Effects of Traffic Related Air Pollution Exposure on Childhood Asthma Onset During the Different Developmental Stages of Childhood

by © Nelson Lau

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Abstract

Childhood asthma is a chronic condition which affects millions of children worldwide. It is associated with many risk factors, including traffic related air pollution (TRAP). While the relationship between TRAP and childhood asthma is well established, the relationship between TRAP and different childhood asthma patterns, or phenotypes is less well studied. This thesis consists of a systematic review and an analysis of a longitudinal cohort study addressing this knowledge gap. The results from our systematic review suggest that TRAP is associated most strongly in early childhood asthma phenotypes, which is supported by the results of our longitudinal cohort study (Quartile 3 OR: 2.11, 95% CI: 1.29, 3.44, Quartile 4 OR: 2.16, 95% CI: 1.27, 3.68 compared to Quartile 1 of NO_2 exposure for early childhood onset asthma phenotype). Our results suggest that younger children are more susceptible to TRAP compared to older children and emphasize the importance of considering asthma phenotypes in future research.

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List of Abbreviations

95% CI – 95% Confidence Interval

ADAM33 – A Disintegrin and Metalloproteinase 33

AERD – Aspirin Exacerbated Respiratory Disease

BHR – Bronchial Hyperresponsiveness

CAAQS – Canadian Ambient Air Quality Standard

CAPI – Computer-Assisted Personal Interviewing

CASP – Critical Appraisal Skills Programme

CLCA3 - Chloride Channel Accessory 3

CO – Carbon Monoxide

CO_2 – Carbon Dioxide

DALY – Disability Adjusted Life Years

DNA – Deoxyribonucleic acid

ECD – Early Childhood Development

FOXP3 – Forkhead Box P3

FSA – Forward Sortation Area

GBD – Global Burden of Disease

GSTP – Glutathione S-transferase P-1

HIC – High Income Countries

HREA – Health Research Ethics Authority

HREB – Health Research Ethics Board

HSES – Household Socioeconomic Score

IgE – Immunoglobin E

IL – Interleukin

ISAAC – International Study of Asthma and Allergies in Children

LFS – Labour Force Survey

LMIC – Lower and Middle Income Countries

LUR – Land Use Regression

NAPS – National Air Pollution Surveillance

NLSCY – National Longitudinal Survey of Children and Youths

NO_2 – Nitrogen Dioxide

NO_x – Nitrogen Oxide

O_3 – Ozone

OR – Odds Ratio

ORMDL3 – Orosomucoid-like Sphingolipid Biosynthesis Regulator 3

$PM_{2.5}$ – Particulate Matter 2.5 (smaller than 2.5 µg in diameter)

PM_{10} – Particulate Matter 10 (smaller than 10 µg in diameter)

PMK – Person Most Knowledgeable

Ppb - Parts per billion

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta Analyses

Q1 – Quartile 1

Q2 – Quartile 2

Q3 – Quartile 3

Q4 – Quartile 4

RR – Relative Risk

SAS – Statistical Analysis Software

SD – Standard Deviation

SNP – Single Nucleotide Polymorphism

SPSS – Statistical Product and Service Solutions

SO_2 – Sulfur Dioxide

SSHRC - Social Sciences and Humanities Research Council

TET1 - Ten-Eleven Translocation Methylcytosine Dioxygenase 1

Th2 – T Helper Type 2

TLR – Toll-Like Receptor

TNF-α – Tumor Necrosis Factor Alpha,

TRAP – Traffic Related Air Pollution

WHO – World Health Organization

YLD – Years Lived with Disability

YLL – Years of Life Lost

Chapter Organization and Declaration of Publication Intent

This manuscript-style thesis focuses on the relationship between traffic related air pollution (TRAP) and the onset of childhood asthma phenotypes. Chapter 1 discusses the background of TRAP, asthma, and asthma phenotypes both in Canada and globally. Chapter 2, "Association between Traffic Related Air Pollution and the Development of Asthma Phenotypes in Children: A Systematic Review," is a systematic review focusing on the relationship between TRAP and childhood asthma phenotypes. Chapter 3 describes the methodology and purpose of the primary dataset used, the National Longitudinal Survey of Children and Youths (NLSCY). The 4th chapter, "Effects of Low Exposure to Traffic Related Air Pollution on Childhood Asthma Onset by Age 10 Years," is a cohort study focusing on TRAP exposure and the timing of asthma onset in Canadian children. The final chapter summarizes the findings of each manuscript and discusses strengths and limitations. The text and tables from Chapter 4 are not currently copyrighted, as it is yet to be published in a scientific journal. However, it will be submitted to the to a peer-reviewed journal for peer-review and eventual publication. The manuscript for Chapter 2 has been previously published. The publication and submission details are as below:

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Chapter 1: Introduction and Background

Asthma is defined by the World Health Organization (WHO) as a chronic condition which occurs due to inflammation of airways in the lungs and can lead to sensitivity, irritation, and narrowing of the airways [1]. Common symptoms of asthma include breathlessness, coughing, and wheezing among others, and these symptoms can be further exacerbated in asthmatics [1, 2]. Currently, there is no known cure for asthma; however, asthma can be managed via medication [2]. The two common medications are bronchodilators, which relax smooth muscle in the airway to counteract airway narrowing, and corticosteroids which treat airway inflammation [2]. These medications are effective in most asthma patients, and those with asthma are often able to maintain a high quality of life with proper treatment [2].

Asthma can develop at any part of the lifespan, but most commonly begins to manifest itself in early childhood [2]. While many preschool aged children who exhibit wheezing no longer do so once they are of school age, there are many children who will continue to exhibit signs of wheezing or asthma into adolescence and adulthood [2]. These differences in asthma pattern, or asthma phenotype, can lead to significantly different outcomes. For instance, children who exhibit frequent or persistent wheezing are more likely to have inflamed airways, airway remodeling and decreased lung function in adulthood [3–5].

Asthma onset has been linked to a combination of genetics and environmental factors, which can act as a trigger for asthma [2, 6–8]. Such environmental factors include allergens like dust mites or pollen, air pollutants such as tobacco smoke or traffic emissions, and chemicals such as paint or varnish [2, 9, 10]. Given that there are over a billion vehicles on roads globally, the role of traffic related air pollutants (TRAP) in asthma onset is particularly important [11]. While TRAP

concentrations have actually decreased in many high-income countries, in lower and middle income countries (LMIC), TRAP concentrations are high in many LMIC urban areas, which may lead to higher rates of childhood asthma in such areas [12, 13].

Childhood Asthma – Epidemiology and Burden

Although asthma is the most common chronic respiratory condition worldwide, it is difficult to establish the current epidemiologic trends of asthma globally. The 2016 Global Burden of Disease (GBD) study estimates that over 330 million people are affected by asthma [2, 14]. The prevalence of asthma varies by country, from under 10% of adults in East Asia to over 25% of adults in Australia [15, 16]. The health burden of asthma across all ages is also significant, with an estimated 13.2 million years lived with disability (YLD) and 10.6 million years of life lost (YLL) due to premature death due to asthma worldwide [2, 14].

Although asthma is a disease which occurs across all age groups and sexes, the burden of asthma does not affect all age groups and sexes equally. The burden of asthma, as measured by Disability Adjusted Life Years (DALY, the sum of YLD and YLL) per 100 000 persons follows a bimodal distribution. In early childhood, the burden of asthma increases until early adolescence (age 10 – 14), after which the burden decreases substantially until age 45 [2]. After age 45, the burden of asthma begins to increase again and peaks in adults age 75 – 79 [2]. Given that asthma onset most commonly occurs in childhood, it is crucial to understand the epidemiology of asthma in children worldwide.

Like adult asthma, it is difficult to establish current epidemiological trends of childhood asthma. The most recent global survey of children with asthma, the International Study of Asthma and Allergies in Children (ISAAC), was conducted between 2000 – 2003 [2]. Nevertheless, available

information shows the burden of asthma in children is also significant. Childhood asthma is the most common chronic condition in children, with an estimated prevalence of 14% globally [17]. The prevalence of children with asthma appears to be stable since peaking around the year 2000 [18]. While the prevalence of childhood asthma varies by country, childhood asthma appears to be more severe in lower income countries [19]. The economic burden is also significant and global annual costs associated with childhood asthma are currently estimated to be over $50 billion [19].

Regardless of age, hospital admissions for asthma are considered to be an indicator for the burden of severe asthma, although the relationship is imperfect and complex. Hospital admissions may occur due to an exacerbation of existing asthma or due to failures in managing already existing asthma conditions. While the self-reported prevalence of childhood asthma has increased in many high-income countries (HIC), the rate of hospitalization for children with asthma has decreased substantially since peaking around 1990 [18]. It is important to note that in many LMICs, high quality data on hospital admissions for asthma is lacking, and thus it is difficult to discern any trends for asthma hospitalizations in these countries [2].

While asthma mortality is rare and in most countries it is believed to account for less than 1% of deaths, it is estimated that over 1000 people die from asthma each day [2]. These rates vary significantly between countries, with mortality rates due to asthma in Fiji being as much as 100 times larger than countries with low asthma mortality such as Finland [2]. Asthma mortality has also decreased by as much as 50% when comparing the period from 2001 – 2005 to 2011 – 2015 [2]. The mortality rate among children also appears to be low, with a hospital mortality rate of 0.02% among children hospitalized for asthma in the United States in 2009 [20]. Despite the low mortality rate, it has been suggested that many of the deaths due to asthma are preventable [2]. A

review of 195 deaths related to asthma in the United Kingdom found that half of those who died did not receive medical care prior to death [21]. While the burden of asthma due to mortality is low, mortality is not necessarily representative of the overall burden of asthma – as previously discussed, much of asthma's global burden of disease can be found in years lived with disability and hospital admissions. [2].

Childhood Asthma – Pathophysiology and Etiology

Asthma is a chronic condition characterized by airway inflammation, airway narrowing, and airway hyperresponsiveness [1]. Common symptoms include breathlessness, coughing, wheezing, and chest tightness [1]. However, the pathophysiology of asthma is complex and there is no single accepted definition for asthma [22]. In fact, multiple pathophysiological pathways are associated with asthma [23].

Several features characterize the pathophysiology underlying asthma. These include remodeling of the airways, airway inflammation, bronchial obstruction, impaired lung function, and bronchial hyperresponsiveness (BHR) [22].

Chronic airway inflammation has long been recognized as a common feature of asthma [23]. It is commonly thought to occur due to allergen sensitization. Inhaling allergens such as pollen, pollutants, mold or dust mites can stimulate the production of T helper type 2 (Th2) cells, which in turn lead to the production of Th2 cytokines and Interleukin (IL)-4, IL-5, and IL-6 [23]. This simulates immune responses including inflammation, and subsequent airway remodeling [23]. While airway remodeling is commonly found in adult asthma, its role in childhood asthma is unclear [22]. Nevertheless, structural changes, such as epithelial cell disruption, sub-epithelial reticular basement layer thickening, proteases and antiprotease imbalance, and neoangiogenesis

(sprouting of new blood vessels from existing vessels), are believed to play a role in airway remodeling in childhood asthma [22].

Airway inflammation, mucosal edema (build-up of tissue fluid) and constriction of the bronchial muscles due to activation of β-adrenergic receptors can all cause bronchial obstruction, which may reduce airway flow [22]. The use of bronchodilators (such as β_2-agonists) can reverse bronchial obstruction, while inhaled corticosteroids can treat the underlying pathophysiology of bronchial obstruction [22].

