (AQHI) for communicating risk from air pollutants

Tools have been developed as a means of communicating the potential health risks of air pollution to the general public. The Air Quality Health Index (AQHI) is a communication tool which is used in Canada to quantify the level of risk from short-term exposure to air pollution [7,124]. The tool uses three pollutants to calculate a single numerical value as the indicator of health risk: PM$_{2.5}$, NO$_2$, and ground level ozone O$_3$ [124,125]. AQHI indicates the health risk from pollution in a scale from 1 to 10. The health risk is classified in categories for the general population by the index as, low risk (1 to 3, ideal), moderate risk (4 to 6, modify activities if symptomatic), high risk (7 to 10; modify outdoor activities to reduce exposure) and very high risk (above 10; reduce outdoor activities) [125]. These risks are altered for at-risk populations including those with pre-existing disease, children and the elderly (low risk is 1 to 3, enjoy; moderate risk is 4 to 6, consider modifying activities; high risk is 7 to 10; reduce outdoor activities; and very high risk is above 10, avoid outdoor activities) [125].

2.5 Summary and gaps from the literature

The thesis literature review discussed research studies on endotoxin as a biological component of particulate matter, its method of detection and its effect on respiratory health. Occupational exposure to endotoxin and its association with both respiratory and systemic pathologies has been well studied as have endotoxin levels in indoor environments. However, very few studies have measured ambient levels of endotoxin, particularly in Canadian rural areas. In addition, and despite, the association between inhaled endotoxin in agricultural settings and respiratory health, very little research on ambient endotoxin levels has been conducted in rural area settings. The study setting was based on prior knowledge, including differences in asthma in children in the two rural study sites. Therefore, this study addresses the need for more knowledge regarding endotoxin levels in rural communities, especially in agriculturally intensive areas with additional industrial operations. Assessing ambient endotoxin levels and possible association with lung function may add important information in this area, particularly for rural Saskatchewan, and this defines the importance and novelty of the thesis topic.
2.6 Objectives of the study

The objectives of this study were to (1) determine the level and distribution of endotoxin in ambient particulate samples of different sizes (PM$_{2.5}$ and PM$_{10}$) obtained from two rural communities (Estevan and Swift Current, Saskatchewan, Canada) and (2) to examine the association between endotoxin levels in the particulate size fractions (PM$_{2.5}$ and PM$_{10}$) and respiratory outcome (FEV$_1$) among a sample of adult residents (50 years and older) in Estevan and Swift Current.

2.6.1 Research Questions and Hypotheses

1. What is the concentration (EU/m$^3$) and load (EU/µg) of endotoxin in particulate matter (PM$_{2.5}$ and PM$_{10}$) in the communities of Estevan and Swift Current?

2. What is the variation in ambient endotoxin distribution in particulate matter (PM$_{2.5}$) and (PM$_{10}$) and does this differ between Estevan and Swift Current?

   Hypothesis 2-1: There are differences in endotoxin levels (concentration and load) between PM$_{2.5}$ dust and PM$_{10}$ dust within each community and each phase.

   Hypothesis 2-2: There are differences in endotoxin levels (concentration and load) in PM$_{2.5}$ and PM$_{10}$ between each community and each phase.

3. What is the association between ambient endotoxin levels (concentration and load) and the forced expiratory volume in 1 second (FEV$_1$)?

   Hypothesis 3-1: Ambient endotoxin levels (concentration and load) in PM$_{2.5}$ and PM$_{10}$ are associated with FEV$_1$ by community.
2.7 References


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99. Statistics Canada, Canadian Cancer Registry (CCR) Database (July 2010 file) and Demography Division (population estimates). CANSIM table no(s): 103-0550 (rates), 103-0553 (age-standardized rates.).


CHAPTER 3 METHODOLOGY

3.1 Overall study methodology

This thesis is a component of a larger study conducted by the Airways Research Group and is in furtherance of previous research by members of the Airways Research Group that studied the prevalence of asthma in children in these two rural communities and reported higher prevalence in Estevan compared to Swift Current [1]. The larger study aimed to assess the impact of airborne environmental contaminants on respiratory public health in Saskatchewan. The thesis herein incorporates only the particulate endotoxin levels and the population measures of FEV\textsubscript{1} from the larger study.

The study has two parts that were conducted simultaneously: a population study and an environmental study. The study was conducted between 2012 and 2014: fall 2012 (phase 1), spring 2013 (phase 2), fall 2013 (phase 3) and spring 2014 (phase 4). Due to technical difficulties with sample collection in the fall of 2012, phase 1 was not included in the thesis and therefore for the thesis the phases were renamed as follows: spring 2013 (phase 1), fall 2013 (phase 2), and spring 2014 (phase 3).

The study was conducted in a small urban centres of Estevan, a primarily industrial (coal mining)-based community, and Swift Current, a predominantly agricultural-based community, both of which are in southern Saskatchewan, Canada. Detailed descriptions of each community are presented in Manuscript 1 (Chapter 4).

The research described in this thesis was supported by grants from the Saskatchewan Health Research Foundation (SHRF) and the University of Saskatchewan.

3.2 Environmental Methods

3.2.1 Particulate sampling

Sampling occurred in the communities of Estevan and Swift Current over each of the study phases to coincide with the population data collection (Phase 1 from April-June 2013, Phase 2 from October-December 2013 and Phase 3 from April-June 2014). Samples were collected over consecutive seven-day periods (sample) for the length of each study Phase.
Sampling equipment was co-located with provincial air monitoring equipment with the site selection based on the Saskatchewan Air Monitoring Directive [2]. The site was chosen to represent an efficient assessment of air quality for the general public in the area taking into account site and power accessibility, air dispersion modelling, topographical effects, local interferences and security. The sampling site in Estevan was located in the southeast of the city, on a hill, at the roof of a one story building, within a secure compound. The sampling site in Swift Current was located in the northwest of the city, on the rooftop of a one-story provincial building near the highway (Figure 3-1).

Ambient particle samples were collected on pre-weighed 25 mm polycarbonate filters (2.0µm, SKC Inc., Eighty-four, PA, USA) using a 2-stage (PM 2.5 and PM 10) sampler (Dekati, ISO 23210, Dekati Ltd, Tampere, Finland) connected to a high-volume pump (Gilian, Aircon2, Sensidyne, FL, USA) that ran at a flow rate of 30 L/minute. Samples were collected over consecutive seven-day periods (sample) for the length of each study Phase. One blank field filter was taken for each five study filters. (Figure 3-2) The blank field filter accounted for any endotoxin accumulation from technical, field, and storage activities. Due to technical difficulties, Phase 2 resulted in a smaller sample size collection. Post sampling, filters were stored in their identity labelled storage cassettes (SKC, Concept Controls, Edmonton, AB, CA), date stamped, placed in a sealed labelled Ziploc bag with desiccation packs, and refrigerated at 4°C until transport and post-weights.
Figure 3-1. Map of Saskatchewan showing the communities and sampling locations. Source: https://www.google.ca/maps
3.2.2 Gravimetric analysis

The weight of filters was measured before and after sampling using a MX5 microbalance (Mettler Toledo, Mississauga, ON, CA) with a resolution of 0.1 µg. Filters were stored with desiccation-pre and post weighing. Before weighing, filters were equilibrated to room temperature and humidity for 30 minutes. Each filter was then placed through a static neutralizer (PRX U Small SET, Haug Static, Richmond Hill, ON, Canada) and weighed in triplicate and the average of the weights (µg) was calculated for each sample (7-day collection period) for each size fraction (PM$_{2.5}$ and PM$_{10}$). PM concentration (µg/m$^3$) was then calculated as follows:

$$\frac{[(W_2 - W_1)] \times 10^3}{V}$$

Where:

- $W_1$ is the mean pre-filter weight (µg)
- $W_2$ is the mean post-sampling weight (µg)
- $V$ is the volume of air sampled (m$^3$)

Volume = sample flow rate (30 L/minute) * (sample time, minutes) / 1000 L/m$^3$
3.2.3 Endotoxin Determination

3.2.3.1 Filter Extraction

Filter extractions were carried out after all samples from all phases were collected (i.e. after August 2014). For extraction each filter was cut in half using a sterile scalpel blade. One half of the filter was placed back in the storage container and stored in the desiccator at 4°C for other analyses. The second half of the filter was immediately extracted for endotoxin analysis. The half filter was placed in a 2 mL bead beating tube containing 2 g of 2 mm Zirconia beads (BioSpec, Bartlesville, OK, USA). One milliliter of 0.05% Tween 20 (Fisher Scientific, Ottawa, ON, Canada) in endotoxin-free water (Hyclone, GE Healthcare, Mississauga, Canada) was added to each tube and bead beaten for 3 min at the highest setting using the Retsch MM 400 Oscillating Mill (Retsch, Newtown, PA, USA). Tubes were placed in an ice box between bead beating and centrifugation. The tubes were centrifuged at 13,000 rpm for 5 min (Thermo Scientific Sorvall Legend Micro 17 Centrifuge, Fisher Scientific, Ottawa, ON, Canada). The supernatant was transferred to 1.5 mL polypropylene tubes (Fisher Scientific, Ottawa, ON, Canada) and centrifuged at 13,000 rpm for 10 min at 4°C (Eppendorf Centrifuge 5430R, Eppendorf Canada, Mississauga, ON, Canada). Finally, the supernatant was removed, aliquoted, and stored at -80°C until endotoxin analysis.

3.2.3.2 Endotoxin analysis

Endotoxin was determined using the Kinetic chromogenic Limulus Amoebocytes Lysate (LAL) assay according to the manufacturer’s recommendations (Lonza, Walkersville, MD, USA). The steps of endotoxin analysis are attached as Appendix A. One aliquot of sample extract was thawed at room temperature. 100 µL of each of undiluted sample extract, LAL reagent water blank, and endotoxin standards (E.coli 055:B5, five standards optimized for the range 0.005 to 50 EU/mL), were dispensed in triplicate into 96-well plates. The plate was then incubated in the ELx808 plate reader (BioTek, Winooski, VT, USA) for 10 minutes at 37°C. After incubation, kinetic LAL reagent was added to each well and the plate was further incubated for another 2 hours.

Absorbance was monitored at 405 nm and the time required for the absorbance to increase to 0.200 absorbance units was determined and considered to be the reaction onset time. The amount
of endotoxin present is inversely proportional to the reaction time. The concentration of endotoxin in the sample extracts (EU/mL) was determined from the standard curve using Gen5 software (BioTek, Winooski, VT, USA).

Endotoxin load (EU/µg) was calculated by multiplying the EU/mL from the assay by the extraction volume and dividing by the quantity of dust on the half filter.

Endotoxin concentration (EU/m³) was calculated as follows:

\[ [(E \times D \times EV) - EB] \times 10^3 / V \] ................................. (3.2)

E is concentration of endotoxin in the sample extracts (EU/ml)

D is dilution factor

EV is extraction volume

EB is endotoxin units per milliliter from the blank sample

V is the volume of air sampled (m³)

Endotoxin concentrations (EU/m³) and endotoxin loads (EU/µg) were reported as a function of particulate size fraction (PM₂.₅ and PM₁₀). The content is expressed in terms of endotoxin unit per microgram of dust (EU/µg) and endotoxin unit per cubic meter (EU/m³) of sampled air for each of the PM₂.₅ and PM₁₀ size fractions.

3.3 Population

3.3.1 Population recruitment and training

The population study targeted adult residents of Estevan and Swift Current who were 50 years and older. Estevan has a total population of 11,054 (census 2011) while Swift Current has a population of 15,503 (census 2011). The three phases of data collection included: Phase 1 from April-June 2013, Phase 2 from October-December 2013 and Phase 3 from April-June 2014. Season of testing was classified as fall (October, November and early December) and spring (April, May and early June). The phased approach was utilized to account for potential variations by time of year (season). During each Phase, adults 50 years and older were recruited in both communities. Older adults were chosen for the study as effects from airborne exposures are likely to be more prevalent in an older population. Recruitment was conducted through advertisements in the community as well as from a calling list made available by the Lung Association of Saskatchewan. Eligibility of participants was confirmed through a screening questionnaire.
Ineligibility included nursing home residence, being absent from the city for more than one week during the study period, and any health issue that impeded the ability to undertake pulmonary testing. Eligible participants were invited by mail and telephone to attend a study orientation and pre-assessment. The recruitment and selection were identical in each phase of the study. The participants could be recruited in any phase and once recruited, the participant could continue in only one or in all phases.

Participants’ pre-assessment and orientation were carried out by a member of the research team and research nurses from the study communities. The orientation included: signing the consent form; instructions on how to complete a diary of daily symptoms; instruction and practice in how to use and report on peak expiratory flow monitoring. Pre-assessment included a breathing test (spirometry), blood pressure, weight, height, waist circumference, and completion of a baseline questionnaire (Appendix B).

3.3.2 Daily diary of exposures, symptoms, and FEV₁

During each study phase subject specific respiratory data were collected using MY Respiratory Health DAILY DIARY (Figure 3-3). This was an easy to fill one-page per day diary. The contents of the daily diary (Appendix C) completed by each participant were based on questions from a standardized questionnaire American Thoracic Society Respiratory Disease Questionnaire [3] which was used in previous studies in Saskatchewan [1, 4].

Figure 3-3. Daily Diary and Piko-1 for FEV₁ monitoring.
Participants completed the information in the diary twice a day, morning and evening, for the duration of each study phase. The diary collected information on exposures, symptoms and peak expiratory flow measures. Information on time spent outdoors and exposure to smoke as well as information on levels of symptoms (cough, phlegm, and wheeze), degree of symptom disturbance were recorded twice a day. Forced expired volume in one second (FEV₁) recordings (5 best measures of a maximum of 7 measures) were recorded at the same time as symptom reporting. FEV₁ was measured using the PIKO-1 meter. The PIKO-1 is a small and portable electronic peak flow/FEV₁ meter device (Ferraris, Pulmonary Data services, INC, Louisville, CO, USA) (Figure 3-3). The FEV₁ was performed as an exhaled maneuver and the testing procedures were conducted according to the American Thoracic Society guidelines. The daily diary contained a detailed reminder guide for undertaking the procedure (Appendix D). FEV₁ measures were conducted in the morning and evening with the five best blows each morning and each evening recorded (litres) in the daily diary for the duration of each study phase.

To align the pulmonary measures to the weekly environmental values, weekly mean, minimum and maximum FEV₁ values were calculated. For each participant the highest daily FEV₁ recorded for morning and evening were used in the calculation of weekly pulmonary measures. Weekly mean FEV₁ (meanFEV₁) was determined by calculating a mean from the highest daily FEV₁ for each day of the week. Weekly minimum (minFEV₁) was the lowest overall FEV₁ for the week from the daily measures. Weekly maximum (maxFEV₁) was the highest overall FEV₁ for the week from the daily measures. Phase (mean, min, max) were determined by calculating the mean of the weekly values (meanFEV₁, minFEV₁, maxFEV₁) for each Phase and each community.

3.4 Variables of Study for the Thesis

3.4.1 Study variables

In this thesis, secondary data of self-measured lung function obtained from the adult study population in Estevan and Swift Current were used. The study also used endotoxin level data measured in particulate samples collected from the study communities. Finally, endotoxin content of PM₂.₅ and PM₁₀ and FEV₁ variables were used in the test for association.
3.4.1.1 Dependent variable

The primary outcome of interest for paper 2 was the forced expiratory volume in the first second of expiration (FEV$_1$). The respiratory health daily diary was used to twice daily record the FEV$_1$ measurements.

3.4.1.2 Independent variables

The primary exposure of interest in this thesis was endotoxin content present in particulate matter (PM) sizes 2.5 and 10 obtained from Estevan and Swift Current during each of the Phases.