Although BHR is considered a characteristic of childhood asthma, it is not always found in childhood asthmatics [22]. Airway inflammation has been associated with BHR, which is consists of two related but distinct components to BHR: fixed or persistent BHR, and variable or episodic BHR [24]. The underlying pathophysiological mechanisms of BHR are not well understood; however, factors such as inflammatory cell infiltration, mast cell accumulation, cytokine and chemokine secretion, airway smooth muscle hypertrophy, hyperplasia, cholinergic nerve activity, and airway inflammation leading to increased parasympathetic nerve activity, have all been associated with persistent BHR [24]. Variable BHR has been associated with acute airway inflammation, eosinophils and mast cells [24].

Many demographic factors have been linked to asthma onset. Numerous studies have shown that sex differences affect the asthma prevalence. In children, males are more likely to develop asthma compared to females, while the reverse is true after puberty [25]. This may be because female newborns generally have more developed lungs than male newborns, and thus are less likely to have reduced lung function after birth and in early childhood [26].

Numerous studies have shown that children of asthmatic parents are also at an increased risk for childhood asthma [6]. However, there is no single gene which is responsible for asthma. Rather, mutations in various genes such as A disintegrin and metalloproteinase 33 (ADAM33), the filaggrin gene, and Orosomucoid-like Sphingolipid Biosynthesis Regulator 3 (ORMDL3), have all been associated with childhood asthma [6]. These genes are associated with various pathophysiological mechanisms leading to asthma, such as BHR (ADAM33), allergen sensitization (filaggrin), and sphingolipid synthesis, which plays a role in cell growth and remodeling (ORMDL3) [6, 27].

In addition to demographic factors, environmental exposures have also been long associated with asthma. Examples of such environmental exposures include allergens such as pollen, dust mites or cockroaches, air pollution, and viral infections [9, 10]. Exposure to allergens or air pollution may lead to an innate immune response, causing airway inflammation BHR [22]. Similarly, exposure to viral infections may lead to production of pro-inflammatory cytokines such as Th1 or cells expressing IL-3 [28].

Traffic Related Air Pollution and Childhood Asthma

Although there are many environmental exposures, a particularly notable exposure is traffic related air pollution (TRAP). The relationship between TRAP and childhood asthma has been well established. Many studies and systematic reviews have consistently found an association between common TRAP particles such as nitrogen dioxide (NO_2), particulate matter between 2.5 and 10 μm ($PM_{2.5}$ and PM_{10}), and carbon monoxide (CO), and childhood asthma [29–34].

The lungs undergo significant development and changes during the postnatal and early childhood periods of life. Exposure to TRAP during this time can have adverse effects on lung development

and lead to asthma susceptibility in children. Studies have found that TRAP exposure during childhood can negatively affect both lung development and lung function. This may be particularly problematic as over 85% of the global population live in areas with ambient pollution levels which exceed WHO guidelines [35]. TRAP exposure is also associated with other detrimental health effects, such as respiratory inflammation and neurotoxicity [36],[37].

There are many biological mechanisms through which TRAP exposure affects the respiratory system and can lead to asthma onset. Children exposed to TRAP show elevated levels of inflammatory cytokines such as Il-6, IL-8, and tumor necrosis factor alpha (TNF-α). These cytokines play a crucial role in the inflammatory process associated with asthma [38]. Additionally, TRAP exposure may lead to epigenetic changes, such as methylation of IL-9, and eosinophil granule major basic protein (MBP), which are associated with airway inflammation, eosinophils, and the Th2/ B cell signaling pathway [39]. Other genes undergoing epigenetic changes after TRAP exposure include Ten-eleven translocation methylcytosine dioxygenase 1 (TET1), which controls DNA methylation, and forkhead box P3 (FOXP3), which helps regulate the immune system [39].

In addition to epigenetic effects, children exposed to TRAP may be more susceptible to TRAP due to genetic variations. Pre-adolescent children with specific variations in Toll like receptor (TLR)-2 and TLR-4, a gene involved in the innate immune response, are more likely to become asthmatic [40]. Variations in other genes, such as Glutathione S-transferase P-1 (GSTP1), which protects cells against oxidative stress, and TNF-α, can also influence asthma susceptibility in children exposed to TRAP [41].

Traffic Related Air Pollution – Global and National Trends

With both the increasing global population and the recent spotlight on climate change, the national Canadian and global trends of TRAP have changed. The exact mixture of TRAP can vary and its exact composition is unknown. However, components include CO, carbon dioxide (CO_2), volatile organic compounds, hydrocarbons, nitrogen oxides (NO_x), and PM [42]. Among these different pollutants, NO_2 has been found to correlate well with the variation found in overall measurements of TRAP exposure [12].

Global trends in ambient TRAP levels vary by pollutant and region. Ambient NO_2 levels appear to have increased from 1995 – 2012 in Northern Africa, Eastern Europe, and particularly in East Asia, where ambient NO_2 has tripled [43]. In contrast, high income North America (USA & Canada), Western and Central Europe, Latin America, Australasia, and Southern Sub-Saharan Africa have all seen declines in ambient NO_2 levels during this period [43]. Overall, on a global scale, ambient NO_2 levels have increased from 1995 – 2012 [43]. The reasons for the diverse regional NO_2 trends are varied and may be reflective of anthropogenic activity in a specific region [43]. For instance, the significant increase in ambient NO_2 in East Asia may be explained due to the significant urban development that has taken place in this region over the time period measured [43]. In regions such as high-income North America, policies designed to reduce ambient levels of nitrogen oxides may contribute to the decreasing trend found in the region [43]. Similar trends have been found for other common TRAP pollutants such as $PM_{2.5}$ and CO [44].

Regional and national measurements of ambient TRAP exposure in Canada agree with the trends observed in global studies. Ambient TRAP levels in Canada have shown a decreasing trend in recent years [45]. Measurements by Environment and Climate Change Canada have found that

the average NO_2 concentration nationally has decreased from 13.7 parts per billion (ppb) in 2002 to 7.8 ppb by 2016 (Table 1) [45]. Peak 1-hour concentration (98^{th} percentile) of NO_2 has also decreased nationally, from 51.6 ppb in 2002 to 38.8 ppb in 2016 [45]. All regions across Canada (Southern Ontario, British Columbia, Southern Quebec, Prairies and Northern Ontario, and Atlantic Canada) have also shown a decreasing trend, although NO_2 concentrations vary greatly (Table 2) [45]. In Southern Ontario, average annual NO_2 concentration has decreased from 18.5 ppb in 2002 to 8.7 ppb in 2016, while in Atlantic Canada, average annual NO_2 concentrations range from 6.9 ppb in 2002 to 3.1 ppb in 2016 [45].

The decreasing trends observed regionally and nationally for ambient NO_2 levels across Canada have also been observed locally, albeit with significant variation. Large urban areas such as Toronto, Vancouver, and Calgary have relatively high average NO_2 concentrations (20.0 ppb, 16.2 ppb, 22.4 ppb in 2002, and 12.2 ppb, 11.2 ppb, 11.0 ppb in 2016 respectively) compared to smaller cities such as Saskatoon or Victoria (11.7 ppb and 10.4 ppb in 2002 and 8.9 ppb and 6.7 ppb in 2016 respectively) [45]. These trends remain consistent for peak NO_2 exposure in urban areas, with larger urban areas generally having higher levels of ambient NO_2 and decreasing trends with time. The magnitude of both the ambient NO_2 level and the decreasing time trend varies significantly depending on the area – for example, in Toronto, NO_2 has decreased from 66.3 ppb in 2002 to 50.7 ppb in 2016 compared to 63.0 ppb in 2002 to 37.0 ppb in 2016 in Oshawa [45].

WHO guidelines for ambient NO_2 exposure have established that annual ambient NO_2 should not exceed 21 ppb [12, 46]. Ambient NO_2 level across Canada at the national, regional, and local levels are well above WHO guidelines as well as the 2020 Canadian Ambient Air Quality Standard (CAAQS), which aims for an annual ambient NO_2 concentration of 17 ppb [47].

Ambient NO_2 levels in Canadian urban areas from the period of 2009 – 2014 are also generally below that of other international cities such as Hong Kong, Denver, London and Paris [48].

Table 1: Regional annual average NO_2 concentrations (parts per billion) in Canada, 2002 – 2016

Year	Atlantic Canada	Southern Quebec	Southern Ontario	Prairies and Northern Ontario	British Columbia
2002	6.9	14.9	18.5	10.6	14.3
2003	7	16.2	17.8	10.9	14
2004	5.5	14.3	15.2	9.2	13.6
2005	5.1	14	15.6	9.1	13
2006	3.3	12	13.3	9	13.2
2007	4	12	12.7	9.4	12.3
2008	4.5	12.2	12.3	9.7	11.8
2009	3.4	11	11.2	8.9	12.1
2010	3.7	10.6	10.5	8.6	10.8
2011	4	11.6	10.5	8	10.4
2012	3.4	9.2	9.4	7.4	10.5
2013	4.3	9.1	9.3	8	10.2
2014	3.8	8.6	9.6	7.8	10.5
2015	3.6	8.5	9.3	7.5	10.1
2016	3.1	8.4	8.7	6.8	9.2
Annual Trend	-0.19	-0.52	-0.66	-0.23	-0.35

Data available from https://www.canada.ca/en/environment-climate-change/services/environmental-indicators/air-quality.html#NO2-average

Table 2: Peak 1-hour NO_2 concentrations (parts per billion) in Canada, 2002 – 2016

Year	Atlantic Canada	Southern Quebec	Southern Ontario	Prairies and Northern Ontario	British Columbia
2002	49.5	59.2	61.9	44.3	45.4
2003	42.4	59.3	62.4	45	44.2
2004	38.8	56.5	62.8	44	43.7
2005	38.7	57.6	61.6	43.9	42.7
2006	35.2	52.6	57.8	43.1	42.9
2007	32.8	50.9	54	42.7	40.8
2008	33.2	49.5	51.5	42.9	40.4
2009	32.5	50.3	49.8	41.2	39.6
2010	33.5	48.1	47.7	43.2	38.7
2011	31.2	48.4	47	43.4	36.3
2012	28.8	45.5	44.5	42	35.1
2013	29.4	42.6	43.4	41.7	35.4
2014	30.5	41.3	43.8	40.8	35.9
2015	30.8	42.2	45.4	40.1	35.3
2016	29.3	42.1	45.1	37.5	35.2
Annual Trend	-0.91	-0.37	-1.66	-0.34	-0.78

Peak 1-hour average NO_2 concentration is defined as the 98^{th} percentile of NO_2 concentration over a 1-hour period. Data available from https://www.canada.ca/en/environment-climate-change/services/environmental-indicators/air-quality.html#NO2-average

Childhood Asthma – Phenotypes

Childhood asthma is a heterogenous disease, with many differences in terms of clinical symptoms, disease prognosis, and response to treatment. There is no one set of criteria or symptoms which can lead to a definitive asthma diagnosis. It has long been observed that some asthma patients may exhibit a certain set of symptoms, but not others [49]. These groupings of observable characteristics, also known as asthma phenotypes, are often found via two approaches. The first is the clinical approach, wherein the asthma phenotype is classified based on the pathology, etiology and response to treatment [50]. The second approach is epidemiological, which is based on the observed symptoms (for instance, period of wheezing) [50].