3.5 Data analysis

Data analyses were carried out using the Statistical Package for the Social Sciences (SPSS) version 22 (IBM, Armonk, NY, USA). Statistical significance was set at p < 0.05 based on two-sided calculations. Specific analysis is described in detail in the two manuscript chapters (Chapters 4 and 5).

As an overview, prior to statistical analysis, endotoxin levels in particulate matter were log-transformed to normalize the variables. Geometric means and geometric standard deviations of dust (µg/m$^3$) were calculated. The analysis was undertaken for the endotoxin contents (EU/µg and EU/m$^3$) by particulate size (PM$_{2.5}$ and PM$_{10}$) in each phase and in each community. Mann-Whitney test was used to compare endotoxin in PM$_{2.5}$ and PM$_{10}$ in each phase and in combined phases (Phases 1-3 combined) for each community. The Mann-Whitney test was applied to this data because of the small sample size, the ordinal nature of the data, and the independency of variables. The median differences were calculated between PM$_{2.5}$ from Estevan and PM$_{2.5}$ from Swift Current, likewise in PM$_{10}$, from Estevan and PM$_{10}$ from Swift Current using Mann-Whitney test. Statistical significance of the median differences is reported.

For the population samples, descriptive analyses were performed for the continuous variables age and height. The association between meanFEV$_1$, minFEV$_1$, and maxFEV$_1$ variables and endotoxin levels (EU/mg and EU/m$^3$) were tested using generalizing estimating equations to account for repeated measurements. Based on the previous studies, the confounders included in the analysis were gender, age, height and smoking status.
3.6 Ethical approval

The University of Saskatchewan Research Ethics Board approved this study (BIO 11-172). The principal investigators and the research team of the project “The impact of airborne environmental contaminants on respiratory public health in Saskatchewan” (Drs. J. Gordon and D. Cockcroft and the ARG research team) approved the use of population study as secondary data for analysis in this thesis and the particulate samples for endotoxin analysis.
3.7 References


CHAPTER 4  PAPER 1: ASSESSMENT OF ENDOTOXIN CONTENT IN PM$_{2.5}$ AND PM$_{10}$ PARTICLE MASS OF AMBIENT AEROSOLS IN TWO SASKATCHEWAN COMMUNITIES

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4.1 Contribution of Paper 1 to the thesis

The work presented in this chapter describes analysis of endotoxin in particulate matter [PM] of size 2.5 and 10 µm from samples collected in Estevan and Swift Current, Saskatchewan. The analysis was done in phases based on the periods of PM collection. The Airways Research Group designed the work, collected the samples and undertook the gravimetric analysis. I set up the research questions specific to this study. I initiated and completed the endotoxin analysis under their supervision. I completed all the experimental work including extraction, sample preparation, and subsequent endotoxin analysis. This paper answered the first and second research questions and likewise addressed the hypotheses:

1. What is the concentration (EU/m³) and load (EU/µg) of endotoxin in particulate matter (PM{sub}2.5 and PM{sub}10) in the communities of Estevan and Swift Current?

2. What is the variation in ambient endotoxin distribution in particulate matter (PM{sub}2.5) and (PM{sub}10) and does this differ between Estevan and Swift Current?

   Hypothesis 2-1: There are differences in endotoxin levels (concentration and load) in PM{sub}2.5 compared to PM{sub}10 within each community and each phase.

   Hypothesis 2-2: There are differences in endotoxin levels (concentration and load) in PM{sub}2.5 and PM{sub}10 between each community and each phase.

In all experimental stages, technical assistant was provided by Brooke Thompson. As first author, I wrote all the draft version of the manuscript and incorporated revision advised by my supervisor, co-supervisor, and the Airways Research Group (ARG) team. This work was fully supervised by Drs. Katselis and Kirychuk.
4.2 Abstract

Globally, particulate matter of various sizes in the air has become an environmental concern. Assessing ambient particulate matter and analysis of its organic composition is not widely studied compared to indoor air. Endotoxin, a component of the gram-negative bacteria cell wall, binds to particulate matter, and triggers airway inflammation when inhaled. This study compared endotoxin levels in particulate matter of 2.5 and 10 µm size fractions in two rural communities, Estevan and Swift Current, in southern Saskatchewan, Canada.

Samples were collected in seven-day increments for three eight-week phases in the two communities. Endotoxin concentrations (EU/m$^3$) and loads (EU/µg) were reported as a function of particulate size fraction (PM$_{2.5}$ and PM$_{10}$).

The highest levels of endotoxin (EU/µg) found in Estevan were 0.02 (Phase 1), 0.03 (Phase 2) and 0.01 (Phase 3), while endotoxin (EU/m$^3$) were 0.04 (Phase 1), 0.07 (Phase 2) and 0.02 (Phase 3). Similar trends were found in Swift Current with 0.04 (Phase 1), 0.10 (Phase 2), and 0.05 (Phase 3) in endotoxin (EU/µg), while 0.07 (Phase 1), 0.13 (Phase 2), and 0.08 (Phase 3) were found in endotoxin (EU/m$^3$).

In Estevan there were no significant differences between PM$_{2.5}$ and PM$_{10}$ for endotoxin load in any of the phases (p > 0.05), but there were significant differences in endotoxin concentrations in all the phases (p < 0.05). In Swift Current, endotoxin loads were significantly different between PM$_{2.5}$ and PM$_{10}$ in Phases 2 and 3 only, while endotoxin concentrations were statistically significant for all phases (p < 0.05).

Comparing the same particulate size in Estevan and Swift Current, endotoxin loads for PM$_{2.5}$ were significantly higher in Swift Current as compared to Estevan (p ≤ 0.05), while differences in PM$_{10}$ endotoxin loads were only seen in Phases 2 and 3. In regards to endotoxin concentration, a statistically significant difference was only found in phase 3 (p < 0.05) with higher concentrations in Swift Current as compared to Estevan for both PM$_{2.5}$ and PM$_{10}$. In general, endotoxin concentrations were higher in PM$_{2.5}$ as compared to PM$_{10}$ and varied by season and community.

Keywords: Ambient endotoxin; Particulate matter; LAL assay; Outdoor air quality; Rural communities
4.3 Introduction

Air pollution is a key problem in both emerging and developed countries giving rise to environmental and health concerns. Most industrialized cities suffer from severe atmospheric air pollution due to inappropriate control of discharges emanating from industries [1]. Industrial development induces pollutants which include particulate matter (PM), sulphur dioxide (SO$_2$), and nitrogen dioxide (NO$_2$). Particulate matter is a complex mixture of solid and liquid particles in the air [2]. Ambient PM with 50% cut-off of particles of aerodynamic size less than 2.5 um (PM$_{2.5}$) and 10 µm (PM$_{10}$) has been shown to have both acute and chronic effects on human health [2-4].

Particulate matter can adsorb other air contaminants such as ammonia and endotoxin [5]. Therefore, the composition of particulate matter, including the assessment of endotoxin on PM, is important in the evaluation of respiratory health risks to the exposure [6]. Endotoxin as a biological agent is embedded in the cell wall of both pathogenic and saprophytic gram-negative bacteria [7]. It consists of lipopolysaccharide (LPS), whose molecules are made up of Lipid A, polysaccharide nucleus, and polysaccharide chains. Lipid A is similar in all species of bacteria and is responsible for the toxic effects of endotoxin [8, 9].

Endotoxin is ubiquitous, and is found in homes, at workplaces, and in both rural and urban environments [10]. Sources of endotoxin at home include kitchen compost bins, humidifiers, and pets [11]. In rural environments, significant sources include animal confinement facilities, grain storage facilities, and crop harvesting [12, 13]. A 2010 study in California’s central valley found the highest concentrations of endotoxin immediately downwind from agricultural activities [14]. Of the pollutants studied, which included PM$_{2.5}$, elemental carbon, and PM$_{10}$, only PM$_{10}$ levels were found to correlate with endotoxin loads [14]. Similar findings were observed in a study of ambient endotoxin in Prince George and Kelowna, British Columbia, Canada where endotoxin concentrations were positively associated with agricultural dust of both PM$_{2.5}$ and PM$_{10}$ size, but the association was stronger with PM$_{10}$ [15]. Studies performed in other countries have reported the same trend of endotoxin bound to particulate matter [10-12]. A study in a German metropolitan area [16] reported higher endotoxin levels in PM$_{10}$, compared to endotoxin in PM$_{2.5}$. This observation was partly associated with potential sources of endotoxin around the collection sites such as trash bins, gardens, sewage and animal facilities areas.

Endotoxin has dual properties; it can cause inflammation of the respiratory tract in adults and children, while it also can modify the immune response, reducing the risk of development of
atopic disease in children [17]. The significant role of endotoxin in development of dust-related respiratory symptoms in agricultural workers has been well studied but the dose-response relationship is yet unclear [18, 19]. Indoor house dust has been widely used to assess the individual level of endotoxin exposure in homes [20, 21], but few studies have measured endotoxin levels in ambient environmental dusts, especially in Canada [10, 22].

A precursor to this study, conducted in 2000 among the children residing in these participating communities found high and differing asthma prevalence rates of 21.4% and 16.2% [23]. In the associations between indoor domestic endotoxin levels and asthma in children, the findings showed that indoor endotoxin was not a major risk factor for current asthma but may aggravate the severity of existing asthma [24]. Few studies have evaluated endotoxin levels in ambient environmental dusts. Therefore, this follow-up study described herein explored the concentration and load of endotoxin in ambient particulate matter in these communities. In the current study, our aim was to determine levels of ambient endotoxin in particulate matter in PM$_{2.5}$ and PM$_{10}$ in two rural communities with differing industrial composition.

4.4 Materials and methods

4.4.1 Sample collection

Sampling occurred in the communities of Estevan (population 11,054), and Swift Current (population 15,503), whose geographical and other relevant characteristics are described in Table 4-1. Sampling equipment was co-located with provincial air sampling equipment with the site based on the Saskatchewan Air Monitoring Directive [25]. The site was chosen to represent an efficient assessment of air quality for the general public in the area taking into account site and power accessibility, air dispersion modelling, topographical effects, local interferences and security [25].

The samples were collected in spring 2013 (Phase 1), fall 2013 (Phase 2), and spring 2014 (Phase 3), which represent the study phases. Ambient particle samples were collected using a 2-stage (PM 2.5 and 10) sampler (Dekati, ISO 23210, Dekati Ltd, Tampere, Finland) connected to a high-volume pump (Gilian, Aircon2, Sensidyne, FL, USA) that ran at a flow rate of 30 L/minute. PM$_{2.5}$ and PM$_{10}$ were collected on pre-weighed 25 mm polycarbonate filters (2.0µm, SKC Inc., Eighty-four, PA, USA). Each sample was collected over a seven-day period (sample), and samples were collected for the length of each study period (Phase). One blank field filter was taken for
each five filters. The blank field filter accounted for any endotoxin accumulation from technical and field, and storage activities. Due to technical difficulties, Phase 2 resulted in a smaller sample size collection.

Post sampling, filters were stored in their identity-labelled clear styrene cassettes (SKC, Concept Controls, Edmonton, AB, CA), date stamped, and placed in a sealed labelled Ziploc bag with desiccation packs, and refrigerated at 4°C until transport and post-weights.

4.4.2 Gravimetric measurement

The weight of filters was measured before and after sampling using a MX5 microbalance (Mettler Toledo, Mississauga, ON, CA) with a resolution of 0.1 µg. Before weighing, filters were equilibrated to room temperature and humidity for 30 minutes. The filters were placed through a static neutralizer (PRX U Small SET, Haug Static, Richmond Hill, ON, Canada) and weighed in triplicate. The average weight and PM concentration (µg/m³) were calculated for each size fraction.

The PM concentration (µg/m³) in the air volume sampled was calculated as follows:

\[
\frac{\left(W_2 - W_1\right) \times 10^3}{V}
\]

Where:

\(W_1\) is the mean pre-filter weight (µg)

\(W_2\) is the mean post-sampling weight (µg)

\(V\) is the volume of air sampled

4.4.3 Filter extraction

Filter extractions were carried out after all samples from all phases were collected (i.e. after August 2014). For extraction each filter was cut in half using sterile scalpel blade. One half of the filter was placed back in the storage container and stored in the desiccator at 4°C for other analyses. The second half of the filter was immediately extracted for endotoxin analysis. This half of the filter was placed in a 2 mL bead beating tube containing 2 g of 2 mm Zirconia beads (BioSpec, Bartlesville, OK, USA). One milliliter of 0.05% Tween 20 (Fisher Scientific, Ottawa, ON, Canada) in endotoxin-free water (Hyclone, GE Healthcare, Mississauga, Canada) was added to each tube and bead beaten for 3 min at the highest setting using the Retsch MM 400 Oscillating Mill (Retsch, Newtown, PA, USA). Tubes were placed in an ice box between bead beating and
centrifugation. The tubes were centrifuged at 13,000 rpm for 5 min (Thermo Scientific Sorvall Legend Micro 17 Centrifuge, Fisher Scientific, Ottawa, ON, Canada). The supernatant was transferred to 1.5ml polypropylene tubes (Fisher Scientific, Ottawa, ON, Canada) and centrifuged at 13,000 rpm for 10 min at 4°C (Eppendorf Centrifuge 5430R, Eppendorf Canada, Mississauga, ON, Canada). Finally, the supernatant was removed, aliquoted, and stored at -80°C until endotoxin analysis.

### 4.4.4 Endotoxin analysis

Endotoxin was determined using the Kinetic chromogenic Limulus Amoebocytes Lysate (LAL) assay according to the manufacturer’s recommendations (Lonza, Walkersville, MD, USA). One aliquot of supernatant (extract) was thawed at room temperature. 100 µL of each undiluted sample extract, a LAL reagent water blank and the endotoxin standards (E.coli 055:B5, five standards optimized for the range 0.005 to 50 EU/mL), were dispensed in triplicate into 96-well plates. The plate was then incubated in the ELx808 plate reader (BioTek, Winooski, VT, USA) for 10 minutes at 37°C. After incubation, kinetic LAL reagent was added to each well and the plate was further incubated for another 2 hours. Absorbance was monitored at 405 nm and the time required for the absorbance to increase to 0.200 absorbance units was determined and considered to be the reaction onset time. The amount of endotoxin present is inversely proportional to the reaction time. The concentration of endotoxin in the sample extracts (EU/ml) was determined from the standard curve using Gen5 software (BioTek, Winooski, VT, USA).

Endotoxin load (EU/µg) was calculated by multiplying the EU/mL by the extraction volume and dividing by the quantity of dust on the half filter. Endotoxin concentration (EU/m³) was calculated as follows:

\[
[(E \times D \times EV) - EB] \times 10^3 / V \]

- E is endotoxin units per milliliter
- D is dilution factor
- EV is extraction volume
- EB is endotoxin units in blank sample
- V is the volume sampled
4.4.5 Statistical methods

All statistical analyses were carried out using SPSS version 22 (IBM Statistics, Armonk, NY, USA). Analyses on environmental variables were completed after log transformation, and geometric means and geometric standard deviations by phase were used to describe the environmental data for both dust and endotoxin. The Mann-Whitney (M-W) test was used to assess significant differences in median endotoxin (EU/µg and EU/m³) between PM_{2.5} and PM_{10} within a community in each phase, and between the communities in the same phases. In all the tests, differences were considered statistically significant at p-value <0.05.

4.5 Results

A summary of characteristics of the communities and sample sites are shown in Table 4.1. The study was comprised of a total of 106 filters; 50 samples (25 PM_{2.5} and 25 PM_{10}) from Estevan and 56 samples (28 PM_{2.5} and 28 PM_{10}) from Swift Current for the three phases.

4.5.1 Estevan

In this community, the dust concentration ranged from 0.34 to 2.72 µg/m³ across the phases. There was a significantly greater concentration of PM_{2.5} than coarse dust (PM_{10}) for all 3 phases (Table 4.2).

As shown in Table 4.3, the highest levels of endotoxin load (geometric mean in EU/µg) found in Estevan were 0.02 (Phase 1), 0.03 (Phase 2) and 0.01 (Phase 3). In all phases, there were no statistically significant differences in endotoxin load between the particle size fractions. Endotoxin concentration (geometric mean in EU/m³) levels were 0.04 (Phase 1), 0.07 (Phase 2) and 0.02 (Phase 3) as shown in Table 4.4. For Estevan, there was significantly greater endotoxin concentration in PM_{2.5} compared to PM_{10} for all phases.

4.5.2 Swift Current

As shown in Table 4.2, in this community, dust concentration ranged from 0.20 to 1.92 µg/m³ across the phases. The dust concentration was significantly greater in the PM_{2.5} size fraction as compared to that of the PM_{10} for all phases in Swift Current.

Highest levels of endotoxin load (geometric mean in EU/µg) found in Swift Current were 0.04 (Phase 1), 0.10 (Phase 2), and 0.05 (Phase 3) (Table 4.3). Endotoxin load was significantly
higher in the PM$_{2.5}$ size fraction as compared to the PM$_{10}$ in Phases 2 and 3 for Swift Current.

For Swift Current, 0.07 (Phase 1), 0.13 (Phase 2), and 0.08 (Phase 3) were found in endotoxin concentration (geometric mean in EU/m$^3$) as shown in Table 4.4. There was significantly greater endotoxin concentration in the PM$_{2.5}$ size fraction as compared to PM$_{10}$ (Table 4.4) for all Phases.

4.5.3 Endotoxin comparisons between communities and by size fraction

The comparison between Estevan and Swift Current communities for endotoxin load (EU/µg), by phase and size fraction, is described in Figure 4.1 (A-C) and Figure 4.2 (A-C). Swift Current had almost three times the endotoxin load as Estevan in Phase 2. Endotoxin load was significantly higher in the PM$_{2.5}$ size fraction as compared to the PM$_{10}$ in Phases 2 and 3 for Swift Current. In comparison, Estevan had no significant differences in endotoxin load between size fractions for any of the phases. There was significantly greater endotoxin load in the PM$_{2.5}$ size fraction in all phases for Swift Current as compared to Estevan. This trend held for PM$_{10}$ for Phases 2 and 3, where there were significantly higher levels in Swift Current as compared to Estevan.

Comparison of the mean endotoxin concentration (EU/m$^3$) by phase and by size fraction between the two communities is described in Figure 4.3 (A-C) and Figure 4.4 (A-C). The mean PM$_{2.5}$ endotoxin concentration was greater in Swift Current as compared to Estevan in all phases, but this difference was significant only in Phase 3 ($p<0.0001$). For PM$_{10}$, the endotoxin concentration was more variable between communities and by community. Only in Phase 3 were the endotoxin concentrations significantly different between communities with Swift Current having a higher level ($p<0.02$). There was significantly greater endotoxin concentration in the PM$_{2.5}$ size fraction as compared to PM$_{10}$ (Table 4-4) for all Phases for both communities.

4.6 Discussion

The purpose of this study was to assess endotoxin distribution by particulate size fraction in two communities with different regional industries. In this study, there were significant differences in the endotoxin concentration between the two size fractions. PM$_{2.5}$ consistently had greater endotoxin concentration as compared to the PM$_{10}$ size fraction. In general, the study findings have revealed that endotoxin is present in both PM$_{2.5}$ and PM$_{10}$. The findings in our study
agree with our hypothesis that more endotoxin are bound to smaller particulate size diameter as compared to coarse particulate. In our study, endotoxin was about 10-fold higher bound to PM$_{2.5}$ as compared to PM$_{10}$. Most previous studies of ambient endotoxin levels reported higher levels of endotoxin bound to PM$_{10}$ compared to PM$_{2.5}$ [15, 16]. Any attached pollutants at PM$_{2.5}$ will have the tendency to penetrate to the inner regions of respiratory tract [3]. The higher endotoxin levels found at PM$_{2.5}$ in the current study could be a reflection of the predominant industry in these communities, which is agriculture, a known source for high levels of endotoxin.

Endotoxin is recognized as an important component of particulate matter associated with occurrence and exacerbation of airway disease [26]. The endotoxin concentrations in this study are many fold lower than 50 EU/m$^3$, which is the occupational exposure limit recommended by the Dutch Expert Committee on Occupational Safety and Health [27,28]. There are no recommendations for ambient endotoxin exposure levels.

Overall, our measurements showed that the mean endotoxin level found in Swift Current, primarily an agricultural environment, were significantly higher compared to Estevan, both an industrial, with open coal mining, and an agricultural community. Our results confirm outcomes from previous studies showing that agricultural environments experience higher levels of endotoxin as compared to other environments. Endotoxin primarily originates from gram-negative bacteria that are predominant in animal confinement areas, crops, and storage facilities [12,17]. For instance, an outdoor ambient endotoxin study carried out in Prince George and Kelowna, BC, Canada between 2005 and 2006 found a positive association between agricultural dust factors in particulate sizes and the level of endotoxin [17]. It is possible that coal mining activities in Estevan could have an impact on the measured endotoxin levels; Sebastian and his co-workers reported that heated particulate matter generated from industry can inactivate endotoxins, such that they possess reduced activities in LAL assays [29].

Endotoxin concentrations in our studies were lower compared to previous studies, for instance, the study in Regina, Saskatchewan, Canada [30]. This is likely due to the differences in the ambient air pollution sources. Regina (216,528), is one of the largest urban centers in Saskatchewan with a significantly larger population size and industrial and vehicular contributions to air quality as compared to the much smaller centers studied herein. Our endotoxin in PM$_{2.5}$ is similar to that found by others, however the endotoxin in PM$_{10}$ is lower than the levels found in other similar studies, as can be seen in Table 4-5. In comparison to other studies our study had
significantly greater collection volumes per filter. Samples were collected at a greater flow rate for much longer sampling periods than comparative studies. These greater volumes, particularly for the low ambient levels we were sampling, provided an adequate sample for the analyses that were undertaken giving strength to the results.

In the current study the highest levels of endotoxin were found in the fall (Phase 2). This is similar to a study from British Columbia that found the highest levels of endotoxin in summer and fall with lowest levels in winter and spring [17]. In the communities we studied, farming activity is at its peak during fall seasons as farmers can be busy with harvesting, hauling bales, putting machinery away, moving cattle and applying fertilizer. It is possible that farming activities might be a contributing factor to elevated endotoxin loads for Swift Current, although higher endotoxin levels may also be due to components derived from plant materials and deposits on leaves surfaces [31]. Based on the previous assumption, Carty et al. studied the ambient endotoxin levels in PM$_{2.5}$ and effects of seasonal variation on endotoxin levels in Germany [32]. The findings showed that predominantly high levels of endotoxin in spring and summer compared to the cold season could be possible due to plants growth during these periods. Furthermore, a study of airborne endotoxin in different background environments and seasons has shown that endotoxin concentration levels were higher in spring compared to winter, but peaked in October [22], while a study in Beijing China has reported that endotoxin concentration was higher in both spring and winter [33]. The observed difference between our study and this last study might be due to various contributors to endotoxin levels including different forms of agricultural practice or climatic variations. However, it is worth mentioning that several studies reported for occupational settings were inconsistent with respect to the seasonal variation of endotoxin [34-36].

Previous studies emphasized several confounders in this type of study which are not different from ours. Differences in climatic conditions, industrial climate, and inter-laboratory procedure are the main confounders. One study indicated that season, sampling sites, and weather conditions were responsible for 24% of the variability in endotoxin concentration [37]. Another study reported on the influence of temperature on the levels of endotoxin [32] while another study also reported highest endotoxin levels during warm weather and moderate humidity [17]. These observations imply that gram-negative bacteria grow better with certain weather conditions or undergo lysis at higher temperature to liberate endotoxin in the air. A strength of this study is that the measurements occurred at the same time periods in both communities, reducing the error
associated with seasonal differences. However, the limited number of collection samples and the low quantity of dust collected may influence the study findings.

4.7 Conclusions

Our study shows that endotoxin in Estevan and Swift Current communities is predominant in the PM$_{2.5}$ size fraction as compared to PM$_{10}$. The finding is important as smaller size fractions have the potential for greater influences on lower respiratory health. Endotoxin, and in particular endotoxin load, was higher in Swift Current as compared to Estevan. The major industrial activities in these communities may have a strong influence on the endotoxin levels.

Acknowledgements
The authors are grateful to Johanne Asselin for assistance with dust sample collection, and Amanda Lambrecht and Brooke Thompson for technical assistance throughout the experimental process. Funding was provided by the Saskatchewan Health Research Foundation.
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Table 4-1. Summary of sample locations and data collections.

<table>
<thead>
<tr>
<th>Study parameter</th>
<th>Sample sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estevan</td>
</tr>
<tr>
<td><strong>Sampling periods</strong></td>
<td></td>
</tr>
<tr>
<td>Phase 1</td>
<td>Spring 2013</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Fall 2013</td>
</tr>
<tr>
<td>Phase 3</td>
<td>Spring 2014</td>
</tr>
<tr>
<td><strong>Sampling location</strong></td>
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<tr>
<td><strong>Population density /km²</strong></td>
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</tr>
<tr>
<td><strong>Number of samples</strong></td>
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</tr>
<tr>
<td>Phase 1</td>
<td>10/10 (PM$<em>{2.5}$/PM$</em>{10}$)</td>
</tr>
<tr>
<td>Phase 2</td>
<td>5/5 (PM$<em>{2.5}$/PM$</em>{10}$)</td>
</tr>
<tr>
<td>Phase 3</td>
<td>10/10 (PM$<em>{2.5}$/PM$</em>{10}$)</td>
</tr>
<tr>
<td><strong>Mean sample volume (m³)</strong></td>
<td></td>
</tr>
<tr>
<td>Phase 1</td>
<td>302</td>
</tr>
<tr>
<td>Phase 2</td>
<td>290</td>
</tr>
<tr>
<td>Phase 3</td>
<td>305</td>
</tr>
</tbody>
</table>

Phase 1 (April-June 2013); Phase 2 (October-December 2013); Phase 3 (April-June 2014); PM$_{2.5}$ particulate matter size fraction 2.5; PM$_{10}$ particulate matter size fraction 10.
Table 4-2. PM$_{2.5}$ and PM$_{10}$ Concentration (µg/m$^3$) by phase and community.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estevan</th>
<th>Swift Current</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>GM (GSD)</td>
</tr>
<tr>
<td>Phase 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>10</td>
<td>2.52 (2.34)</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>10</td>
<td>0.34 (1.81)</td>
</tr>
<tr>
<td>Phase 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>5</td>
<td>2.72 (1.58)</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>5</td>
<td>0.59 (1.42)</td>
</tr>
<tr>
<td>Phase 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>10</td>
<td>2.09(1.95)</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>10</td>
<td>0.36(1.45)</td>
</tr>
<tr>
<td>Combined Phases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>25</td>
<td>2.38 (2.01)</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>25</td>
<td>0.39 (1.65)</td>
</tr>
</tbody>
</table>

Phase 1 (April-June 2013); Phase 2 (October-December 2013); Phase 3 (April-June 2014); Combined Phases (Phases 1-3 combined); n number of samples; min minimum; max maximum; GM geometric mean; GSD geometric standard deviation; significance P-value <0.05.
Table 4-3. PM$_{2.5}$ and PM$_{10}$ endotoxin load (EU/µg) by phase and community.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estevan</th>
<th>Swift Current</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GM (GSD)</td>
<td>Min-Max</td>
</tr>
<tr>
<td>Phase 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>0.02 (1.61)</td>
<td>0.01-0.04</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>0.02 (6.10)</td>
<td>0.0014-1.24</td>
</tr>
<tr>
<td>Phase 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>0.03 (2.09)</td>
<td>0.01-0.05</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>0.01 (4.26)</td>
<td>0.001-0.03</td>
</tr>
<tr>
<td>Phase 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>0.01 (1.97)</td>
<td>0.003-0.025</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>0.01 (2.15)</td>
<td>0.00-0.01</td>
</tr>
<tr>
<td>Combined Phases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>0.01 (2.15)</td>
<td>0.00-0.05</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>0.01 (4.44)</td>
<td>0.01-1.24</td>
</tr>
</tbody>
</table>

Phase 1 (April-June 2013); Phase 2 (October-December 2013); Phase 3 (April-June 2014); Combined Phases (Phases 1-3 combined); min minimum; max maximum; GM geometric mean; GSD geometric standard deviation; significance P-value <0.05; EU/µg Endotoxin unit in 1 µg of dust.
Table 4-4. PM$_{2.5}$ and PM$_{10}$ endotoxin concentration (EU/m$^3$) by phase and community.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estevan</th>
<th>Swift Current</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GM (GSD)</td>
<td>Min-Max</td>
</tr>
<tr>
<td>Phase 1</td>
<td>PM$_{2.5}$</td>
<td>0.04 (1.94)</td>
</tr>
<tr>
<td></td>
<td>PM$_{10}$</td>
<td>0.01 (7.75)</td>
</tr>
<tr>
<td>Phase 2</td>
<td>PM$_{2.5}$</td>
<td>0.07 (2.53)</td>
</tr>
<tr>
<td></td>
<td>PM$_{10}$</td>
<td>0.004 (5.38)</td>
</tr>
<tr>
<td>Phase 3</td>
<td>PM$_{2.5}$</td>
<td>0.02 (1.58)</td>
</tr>
<tr>
<td></td>
<td>PM$_{10}$</td>
<td>0.002 (2.69)</td>
</tr>
<tr>
<td>Combined phases</td>
<td>PM$_{2.5}$</td>
<td>0.03 (2.36)</td>
</tr>
<tr>
<td></td>
<td>PM$_{10}$</td>
<td>0.003 (5.32)</td>
</tr>
</tbody>
</table>

Phase 1 (April-June 2013); Phase 2 (October-December 2013); Phase 3 (April-June 2014); Combined Phases (Phases 1-3 combined); min minimum; max maximum; GM geometric mean; GSD geometric standard deviation; significance P-value <0.05; EU/m$^3$ endotoxin unit in cubic meters.
Table 4-5. Comparison of ambient endotoxin measured in studies reported in literature.