The clinical approach to asthma phenotyping has traditionally divided childhood asthma into eosinophilic (allergic) and non-eosinophilic asthma phenotypes [49]. Eosinophilic asthma is triggered by environmental allergens and is characterized by the activation of eosinophilic Th2 and inflammation of the respiratory system [49, 51]. Eosinophilic asthma tends to respond well to corticosteroid treatment [51]. Eosinophilic asthma is a broad phenotype which contains distinct phenotypes [49]. These include early-onset allergic asthma and late-onset persistent eosinophilic asthma, each of which have distinctive characteristics [49]

Asthma which originates in childhood and remains persistent tends to have an atopic or allergic trigger [49]. Most early-onset asthma cases are associated with Th2, although there are exceptions [52, 53]. Early-onset allergic asthma tends to be associated with other atopic diseases such as allergic rhinitis and atopic dermatitis [54–57]. Those with early-onset allergic asthma tend to have higher Immunoglobin E (IgE) levels than those with late-onset or adult onset

asthma, and higher eosinophil, mast cell, and IgE levels than non Th2 related asthma phenotypes [49]. People of African descent are at greater risk for this type of asthma, and there are likely both genetic and environmental factors involved with this type of asthma [49, 57]. The preferred therapy of choice for early-onset eosinophilic asthma is corticosteroid treatment [58–60].

Similar to early-onset Th2 asthma, late-onset (adult) persistent eosinophilic asthma is also associated with high eosinophil counts [61–63]. This phenotype is associated with sinusitis, nasal poylps and aspirin exacerbated respiratory disease (AERD), but there is likely no association with allergic responses [55]. There is also little evidence of a genetic or family connection in contracting this phenotype [55, 57]. Corticosteroids are less effective in treating this phenotype despite eosinophils usually being responsive to corticosteroid treatment [64]. However, high doses of corticosteroids tend to be effective in treating late-onset eosinophilic asthma [65]. Leukotriene modifiers can be effective in treating symptoms of this phenotype among those who have AERD [66, 67].

Compared to eosinophilic asthma, less is understood about the non-eosinophilic (non-Th2) asthma phenotype [49]. However, this phenotype may be present in over half of asthmatic individuals who do not respond to corticosteroid treatment [68, 69]. Broadly speaking, two common, more specific phenotypes associated with non Th2 asthma are obesity-related asthma and neutrophilic asthma [70–72]. Corticosteroid treatment tends to be less effective in treating either of these phenotypes and alternate therapies (such as weight loss or antibiotics respectively) may be preferred [49, 73].

Epidemiological approaches to classifying childhood asthma phenotypes commonly classify asthma phenotypes based on the timing of observed wheezing symptoms. A seminal study focusing on these phenotypes found that children could be divided into no wheezing, early

transient wheezing (wheezing before age 3, but not by age 6), persistent wheezing (wheezing both before age 3 and at age 6) and late-onset wheezing (no wheezing before age 3 but wheezing by age 6) phenotypes [74]. Grouping phenotypes based on the timing of observed wheezing symptoms can reveal crucial differences [74],[75].

These differences may be due to several factors. It is known that the lungs of newborn children undergo substantial structural and development changes during the first 18 months of life, and lung damage during this period may lead to complications [76–78]. Due to this, younger children may also be more susceptible to asthma risk factors compared to older children [32]. The timing of childhood wheezing or asthma onset can also inform the asthma prognosis. Early-onset wheezing has been associated with lower lung function and a higher exhaled nitric oxide fraction in adolescence compared to children with late-onset wheezing. Finally, as the timing of wheezing onset indicates a specific phenotype, each subgroup may require specialized therapeutic strategies [79, 80].

Thesis Objectives

The objective of this thesis is to assess the effect of TRAP on the onset of childhood asthma phenotypes using data from Canadian children. This thesis produced two manuscripts which focused on this research objective:

1) The first manuscript (Chapter 2) involved a systematic review following Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) guidelines on the current knowledge regarding the relationship between TRAP and childhood asthma phenotypes. This study focused on childhood asthma phenotypes based on the longitudinal pattern of wheezing, such as early wheezing or persistent wheezing.

2) The second manuscript (Chapter 4) is a longitudinal cohort study to assess the effect of TRAP on the development of asthma phenotypes in Canadian children. Data from a Canadian longitudinal infant cohort from Statistics Canada and pollution exposure data taken from a nationwide air pollution monitoring program were used to fulfill the objectives of this manuscript.

Chapter 3 describes the primary dataset used in Chapter 4, the National Longitudinal Survey of Children and Youths (NLSCY).

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Chapter 2: Association between Traffic Related Air Pollution and the Development of Asthma Phenotypes in Children: A Systematic Review

International Journal of Chronic Diseases

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Author Contribution Statement

As the primary author, N.L. was involved in developing the research question, developing the inclusion and exclusion criteria, the literature review, data extraction, and writing of the manuscript. Z.G. was responsible for the original research question, developing the inclusion and exclusion criteria, and guiding N.L. and A.N. as the supervising author. A.N. was involved in the literature review and data extraction. A.S. and M.J.S. were involved in supervising this project and advising N.L and A.N. All authors reviewed and approved the final manuscript prior to submission for publication.

Abstract

Introduction. Traffic related air pollution (TRAP) has long been associated with the onset of childhood asthma. Less understood is the relationship between TRAP exposure and the development of childhood asthma phenotypes. To better understand this relationship, we performed a systematic review of the literature studying childhood TRAP exposure and the development of childhood asthma and wheezing phenotypes (transient, persistent, and late-onset asthma/wheezing phenotypes). *Methods.* A literature search was performed in Pubmed, Embase and Scopus databases for current literature, returning 1706 unique articles. After screening and selection, 7 articles were included in the final review. Due to the low number of articles, no meta-analysis was performed. *Results.* TRAP exposure appears to be associated with both transient and persistent asthma/wheezing phenotypes. However, there was little evidence to suggest a relationship between TRAP exposure and late-onset asthma/wheezing. The differing results may be in part due to the heterogeneity in study methods and asthma/wheezing phenotype definitions, in addition to other factors such as genetics. *Conclusion.* TRAP exposure may be associated with transient and persistent asthma/wheezing phenotypes in children. The low number of studies and differing results suggest that further studies are warranted.

1. Introduction

Childhood asthma is the most common chronic disease in children, with an estimated prevalence of 14% in children worldwide [1,2]. This high prevalence is also associated with significant economic burden. Asthma in school-aged children in the United States alone is estimated to cost nearly $6 billion annually in healthcare expenditures [3]. Given the high burden of disease as well as the complex and heterogeneous nature of childhood asthma, it is essential to investigate further beyond the incidence and outcomes associated with childhood asthma [4].

Among the first studies to investigate the differences between childhood asthma symptoms was the Tucson Children's Respiratory Study, which identified 4 separate wheezing phenotypes based on the longitudinal pattern of wheezing that was observed [5]. These phenotypic groups were based on the age of wheezing onset and the duration of wheezing, and include the following groups: 1) no wheezing, 2) early transient wheezing (wheezing before age 3, but not at age 6 years), 3) persistent wheezing (wheezing both before age 3 and at age 6 years) and 4) late-onset wheezing (no wheezing before age 3 but wheezing by age 6 years) [5]. The existence of these phenotypes has been supported by further studies, using methods such as latent class analysis and group-based trajectory modelling [4, 6-8]. Currently, childhood asthma consists of many different phenotypes, each associated with differing clinical and genetic markers, risk factors, outcomes and response to medications [9, 10]. Thus, understanding the different clinical phenotypes of childhood asthma and wheeze may lead to several benefits in diagnosis and treatment. These include knowledge of probable outcomes and prognosis, personalized treatments for patients, and understanding how environmental exposures can modify the risk of developing different childhood asthma or wheezing phenotypes [11].

Numerous studies have found traffic related air pollution (TRAP) to be associated with the onset of childhood asthma [12-16]. These results are further supported by a systematic review which showed strong associations between exposure to black carbon (BC), NO_2, $PM_{2.5}$ (atmospheric particulate matter less than 2.5 μm in diameter), and PM_{10} (atmospheric particulate matter less than 10.0 μm in diameter) with the onset of childhood asthma [17]. Less well understood is the association between TRAP and different childhood asthma phenotypes. Earlier reviews have focused primarily on the association between TRAP and the onset of childhood asthma, rather than the development of the asthma phenotype [17,18]. Nevertheless, the effect of TRAP exposure on the development of different childhood asthma phenotypes may be significantly different. One study found no association between NO_2 exposure with either early transient wheeze nor persistent wheeze phenotypes in children [14]. These findings conflict with another study which found an association between childhood NO_2 exposure and persistent wheeze in children [19]. These associations may also change with the pollutant being studied: although one study found no association between childhood NO_2 exposure and early transient wheezing, it found an association between childhood $PM_{2.5}$ exposure and early transient wheezing [14].

The purpose of this systematic review is to synthesize the results of observational epidemiological studies studying the association between TRAP exposure and the development of childhood asthma/wheezing phenotypes, namely transient asthma/wheezing, late-onset asthma/wheezing and persistent asthma/wheezing in children aged 0-18.

2. Methods

2.1 Selection Criteria. This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement for reporting

systematic reviews and meta-analyses [20]. The review included cross-sectional, case-control and cohort studies which studied the association between TRAP exposure and the development of childhood asthma phenotypes, namely early-transient asthma, late-onset asthma and persistent asthma in children aged 0-18.

Studies were included if they:

1) Were epidemiological or observational studies such as cross-sectional, cohort or case-control studies;
2) Had some measure of TRAP (CO, $PM_{2.5}$, PM_{10}, NO_2) exposure [21] for children within the early life period between fetal stage to age 12 (either through modelling or direct measurement);
3) Examined the association between TRAP exposure and development of asthma or wheeze outcomes when the child is aged 0-18;
4) Explicitly included at least one type of asthma/wheezing phenotype (late onset asthma/wheeze, persistent asthma/wheeze, transient asthma/wheeze) in their outcomes.

Studies were excluded if they:

1) Measured TRAP exposure was only when children were age > 12;
2) Were reviews, commentaries, experimental studies, letters to the editor, et. cetera.;
3) Were studies which only examine the association between TRAP exposure and asthma development without specifying the phenotype, or look at exacerbation of asthma/wheeze, allergies, etc. as the outcome;
4) Measured exclusively pollution exposure to non-TRAP pollutants, such as O_3 or SO_2;
5) Were non-English language studies;

No studies were excluded on the basis of publication year.

2.2 Health Outcomes. The primary health outcomes assessed were childhood asthma/wheezing phenotypes. Articles with either wheezing phenotypes or asthma phenotypes as outcomes were included for analysis. Although wheezing is a non-specific symptom which is not always associated with childhood asthma, wheezing phenotypes have long been used to characterize the corresponding childhood asthma phenotypes [18, 22, 23]. To account for the differing follow-up times between studies, asthma/wheezing phenotypes were divided into 3 groups with the following modified definitions based on the Tucson Children's Respiratory Study [5]:

1) Transient asthma/wheezing: onset of asthma or wheezing before or at age 3, and no asthma or wheezing after age 3
2) Persistent asthma/wheezing: onset of asthma or wheezing before or at age of 3, with evidence of asthma or wheezing after age 3
3) Late-onset asthma/wheezing: onset of asthma or wheezing after age 3

2.3 Search Strategy. Searches were performed in the Pubmed, Embase, and Scopus databases for relevant articles. Search strings containing terms for "asthma", "vehicle emissions" and "children" were used. An example search string for Pubmed is given below:

("Asthma"[Mesh] OR asthma OR wheeze) AND ("Motor Vehicles"[Mesh] OR "Vehicle Emissions"[Mesh] OR traffic OR car OR truck OR bus OR motorcycle OR automobile OR vehicle OR exhaust) AND ("Child"[Mesh] OR "Infant"[Mesh] OR childhood OR children OR infant OR baby OR paediatric OR pediatric OR paediatrics OR pediatrics).

The search was performed in May 2018 and included papers published until May 2018.

2.4 Quality Assessment. The Critical Appraisal Skills Programme (CASP) checklist for cohort studies was used to assess the quality of applicable studies [24]. CASP consists of 12 questions used to evaluate the quality of cohort studies. The CASP criteria was used to evaluate cohort studies for: 1) selection bias in the cohorts used, 2) measurement, classification or recall bias in exposures, 3) measurement or classification bias in outcomes, 4) adjustment for appropriate confounders, 5) length and completeness of follow-up, 6) potential validity of results. Two reviewers (N.L. and A.N.) independently assessed each article using the CASP criteria.

2.5 Data Extraction. Relevant information was extracted independently by two reviewers (N.L. and A.N.). Information was extracted from supplementary material when deemed necessary. Disagreements on what information to extract were resolved via consensus by both reviewers. Extracted information included: authors, study location, year of publication, study design, study population, pollutant and exposure information, asthma and wheezing phenotype definitions, and outcome data.

3. Results

3.1 Search Results. A literature search was conducted in the Pubmed, Embase, and Scopus databases, yielding 1706 unique articles. After initial screening, 233 articles were chosen for full text review, using the selection criteria and 7 articles were deemed suitable for inclusion [4, 14, 19, 25-28]. Figure 1 represents the PRISMA flow diagram for article selection in this study.

3.2 Study Characteristics. The 7 studies included were published from 2007 to 2018, with all 7 studies included being cohort studies [4, 14, 19, 25-28]. Among the 7 studies, one birth cohort was

utilized twice in separate studies [14, 19]. 111 038 individuals across these 7 studies were included (duplicated cohorts were counted twice). The sample size in the included studies ranged from 2871 to 68 195 individuals. Studies were conducted in Canada, France, USA, Sweden, The Netherlands and Norway, and were all English language studies. The length of follow-up varied among the 7 studies, with all studies starting from birth, and the end of follow-up ranging from age 4-12. To estimate pollutant exposure, 3 studies utilized Land Use Regression (LUR) and 4 utilized dispersion modelling. CO, NO_2, NO_x, $PM_{2.5}$, PM_{10} were the traffic related air pollutants assessed. The number of studies measuring each individual pollutant are as follows:

- CO: 1 study
- NO_2: 4 studies
- NO_x: 3 studies
- $PM_{2.5}$: 4 studies
- PM_{10}: 1 study

Due to the differing follow-up times among the included studies, phenotypic definitions varied by study. 5 studies reported results in the form of odds ratios for asthma phenotype risk per unit of pollutant exposure ($\mu g/m^3$) [14, 19, 25, 26, 28]. One study, Sbihi et al., divided the cohort into quartiles based on exposure quartiles to the lowest quartile of pollutant exposure [4]. The last study, Pennington et al., reported asthma phenotype risk in the form of absolute risk difference between different exposure groups [27]. Complete study characteristics, including phenotypic definitions, can be found in Table 1, while the individual results for each study can be found in Table 2. Due to the low number of included studies, we were unable to conduct a meta-analysis.

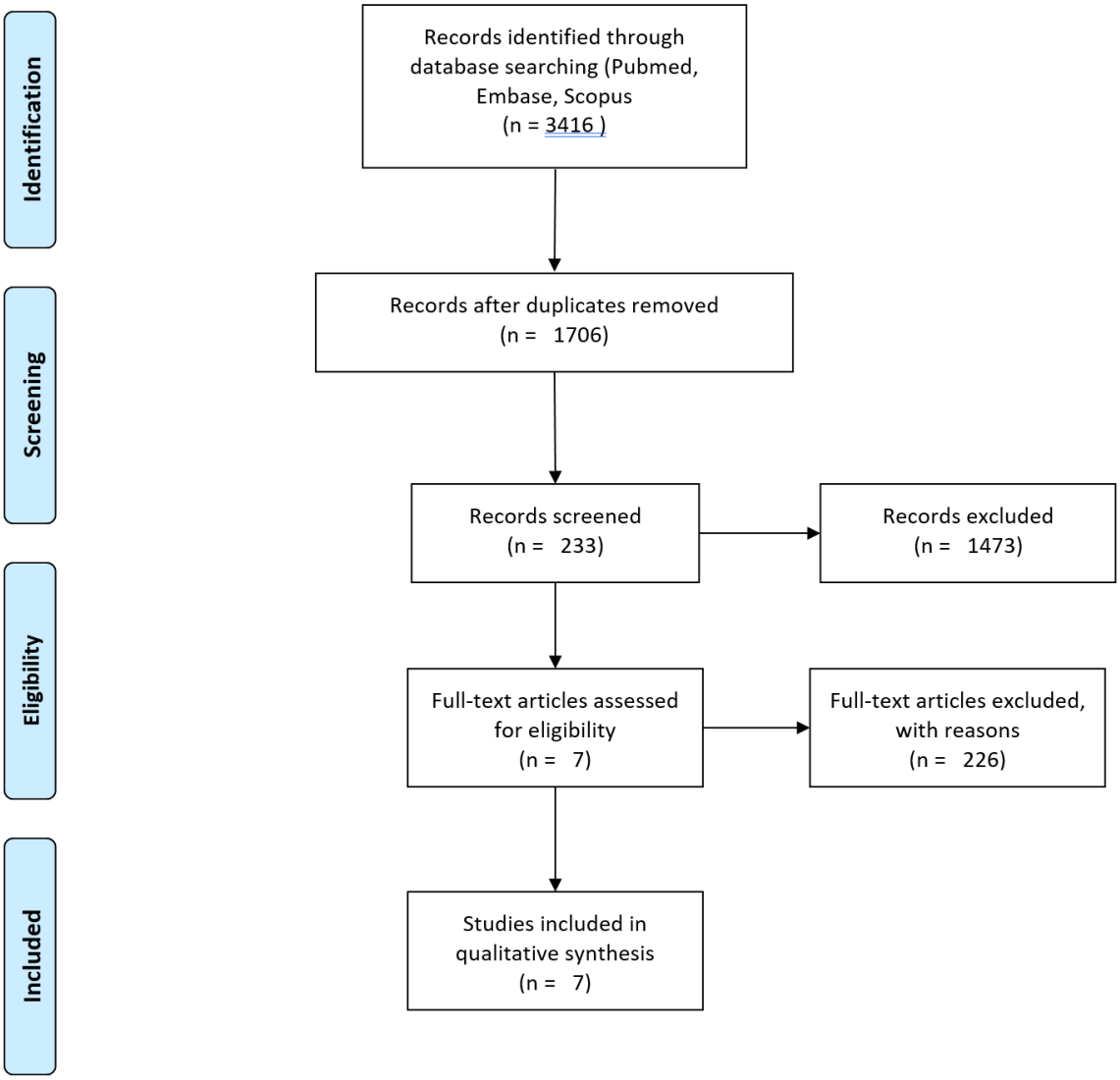

Figure 1: Preferred Reporting Item for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram for article selection

TABLE 3: Characteristics of included studies

Study Reference and Setting	Study Design	Age Group	Partici-pants included	Expo sure Asses sment	Traffic Related Pollutan-ts	Traffic related pollutants measured	Asthma Assessmen t	Transient Asthma/ Wheezing Definition	Persistent Asthma/ Wheezing Definition	Late-Onset Asthma/ Wheezing Definitio n	Adjustment Variables	CASP Comments
(Brauer et al., 2006), Utrecht, Netherlands [19]	Birth Cohort (PIAM A)	Birth – 4 years	4146	LUR Mode l	$PM_{2.5}$, NO_2	$PM_{2.5}$ mean: 16.9, range: [13.5, 25.2] $\mu g/m^3$; NO_2 mean: 25.4, range: [12.6, 58.4] $\mu g/m^3$	Parental reporting of asthma/ wheeze	Report of wheezing at age 3 but not at age 4	Report of wheezing at age 3 as well as at age 4	No report of wheezing at age 3 but wheezing reported at age 4 [a]	Sex, Study arm, allergic mother /father, mother /father's education, maternal smoking during pregnancy, breastfeedi ng at 3 months, gas stove, unvented gas water heater, siblings at birth, smoking at home, dampness in living room/child' s bedroom, pets, daycare	Pollutant levels only measured for four 2 week periods in a single year, risk of recall bias, no adjustment for familial history of asthma, race, or socio-economic status (outside of education)

											attendance, Dutch nationality, moving houses before age 8	
(Gehring et al., 2010), Utrecht, Netherlands [14]	Birth Cohort (PIAMA)	Birth – 8 years	3863	LUR Model	$PM_{2.5}$, NO_2	$PM_{2.5}$ mean: 16.9, range: [13.5, 25.2] $\mu g/m^3$; NO_2 mean: 25.4, range: [12.6, 58.4] $\mu g/m^3$	Parental report of wheezing	Report of wheezing before age 3 but no wheezing after age 6	Report of wheezing before age 3 as well as after age 6	No report of wheezing before age 3 but wheezing at age 6 or later	Sex, Study arm, allergic mother /father, mother /father's education, maternal smoking during pregnancy, breastfeeding at 3 months, gas stove, unvented gas water heater, siblings at birth, smoking at	Pollutant levels only measured for four 2 week periods in a single year, risk of recall bias, no adjustment for familial history of asthma, race, or socioeconomic status (outside of education)

											home, dampness in living room/child's bedroom, pets, daycare attendance, Dutch nationality, moving houses before age 8	
(Nordling et al., 2007), Stockholm, Sweden [25]	Birth Cohort (BAMSE)	Birth – 4 years	3515	Dispersion Model	PM_{10}, NO_X	PM_{10} mean: 3.9, 5^{th} – 95^{th} percentile: [0.94, 6.8] $\mu g/m^3$; NO_X mean: 23.1, 5^{th} – 95^{th} percentile: [4.7, 48.7] $\mu g/m^3$	Parental report of wheezing	At least 3 episodes of wheezing before age 2, but no episodes between age 3 and 4;	At least 1 wheezing episode before age 2 and at least 1 wheezing episode between age 3 and 4	No episode of wheezing before age 2, but at least 1 episode of wheezing between age 3 and 4	Municipality, socioeconomic status, heredity, mother's smoking during pregnancy and infancy, year that house was built, damp or mold in the home at birth, and sex of the child	Risk of recall bias, no adjustment for race, endpoint is early for persistent asthma diagnosis
(Oftedal et al., 2009), Oslo, Norway [26]	Birth Cohort (Oslo)	Birth – 10 years	2871	Dispersion Model	NO_2	NO_2 range: (1.4, 65.1),	Parental reporting of doctor	None	None	Onset of doctor diagnosed	Sex, parental atopy,	Risk of recall bias, no