<table>
<thead>
<tr>
<th>Author</th>
<th>Sampling location</th>
<th>Sampling period</th>
<th>Particulate size</th>
<th>Findings in EU/m$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen et al., [15]</td>
<td>Urban-Prince George; pulp mills, saw mills, refinery and other industrial plants</td>
<td>Oct. 2005- Sept. 2006</td>
<td>PM$_{2.5}$</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PM$_{10}$</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Urban – Kelowna; agricultural area that produce fruit &amp; vegetable crop</td>
<td></td>
<td>PM$_{2.5}$</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PM$_{10}$</td>
<td>0.67</td>
</tr>
<tr>
<td>Heinrich et al., [10]</td>
<td>Small towns; livestock - pigs, sheep, cows, lay hens and broilers</td>
<td>Jan-June 2002</td>
<td>PM$_{2.5}$</td>
<td>1$^{st}$ City 0.006 ; 2$^{nd}$ City 0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PM$_{10}$</td>
<td>1$^{st}$ City 0.063; 2$^{nd}$ City 0.071</td>
</tr>
<tr>
<td>Morgenstern et al., [16]</td>
<td>Urban; no large scale agricultural or potential (what can be called) industry in the study areas</td>
<td>Mar. 1999-July 2000</td>
<td>PM$_{2.5}$</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PM$_{10}$</td>
<td>0.081</td>
</tr>
<tr>
<td>Nilsson et al., [38]</td>
<td>Urban; both traffic sites and non-traffic sites</td>
<td>May - Sept. 2009</td>
<td>PM$_{2.5}$</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PM$_{10}$</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Figure 4-1 A): Comparison of mean endotoxin load [lnEU/µg] in PM$_{2.5}$, Phase 1, between Estevan and Swift Current. P-value =0.05 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
Figure 4-1 B): Comparison of mean endotoxin load [InEU/μg] in PM$_{2.5}$, Phase 2, between Estevan and Swift Current. P-value =0.01 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
Figure 4-1 C): Comparison of mean endotoxin load [InEU/µg] in PM$_{2.5}$, Phase 3, between Estevan and Swift Current. P-value <0.001 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
Figure 4-2 A): Comparison of mean endotoxin load [InEU/µg] in PM$_{10}$, Phase 1, between Estevan and Swift Current. P-value >0.05 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
Figure 4-2 B): Comparison of mean endotoxin load [lnEU/µg] in PM$_{10}$, Phase 2, between Estevan and Swift Current. P-value = 0.03 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
Figure 4-2 C): Comparison of mean endotoxin load [lnEU/µg] in PM$_{10}$, Phase 3, between Estevan and Swift Current. P-value = 0.02 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
Figure 4-3 A): Comparison of log mean endotoxin concentration [lnEU/m³] in PM$_{2.5}$, Phase 1, between Estevan and Swift Current. P-value >0.05 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
Figure 4-3 B): Comparison of log mean endotoxin concentration [lnEU/m^3] in PM_{2.5}, Phase 2, between Estevan and Swift Current. P-value >0.05 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
Figure 4-3 C): Comparison of log mean endotoxin concentration [InEU/m$^3$] in PM$_{2.5}$, Phase 3, between Estevan and Swift Current. P-value <0.001 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
Figure 4-4 A): Comparison of log mean endotoxin concentration [lnEU/m³] in PM$_{10}$, Phase 1, between Estevan and Swift Current. P-value >0.05 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
Figure 4-4 B): Comparison of log mean endotoxin concentration [lnEU/m$^3$] in PM$_{10}$, Phase 2, between Estevan and Swift Current. P-value >0.05 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
Figure 4-4 C): Comparison of log mean endotoxin concentration [InEU/m$^3$] in PM$_{10}$, Phase 3, between Estevan and Swift Current. P-value <0.02 using Mann-Whitney test. Y-axis represents mean log of endotoxin concentration and 95% confidential interval; X-axis represents study communities.
CHAPTER 5  PAPER 2: ASSOCIATION BETWEEN AMBIENT ENDOTOXIN AND LUNG FUNCTION AMONG THE ADULT RESIDENTS IN TWO SASKATCHEWAN COMMUNITIES

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\textsuperscript{b}Department of Medicine, Canadian Centre for Health and Safety in Agriculture, College of Medicine, University of Saskatchewan, Saskatoon, Canada
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\textsuperscript{*}Corresponding Author
5.1 Contribution of Paper 2 to the thesis

The work presented in this chapter describes the association between endotoxin levels (load and concentration) and forced expiratory volume in 1 second (FEV₁) among the adult residents in Estevan and Swift Current. Endotoxin data from the first manuscript was used in the test for association with secondary FEV₁ data using generalizing estimating equations under the supervision of Dr. Joshua Lawson. I completed the endotoxin analysis and generated data, while FEV₁ data was obtained by the ARG research team. Dr. George Katselis and Dr. Shelley Kirychuk also checked the produced data and its quality. I set up the research question specific to this study. This paper answered my third research question and the hypothesis.

3. What is the association between ambient endotoxin levels (concentration and load) and the forced expiratory volume in 1 second (FEV₁)?

I wrote the draft version of the manuscript and incorporated the version advised by all authors. This work was supervised by Drs. Kirychuk, Lawson, and Katselis.
5.2 Abstract

Exposure to endotoxin, a major biological component of particulate matter, has been found to be associated with wheeze, cough, phlegm, and decline in lung function. Indoor endotoxin at the household and occupational exposure level has been commonly studied while ambient endotoxin has rarely been examined in relation to lung function.

This study aimed to assess the association between ambient endotoxin level and lung function among adult residents in two Saskatchewan communities, Estevan and Swift Current. Adult participants were selected from the general population with a screening questionnaire. During the three phases beginning in April 2013, Forced Expiratory Volume in 1s (FEV₁) was recorded in a daily diary, and weekly assessments of endotoxin in a 50% cut-off aerodynamic diameter of 2.5 µm and 10 µm of particulate matter were conducted. Associations were examined using generalizing estimating equations adjusting for confounders. Interaction of endotoxin and smoking was assessed.

There were 209 adult residents included in the present study. The geometric means of endotoxin in dust sampled (EU/µg) and in the sampled air (EU/m³) were less than 0.2 in the two communities. However, despite low levels of endotoxin, in Swift Current, mean and max FEV₁ was associated significantly with PM₂.₅ endotoxin concentration and load in Phase 3. There was only significant interaction with smoking for Swift Current in endotoxin concentration PM₂.₅.

In this study, there is an inconsistent association between exposure to endotoxin in ambient air and the outcome measurements of lung function. Results indicated that low endotoxin levels present might not affect the lung function of adults in both communities.

Key words: Adults; Endotoxin; Lung function; Community; Smoking
5.3 Introduction

Respiratory illnesses are one of the most prevalent disease conditions affecting adults [1]. According to Health Canada, respiratory problems affect more than 3 million Canadians [2]. In the past decade in Canada, respiratory diseases accounted for approximately 10% of all admission in the hospital and about 37,000 deaths [2]. Within the same period in Saskatchewan, 8.5% of the adult population reported physician-diagnosed asthma [3]. Many studies have established the relationship between tobacco smoking and respiratory problems [2]. Short and long term exposure to outdoor air pollution has an important influence on the respiratory health of Canadians [4,5]. Among 34 countries including Canada, effects of outdoor pollution, on the children’s respiratory health cost 1.7 trillion US dollars [6]. Adults are at increased risk from air pollution due to normal and pathological aging and other processes [7].

Endotoxin, one of the constituents of particulate air pollution has been singled out as the most significant biological component associated with the development and exacerbation of respiratory diseases in persons exposed to agricultural environments [8]. Endotoxin is regarded as a biological agent that can be hazardous to respiratory health [9, 10].

Inhalation of endotoxin has a contributing role in respiratory tract obstruction, measured by a decrease in forced expiratory volume in 1s (FEV₁) [11]. FEV₁ is a common spirometric measure of lung function that can indicate chronic respiratory problems and systemic inflammation [12]. In other reviews of the roles of endotoxin in both children and adults’ respiratory problems, endotoxin was found to be protective in childhood asthma and responsible for exacerbation of asthma in adults [13].

According to previous epidemiological studies in the same study locations, in which asthma prevalence was higher in Estevan with 21.4 % as compared to Swift Current (16.2%) [14], further studies in the children’s population reported a significant association between indoor endotoxin load and asthma severity among children [15]. The study described herein further examined these communities and the relationships between ambient endotoxin exposure in particulate pollution and lung function in older adults in the communities.

The aim of this study was to determine the associations between endotoxin load (EU/μg), endotoxin concentration (EU/m³), and FEV₁ in two small urban communities in Saskatchewan,
Estevan and Swift Current. Associating lung function with outdoor endotoxin levels has rarely been considered in previous studies, and to date, no other study has evaluated the role of ambient endotoxin in this region.

5.4 Materials and methods

5.4.1 Recruitment and study design

A study of adults residing in the Estevan and Swift Current communities in Saskatchewan was conducted between 2013 and 2014. Estevan has coal mining, coal-based power generation, and agricultural industries with a total population of 11,054 (census 2011). Swift Current is a primarily agriculture-based region with a population of 15,503 (census 2011). The study involved three phases of data collection: April-June 2013, October-December 2013 and April-June 2014. Season of testing was classified as fall (October, November and early December) and spring (April, May and early June). A three phased approach was used to account for potential variations by time of year (season). During each Phase, adults 50 years and older were recruited in both communities. Older adults were chosen for the study as effects from airborne exposures would be more prevalent in an older population as compared to a younger adult population. Recruitment was by convenience sampling and included response from advertisements and from the Lung Association volunteer call list in both communities. The investigators confirmed eligibility of participants through a screening questionnaire. Ineligibility included nursing home residence, the absence of more than one week during the study period, and any health issue that impeded the ability to undertake pulmonary testing. Eligible participants were invited by mail and telephone to attend the study orientation and assessment. The recruitment and selection were similar in each phase of the study.

Participants’ pre-assessment and orientation were carried out by a member of the research team and research nurses from the study communities. At the orientation, the consent form was signed and instructions on how to complete a diary of daily symptoms and how to use a peak expiratory flow monitoring device (twice a day over the study period) were given. Pre-assessment included a breathing test (spirometry), blood pressure, weight, height, waist circumference, and completion of a baseline questionnaire. Biomedical Research Ethics Board from the University of
Saskatchewan, BIO11-172 approved the study. The participants could be recruited in any phase and once recruited, the participant could continue in all phases.

5.4.2 Exposure assessment

5.4.2.1 Sample collection

Sampling occurred in the communities of Estevan (11,054) and Swift Current (15,503). Sampling equipment was co-located with provincial air sampling equipment with the site selection based on the Saskatchewan Air Monitoring Directive. The site was chosen to represent an efficient assessment of air quality for the general public in the area taking into account site and power accessibility, air dispersion modelling, topographical effects, local interferences, and security. The sampling site in Estevan was located in the southeast of the city, on a hill, at the roof of a one story building, within a secure compound. The sampling site in Swift Current was located in the northwest of the city, on the rooftop of a one-story provincial building near the highway.

The samples were collected in spring 2013 (Phase 1), fall 2013 (Phase 2), and spring 2014 (Phase 3), which represent the study phases. Ambient particle samples were collected using a 2-stage (PM 2.5 and 10) sampler (Dekati, ISO 23210, Dekati Ltd, Tampere, Finland) connected to a high-volume pump (Gilian, Aircon2, Sensidyne, FL, USA) that ran at a flow rate of 30 L/minute. PM$_{2.5}$ and PM$_{10}$ were collected on pre-weighed 25 mm polycarbonate filters (2.0µm, SKC Inc., Eighty-four, PA, USA). Each sample was collected over a seven-day period (sample), and samples were collected for the length of each study period (Phase). One blank field filter was taken for each five filters. The blank field filter accounted for any endotoxin accumulation from technical and field, and storage activities. Due to technical difficulties, Phase 2 resulted in a smaller sample size collection.

Post sampling, filters were stored in their identity labelled clear styrene cassettes (SKC, Concept Controls, Edmonton, AB, CA), date stamped, placed in a sealed, labelled Ziploc bag with desiccation packs, and refrigerated at 4°C until transport and post-weights.

5.4.3 Gravimetric measurements

The weight of filters was measured before and after sampling using a MX5 microbalance (Mettler Toledo, Mississauga, ON, CA) with a resolution of 0.1 µg. Before weighing, filters were equilibrated to room temperature and humidity for 30 minutes. The filters were placed through a
static neutralizer (PRX U Small SET, Haug Static, Richmond Hill, ON, Canada) and weighed in triplicate. The average weight and PM concentration (µg/m$^3$) were calculated for each size fraction.

The PM concentration (µg/m$^3$) in the air volume sampled was calculated as follows:

\[ [(W2 - W1)] * 10^3 / V \]

Where:
- $W_1$ is the mean pre-filter weight (µg)
- $W_2$ is the mean post-sampling weight (µg)
- $V$ is the volume of air sampled

5.4.4 Filter extraction

Filter extractions were carried out after all samples from all phases were collected (i.e., from June to August 2014). For extraction, each filter was cut in half using a sterile scalpel blade. One half of the filter was placed back in the storage container and stored in the desiccator at 4°C for other analyses. The second half of the filter was immediately extracted for endotoxin analysis. The half filter was placed in a 2 mL bead beating tube containing 2 g of 2 mm Zirconia beads (BioSpec, Bartlesville, OK, USA). One milliliter of 0.05% Tween 20 (Fisher Scientific, Ottawa, ON, Canada) in endotoxin-free water (Hyclone, GE Healthcare, Mississauga, Canada) was added to each tube and bead beaten for 3 min at the highest setting using the Retsch MM 400 Oscillating Mill (Retsch, Newtown, PA, USA). Tubes were placed in an ice box between bead beating and centrifugation. The tubes were centrifuged at 13,000 rpm for 5 min (Thermo Scientific Sorvall Legend Micro 17 Centrifuge, Fisher Scientific, Ottawa, ON, Canada). The supernatant was transferred to 1.5 mL polypropylene tubes (Fisher Scientific, Ottawa, ON, Canada) and centrifuged at 13,000 rpm for 10 min at 4°C (Eppendorf Centrifuge 5430R, Eppendorf Canada, Mississauga, ON, Canada). Finally, the supernatant was removed, aliquoted, and stored at -80°C until endotoxin analysis.

5.4.5 Endotoxin analysis

Endotoxin was determined using the Kinetic chromogenic Limulus Amoebocytes Lysate (LAL) assay according to the manufacturer’s recommendations (Lonza, Walkersville, MD, USA).
One aliquot of supernatant (extract) was thawed at room temperature. 100 µL of each undiluted sample extract, a LAL reagent water blank and the endotoxin standards (E.coli 055:B5, five standards optimized for the range 0.005 to 50 EU/mL), were dispensed in triplicate into 96-well plates. The plate was then incubated in the ELx808 plate reader (BioTek, Winooski, VT, USA) for 10 minutes at 37°C. After incubation, kinetic LAL reagent was added to each well and the plate was further incubated for another 2 hours. Absorbance was monitored at 405 nm and the time required for the absorbance to increase to 0.200 absorbance units was determined and considered to be the reaction onset time. The amount of endotoxin present is inversely proportional to the reaction time. The concentration of endotoxin in the sample extracts was determined from the standard curve using Gen5 software (BioTek, Winooski, VT, USA).

Results for each half filter were calculated as endotoxin units per milliliter (EU/mL). Endotoxin load (EU/µg) was calculated by the EU/mL multiplied by extraction volume and divided by the quantity of dust on the half filter. Endotoxin concentration (EU/m³) was calculated as follows:

\[ \frac{(E \times D \times EV) - EB}{V} \times 10^3 \]

E is endotoxin units per milliliter
D is dilution factor
EV is extraction volume
EB is endotoxin units in blank sample
V is the volume sampled

5.4.6 Lung function assessment

A daily diary was developed based on a standardized questionnaire [Appendix B; 17] which was used in previous studies in Saskatchewan [14,15,18]. The diary includes information such as respiratory symptoms, degree of symptoms disturbance, current smoking, and FEV₁ recordings. The present study analyzed only FEV₁ as the outcome. During the study period, participants measured and recorded FEV₁ twice daily using a PIKO-1 meter, according to the manufacturer’s instruction (Ferraris, Pulmonary Data Services, INC) and in line with American Thoracic Society guidelines [19]. Participants were trained during an orientation in the proper FEV₁ measuring techniques using the PIKO meter. This meter has the ability to reject poor blows or coughs.
Written instructions with diagrams were provided to each participant in the daily diary. Five best blows were recorded from at least seven maneuvers in the morning and in the evening before bed. FEV$_1$ is regarded as a yardstick for measuring airflow capacity and reflects the quality of both large and small airways [20].

**5.4.7 Statistical methods**

For the population study, descriptive analyses were performed for categorical variables using frequencies and percentages while arithmetic means and standard deviations were used for the continuous variables. The differences between the two communities were tested using the t-test analysis. Data analyses were carried out using the Statistical Package for the Social Sciences (SPSS) version 22 (IBM, Armonk, NY, USA). Statistical significance was set at $p < 0.05$ based on two-sided calculations.