				l (EPISODE)		mean: 25.3 µg/m^3;	diagnosed asthma/wheeze			asthma after age 4 years	maternal smoking in pregnancy, paternal education, and maternal marital status at the child's birth. Parental atopy was defined as a history of maternal or paternal asthma, hay fever, or eczema	adjustment for race nor socioeconomic status (except education and marital status)
(Pennington et al., 2018), Atlanta, Georgia, USA [27]	Birth Cohort (KAPPA)	Birth – 6 years	24 608	Dispersion Model (RLINE)	CO, $PM_{2.5}$, NO_X	CO median: 0.59 ppm; NO_x median: 55.5 ppb $PM_{2.5}$ range: (0.06, 13.8), median 1.55 µg/m^3	At least one doctor diagnosis of asthma and one asthma-related medication dispensing after the first year of life from medical records.	None	Evidence of incident asthma who also had evidence of asthma in the past year at each follow-up age up to age 5 years.	None	Sex, race, ethnicity, maternal asthma, maternal age, parental education, maternal marital status, neighborhood socioeconomic status (SES),	Results presented as absolute risk difference – difficult to interpret

											birth year, and city region.	
(Rancière et al., 2017), Paris, France [28]	Birth cohort (PARIS)	Birth – 4 years	3840	Dispersion Model (ExTra Index)	NO_x	NO_2 range: (39.0, 257.0), median: 75 µg/m^3	Parental reporting of doctor diagnosed asthma or wheezing in the past 12 months at ages 1, 2, 3, 4	Wheezing occurring between 0 and 2 years of age and not till age 4	Wheezing occurring between 0 and 2 years of age and persisting till age 4.	Wheezing occurring between 2 and 4 years of age	Sex, birth weight, family socioeconomic status, maternal education level, maternal history of asthma, allergic rhinitis, or eczema, paternal history of asthma, allergic rhinitis, or eczema, maternal smoking during pregnancy, exposure to environmental tobacco smoke at home during the first year,	Potential for recall bias, no adjustment for race, endpoint is early for persistent wheezing diagnosis

											exclusive breastfeeding during the first 3 months, type of child care during the first 6 months, stressful family events during the first 2 years, body mass index ≥ 85th percentile for age and sex at 2–3 years, use of gas for cooking or heating in the home, and visible mold in the home	
(Sbihi et al., 2017), Vancouver, British Columbia, Canada [4]	Birth cohort	Birth – 10 years	68 195	LUR Model	NO_2, $PM_{2.5}$	NO_2 range: (15.0, 53.7), median: 33.3 $\mu g/m^3$;	At least two primary care physician diagnoses within a	Asthma definition is met by age 1 with asthma prevalence peaking	Asthma develops by age 3 with asthma prevalence peaking	Asthma develops by age 3 with asthma prevalence peaking	Sex, parity, breastfeeding initiation, birth weight, delivery	Did not adjust for familial history of asthma, race, ethnicity

						$PM_{2.5}$ range: (3.2, 7.6), median: 5.4 $\mu g/m^3$	12-month period or a minimum of one hospital admission were identified as asthma cases each year.	among the group by age 2, and no asthma activity after age 6.[b]	among the group by age 4 that is sustained until the end of follow-up.[b]	among the group by age 6 and is sustained until the end of follow-up.[b]	mode, maternal smoking and educational attainment, and household income	Odds ratios reported only to 1 decimal place, only study to find an association between TRAP and late-onset asthma

[a] No result on the association between pollutant exposure and late-onset asthma phenotype was reported.

[b] Asthma phenotypes were defined based on group based trajectory modelling.

3.3 Quality Assessment of Studies. All included studies were considered to be of sufficient quality for inclusion. The most common limitations identified from the CASP checklist were the potential for recall bias in studies where outcomes were reported via questionnaire, and not adjusting for potential confounders.

3.4 Effect of CO on Childhood Asthma Phenotype Development. The sole study which measured CO exposure measured only persistent childhood asthma by age 5 as a phenotypic outcome [27]. Prenatal exposure to CO was associated with an absolute risk increase of 3.5% for persistent asthma at age 5. Exposure to CO during the 1st year of life was associated with an absolute risk increase of 3.9% for persistent asthma at age 5.

3.5 Effect of NO_2 on Childhood Asthma Phenotype Development. Of the four studies which measured the association between NO_2 and the development of childhood asthma phenotypes, two reported associations for transient, persistent, and late-onset asthma/wheeze, one reported associations for transient and persistent wheeze, and one study reported solely the association for late-onset asthma [4, 14, 19, 26]. Two studies, reported results from an identical study cohort (PIAMA) [14, 19].

Two of the studies which listed transient asthma/wheezing as an outcome found a significant association between NO_2 exposure and transient wheezing [4, 19], with the third study reporting no significant association [14]. However, a significant association was reported by Sbihi *et al.* only when the second and fourth exposure quartiles were compared to the lowest quartile. No association was observed between NO_2 exposure and transient wheezing when the third exposure quartile was compared to the lowest quartile [4].

Of the three studies which reported persistent asthma/wheezing, two studies reported no association between NO_2 and persistent wheezing [14, 19]. The third study reported significant associations for the second and third exposure quartiles compared to the reference quartile; but the highest exposure quartile was not associated with persistent asthma [4].

Two of three studies found no association between NO_2 and late-onset asthma/wheezing [14, 26]. Significant associations for the second and third exposure quartiles with late-onset asthma were reported by the third study, but the highest exposure quartile was not associated with late-onset asthma [4].

3.6 Effect of NO_x on Childhood Asthma Phenotype Development. Among the three studies which measured the association between NO_x exposure and the development of childhood asthma phenotypes, two studies studied transient wheezing, persistent wheezing, and late-onset wheezing as outcomes [25, 28]. One study studied solely persistent asthma [27].

Pennington *et al*. found prenatal exposure to NO_x was associated with an absolute risk increase of 3.8% for persistent asthma at age 5, while exposure to NO_x during the 1st year of life was associated with an absolute risk increase of 4.0% for persistent asthma [27].

Both studies which reported odds ratios for phenotypic outcomes found a significant association between NO_x exposure and persistent wheezing, with no association found with NO_x and either transient nor late-onset wheezing [25, 28].

3.7 Effect of $PM_{2.5}$ on Childhood Asthma Phenotype Development. Among the four studies which measured the association between $PM_{2.5}$ exposure and the development of childhood asthma phenotypes, two studies studied transient wheezing, persistent wheezing, and late-onset asthma or

wheezing as outcomes [4, 14]. One study reported associations for transient and persistent wheeze [19]. The final study contained solely persistent asthma as a phenotypic outcome [27]. Two studies, Brauer *et al.* and Gehring *et al.*, reported results from an identical study cohort (PIAMA) [14, 19]. Pennington *et al*. found prenatal exposure to $PM_{2.5}$ was associated with an absolute risk increase of 4.4% for persistent asthma at age 5 [27]. Exposure to $PM_{2.5}$ during the 1st year of life was associated with an absolute risk increase of 4.5% for persistent asthma at age 5.

Among the three studies which reported associations between $PM_{2.5}$ and transient asthma or wheezing, two reported a significant association [14, 19]. The third, Sbihi *et al*. reported a significant association between transient asthma and the second and third exposure quartiles [4]. However, the highest exposure quartile was not associated with transient asthma.

Two of three studies found no association between $PM_{2.5}$ exposure and persistent wheezing or asthma phenotype [14, 19]. Sbihi *et al*. found that the second exposure quartile of $PM_{2.5}$ was associated with persistent asthma, but there was no association between $PM_{2.5}$ and the third and fourth quartiles [4].

Gehring *et al.* reported an association between $PM_{2.5}$ and late-onset wheezing [14]. Sbihi *et al*. also reported that the second and third exposure quartiles were associated with late-onset asthma, although there was no association with the highest exposure quartile [4].

3.8 Effect of PM_{10} on Childhood Asthma Phenotype Development. Nordling *et al.* reported the association between $PM_{2.5}$ exposure and the development of transient wheezing, persistent wheezing, and late-onset wheezing as outcomes [25]. No significant association was found between PM_{10} and any of the three wheezing phenotypes.

TABLE 4**:** Effect of TRAP and Childhood Asthma Phenotypes in Included Studies.

Study Reference and Setting	Traffic Related Pollutant	Transient Asthma/Wheezing [a]	Persistent Asthma/Wheezing [a]	Late-onset Asthma/Wheezing [a]
Brauer et al., 2006 (Total n = 4146; Pollutant Exposure n = 2588) [19]	$PM_{2.5}$	**OR: 1.16, 95% (CI: 1.00 - 1.34 per 4.4 µg/m^3 increase of $PM_{2.5}$**	OR: 1.19 (95% CI: 0.96 - 1.48) per 3.3 µg/m^3 increase of $PM_{2.5}$	None
	NO_2	**OR: 1.13 (95% CI: 1.00 - 1.28) per 10.6 µg/m^3 increase of NO2**	OR: 1.13 (95% CI: 0.99 - 1.29) per 10.4 µg/m^3 increase of NO_2	None
Gehring et al., 2010 (n = 3863; NO_2 n = 2668) [14]	$PM_{2.5}$	**OR: 1.29 (95% CI: 1.04 - 1.62) per 3.2 µg/m^3 increase of $PM_{2.5}$**	OR: 1.37, 95% CI: 0.99 - 1.91 per 3.2 µg/m^3 increase of $PM_{2.5}$	**OR: 1.18, 95% CI: 1.01 - 1.37 per 3.2 µg/m^3 increase of $PM_{2.5}$**
	NO_2	OR: 1.17 (95% CI: 0.97 - 1.41) per 10.4 µg/m^3 increase of NO2	OR: 1.30 95% (CI: 0.99 - 1.72) per 10.4 µg/m^3 increase of NO2	OR: 1.13 95% (CI: 0.99 - 1.35) per 10.4 µg/m^3 increase of NO2
Nordling et al., 2007 (n = 3515) [25]	PM_{10}	OR: 0.90 (95% CI: 0.45 - 1.81) per 6 µg/m^3 increase of $PM_{2.5}$	OR: 1.64 (95% CI: 0.90 - 3.00) per 3 µg/m^3 increase of NO_x	OR: 0.94 (95% CI: 0.42 - 2.11) per 6 µg/m^3 increase of $PM_{2.5}$
	NO_X	OR: 0.82 (95% CI: 0.48 - 1.40) per 44 µg/m^3 increase of NO_x	**OR: 1.60, 95% (CI: 1.09 - 2.36) per 44 µg/m^3 increase of NO_x**	OR: 0.87, 95% (CI: 0.47 - 1.60) per 44 µg/m^3 increase of NO_x
Oftedal et al., 2009 (n= 2871, NO_2 n = 2329) [26]	NO_2	None	None	OR: 1.05 (95% CI: 0.64 - 1.72) per 27.3 µg/m^3 increase of NO2
Pennington et al., 2018 (Total n = 24 608; prenatal exposure n = 6795; 1st year of life n = 7755) [27]	CO	None	**Prenatal exposure absolute risk increase: 3.5% (95% CI: 1.5%, 6.2%) per 2.7-fold increase CO** **Age 1 exposure absolute risk increase: 3.9% (95% CI: 1.5%, 6.2%) per 2.7-fold increase CO**	None

	$PM_{2.5}$	None	**Prenatal exposure absolute risk increase: 4.4% (95% CI: 2.3%, 6.4%) per 2.7-fold increase $PM_{2.5}$** **Age 1 exposure absolute risk increase: 4.5% (95% CI: 2.3%, 6.6%) per 2.7-fold increase CO**	None
	NO_x	None	**Prenatal exposure absolute risk increase: 3.8% (95% CI: 1.7%, 5.9%) per 2.7-fold increase NO_x** **Age 1 exposure absolute risk increase: 4.0% (95% CI: 1.8%, 6.1%) per 2.7-fold increase NO_x**	None
Rancière et al., 2017 (n = 3840, NO_x n = 698) [28]	NO_x	OR: 1.03, 95% CI: 0.91 - 1.17 per 26 µg/m^3 increase of NO_2 equivalent	**OR: 1.27, 95% CI: (1.09 - 1.47) per 26 µg/m^3 increase of NO_2 equivalent**	OR: 1.19, (95% CI: 0.89 - 1.33) per 26 µg/m^3 increase of NO2 equivalent
Sbihi et al., 2017 (n = 68 195, NO_2 n = 68 024) [4 b]	NO_2	**1 vs 0 OR: 1.10 (95% CI: 1.0 - 1.3)** 2 vs 0 OR: 1.04 (95% CI: 0.9 - 1.2) **(3 vs 0 OR: 1.10 (95% CI: 1.0 - 1.3)**	**1 vs 0 OR: 1.42 (95% CI: 1.1 - 1.8)** **2 vs 0 OR: 1.20 (95% CI: 1.0 - 1.5)** 3 vs 0 OR: 1.05 (95% CI: 0.9 - 1.2)	**1 vs 0 OR: 1.18 (95% CI: 1.0 - 1.4)** **2 vs 0 OR: 1.29 (95% CI: 1.1 - 1.5).** 3 vs 0 OR: 1.00 (95% CI: 0.8 - 1.2).
	$PM_{2.5}$	**1 vs 0 OR: 1.15 (95% CI: 1.0 - 1.3)**	**1 vs 0 OR: 1.17 (95% CI: 1.0 - 1.4)**	**1 vs 0 OR: 1.13, 95% CI: 1.0 - 1.3**

		2 vs 0 OR: 1.21 (95% CI: 1.1 - 1.4) 3 vs 0 OR: 1.05 (95% CI: 0.9 - 1.2)	2 vs 0 OR: 1.0 (95% CI: 0.8 - 1.2) 3 vs 0 OR: 0.86 (95% CI: 0.7 - 1.1)	**2 vs 0 OR: 1.25, 95% CI: 1.1 - 1.5** 3 vs 0 OR: 0.97, 95% CI: 0.8 - 1.1)

[a] Results in boldface indicate significant results at the 95% confidence level.

[b] Reported ORs compared higher exposure quartiles (groups 1, 2, and 3) to the lowest exposure quartile (reference group = 0). NO_2 exposure ranged from 15 to 53.7 µg/m^3, $PM_{2.5,}$ exposure ranged from 3.2 to 7.6 µg/m^3

4. Discussion

Although previous studies have looked at TRAP and the onset of childhood asthma, to our knowledge this is the first attempt to systematically evaluate the available literature on the effect of TRAP exposure with the development of childhood asthma or wheezing phenotypes. 7 studies were included for final analysis. The results suggest that TRAP is associated with the development of childhood transient and persistent asthma/wheezing phenotypes but may not be associated with late-onset asthma/wheezing. Nevertheless, the significance of these associations is inconsistent among the included studies and any interpretation of the results should be drawn cautiously. Stratifying studies by pollutant reduced the number of eligible studies per pollutant and made a meta-analysis unfeasible.

Early childhood exposure to TRAP has significant impacts on the development of lungs [16]. Starting from the early postnatal period till about 1.5 years of age, bulk alveolar formation in the lungs leads to substantial structural remodeling of the lung parenchyma [29, 30]. Microvascular maturation in the lungs also occurs starting from the early postnatal period till about 2-3 years of age. Damage to the lungs during the developmental periods has been associated with the development of long-term sequelae [31-33]. Additionally, compared to adults, children are more likely to be outdoors and active, have a higher ventilation rate, and are more likely to inhale pollutants into the distal lung, with accordingly higher exposure to TRAP [34].

NO_2, $PM_{2.5}$, and PM_{10} have been reported to be the primary constituents of TRAP [35], and long-term exposure to these pollutants in mice has shown to lead to elevated levels of interleukin-6, a pro-inflammatory cytokine associated with inflammation and pulmonary diseases such as asthma [35-38]. TRAP exposure has also been associated with elevated expression of the Clca3 gene [35]. In animal models, expression of Clca3 has led to mucous cell metaplasia and airway

hyperreactivity, leading to the development of episodic recurrent airway obstruction [39-42]. As the mucous cell metaplasia developed, it was observed that Muc5ac was the primary airway mucin expressed, which is also characteristic of human asthma [38, 39]. Consequently, it has been suggested that the association of TRAP with asthma onset may be due to the expression of Clca3 [35].

Genetic factors may also, in part, explain the heterogeneity in asthma and wheezing phenotype results presented in this review. Among children exposed to NO_2, those with either a GSTP1 rs1138272 or rs1695 single nucleotide polymorphism (SNP) were found to the be at an increased risk for asthma in a study combining multiple birth cohorts [44]. Additionally, high exposure to diesel exhaust particles (DEP) in children with the GST-P1 Val^{105} polymorphism was associated with a high risk of persistent wheezing [45]. Thus, differing genotypes among those exposed to TRAP may lead to differences in asthma or wheezing phenotypic outcomes.

Childhood asthma is a complex disease which involves many genetic and environmental factors, as well as interplay between these factors. Male children are at higher risk of childhood asthma than females, although this is reversed after puberty [46-49]. Male sex has also been shown to modify the association between prenatal $PM_{2.5}$ exposure and childhood asthma onset [50]. It is uncertain whether similar interactions between sex and other forms of TRAP exist for childhood asthma onset. Exposure to other allergens such as mites is also associated with childhood asthma and can modify the risk of childhood asthma associated with TRAP [51]. Other environmental exposures such as prenatal smoke exposure, home dampness, and prenatal acetaminophen use can modify the association of genetic risk factors with childhood asthma onset [52-54].

Several limitations of this systematic review must be acknowledged. Firstly, the low number of eligible studies makes it difficult to draw any firm conclusions. Given that we assessed each

pollutant separately, the number of studies per pollutant was reduced even further. Secondly, the heterogeneity in phenotype definitions and in study follow-up length may be a source of bias. The original phenotypic definitions from the Tucson Children's Respiratory Study measured outcomes at ages 3 and 6 to define wheezing phenotype [5]. Given the differing follow-up length across the available studies, a child's phenotypic classification may differ between studies based on the definition used. A child with wheezing at ages 3 and 4 but not at age 6 would be classified as transient wheeze by Gehring *et al.,* but would be persistent wheeze under Nordling *et al.*, as follow-up ends at age 4. It is therefore important to account for these differences in phenotype definition between studies. Finally, all but two studies used parental reporting of wheezing or asthma symptoms via questionnaire response to report asthma or wheezing in children. Although standard, this may lead to recall bias in the results. These limitations suggest that further studies studying TRAP exposure and the onset of childhood asthma and wheezing phenotypes are warranted.

5. Conclusion

Based on the results of this systematic review, there is evidence to suggest an association between TRAP exposure and transient as well as persistent childhood asthma/wheezing phenotypes. Conversely, TRAP may not be associated with late onset asthma/wheezing phenotype. However, results remain inconsistent among different studies. The low number of studies per pollutant, as well as the heterogeneity in study methods such as follow-up length and in the phenotypic definitions of asthma and wheezing used, indicate the need for further studies on this topic.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Chapter 3: The National Longitudinal Survey of Children and Youths (NLSCY)

Background and Objectives of the NLSCY

This chapter describes the methodology behind the National Longitudinal Survey of Children and Youths (NSLCY), which is the primary dataset used for this study. The NLSCY is one of the first long-term cohort studies to follow the development and well-being of Canadian children from birth to early adulthood and is conducted by Statistics Canada [1]. It is a biennial survey designed to collect information about the factors affecting social, emotional, behavioral development of children [1]. These factors cover many different aspects of a child's life, from their health, physical development, social and home environment (such as friends and family), learning, and behavior [1].

In addition to collecting information on the various factors affecting the development of Canadian Children, the NLSCY fulfills several other objectives. These are:

1) To monitor the impact and prevalence of the aforementioned factors, life events, and protective factors of youth [1];
2) To understand how these risk factors, life events, and protective factors affect child and youth development [1];
3) To use the data gathered by the NLSCY to help develop meaningful policies and programs for children and youths [1];
4) To ensure that the lack of information regarding the experiences and characteristics of Canadian children, particularly young ones, is properly addressed [1];

To gather data on many different aspects of Canadian children, incorporating information from home, school, and the wider community as well as other biological, social and economic information [1].

Definitions

This section defines some of the concepts crucial to the NLSCY.

The **Adult component** of the NLSCY is created to gather information for the Person Most Knowledgeable (PMK) about a child and their spouse/partner. It is equivalent to the **Parent questionnaire** in Cycles 1 – 3 [1,2].

The **Child component** was created to gather information for each selected child between 0 – 11 for cycle 1, and age 0 – 17 by cycle 5. This was generally answered by the PMK of the child. It is equivalent to the **Child questionnaire** in cycles 1 – 3 [1,2].

The **Youth component** was created to gather information for 16 – 19 year old adolescents. This was completed by the adolescent rather than the PMK. This component was introduced in cycle 4 as the **Adolescent component** [2,3]**.**

The **Person Most Knowledgeable (PMK)** refers to the person within a NLSCY household who was deemed to be the person most knowledgeable about the selected child [1]. The PMK was determined via response to a question and was responsible for providing information about the selected children, spouse or partner, and himself or herself [1]. This was usually the biological mother of the child [1].

A **cross-sectional respondent** is a child for whom the Adult or Child component of the NLSCY has been completed. These respondents represent the population at the time of data collection [2].

A **longitudinal respondent** is a child who was introduced in a previous cycle and for whom either the Adult, Child, or Youth component has been completed [2]. Although the NLSCY contains longitudinal cohorts which began in cycle 2, 3 and 4, for our purposes, this term will refer to the children who were introduced in NLSCY cycle 1 (1994/1995) [2].

A **respondent household** is a household in which an Adult, Child, or Youth component has been completed [2].

A **respondent child** is a child who has completed Adult, Child, or Youth component [2].

An **economic family** is defined as families with all members being related either by blood, marriage, common-law, or adoption [2].

Sample

The focus of the NLSCY was to survey a representative sample of Canadian children, and to monitor these children into adulthood [1]. The NLSCY consists of several cohorts. These include:

1) The original longitudinal cohort consisting of 22 831 children aged 0 – 11 years as of December 31, 1994 and who were living in any province at the time of the initial data collection (Cycle 1) [1]. These children were selected from the Labour Force Survey (LFS), and data collection occurred between fall of 1994 and spring of 1995 [1]. Data from this birth cohort was then collected again every two years for 8 cycles [4]. By cycle

8 (December 2008), the children in this birth cohort would be between ages 14 – 25 [4]. Only children from the initial cycle 1 cohort who responded in cycle 2 were included [5]. This meant that only 16 903 children were included in this cohort [5]. The number of respondents for the longitudinal cohort were: Cycle 1 – 16 903, Cycle 2 – 15 468 (91.5%), Cycle 3 – 14 997 (88.7%), Cycle 4 – 13 310 (78.7%), Cycle 5 – 12 523 (74.1%), all cycles until cycle 5: 11 136 (65.9%) [2]. Longitudinal sampling weights for this cohort were produced for each cycle [4]. The longitudinal cohort up to Cycle 5 was analyzed for Chapter 4.