The outcome of interest, absolute FEV$_1$, was taken in the morning and evening. The highest daily FEV$_1$ recordings in the morning and evening were used for each participant. To align the weekly environmental values to weekly pulmonary measures mean, minimum and maximum weekly morning and evening FEV$_1$ values were calculated. Weekly mean FEV$_1$ (meanFEV$_1$) is determined by calculating a mean from the highest daily FEV$_1$ for each day of the week for each week of the study. Weekly minimum (minFEV$_1$) is the lowest FEV1 for the week from the daily measures. Weekly maximum (maxFEV$_1$) is the highest FEV1 for the week from the daily measures. Phase (mean, min, max) were determined by calculating the mean of the weekly values for each Phase and each community.

Prior to statistical analysis, endotoxin contents in particulate matter were log-transformed to normalize the variables. The test for association between meanFEV$_1$, minFEV$_1$, and maxFEV$_1$ variables and endotoxin were performed using generalizing estimating equations to account for repeated measurements. Based on the previous studies, the potential confounders included in the analysis were gender, age, height and smoking status.

**5.5 Results**

A total of 233 adults were screened for the study, with 209 completing at least one or more of the three study phases: 105 (50.2%) from Estevan and 104 (49.8%) from Swift Current. Characteristics of the populations by study phase are summarized in Table 5-1. There were no
statistically significant differences between adults from Estevan and those from Swift Current for age, gender, height and smoking status. There was a greater percentage of female participants in both communities as compared to the general population ratios (Based on 2011 Census, Estevan: male – 49%, female – 51%; Swift Current: male – 48%, female – 52%).

Ambient endotoxin levels by phase and community are shown in Table 5-2. The endotoxin geometric means varied from 0.01 to 0.03 EU/µg and 0.002 to 0.07 EU/m³ in Estevan, while the range is from 0.02 to 0.10 EU/µg and 0.003 to 0.13 EU/m³ in Swift Current. Endotoxin results by community were discussed in the first manuscript.

Similar trends of FEV₁ were found in both communities, with higher estimates observed in the morning (Table 5-3). A Phase-specific association between endotoxin and measurements of FEV₁ are presented in Tables 5-4 to 5-7. There is no consistent pattern of association in this study. In Estevan, the associations between FEV₁ and endotoxin were observed in Phase 3 evening and Phase 1 morning (EU/m³; PM₂.₅). The statistical significance was found only in min FEV₁ Phase 1, while it was borderline (p=0.08) in mean and min FEV₁ Phase 3. Similar association was observed in Phase 3 morning mean and max in Swift Current (Table 5-4).

The association with endotoxin load of PM₂.₅ was statistically significant only in Phase 3 for mean and max FEV₁ of both morning and evening (Table 5-6). In combined phase analysis (Tables F1-F4; Appendix F), only mean FEV₁ morning in Swift Current was significant for endotoxin concentration PM₂.₅. Similarly, endotoxin load PM₂.₅ was significantly associated with max FEV₁ (Table F4). No overall pattern of association could be established.

In the analysis for interaction (endotoxin*smoking status) using the combined phases, there was a significant interaction for Swift Current between smoking and endotoxin concentration EU/m³ in PM₂.₅. However, the stratified estimate was considered too unstable due to the small sample size (n=7 smokers) and not used (Tables G1-G4; Appendix G).

5.6 Discussion

Outdoor air quality is an important contributing factor in the development of respiratory illnesses especially in developing and industrial communities [21]. Exposure to endotoxin has been associated with changes in lung function at various workplaces, but few studies have explored the effects of ambient endotoxin on health and specifically lung function [22]. This study assessed
associations between endotoxin, as a component of particulate air pollution, and older adults’ lung function in two different small urban communities in Saskatchewan.

Endotoxins are complex chemical components of the outer membrane of Gram-negative bacteria and are released into the air during cell growth and after the occurrence of bacterial death [22]. Endotoxin has been known to have health impacts especially when organic dust is inhaled. [23]. Studies on inhalation have shown that endotoxin can be associated with a headache, nose and throat irritation, nausea, shortness of breath, chest tightness, acute airflow obstruction and airway inflammation [24]. In our study, it was hypothesized that endotoxin bound with different particulate sizes (PM$_{2.5}$ and PM$_{10}$) are associated with lung function measurements from the adult residents of two rural communities. Particulate levels and associated endotoxin levels were described in Manuscript one (Chapter 4). Estevan and Swift Current populations were demonstrated to be similar demographically but not with respect to exposure. Overall, our measurements showed that the mean endotoxin level found in Swift Current, primarily an agricultural environment, was significantly higher as compared to Estevan, which has both industrial activities, with open coal mining activities, and agricultural production.

In this study of older adults in two rural communities, we found no overall association of FEV$_1$ with ambient endotoxin levels. To the best knowledge of the authors, there is no study in which the association of ambient endotoxin and varied FEV$_1$ measurements has been examined. The authors did not find an overall association of FEV$_1$ with ambient endotoxin, even though some literature suggests that changes in FEV$_1$ are associated with workplace endotoxin [11]. This might be due to higher exposure at workplaces than from the ambient air and limits a direct comparison of our findings with other studies.

The present study showed that there was no consistent pattern of associations between FEV$_1$ and endotoxin in either PM$_{2.5}$ or PM$_{10}$. This is an indirect comparison with a study by Van der Zee et al., (2000) that found no consistent associations between air pollution (PM and gases) and health indictors (respiratory symptoms and pulmonary function) [25]. It is challenging to find a comparable literature report to our study in terms of ambient endotoxin. Most studies measured indoor endotoxin, ambient air pollutants excluding endotoxin, used children in their sample or measured Peak Expiratory Force (PEF) instead of FEV$_1$ as the parameter [11, 12, 25-29].
The results for Estevan showed no consistent pattern of association between endotoxin and FEV₁. When phases were combined, only the endotoxin in PM_{2.5} was associated with evening FEV₁. This pattern of association in Estevan might be due to the study environment. Estevan is a predominantly coal-mining, power generating, and oil and gas producing environment and such activities involve heat-producing processes. Sebastian et al. [30] found that heat has the capacity to inactivate the effectiveness of bioactive endotoxin, which might lead to underestimating the level of endotoxin observed, and therefore accounted for the observed association found in Estevan.

In Swift Current, FEV₁ values in Phase 3 and when phases were combined were significantly associated with endotoxin in PM_{2.5}. However, the observed consistent morning FEV₁ decrease as endotoxin increased in Swift Current may be explained by the season of data collection, which marks the beginning of agricultural practices in this area. Previous studies have found high concentration of endotoxin in a predominantly farming environment [31,32].

The study of endotoxin and its association with respiratory illnesses in a general population has been inconclusive. In most studies, indoor endotoxin was found to be a contributing factor in exacerbation of respiratory illnesses and decline of FEV₁ in homes and at workplaces among the children and adults [15, 33], while in children populations the findings sometimes are protective [34].

The lack of association in our study could be due to the lower concentrations of endotoxin we found compared to concentrations previously reported to cause respiratory tract inflammation. Several studies from experimental to epidemiological, with concentrations between 50 and 100 EU/m³, were reported to influence respiratory tract in human [35-38].

One source of differences in the level of endotoxin between our study and previous ones might be in location and in the extraction or analysis procedure [39]. The observed pattern of association in this study will require further study to better understand if the association is true. Another explanation is exposure assessment which was based on a fixed site ambient endotoxin concentrations measured at one location in each community. It could be asked whether exposure to air pollution was adequately characterized, if not, the resulting non-differential misclassification would probably result in a bias of observed association. Unlike children, adults spend less time outside and therefore the magnitude of misclassification in exposure assessment may be large.
While it has been reported that endotoxin maybe protective against the development of respiratory illnesses such as asthma, this does not exempt a possible negative impact of endotoxin exposure on FEV₁ as reported in the present study.

The use of an unsupervised method of obtaining FEV₁ data might result in a lack of compliance and possible falsification of results among the adults. A low number of the participants in each phase could be another factor affecting the associations. To detect endotoxin estimates resulting in small changes in lung function, a higher number of adults would be needed to get sufficient statistical power. Furthermore, the poor association may be due to the sugar and fatty acid molecular structures of endotoxin present in these environments. Some other studies found that endotoxin with more lactose and hexose units in the sugar chain was more potent to trigger biological activities [39-41].

One of the strengths of this study was the use of objective measures of endotoxin as well objective measures of lung function. The use of a 7-day monitoring period of dust provided a more valid assessment of endotoxin levels. Further research might be considered to examine the use of personal exposure sampling.

5.7 Conclusions

The study of the impact of airborne environmental contaminants on respiratory public health in Saskatchewan did not show clear effects of endotoxin on FEV₁. The observed pattern of association, which may indicate at least a weak effect of outdoor endotoxin, in this study should be interpreted with caution. Adult populations in Estevan and Swift Current are exposed to lower concentrations of endotoxin than what is found in other environments, especially in industrial areas.
5.8 References


Table 5-1. Characteristics of the study population by phase.

<table>
<thead>
<tr>
<th></th>
<th>Estevan</th>
<th>Swift Current</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Original sample size (Final), n</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 1</td>
<td>35 (31)</td>
<td>36 (28)</td>
<td></td>
</tr>
<tr>
<td>Phase 2</td>
<td>45 (36)</td>
<td>45 (37)</td>
<td></td>
</tr>
<tr>
<td>Phase 3</td>
<td>40 (38)</td>
<td>42 (39)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 1</td>
<td>32.2</td>
<td>35.7</td>
<td>0.78</td>
</tr>
<tr>
<td>Phase 2</td>
<td>36.1</td>
<td>35.1</td>
<td>0.41</td>
</tr>
<tr>
<td>Phase 3</td>
<td>36.8</td>
<td>33.3</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Age, years, Mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 1</td>
<td>64.52 (9.67)</td>
<td>62.50 (10.93)</td>
<td>0.46</td>
</tr>
<tr>
<td>Phase 2</td>
<td>64.72 (10.76)</td>
<td>63.59 (9.92)</td>
<td>0.64</td>
</tr>
<tr>
<td>Phase 3</td>
<td>63.13 (10.34)</td>
<td>64.44 (9.82)</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Height, cm, Mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 1</td>
<td>164.65 (8.77)</td>
<td>166.16 (12.93)</td>
<td>0.60</td>
</tr>
<tr>
<td>Phase 2</td>
<td>166.11 (8.99)</td>
<td>166.34 (11.43)</td>
<td>0.93</td>
</tr>
<tr>
<td>Phase 3</td>
<td>165.85 (8.18)</td>
<td>165.35 (11.43)</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>Current smoking, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 1</td>
<td>4 (12.9)</td>
<td>1 (3.6)</td>
<td>0.15</td>
</tr>
<tr>
<td>Phase 2</td>
<td>9 (13.9)</td>
<td>3 (8.1)</td>
<td>0.46</td>
</tr>
<tr>
<td>Phase 3</td>
<td>4 (10.5)</td>
<td>3 (7.7)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

# The number of participants that completed the study.
Table 5-2. Summary of endotoxin geometric means by phase and community.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estevan</th>
<th></th>
<th>Swift Current</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endotoxin load</td>
<td>Endotoxin concentration</td>
<td>Endotoxin load</td>
</tr>
<tr>
<td></td>
<td>GM (GSD)</td>
<td>Min-Max</td>
<td>GM (GSD)</td>
</tr>
<tr>
<td>Phase 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>0.02 (1.61)</td>
<td>0.01-0.04</td>
<td>0.04 (1.94)</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>0.02 (6.10)</td>
<td>0.0014-1.24</td>
<td>0.01 (7.75)</td>
</tr>
<tr>
<td>Phase 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>0.03 (2.09)</td>
<td>0.01-0.05</td>
<td>0.07 (2.53)</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>0.01 (4.26)</td>
<td>0.001-0.03</td>
<td>0.004 (5.38)</td>
</tr>
<tr>
<td>Phase 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>0.01 (1.97)</td>
<td>0.003-0.025</td>
<td>0.02 (1.58)</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>0.01 (2.15)</td>
<td>0.00-0.01</td>
<td>0.002 (2.69)</td>
</tr>
<tr>
<td>Combined Phases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>0.01 (2.15)</td>
<td>0.00-0.05</td>
<td>0.03 (2.36)</td>
</tr>
<tr>
<td>PM$_{10}$</td>
<td>0.01 (4.44)</td>
<td>0.01-1.24</td>
<td>0.003 (5.32)</td>
</tr>
</tbody>
</table>

Phase 1 (April-June 2013); Phase 2 (October-December 2013); Phase 3 (April-June 2014); Combined Phases (Phases 1-3 combined); min minimum; max maximum; GM geometric mean; GSD geometric standard deviation.
Table 5-3. Forced Expiratory Volume in 1 second (FEV$_1$) by community.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Estevan Morning L (95% CI)</th>
<th>Evening L (95% CI)</th>
<th>Swift Current Morning L (95% CI)</th>
<th>Evening L (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>Mean</td>
<td>2.27 (2.07-2.47)</td>
<td>2.22 (2.04-2.40)</td>
<td>2.34 (2.14-2.53)</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>2.14 (1.95-2.32)</td>
<td>2.08 (1.90-2.26)</td>
<td>2.18 (1.98-2.38)</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>2.43 (2.05-2.81)</td>
<td>2.38 (2.19-2.57)</td>
<td>2.49 (2.30-2.68)</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Mean</td>
<td>2.42 (2.24-2.61)</td>
<td>2.33 (2.16-2.50)</td>
<td>2.50 (2.33-2.67)</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>2.28 (2.10-2.45)</td>
<td>2.22 (2.05-2.39)</td>
<td>2.39 (2.21-2.57)</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>2.73 (2.37-3.09)</td>
<td>2.44 (2.26-2.62)</td>
<td>2.61 (2.45-2.78)</td>
</tr>
<tr>
<td>Phase 3</td>
<td>Mean</td>
<td>2.41 (2.23-2.59)</td>
<td>2.35 (2.19-2.51)</td>
<td>2.48 (2.31-2.65)</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>2.29 (2.12-2.46)</td>
<td>2.22 (2.06-2.39)</td>
<td>2.37 (2.20-2.54)</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>2.52 (2.18-2.86)</td>
<td>2.49 (2.32-2.66)</td>
<td>2.61 (2.44-2.77)</td>
</tr>
</tbody>
</table>
Table 5-4. Association between endotoxin concentration (EU/m$^3$) in PM$_{2.5}$ and FEV$_1$ (L) by community.

<table>
<thead>
<tr>
<th></th>
<th>Estevan</th>
<th></th>
<th>Swift Current</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>(SE)</td>
<td>P-value</td>
<td>$\beta$</td>
</tr>
<tr>
<td><strong>Phase 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>-0.005</td>
<td>0.005</td>
<td>0.34</td>
<td>-0.003</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.017</td>
<td>0.008</td>
<td><strong>0.05</strong></td>
<td>-0.003</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.009</td>
<td>0.013</td>
<td>0.51</td>
<td>-0.005</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>0.002</td>
<td>0.006</td>
<td>0.73</td>
<td>0.002</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.008</td>
<td>0.008</td>
<td>0.28</td>
<td>0.012</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.006</td>
<td>0.018</td>
<td>0.75</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Phase 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>0.04</td>
<td>0.043</td>
<td>0.36</td>
<td>-0.001</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.001</td>
<td>0.008</td>
<td>0.86</td>
<td>0.002</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.28</td>
<td>0.300</td>
<td>0.35</td>
<td>-0.003</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td>1.00</td>
<td>-0.003</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.008</td>
<td>0.006</td>
<td>0.15</td>
<td>0.008</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.009</td>
<td>0.008</td>
<td>0.24</td>
<td>-0.029</td>
</tr>
<tr>
<td><strong>Phase 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>-0.009</td>
<td>0.007</td>
<td>0.20</td>
<td>-0.03</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.001</td>
<td>0.012</td>
<td>0.96</td>
<td>-0.02</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>-0.007</td>
<td>0.016</td>
<td>0.67</td>
<td>-0.05</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>-0.022</td>
<td>0.013</td>
<td>0.08</td>
<td>-0.03</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.023</td>
<td>0.013</td>
<td>0.08</td>
<td>-0.01</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>-0.026</td>
<td>0.028</td>
<td>0.35</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, height and smoking status.
Table 5-5. Association between endotoxin concentration (EU/m$^3$) in PM$_{10}$ and FEV$_1$ (L) by community.