2) Longitudinal early childhood development (ECD) cohorts which were based on subsequent cycles [2]. These cohorts include children who are aged 0 – 1 at the time of the data collection and who live in any province [1]. For instance, the Cycle 2 longitudinal cohort included children aged 0 – 1 as of December 31, 1996 [3]. In contrast to the Cycle 1 longitudinal cohort, these children will be followed only for two or three subsequent cycles [2–6].
3) A cross-sectional cohort for each NLSCY Cycle (every 2 years) following cycle 1 [2-6]. These cross-sectional cohorts included a combination of children from previous cycles as well as the current cycle [2-6]. For example, the cross-sectional cohort for NLSCY cycle 3 combines the ECD cohort of children aged 0 – 1 selected for cycle 3, children aged 2 – 3 years old returning from the age 0 -1 cohort from cycle 2, 5 year old children selected from the birth registry, and 4 – 15 year old children and youths from the original longitudinal cohort in cycle 1 [6].

Data Collection

Data collection for the original NLSCY cycle 1 longitudinal cohort occurred between spring of 1994 and fall of 1995 [1]. Data from subsequent cycles was collected every 2 years afterwards [1]. NLSCY Data collection occurred in two parts: the Household Collection and the School Collection [1].

The first step for the Household Collection involved a knowledgeable person in each household fulfilling a household roster, which asked for basic demographic and dwelling information [1]. This household roster was then used to derive a relationship grid which established the relationships of each individual in a household compared to all other household individuals and to derive family related variables (single or double parent household, stepfamily, etc.) [1]. Following this, one child aged 0 – 11 was selected in each household, and a question asking for the Person Most Knowledgeable about the child was sent [1]. This individual was designated the PMK and was usually the mother of the child [1].

Information for each child was then collected via three separate questionnaires [1]. The first questionnaire was the Parent Questionnaire, which gathered information on the PMK and their spouse (if applicable), as well as information on the neighborhood and social environment of the child [1]. The second questionnaire was the General Questionnaire, which collected socio-economic information for each household (such as income and education) [1]. The final questionnaire was the Child Questionnaire, which gathered information on up to a maximum of four children in each household [1]. The designated PMK responded to this questionnaire [1]. Other aspects of the Household Collection data collection included a verbal test of children aged 4 – 5 years old used to assess whether literacy affected a child's responses, a questionnaire which

was answered by children aged 10 – 11 years, and neighborhood observation by an interviewer, wherein the interviewer would collect data on their perception of the neighborhood of the selected household [1].

In addition to the Household Collection, the PMK was asked to give written permission for interviewers to collect information from teachers and principal of each selected child's school for the School Collection [1]. This part of the NLSCY consisted of three questionnaires. The first questionnaire was a Math Computation Test, designed to assess mathematical academic achievement for children in Grade 2 or higher [1]. The second questionnaire was the Teacher's Questionnaire, which gathered information on a child's academic achievement, behavior at school as well as information on the teacher's teaching practices and the child's class [1]. The final questionnaire was the Principal's Questionnaire, which collected information on school information and policies, but not about the individual child [1].

Computer-assisted personal interviewing (CAPI) technology was a crucial component of data collection and consisted of both Case Management and a survey specific section [1]. Case Management refers to the case assignment and transmission of survey data and was involved in recording information in terms of contacting respondents and encryption of transmitted data [1]. The survey-specific part helps generate the appropriate questions based on household composition and selection procedures [1]. Using CAPI allows for the collection of high-quality data. For instance, the aforementioned relationship grid of all household members was collected by CAPI, which allowed for a detail analysis of the household family structure [1].

Initially, there were four periods of data collection for the Household Collection: November 1994, December 1994, February 1995, and March 1995 [1]. However, due to the lower than expected response rate, households which did not respond in earlier data collection periods

(November, and December) were contacted again in an attempt to collect information [1]. Further attempts were made in June 1995 to collect information once more from households which did not respond to improve data response [1].

The school collection occurred between March and June 1995 [1]. Questionnaires were mailed to teachers and principals with instructions on how to complete the questionnaires [1]. Postcards were sent one week afterwards, to thank respondents and remind those who had not responded to complete the questionnaire [1]. Two weeks afterwards, a second set of questionnaires was sent to teachers and principals who had not yet responded [1]. Finally, principals and teachers who had yet to responded were contacted by telephone to encourage them to participate [1]. School collection for households with information collected during the June 1995 follow-up was not performed, as the school year would be almost over [1].

Similar procedures for data collection were used in subsequent NLSCY Cycles [2–6].

Interviewing

All interviews were conducted via telephone conversation using computer assisted interviewing or face-to-face, save for the age 10 – 11 questionnaire [1]. Interviews were conducted by LFS interviewers, who were supervised by senior interviewers [1]. Senior interviewers were responsible for ensuring that NLSCY interviewers were familiar with the procedures of the survey as was as monitoring interviewers and reviewing completed documents [1]. They were also responsible for follow-up action for refusals and non-responses [1]. Senior interviewers are supervised by LFS program managers, who are based in the regional offices of Statistics Canada [1].

Median interview time for responding households was 119 minutes for all questionnaires [1]. The child questionnaire required a median of 44 minutes, and all major components (child, parents, general, vocabulary test), required a median interview time of 74 minutes [1]. The most important factor in determining median interview time was the number of selected children for each household [1]. Households with four selected children required a median interview time of 200 minutes, and extreme cases required over four hours [1]. Due to this, in subsequent cycles, a maximum of two children per household were interviewed to reduce the interview time needed [1].

Data Processing

There were many processing steps to create the final clean NLSCY microdata file which is accessed by researchers. This section discusses some of the steps involved in data processing.

Despite the efforts of Statistics Canada, information was not collected for all selected households. Reasons for this include being unable to contact a household during the collection period, a household refusing to participate, or special circumstances such as illness or recent death in a family [1]. Such households were removed and sampling weights for other households were adjusted to compensate for removed households [1]. This procedure is discussed further in detail in the next section on sample weighting.

In many cases, an interview was carried out, but data collection was incomplete. One such reason for incomplete data collection include households only willing to be interviewed for a certain amount of time [1]. Another reason is that a household may have agreed to continue the interview in the future, but the interviewer was unable to contact the household [1]. To address this, criteria to assess these “partial” interviews was necessary [1]. Assessing the data quality was

done by looking at whether data had been collected for 7 to 8 “key” questions for at least one child in each household [1]. Households with children which met these criteria were deemed to have adequate information and were remained in the survey sample [1]. Variables with missing information were either left as “not stated” or imputed [1]. Households without adequate information for at least one child were dropped from the survey sample and were considered as non-response households [1]. In total, 22 746 child records from 13 439 were deemed to have adequate information and remained in the survey sample, while 140 child records were removed due to a lack of information [1].

In the data processing stage, surveys underwent both pre-editing and consistency editing [1]. Pre-editing consisted of formatting and preliminary editing [1]. For example, different wording would be used for different age groups [1]. A question for households with children aged 0 – 2 asked “How easy is it for you to know what’s bothering him/her when he/she cries or fusses?”; whereas for households with children who were aged 3, this question was “How easy is it for you to know what’s bothering him/her when he/she is irritable [1]?” Such questions were combined into a single variable during the pre-editing process [1]. Consistency editing occurred after pre-editing and verified the relationship between multiple variables [1]. For example, among children who immigrated to Canada, the year in which they immigrated to Canada was compared to the year of birth for the child [1]. If the immigration year was before the year of birth, then the year of immigration would be set to not-stated [1].

Variables were labelled with an alphanumeric code in the data processing stage so that the dataset could be more easily used with statistical software such as SPSS and SAS [1]. Variables names were a maximum of eight characters, and were formatted as following:

“A SE C Q nnx”

Wherein

1) “A” refers to the cycle – A refers to cycle 1, B refers to cycle 2, etc [1].
2) SE refers to the section of the questionnaire under which the question was asked from which the variable was derived [1]. For instance “SD” would refer to a variable derived under the socio-demographics section of the NLSCY [1].
3) C refers to the unit to which the variable references. For instance, C would indicate a variable is based on the child, while P would refer to a variable based on the PMK [1].
4) Q refers to the variable type [1]. For example, Q refers to a question that was directly asked on the NLSCY, while D refers to a variable which was derived from questions asked on the NLSCY [1].
5) Nnx identifies the question or variable [1]. Nn is a sequential number assigned to the variable, while x is an alphabetical indicator used to distinguish variables of a similar type [1].

In cases where a question or variable had no response, different codes were used to describe the situation [1]. The first was “Refusal”, which indicates a respondent refused to properly answer a specific question [1]. The second was “Don’t Know”, indicating a respondent did not know the answer to a particular question [1]. The third was “Not applicable”, which was used in cases where a question was not applicable to the respondent [1]. For some respondents, this was restricted to specific questions or a series of questions, while in other cases, an entire section of a question, or even an entire questionnaire itself was not applicable [1]. The fourth and final code was “Not-stated” which was used when the answer to an item was unknown [1]. This could occur due to 1) skipping parts of the questionnaire which were deemed sensitive and to which the respondent would likely not answer, 2) premature ending while responding to a specific

questionnaire, which could be due to an interruption or the respondent deciding to terminate the interview, or 3) due to consistency edits, where inconsistent values would be coded as not-stated [1].

Weighting

As the NLSCY is a survey which aims to gather information on a representative sample, each person included in the survey "represents" other persons not selected in the survey sample. For a simplified example, if 2% of the population were sampled, each sampled person would represent 50 other persons from the overall population [1]. The number of persons in the population which each sampled respondent represents is calculated in the weighting phase. These weights are present in final NLSCY microdata file and must be incorporated into any statistical procedures to receive accurate and meaningful estimates [1]. As the NLSCY contains both cross-sectional and longitudinal cohorts, separate sampling weights were calculated for each cohort. The derivation of cross-sectional weights follows the LFS weighting procedure closely, as the NLSCY is based on the LFS [1].

The subweight attached to each respondent of the NLSCY is the product of four parts: the basic weight, the cluster sub-weight, the balancing factor for non-response, and the rural-urban factor [1]. The basic weight is determined by the sample design and is calculated by taking the inverse of the probability that a respondent is selected [1]. In the aforementioned example wherein 2% of the population was, the probability would be 0.02, and thus the basic weight would be 1/0.02 = 50 [1]. Cluster subweights refers to derived weights due to sub-sampling of dwelling clusters [1]. This occurs since interviewers are assigned clusters of dwellings to sample from [1]. However, significant population growth in dwelling clusters can lead to an excessive workload for

interviewers [1]. Dwelling clusters are therefore sub-sampled to ensure interviewers are not overwhelmed and this is used to derive the cluster sub-weight [1].