<table>
<thead>
<tr>
<th></th>
<th>Estevan</th>
<th>Swift Current</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>(SE)</td>
</tr>
<tr>
<td><strong>Phase 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.005</td>
<td>0.006</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.015</td>
<td>0.009</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>0.005</td>
<td>0.004</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.004</td>
<td>0.005</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.019</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>Phase 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>-0.026</td>
<td>0.027</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.205</td>
<td>0.184</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>0.005</td>
<td>0.006</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>$&lt;$0.001</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>Phase 3</strong></td>
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<td></td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>0.012</td>
<td>0.007</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.011</td>
<td>0.006</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.017</td>
<td>0.018</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
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<td>0.009</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.003</td>
<td>0.008</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.030</td>
<td>0.023</td>
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</table>

Adjusted for age, gender, height and smoking status.
Table 5-6. Association between endotoxin concentration (EU/µg) in PM$_{2.5}$ and FEV$_1$ (L) by community.

<table>
<thead>
<tr>
<th></th>
<th>Estevan</th>
<th></th>
<th></th>
<th>Swift Current</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>(SE)</td>
<td>P-value</td>
<td>β</td>
<td>(SE)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Phase 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning Mean FEV$_1$</td>
<td>0.006</td>
<td>0.015</td>
<td>0.61</td>
<td>-0.003</td>
<td>0.002</td>
<td>0.34</td>
</tr>
<tr>
<td>Min FEV$_1$</td>
<td>0.002</td>
<td>0.019</td>
<td>0.90</td>
<td>&lt;0.001</td>
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<td>Max FEV$_1$</td>
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<td>0.18</td>
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<td>0.006</td>
<td>0.76</td>
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<td>0.85</td>
<td>0.007</td>
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<tr>
<td>Max FEV$_1$</td>
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<td>0.043</td>
<td>0.24</td>
<td>0.005</td>
<td>0.011</td>
<td>0.63</td>
</tr>
<tr>
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<td>0.009</td>
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<td>-0.07</td>
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Adjusted for age, gender, height and smoking status.
Table 5-7. Association between endotoxin concentration (EU/µg) in PM$_{10}$ and FEV$_1$ (L) by community.

<table>
<thead>
<tr>
<th></th>
<th>Estevan</th>
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<th>Swift Current</th>
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<td></td>
<td>β</td>
<td>(SE)</td>
<td>P-value</td>
<td>β</td>
<td>(SE)</td>
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<tr>
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<td>0.005</td>
<td>0.22</td>
<td>-0.022</td>
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<tr>
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<td>-0.035</td>
<td>0.034</td>
<td>0.31</td>
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<td>0.58</td>
<td>0.006</td>
<td>0.006</td>
<td>0.31</td>
</tr>
<tr>
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<td>0.254</td>
<td>0.27</td>
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<td>0.88</td>
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</tr>
<tr>
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<td>0.14</td>
<td>-0.002</td>
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<td>-0.003</td>
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</tr>
<tr>
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<td>0.015</td>
<td>0.022</td>
<td>0.49</td>
<td>0.004</td>
<td>0.006</td>
<td>0.47</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>0.013</td>
<td>0.011</td>
<td>0.24</td>
<td>0.003</td>
<td>0.005</td>
<td>0.63</td>
</tr>
<tr>
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<td>0.005</td>
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<td>0.001</td>
<td>0.004</td>
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<td>0.025</td>
<td>0.20</td>
<td>0.017</td>
<td>0.014</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, height and smoking status.
CHAPTER 6 DISCUSSION

6.1 Summary and bridge of the two manuscripts

This project used data from a panel study that was designed to assess the impact of airborne environmental contaminants on respiratory health in Saskatchewan, focusing on the communities of Estevan and Swift Current. This thesis had two objectives: 1) to determine the levels of endotoxin and its distribution between the particulate sizes in two rural communities in Saskatchewan, Canada and 2) to examine the associations between endotoxin in PM (2.5 & 10) and FEV₁ from lung function tests. Results from the first objective focused on levels of endotoxin while the second objective extended the investigation to look at the associations between those endotoxin levels and lung function.

Previous studies have shown that endotoxin has the potential to increase the toxicity of particulate matter and upon inhalation impact the FEV₁ [1-3]. Earlier studies have shown that endotoxin levels in an agricultural environment or agricultural facility are higher compared to other environments [4-6] but this has not been studied in rural Saskatchewan communities. A previous study revealed that asthma prevalence was higher in Estevan compared to Swift Current among children [7]. Estevan is a small urban community with a composition of few industries (such as oil and gas exploration, mining and power generation industry), that have the capacity to generate particulate matter, and endotoxin has become an important component of particulate matter associated with respiratory health [3].

The first objective was to identify the amount of endotoxin present in these regions. In Estevan, the highest geometric mean of endotoxin found was 0.03 EU/µg and 0.07 EU/m³. In Swift Current, the highest value was 0.10 EU/µg and 0.13 in EU/m³. In general, our measurements showed that the mean endotoxin level found in Swift Current, primarily an agricultural environment, was significantly higher compared to Estevan, an industrial community with open coal mining and minor agricultural activities. This confirms results from previous studies showing that agricultural environments experience higher levels of endotoxin as compared to other environments [4, 8]. In this study the highest levels of endotoxin were found in the fall (Phase 2).
These results are similar to a study from British Columbia that found the highest levels of endotoxin during fall with lowest levels in spring [9]. This might be due to seasonal agricultural activities. The observed endotoxin levels found in Estevan might result from Sebastian et al. 's observation that heated particulate matter generated from industry has the capacity to inactivate the bioactive potency of endotoxin, therefore, resulting in low bioactive response to LAL assay.

The first objective further identified the differences in particulate size fraction of endotoxin which might indicate lung deposition characteristics and useful to explain differences in respiratory outcomes observed in both communities. However, only one outcome was studied in this thesis. In Estevan, there were no statistically significant differences between endotoxin in PM$_{2.5}$ and endotoxin in PM$_{10}$ (lowest p-value 0.15) as measured in endotoxin unit per microgram. In Swift Current, statistically significant differences between PM$_{2.5}$ and PM$_{10}$ were found in Phases 2 and 3 (measured in EU/µg). Considering measurement of endotoxin in EU/m$^3$, there were significant differences in all phases and both communities (p-values < 0.001). In comparing endotoxin PM$_{2.5}$ (EU/µg) between the two communities, there were significant differences, with higher levels in Swift Current than in Estevan (p-value < 0.001); a similar trend was found in endotoxin PM$_{10}$ (EU/µg) with the exception of Phase 1 (p>0.05). Endotoxin PM$_{2.5}$ and PM$_{10}$ in EU/m$^3$ were similar with statistically significant difference found only in Phase 3 of the study (P<0.05). This supports the hypothesis that there are differences in the particulate size fraction of endotoxin with higher levels in PM$_{2.5}$ than PM$_{10}$. Most previous studies showed higher levels in PM$_{10}$ than PM$_{2.5}$ (summary in table 4-5). The reason for the observed distribution in this study is not clear.

The second objective furthered the results by assessing possible association(s) between ambient endotoxin and FEV$_1$ in the two communities. In Estevan, no consistent association was found for endotoxin with either PM measured by EU/m$^3$ or EU/µg. By combining the phases, an association was found in the evening FEV$_1$ for PM$_{2.5}$, p=0.05 (EU/m$^3$ and EU/µg). However, endotoxin (PM$_{2.5}$) in Swift Current was found to be associated with morning and evening FEV$_1$ in Phase 3, with both mean and maximum FEV$_1$, being statistically significant. In the combined phases, only morning FEV$_1$ was associated with PM$_{2.5}$ in both units of endotoxin. Overall, however, there is no consistent pattern of association in this study. One of the reasons for the lack
of association might be the low levels of endotoxin observed in the study described in the first manuscript. Although the association has been established in some studies between workplace endotoxin and FEV$_1$ (summary in Table 2-4) which might be due to higher exposure and proximity to the source than from the ambient air. For instance, a study among the sewage workers by Cyprowski et al. found the concentration of endotoxin ranged from 0.68 to 214 EU/m$^3$. In an association with the changes in pulmonary function, they concluded that exposure to low endotoxin levels causes a significant decline in FEV$_1$ [11]. The higher levels of endotoxin found in PM$_{2.5}$ compared to PM$_{10}$, reported in the study described in the first manuscript, might contribute to the few associations observed between endotoxin in PM$_{2.5}$ and FEV$_1$, while endotoxin in PM$_{10}$ showed a negligible association. In general, endotoxins in this study, which can impact FEV$_1$, were very low compared to the reported limits in other similar studies. For instance, Donham et al. in an occupational exposure study, such as poultry farming, recommended an exposure limit of 14 EU/m$^3$ for total endotoxin while 7.15 EU/m$^3$ for respirable endotoxin [12]. Additionally, Rylander also calculated the limit of exposure to be 33 ng/m$^3$ [13]. This observation is not in line with our hypothesis that endotoxins in this two communities are associated with the FEV$_1$ measured among adult residents.

6.2 Study validity

6.2.1 Internal validity

6.2.1.1 Selection bias

In the first manuscript, selection bias is not a major problem. The period of study was selected based on the three years’ historical pattern of air pollution in the province with maximum pollution observation in April-June and October-December. Also, the site of sampling in each community was based on the Saskatchewan Air Monitoring directive. In the second manuscript, the source population for this study was adult residents in the two communities. The adults were restricted to age 50 and above, residing in the study locations and not living in a nursing home. This is to capture the age range that is more susceptible to develop acute respiratory illness and be
potentially more responsive to air pollutants. It also includes adults that can walk outside their homes and were likely exposed to ambient endotoxin.

Despite the use of volunteers from the call lists of Lung Association, the descriptive analysis did not show any significant difference between participants from each community. However, there is the possibility of underestimation of association if the adults that refused to participate had higher exposure or were more likely to exhibit lower FEV\(_1\) as compared to adults that participated. The percentage of adults that refused to participate from the source population was not known. Furthermore, selective attrition due to drop out may occur as a result of moving from one area to the other or becoming homebound due to sickness or just lack of interest [14]. The observed percentage of drop out from the study was quite similar in Estevan and Swift Current communities except in Phase 1 (Phase 1: 11% vs 22%, Phase 2: 16% vs 18%, Phase 3: 5% vs 7%). Among the participants, the ‘healthy workers (adults) effect’ is unlikely to distort the association because of the age range and most are in retirement stage (above 60).

6.2.1.2 Information bias

Sampling equipment was co-located with provincial air monitoring equipment with the site selection based on the Saskatchewan Air Monitoring Directive, a strength for the environmental study results. The environmental sampling site represents an efficient assessment of air quality for the general public in the area (i.e. Estevan and Swift Current) taking into account important variables in exposure including air dispersion, topographical effects, and local interferences. This is the common measure for ambient exposure, although not predictive of personal exposure. The assessment was objectively measured using identical equipment for each site and quantitative kinetic chromogenic limulus amoebocytes lysate method that has been widely used in other studies and to detect low levels of bioactive endotoxin. The residents were blinded, in other words, not aware of the simultaneous environmental sample collection, which could have led to overestimation of respiratory outcomes. The FEV\(_1\) outcome was also objectively measured using daily diary and the peak flow meter (FEV\(_1\) range from 0.15 to 9.99 and the accuracy is ±3.5% or 0.05 L). The use of daily diary reduced the error due to recall, as participants recorded the results
immediately after being measured. The content of the diary was based on the past questionnaire used in the same province.

6.2.1.3 Confounding factors

The etiology of respiratory illnesses is complex, but in this thesis, a few previously studied confounders including age, height, gender, and smoking status were adjusted in the GEEs analysis. However, as in all epidemiological research, the overall study had practical limitations on the data collection used and the amount of data obtained from each participant. This could result in residual confounders that might be misclassified, or not available for inclusion in the analysis such as migration, health status, home dampness, mould, indoor endotoxin levels could have influenced some of the associations. For instance, based on characteristics of this study that involved time-series, it could be difficult to draw associations due to challenges in determining actual pollutant concentrations to which people are exposed, possibly allowing the potential for misclassification of exposure.

6.2.2 External validity

External validity refers to how the findings from a study can be extended to other populations [15]. Generalization of these results should be done with caution, as this type of study design usually requires a lengthy time and is often expensive. Therefore, only a small group of people are usually available [16]. This might make it difficult to apply the results to a larger population. For example, one potential threat to external validity could be that adults residing in urban communities or busy cities or moving to-and-from to other cities were not included in this study. Also, the study recruited adults from rural communities, thereby limiting the generalizability of the current study findings to urban residents.

Time series design has shown that ambient air quality might vary over a short distance in an urban area, which may lead to underestimation of exposure and related health impacts. Therefore, our results might not be generalizable to those populations, since our study is in a rural, small industrial area. It is possible the participants are exposed to other pollutants which may complicate or further lower their FEV₁. In general, levels of endotoxin and the observed associations may be
generalized to other adults within Canada with similar industrial and agricultural activities. Since endotoxin is strongly associated with agricultural environments, generalization should be undertaken cautiously in other countries with different industrial settings.

6.2.3 Other strengths and limitations

The studies described in both manuscripts share some common strengths and limitations, a few of which were mentioned in Chapters 4 and 5. A broad range of variables was collected in the original study. Most variables used in this study were obtained objectively, that is, endotoxin, FEV\textsubscript{1} and height, therefore reducing the chance for error due to measurement. The investigation of outdoor endotoxin was a strength of this study. As stated in Chapters 2 and 5, most previous studies evaluated indoor or workplace endotoxins. Furthermore, the present study further examined endotoxin levels and made comparisons between particulate sizes. Endotoxin research often features only one of the particulate sizes, or both but without statistical comparison. The current study contributes to the rare research on the potential effects ambient endotoxin might have on adult lung function.

As mentioned earlier, the objective assessment of endotoxin using the KQCL test as opposed to other methods such as kinetic turbidimetric LAL or recombinant Factor C is important in this study. Apart from reducing measurement error, it also allows for comparison with other studies.

However, it is important to consider some limitations in our laboratory tests. Interference of β-1, 3-glucan from fungus could activate Factor G in the reactions which might affect endotoxin levels [8, 17] and eventually distort the association. Another factor that might influence the level of endotoxin is storage time, that is, the time between collection of dust samples and the time of laboratory analysis. Laitinen found that endotoxin content dropped 10%, 30%, and 70% after 2, 7, and 14 days of storage, respectively [17]. This could result in underestimations in the quantity of our endotoxin.

Several epidemiological studies were targeted towards children respiratory health but this study focused on rural adult residents, which is an important strength. Based on the study design, it will be difficult to establish a temporal relationship between the exposure and the outcome because the measurement of exposure and determination of outcome occurred simultaneously for
each participant. A definite causal inference is therefore not possible in this study. A dose-response relationship between the independent variable and dependent variable can be important in the determination of illnesses. Previous studies linked endotoxin and FEV\textsubscript{1} but dose-response has not been established [18].

6.2.4 Future research recommendation

Recommendations for future studies are necessary. First, in our study, we found comparable but slightly lower endotoxin values as compared to other studies. This study can be repeated with the intention of having a larger sample size. Likewise, multiple sites for sample collection will also expand the knowledge of endotoxin pollution which might vary from one street to another based on the proximity to the source. The use of personal sample collection could be suggested but might be difficult to achieve due to time factors.

Another method that merits consideration in the future is Glickman’s method of spatial buffering [19], in which participants are assigned to a particular exposure group if their home address is within a pre-defined distance from industry or busy traffic road or agricultural facilities, referred to as “buffer zone”, while residents with addresses outside the zone can be considered unexposed. This simple method would allow us to further compare the impact of endotoxin in different neighbourhoods and determine the effects of proximity. Thirdly, to have a comprehensive knowledge of the endotoxin in Saskatchewan air, a chemical analytical method (Gas Chromatography-Mass Spectrometry, GC-MS) could be used to determine the total and bioactive endotoxin components of the dust. This could contribute and generate a predictable or estimate level of toxin, and account for the presence of biologically active yet unexposed endotoxins which likely have endotoxic effects when inhaled.

6.2.5 Conclusion of the thesis

The justification of this thesis is to continue the investigation of lung health in the study regions. Bacterial endotoxin is one of the biologically active constituents of organic particulate matter that is known to trigger inflammation along the respiratory tract. Endotoxins are present in rural communities in Saskatchewan but in low amounts. They were similar to levels found in a
few other studies. In general, the associations between endotoxins and FEV₁ were weak in this study, though few positive associations were found in Swift Current. Findings from this thesis may add to the development of appropriate air quality guidelines in industrial communities.
6.3 References


APPENDICES

Appendix A: Summary of the endotoxin analysis

Dust Extraction

1. Add 2 mg of 2 mm Zirconia beads into 2 mL bead beading tube.
2. Place half of the filter in the 2 mL bead beading tube after taking the measurement of the filter using sterile forceps.
3. Add 1 mL of 0.05% Tween 20 in LPS free water.
4. Place the tube in ice box.
5. Bead beat each tube once for 3 mins on highest setting. At interval, place the tube in ice.
6. Spin the tubes at Max (13,200 rpm) for 5 min.
7. Transfer the supernatant into a new 1.5 mL tube.
8. Spin the supernatant again at Max (13,200 rpm) for 10 min.
9. Remove the supernatant and aliquot. Aliquot stores at -80%.

Endotoxin Analysis

1. Prepare standard solutions:
   0.1 mL of 50 EU/mL + 0.9 mL = 5 EU/mL
   0.1 mL of 5 EU/mL + 0.9 mL = 0.5 EU/mL
   0.1 mL of 0.5 EU/mL + 0.9 mL = 0.05 EU/mL
   0.1 mL of 0.05 EU/mL + 0.9 mL = 0.005 EU/mL
   Vigorously vortex for at least 1 min before each proceeding.
2. Dispense 100 µL of the endotoxin standard, LAL reagent water blank, and samples (in triplicate).
3. Place the filled plate in the microplate reader and close the lid.
4. Pre-incubate the plate for 10 minutes @ 37°C.
5. Toward the end of preincubation, reconstitute each of the reagent vials with 2.6 mL reagent
water/vial. Mix gently but thoroughly.
6. Pool the reagents into a reagent reservoir and mix by gently rocking the reservoir from side to side.
7. Using an 8-channel multipipettor dispense 100 µL of the Kinetic-QCL reagent into all wells of the microplate beginning with first column and proceeding in sequence to the last column used. Add reagents as quickly as possible. Avoid bubbles.
8. Immediately click on the OK button on the computer keyboard to initiate the test
The Airways Research Group at the University of Saskatchewan is conducting this survey to learn more about the respiratory health of adults living in Saskatchewan.

Thank you for your willingness to participate in this study. As part of the study we would like to ask some questions about your past and current health and your indoor and outdoor living environments. Please answer the questions to the best of your ability, but you do not have to answer all of the questions if you choose not to. Please answer the questions as accurately as possible.

All information obtained in the study will be kept confidential and used for medical research only.

Read the instructions carefully. Some of the questions will direct you to other questions in the survey. The completed questionnaire should be brought to the orientation meeting on

_________________, 2012 at ___________________________
PERSONAL CONTACT INFORMATION (please print)

This information will be removed from your questionnaire upon the end of this study to ensure confidentiality.

Name: ____________________________ Age: __________

☐ Male  ☐ Female

Spouse's Name: ____________________________

Mailing Address: ____________________________

_________________________________________

_________________________________________

Street Address: ____________________________

Telephone Numbers (check most preferred):

☐ Work ________________

☐ Home ________________

☐ Cell ________________

Email Address: ____________________________

If you have any questions or concerns, please feel free to contact us at the

Canadian Centre for Health and Safety in Agriculture:

103 Hospital Drive
RUH Box 120
University of Saskatchewan
Saskatoon, SK S7N 0W8
Ph: (306) 966-1356
Fax: (306) 966-8799
Email: airways.health@usask.ca
HEALTH

PLEASE ANSWER THE FOLLOWING QUESTIONS BY FILLING IN A BLANK OR MARKING THE APPROPRIATE BOX REGARDING YOURSELF AND YOUR SYMPTOMS.

COUGH
1. Do you usually have a cough?
   ☐ Yes
   ☐ No

2. Do you cough at all on getting up, or first thing in the morning?
   ☐ Yes
   ☐ No

3. Do you usually cough at all during:
   the rest of the day?
   ☐ Yes
   ☐ No
   at night?
   ☐ Yes
   ☐ No

   If NO to 1, 2, and 3, go to 7.

4. Do you usually cough like this on most days for 3 consecutive months or more during the year?
   ☐ Yes
   ☐ No

5. For how many years have you had this cough?
   _____ years

6. Which month(s) does your cough give you the most trouble?

PHLEGM
7. Do you usually bring up phlegm from your chest?
   ☐ Yes
   ☐ No

8. Do you bring up phlegm at all on getting up, or first thing in the morning?
   ☐ Yes
   ☐ No

9. Do you usually bring up phlegm at all during:
   the rest of the day?
   ☐ Yes
   ☐ No
   the night?
   ☐ Yes
   ☐ No

   If NO to 7, 8, and 9, go to 14.

10. Do you usually bring up phlegm like this on most days for 3 consecutive months or more during the year?
    ☐ Yes
    ☐ No

11. For how many years have you brought up phlegm?
    _____ years

12. Have you had periods or episodes of (increased) cough and phlegm lasting for 3 weeks or more each year?
    ☐ No
    ☐ Yes, during the last 3 years
    ☐ Yes, for more than 3 years

13. Which month(s) does your phlegm give you the most trouble?

WHEEZE
14. Does your chest ever sound wheezy or whistling:
    (check all that apply)
    ☐ 1. When you have a cold?
    ☐ 2. Apart from colds?
    ☐ 3. Most days or nights

    If YES to 1, 2, or 3, for how many years has this been present?
    _____ years

15. Have you ever had an attack of wheezing that has made you feel short of breath?
    ☐ Yes
    ☐ No

    If YES, have you ever required medicine or treatment for the attack(s)?
    ☐ Yes
    ☐ No

16. Which month(s) does your wheezing give you the most trouble?
BREATHLESSNESS

17. Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?
   ✔ Yes
   ❌ No

If NO, proceed to 25.

18. Do you have to walk slower than people of your age because of breathlessness?
   ✔ Yes
   ❌ No

19. Do you ever have to stop for breath when walking at your own pace on level ground?
   ✔ Yes
   ❌ No

20. Do you ever have to stop for breath after walking about 100 yards (or after a few minutes) on level ground?
   ✔ Yes
   ❌ No

21. Are you too breathless to leave the house or breathless on dressing or undressing?
   ✔ Yes
   ❌ No

22. Which month(s) does your breathlessness give you the most trouble?

23. Please list any pills, tablets, capsules, or any medication you have used in the past 12 months to help your breathing: (If you need more room, use the last page of the questionnaire)

   1. 

   2. 

   3. 

   4. 

   5. 

ASTHMA

24. During the past 12 months, how many times have you required services for your breathing from the following places?
   1. Hospital inpatient:_____ times
   2. Emergency room outpatient:_____ times
   3. Doctor's office:_____ times

25. Has a doctor ever diagnosed you with asthma?
   ✔ Yes
   ❌ No

   If NO, proceed to 32.

26. At what age did it start?
   ___ years

27. Do you still have it?
   ✔ Yes
   ❌ No

   If NO, proceed to 32.

28. Which month(s) does your asthma give you the most trouble?

29. If you no longer have it, at what age did it stop?
   ___ years

30. How many times have you required services for asthma from the following places during the past 12 months?

   Hospital inpatient:_____ times
   Emergency room outpatient:_____ times
   Doctor's office:_____ times

31. How often did you use your asthma medication in the past 12 months?
   ☐ Never
   ☐ At least once
   ☐ At least once per month
   ☐ At least once per week
   ☐ Every day

   Asthma Medication: 

---

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CHEST ILLNESS

32. Has a doctor ever said you had any of the following chest illnesses?

<table>
<thead>
<tr>
<th>Chest Illness</th>
<th>During the Past 12 Months</th>
<th>Ever in Your Life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchitis</td>
<td>□ Yes □ No</td>
<td>□ Yes □ No</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>□ Yes □ No</td>
<td>□ Yes □ No</td>
</tr>
<tr>
<td>Rhinitis (Hay fever)</td>
<td>□ Yes □ No</td>
<td>□ Yes □ No</td>
</tr>
<tr>
<td>Emphysema</td>
<td>□ Yes □ No</td>
<td>□ Yes □ No</td>
</tr>
<tr>
<td>COPD (Chronic Obstructive Pulmonary Disease)</td>
<td>□ Yes □ No</td>
<td>□ Yes □ No</td>
</tr>
<tr>
<td>Sleep Apnea</td>
<td>□ Yes □ No</td>
<td>□ Yes □ No</td>
</tr>
<tr>
<td>Other Chest Illness (Example: chest operation)</td>
<td>□ Yes □ No</td>
<td>□ Yes □ No</td>
</tr>
</tbody>
</table>

Please specify: ____________________________

ALLERGIES

33. Have you ever had an allergic reaction to any of the following: (check all that apply)

☐ House dust
☐ Cats
☐ Dogs
☐ Farm animals
☐ Grass
☐ Pollen
☐ Molds
☐ Trees
☐ Others,

Please specify: ____________________________

34. In the past 12 months, have you been bothered by sneezing, or a runny or blocked nose, when you did not have a cold or the flu?

☐ Yes
☐ No

If NO, proceed to 38.

35. Has it been accompanied by itchy or watery eyes?

☐ Yes
☐ No

36. How do you treat your runny nose?

☐ No treatment
☐ Anti-histamines
☐ Nasal wash
☐ Other sprays,

Please specify: ____________________________

37. Which month(s) did sneezing or a runny or blocked nose give you the most trouble?

GENERAL HEALTH STATUS

38. In general would you say your health is:

☐ Excellent
☐ Very good
☐ Good
☐ Fair
☐ Poor

39. During the past 12 months, were you seen by a doctor or other primary care giver for:

☐ Stomach acidity or reflux __Yes __No
☐ An injury __Yes __No

40. Has a doctor or primary care giver ever said you have:

<table>
<thead>
<tr>
<th>Illness</th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart attack</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardening of the arteries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If YES to cancer, please specify type(s): ____________________________

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41. Please list any medications you take regularly?

1. __________________________

2. __________________________

3. __________________________

4. __________________________

5. __________________________

42. Has anyone noticed that you stop breathing during your sleep?
- Yes
- No

43. Do you snore?
- Yes
- No
- Don’t know

If NO or DON’T KNOW proceed to 46.

44. Your snoring is:
- Slightly louder than breathing
- As loud as talking
- Louder than talking
- Very loud – can be heard in adjacent rooms

45. Has your snoring ever bothered other people?
- Yes
- No

46. How likely are you to doze off or fall asleep in the situations described below, in contrast to just feeling tired? This refers to your usual way of life in recent times. Even if you haven’t done some of these things recently, try to work out how they would have affected you. (Please check one response choice for each situation.)

<table>
<thead>
<tr>
<th>Situation</th>
<th>Would never doze</th>
<th>Slight chance of dozing</th>
<th>Moderate chance of dozing</th>
<th>High chance of dozing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting and reading</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watching TV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sitting inactive in a public place (e.g., a meeting)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As a passenger in a car for an hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lying down to rest in the afternoon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sitting and talking to someone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sitting quietly after lunch without alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In a car, while stopped in traffic for a few minutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

47. Which statement best describes your activity?
- Inactive: No regular physical activity
- Light: Walking daily for about 30 minutes
- Moderate: Sporadically involved in recreational activities such as occasional swimming or cycling
- Heavy: Participates in recreational activities such as swimming, baseball, soccer, hockey etc. at least 3 times a week for 30 to 60 minutes per session
- Vigorous: participation in extensive physical activity for 60 minutes or more at least 4 days per week

48. In a typical week in the past 3 months, how much time did you usually spend watching television or videos or on a computer, including using the internet, or playing computer games? (Please do not include time spent at work or at school)
- None
- Less than 1 hour
- 1-2 hours
- 3-5 hours
- 6-10 hours
- 11-14 hours
- 15-20 hours
- More than 20 hours

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ENVIROMENT

49. Have you ever lived on a farm?
   • Yes
   • No
   • Don’t know

50. Did you live on a farm during the first year of your life?
   • Yes
   • No
   • Don’t know
   If NO or DON’T KNOW proceed to 52.

51. What type of farm was it?
   • Grain ___Yes ___No
   • Livestock ___Yes ___No
   • Other, Please specify: ______________________

TOBACCO SMOKING

52. Do you smoke cigarettes now?
   • Yes
   • No

53. Have you smoked more than 20 packs of cigarettes in your lifetime?
   • Yes
   • No
   If NO to both 52 and 53, proceed to 56.

54. For how many years have you smoked in total? _______ years

55. Of the entire time you smoked, how many cigarettes did you smoke per day on average?
   _______ cigarettes per day

56. Did your mother smoke while she was pregnant with you?
   • Yes
   • No
   • Don’t know

TIME SPENT OUTDOORS

57. What time of day in the summer do you spend the most time outside?
   • Morning (5am-11am)
   • Around noon (11am-1pm)
   • Afternoon (1pm-5pm)
   • Evening (5pm-9pm)
   • Night (9pm-5am)

58. What time of day do you spend most time outside in the fall?
   • Morning (5am-11am)
   • Around noon (11am-1pm)
   • Afternoon (1pm-5pm)
   • Evening (5pm-9pm)
   • Night (9pm-5am)

59. What time of day do you spend most time outside in the winter?
   • Morning (5am-11am)
   • Around noon (11am-1pm)
   • Afternoon (1pm-5pm)
   • Evening (5pm-9pm)
   • Night (9pm-5am)

60. What time of day do you spend most time outside in the spring?
   • Morning (5am-11am)
   • Around noon (11am-1pm)
   • Afternoon (1pm-5pm)
   • Evening (5pm-9pm)
   • Night (9pm-5am)

61. What is your primary activity outside?
   • Exercise (walking, biking, etc)
   • Yard work (gardening, etc)
   • Working (occupation)
   • Farming
   • Relaxing
   • Other, Please specify: ______________________

OCCUPATIONAL HISTORY

62. What is your current occupational status?
   • employed full time
   • employed part time
   • retired
   • unemployed
   • volunteer

63. Please list any occupations and/or volunteer activities which you are currently involved with, including duration:

   1. ________________________________ years

   2. ________________________________ years

   3. ________________________________ years
64. How many days of work have you missed because of asthma, shortness of breath or wheezing in the last 12 months, if any? 
   _____days

65. Were you forced to give up working because of asthma, wheezing or shortness of breath in the last 12 months?  
   □ Yes  
   □ No

66. How many days have you had to give up activities other than work because of your asthma, wheezing or shortness of breath in the last 12 months, if any?  
   _____days

67. How many days on average each month have you missed these regular activities in the last 12 months? 
   _____days per month

68. How often have you been exposed to the following in your work place? 

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Daily</th>
<th>Weekly</th>
<th>Monthly</th>
<th>Occasionally</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain dust</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mine dust (e.g. potash, coal)</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Asbestos dust</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood dust</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other dust</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Livestock</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoke from stubble burning</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Diesel fumes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solvent fumes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil/Gas well fumes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbicides/pesticides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Welding fumes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BIOGRAPHICAL INFORMATION

69. Date of birth: MM____DD_____YY_____

70. What is your marital status?  
   □ Married  
   □ Common law/living together  
   □ Widowed  
   □ Divorced/separated  
   □ Single, never married

71. What is the highest level of education you have completed?  
   □ Less than high school  
   □ Completed high school  
   □ Completed university  
   □ Completed post-secondary education other than above

72. What was your birth weight?  
   _____ pounds _____ounces or _____grams  
   □ Don't know

73. Were you breastfed as a child?  
   □ Yes  
   □ No  
   □ Don't know  

   If YES, was it for 6 months or longer?  
   □ Yes  
   □ No

74. What is your ethnic background?  
   □ Caucasian  
   □ First Nation  
   □ Métis  
   □ Asian  
   □ Other,  

   Please specify:____________________
LIVING ENVIRONMENT

INDOOR

75. How long have you lived in your current home?
   _____ years

76. How long have you lived in the town in which you currently live?
   _____ years

77. Which best describes the building in which you live?
   □ Mobile home/trailer
   □ One family house not attached to any other house
   □ A building for 2 families
   □ A building for 3 families or more
   □ Other,
   ✔ Please specify: ____________________________

78. Which category of years do you think most closely matches when the building was built?
   □ 1999-present
   □ 1978-1998
   □ 1961-1977
   □ 1946-1960
   □ 1940-1945
   □ 1939 or before
   □ Don't know

79. What fuel is used most for cooking in your home?
   □ Gas
   □ Electricity
   □ Other,
   ✔ Please specify: ____________________________

80. Is there a fan that draws air from the stove to the outside of the building?
   □ Yes
   □ No
   □ Don't know

81. What is the main type of fuel source used to heat your home?
   □ Gas
   □ Electricity
   □ Wood
   □ Other,
   ✔ Please specify: ____________________________
   □ Don't know

82. Does your home have air conditioning?
   □ Yes
   □ No
   □ Don't know

   If YES, please check one:
   □ Central
   □ Room
   □ Both

83. Is a humidifier or vaporizer used in your home?
   □ Yes
   □ No
   □ Don't know

84. Is a dehumidifier used in your home?
   □ Yes
   □ No
   □ Don't know

85. Is there an air filtration device in your home, such as HEPA filtration system or some other special filter?
   □ Yes
   □ No
   □ Don't know

86. What type of vacuum system do you have?
   □ Central vacuum to outside
   □ Central vacuum not vented
   □ Upright or floor model

87. Does your vacuum system have a HEPA filter?
   □ Yes
   □ No
   □ Don't know

88. On average, how often per month are the floors cleaned or the carpet vacuumed in your home?
   _____ times per month

89. Does your home (including basement) frequently have a mildew odour or musty smell?
   □ Yes
   □ No
   □ Don't know

90. During the past 12 months, has there been water damage or dampness in your home from broken pipes, leaks, heavy rain, or floods?
   □ Yes
   □ No
   □ Don't know
91. Within the past 12 months, have there been any of the following major renovations done on your residence:

☐ Dry wall
☐ New carpeting/flooring
☐ Painting
☐ Other,

☐ Please specify: __________________________

92. In the past 12 months, have you had any problems with the following?

☐ Cockroaches
☐ Silverfish
☐ Mice or rats
☐ Ants

93. In the past summer and fall, how many hours a day did you keep the windows or doors open in your home?

☐ Constantly
☐ Often
☐ Seldom
☐ Never

94. Do you sleep with the window open at night during:
(check all that apply)

☐ November-March?
☐ April-May?
☐ June-August?
☐ September-October?

95. In the past 12 months, have you had any of the following pets living in your home? Please check Yes or No for each type of pet.

☐ Cat
☐ Dog
☐ Bird

☐ Other pets, please specify: __________________________

96. Other than you, is there anyone who smokes IN YOUR HOME?

☐ Yes
☐ No

HOUSEHOLD INCOME:

97. At the end of the month, how much money do you have left over? (Please check only one)

☐ Some money
☐ Just enough money
☐ Not enough money

OUTDOOR

98. How many hours per week are you exposed to smoke outside the home?

_________ hours per week

99. How often do vehicles pass by your house?

☐ Constantly
☐ Often
☐ Seldom
☐ Never

100. How often do heavy vehicles (trucks/buses etc.) pass by your house?

☐ Constantly
☐ Often
☐ Seldom
☐ Never

101. Do you live nearby a highway?

☐ Yes
☐ No

102. How much are you annoyed by outdoor air pollution (from traffic, industry, etc) if you keep the windows open? (This scale allows you to rate your personal opinion regarding the following question on annoyance from air pollution. You can indicate your level of annoyance on this scale between 0 and 10 where 0 means ‘does not annoy at all’ and 10 means ‘intolerable annoyance’)

INTOLERABLE ANNOYANCE

10 ______
9 ______
8 ______
7 ______
6 ______
5 ______
4 ______
3 ______
2 ______
1 ______

DOES NOT ANNOY AT ALL

0 ______

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Appendix C: Daily diary

Today’s Date: _______________________

1. Did you experience any of the following symptoms since you went to bed last night?

<table>
<thead>
<tr>
<th></th>
<th>Night</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheeze</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortness of breath</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phlegm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest tightness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Please specify:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Overall, to what degree did these symptoms disturb you today? (Please indicate with an X on the line)

<table>
<thead>
<tr>
<th></th>
<th>Not at all</th>
<th>Badly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limited your activities</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Caused you to miss work</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Today, do you feel that these symptoms:

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hay fever</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Did you have any of the following today or last night?

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hay fever</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Please list any breathing medications taken since last night:

<table>
<thead>
<tr>
<th>Name of Medication</th>
<th>Strength (eg. 200 mcg)</th>
<th>Number of “puffs”, pills or tablets</th>
<th>Time Taken</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6. Were you exposed to any of the following today or last night?

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>For how long?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco smoke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood smoke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other dusty or gaseous exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Please specify:</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

7. Have you smoked since last night entry?
   - Yes → If YES, how many cigarettes? ______
   - No

8. How much time did you spend outside last night and today?
   ______ hours ______ minutes
   When did you spend most of the time outside?
   - Morning
   - Afternoon
   - Evening
   - Night

9. How would you rate your overall well-being today?
   (Please indicate with an X on the line)

<table>
<thead>
<tr>
<th>Very Low</th>
<th>Very High</th>
</tr>
</thead>
</table>

Are you in your home town today?  ____Yes  ____No

**FEV1 RECORDINGS (fill in values from all blows)**
Complete before taking breathing medication

<table>
<thead>
<tr>
<th>Time</th>
<th>Blow 1</th>
<th>Blow 2</th>
<th>Blow 3</th>
<th>Blow 4</th>
<th>Blow 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening</td>
<td>______</td>
<td>______</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
</tbody>
</table>

Comments: ____________________________________________
Appendix D: Instructions for FEV 1 monitoring

1. Stand up

2. Hold the peak flow monitor lightly and level

3. Do NOT cover the vent holes

4. Inhale fully

5. Make a tight seal around the mouthpiece

6. Blow into mouthpiece as hard as you can for about 3 seconds

7. Do NOT puff cheeks, flex your neck or bend over
Appendix E: Template 96 – well plate

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
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<td></td>
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</tr>
<tr>
<td>B</td>
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<td></td>
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<tr>
<td>C</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>D</td>
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</tr>
<tr>
<td>E</td>
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<tr>
<td>F</td>
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<tr>
<td>G</td>
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<td></td>
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<tr>
<td>H</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix F: Association between endotoxin and the lung function in a combined phase

Table F1. Association between endotoxin concentration (EU/m$^3$) in PM$_{2.5}$ and FEV$_1$ (L) by community in a combined phase.

<table>
<thead>
<tr>
<th></th>
<th>Estevan</th>
<th></th>
<th>Swift Current</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$ (SE)</td>
<td>P-value</td>
<td>$\beta$ (SE)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Combined Phases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>0.013 (0.006)</td>
<td>0.44</td>
<td>-0.007 (0.004)</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.007 (0.005)</td>
<td>0.15</td>
<td>-0.006 (0.004)</td>
<td>0.13</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.117 (0.120)</td>
<td>0.31</td>
<td>-0.011 (0.006)</td>
<td>0.07</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>-0.005 (0.011)</td>
<td>0.26</td>
<td>-0.004 (0.007)</td>
<td>0.36</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.006 (0.010)</td>
<td>0.23</td>
<td>0.007 (0.014)</td>
<td>0.52</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>-0.001 (0.021)</td>
<td>0.96</td>
<td>-0.007 (0.009)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, height, phase and smoking status.

Table F2. Association between endotoxin concentration (EU/m$^3$) in PM$_{10}$ and FEV$_1$ (L) by community in a combined phase.

<table>
<thead>
<tr>
<th></th>
<th>Estevan</th>
<th></th>
<th>Swift Current</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$ (SE)</td>
<td>P-value</td>
<td>$\beta$ (SE)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Combined Phases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>0.002 (0.006)</td>
<td>0.74</td>
<td>-0.004 (0.003)</td>
<td>0.16</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.003 (0.004)</td>
<td>0.47</td>
<td>&lt;0.001 (0.003)</td>
<td>0.89</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>-0.013 (0.027)</td>
<td>0.62</td>
<td>-0.003 (0.003)</td>
<td>0.26</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>0.008 (0.008)</td>
<td>0.05</td>
<td>-0.007 (0.005)</td>
<td>0.13</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.004 (0.004)</td>
<td>0.34</td>
<td>-0.002 (0.003)</td>
<td>0.57</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.021 (0.011)</td>
<td>0.05</td>
<td>-0.02 (0.021)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, height, phase and smoking status.
Table F3. Association between endotoxin concentration (EU/µg) in PM$_{2.5}$ and FEV$_1$ (L) by community in a combined phase.

<table>
<thead>
<tr>
<th></th>
<th>Estevan</th>
<th></th>
<th>Swift Current</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>(SE)</td>
<td>P-value</td>
<td>β</td>
</tr>
<tr>
<td><strong>Combined Phases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>0.007</td>
<td>0.015</td>
<td>0.65</td>
<td>-0.006</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.001</td>
<td>0.007</td>
<td>0.86</td>
<td>-0.002</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.063</td>
<td>0.088</td>
<td>0.47</td>
<td>-0.012</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>-0.001</td>
<td>0.011</td>
<td>0.92</td>
<td>-0.007</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.002</td>
<td>0.008</td>
<td>0.83</td>
<td>0.005</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>-0.004</td>
<td>0.024</td>
<td>0.87</td>
<td>-0.015</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, height, phase and smoking status.

Table F4. Association between endotoxin concentration (EU/µg) in PM$_{10}$ and FEV$_1$ (L) by community in a combined phase.

<table>
<thead>
<tr>
<th></th>
<th>Estevan</th>
<th></th>
<th>Swift Current</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>(SE)</td>
<td>P-value</td>
<td>β</td>
</tr>
<tr>
<td><strong>Combined Phases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>0.003</td>
<td>0.007</td>
<td>0.65</td>
<td>-0.003</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.003</td>
<td>0.006</td>
<td>0.57</td>
<td>-0.001</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>-0.012</td>
<td>0.029</td>
<td>0.69</td>
<td>-0.001</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>0.009</td>
<td>0.005</td>
<td>0.07</td>
<td>-0.005</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.005</td>
<td>0.006</td>
<td>0.41</td>
<td>-0.001</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.026</td>
<td>0.013</td>
<td>0.05</td>
<td>-0.008</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, height, phase and smoking status.
Appendix G: Interaction analysis results

Table G1. Association between endotoxin concentration (EU/m$^3$) in PM$_{2.5}$ and FEV$_1$ with interaction variable.

<table>
<thead>
<tr>
<th>Combined phases</th>
<th>Estevan</th>
<th></th>
<th>Swift Current</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>P-value</td>
<td>β</td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>0.009</td>
<td>0.03</td>
<td>0.77</td>
<td>-0.023</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.017</td>
<td>0.04</td>
<td>0.67</td>
<td>-0.03</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.161</td>
<td>0.16</td>
<td>0.31</td>
<td>-0.026</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>-0.04</td>
<td>0.03</td>
<td>0.18</td>
<td>-0.016</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.035</td>
<td>0.04</td>
<td>0.35</td>
<td>-0.004</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>-0.052</td>
<td>0.03</td>
<td>0.07</td>
<td>-0.014</td>
</tr>
</tbody>
</table>

Table G2. Association between endotoxin concentration (EU/m$^3$) in PM$_{10}$ and FEV$_1$ with interaction variable

<table>
<thead>
<tr>
<th>Combined phases</th>
<th>Estevan</th>
<th></th>
<th>Swift Current</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>P-value</td>
<td>β</td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>0.004</td>
<td>0.02</td>
<td>0.80</td>
<td>0.004</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.009</td>
<td>0.02</td>
<td>0.69</td>
<td>0.011</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.04</td>
<td>0.03</td>
<td>0.16</td>
<td>0.013</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>-0.016</td>
<td>0.01</td>
<td>0.26</td>
<td>-0.005</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.014</td>
<td>0.02</td>
<td>0.49</td>
<td>0.005</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>-0.014</td>
<td>0.02</td>
<td>0.35</td>
<td>-0.028</td>
</tr>
</tbody>
</table>
Table G3. Association between endotoxin load (EU/µg) in PM$_{2.5}$ and FEV$_1$ with interaction variable.

<table>
<thead>
<tr>
<th>Combined phases</th>
<th>Estevan</th>
<th></th>
<th>Swift Current</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
<td>P-value</td>
<td>β</td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>0.019</td>
<td>0.03</td>
<td>0.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.02</td>
<td>0.05</td>
<td>0.96</td>
<td>0.01</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.154</td>
<td>0.15</td>
<td>0.31</td>
<td>-0.013</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>-0.035</td>
<td>0.04</td>
<td>0.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.026</td>
<td>0.05</td>
<td>0.56</td>
<td>0.02</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>-0.046</td>
<td>0.04</td>
<td>0.21</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table G4. Association between endotoxin load (EU/µg) in PM$_{10}$ and FEV$_1$ with interaction variable

<table>
<thead>
<tr>
<th>Combined phases</th>
<th>Estevan</th>
<th></th>
<th>Swift Current</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
<td>P-value</td>
<td>β</td>
</tr>
<tr>
<td>Morning MeanFEV$_1$</td>
<td>0.009</td>
<td>0.02</td>
<td>0.59</td>
<td>0.002</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>0.019</td>
<td>0.03</td>
<td>0.52</td>
<td>0.013</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>0.051</td>
<td>0.04</td>
<td>0.15</td>
<td>0.006</td>
</tr>
<tr>
<td>Evening MeanFEV$_1$</td>
<td>-0.015</td>
<td>0.02</td>
<td>0.39</td>
<td>-0.005</td>
</tr>
<tr>
<td>MinFEV$_1$</td>
<td>-0.013</td>
<td>0.02</td>
<td>0.57</td>
<td>0.004</td>
</tr>
<tr>
<td>MaxFEV$_1$</td>
<td>-0.013</td>
<td>0.02</td>
<td>0.55</td>
<td>-0.011</td>
</tr>
</tbody>
</table>