Non-response is inevitable in a large sample survey, which remains true for the NLSCY. In certain cases of non-response, data from prior interviews will be carried forward and used as the data for the current month [1]. However, an alternate method is to proportionally increase the weights of participating households to compensate for non-responses [1]. Weights of participating households are increased by the following ratio:

$$\frac{\#\ of\ households\ to\ be\ interviewed}{\#of\ households\ actually\ interviewed}$$

This weighting adjustment is calculated separately for each geographic area [1]. The balancing factor for non-response adjustment assumes the responding households are representative of the non-responding households [1]. The final weighting adjustment is the rural-urban factor, which accounts for either under or over-representation of selected primary sampling units (PSUs) when compared to actual rural or urban census counts [1]. In sum, the product of these four weighting factors is the subweight of a household, with each member of a household having the same subweight [1].

Following this, further adjustments to LFS derived weights were made for the NLSCY [1]. Firstly, the LFS contains 6 groups of households which are “rotated” in over time for data collection [1]. For the NLSCY however, there are 6 groups used in all provinces except Ontario and Alberta, which used 5 [1]. Thus, a correction was made for the number of rotation groups [1]. Secondly, approximately three months elapsed between sample selection and data collection [1]. This led to households without children and which were not eligible during the sample selection becoming eligible during data collection or vice versa, which in turn required a

correction of the subweight [1]. Third, certain households contained children who belonged to multiple economic families [1]. While only one of the economic families was chosen for data collection, an adjustment was made to compensate for the economic family which was not selected [1].

Fourth, as previously mentioned, the maximum number of children aged 0 – 11 selected per household was four [1]. However, it was necessary to account and correct for households with more than four children aged between 0 –11 [1]. Fifth, as previously stated, household non-responses were compensated for by responding households during the weighting process [1]. Sixth, the sub-weights after adjustment for the first five factors underwent post-stratification to ensure population estimates matched those of the provincial and national demographic estimates for children aged 0 – 11 [1]. Children were post-stratified by province, age group, sex of child, and census metropolitan area [1]. Finally, since the NLSCY sample was derived from multiple sources or sampling frames (such as the LFS and National Public Health Survey), it was necessary to adjust for the relative contributions of each sampling frame [1].

Longitudinal weights were adjusted in the following manner: Firstly, the initial weight was determined, which for NLSCY cycle 5 was based on the longitudinal weight in cycle 4 to account for attrition between cycles 1 – 4 [1]. Weights were then adjusted for household non-response and post-stratification [1].

The NLSCY population consists of only a sample of the overall population, which can cause sampling variance in the derived estimates [2] The sampling variance can change depending on the sample which is used for an estimate, but is used to describe the quality of the estimate [2]. Due to the complex design and heterogenous population of the NLSCY, the Bootstrap method was used to estimate the sampling variance [2]. This method involves repeatedly subsampling

the full NLSCY sample in a manner which reflects the overall design of the full sample [2]. Survey weights for each subsample are then calculated in the same manner as for the full sample (as previously described), which can then be used to calculate estimates for each variable in each subsample [2]. The variance among these subsample estimates is then used to estimate the sampling variance in the overall sample [2]. The NLSCY contains 1000 bootstrap weight estimates to account for sampling variance [2].

Variables

The NLSCY was designed to gather information on a wide range of factors which play a role in child development. A wide-ranging and multidisciplinary approach was taken to ensure the NLSCY addressed as many of the relevant factors affecting child development as possible [1]. Advice and input were gathered from 1) the NLSCY expert advisory group, which consists of child development and social science researchers, 2) relevant federal departments, and 3) provincial and territorial representatives who were responsible for their respective regions' child development programs [1]. This led to the NLSCY encompassing a broad set of characteristics and factors affecting child development and growth - including information about the child, their parents, family, neighbourhood, school and school experiences [1].

The household component gathered basic demographic information for each household member, as well as relationship information for each person relative to everyone else in the household [1]. Information from this component was used to create a relationship grid for each household and to describe each child's family situation [1]. Extensive editing was performed on this set of data – for example, ensuring that a birth parent was at least 12 years but not more than 55 years older

than a birth child. This section discusses variables from the adult and child questionnaires, as these were the sections used for Chapter 5.

The adult questionnaire contained multiple sections focusing on the PMK and spouse or partner. The first was the Education section, which focused on years of completed schooling, educational attainment, and whether the PMK or spouse/partner were currently attending an educational institution [1]. The second section was the Labour Force section, which included questions on current or recent occupation, periods of absence from work, hours worked and work arrangements the previous year [1]. The Income section focused on income sources and income collected for each household, as well as perceptions of financial stability [1]. This data was also used to create derived variables to classify a family as low income or otherwise [1]. The Adult Health section asked about overall health, chronic conditions such as asthma or high blood pressure, whether activities are restricted at home, school or work, pregnancy history, drinking, smoking, and also included a depression scale [1]. Other sections of the Adult component gathered information on family functioning, safety and satisfaction with the neighbourhood, social support from friends, family, and other community members, and sociodemographic characteristics such as immigration, ethnicity, and religious affiliation for each household [1].

As the primary focus of the NLSCY, the Child questionnaire was extensive and gathered a wide range of information regarding each child. The Education (Child) section gathered information about each child's educational experience, which included grade level, type of school, language of instruction, the child's perception of going to school and more [1]. Older children were also asked about other aspects such as academic achievement and special education [1]. The Health (Child) section gathered information on a child's physical health [1]. This included general health, injuries, limitations, chronic conditions, mental health, emotions, and cognition among

other aspects [1]. The Ages and Stages Questionnaires and Milestones section focused on gathering information on aspects such as communication, problem solving, motor skills, and developmental milestones such as a child's first words or first steps [1]. Other sections assessed the temperament of the child, literacy, oral communication abilities, the child's interests, overall behaviour, sleep patterns, relationships, parenting, and sociodemographic information [1].

Statistical Methods – Purposeful Selection

Sampling weights were applied in all statistical analyses in Chapter 4, and complex survey design issues such as stratification and clustering were accounted for by using sampling weights and 1000 bootstrap weights to calculate unbiased estimates and standard errors. The final models used in Chapter 4 were built using purposeful selection. This method fits a univariate logistic regression model for each independent variable as the first step. Then, an interim multivariate model is fitted which includes all statistically significant independent variables at the $p = 0.20$ level from the univariate regression models. Both statistically significant and clinically important variables were included in the final model.

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Chapter 4: Effects of Low Exposure to Traffic Related Air Pollution on Childhood Asthma Onset by Age 10 Years

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Author Contribution Statement

As the primary author, N.L. was involved in data processing, data analysis, and writing of the manuscript. Z.G. was responsible for developing the research question, developing the research protocol

and guiding N.L. as supervising author. A.S. co-supervised this project and provided expertise in environmental health. M.J.S. co-supervised this project and provided expertise in pediatric asthma. All authors reviewed and approved the final manuscript prior to submission for publication.

Abstract

Introduction: Although NO_2, a major traffic related air pollutant, has been associated with onset of childhood asthma, young children may be more susceptible to traffic related air pollution exposure compared to other individuals.

Methods: We linked data from National Longitudinal Survey of Children and Youths (NLSCY) Cycle 1 – 5 (1994 – 2003) and the National Air Pollution Surveillance Program (NAPS) to determine the association between NO_2 exposure and either early or late onset childhood asthma phenotypes. Children diagnosed with asthma from age 0 – 3 were defined as having early onset asthma. Children diagnosed with asthma from age 4 – 9 were defined as having late onset asthma.

Results: Mean NO_2 exposure for each quartile was 6.31 ppb, 9.45 ppb, 11.83 ppb, and17.9 ppb. Higher levels of NO_2 exposure were more strongly associated with early childhood asthma (Quartile 3 OR: 2.11, 95% CI: 1.29, 3.44, Quartile 4 OR: 2.16, 95% CI: 1.27, 3.68) compared to the lowest level of NO_2 exposure (Quartile 1). No such association was observed with risk of late childhood asthma onset.

Discussion: Asthma susceptibility to NO_2 exposure may vary with the childhood developmental stage, and young children may be susceptible to NO_2 exposure at levels well below national and international guidelines. Our study emphasizes the importance of considering the timing of childhood asthma onset in future studies and confirms the increased risk of early onset of childhood asthma associated even with relatively low NO_2 exposure levels.

Keywords: Asthma, Asthma Phenotypes, Traffic Pollution, Children, Nitrogen Dioxide

Introduction

Childhood asthma is one of the most common diseases worldwide, with an estimated global prevalence as high as 14% and an economic burden of $6 billion among school-aged children in the United States alone (Ferrante & La Grutta, 2018; Sullivan et al., 2017). A major risk factor for childhood asthma is exposure to traffic related air pollution (TRAP) such as nitrogen dioxide (NO_2), particulate matter ($PM_{2.5}$), and PM_{10} (Achakulwisut et al., 2019; Bowatte et al., 2015; Favarato et al., 2014; Khreis et al., 2017). A systematic review focusing on the association between TRAP and childhood asthma found multiple types of TRAP were associated with childhood asthma (Khreis et al., 2017). Exposure to NO_2, which is the most widely used proxy for TRAP exposure and accounts for up to 80% of ambient traffic emissions, has been associated with up to four million new cases of childhood asthma annually with 64% of such cases occurring in urban centers (Achakulwisut et al., 2019; Beckerman et al., 2008; Levy, Mihele, et al, 2014).

Childhood asthma remains a complex and heterogeneous disease which is not fully understood (Deliu, et al., 2017; Reddy & Covar, 2016; Scherzer & Grayson, 2018). Childhood asthma is associated with many different phenotypes, which can differ in risk factors, symptoms, and clinical treatment (Duijts et al., 2016; Just, Bourgoin-Heck, & Amat, 2017; Krautenbacher et al., 2019; Owora et al., 2018; Raedler et al., 2015; Reddy & Covar, 2016). Many studies have classified childhood wheezing based on the timing and the duration of symptoms, while other studies have focused on clinical and physiological characteristics (Duijts et al., 2016; Granell et al., 2016; Henderson et al., 2008; Just et al., 2017; Krautenbacher et al., 2019; Martinez et al., 1995; Raedler et al., 2015; Reddy & Covar, 2016; Savenije et al., 2011; Spycher et al., 2008). Regardless, many of the risk factors and outcomes (such as lung function in adolescence) differ based on the

childhood asthma phenotype (Landgraf-Rauf et al., 2016; Reddy & Covar, 2016; Siroux & Bouzigon, 2019).

Current World Health Organization (WHO) guidelines written in 2005 suggest annual mean exposure to ambient NO_2 should not exceed 40 $\mu g/m^3$ or 21 ppb (Achakulwisut et al., 2019; World Health Organization, 2005). More recent guidelines such as the 2020 Canadian Ambient Air Quality Standard (CAAQS) suggest an annual ambient NO_2 concentration of 17 ppb (Environment and Climate Change Canada, 2016). However, younger children may be more prone to differences in susceptibility to asthma risk factors and may be vulnerable to TRAP even at levels mandated by national and international guidelines (Khreis et al., 2017). To study whether this was the case, we used a Canadian prospective cohort, the National Longitudinal Survey of Children and Youths (NLSCY) Cycles 1–5, and the National Air Pollution Surveillance (NAPS) program to investigate the association between NO_2 exposure and both early-onset and late-onset asthma phenotypes in Canadian children.

Materials and Methods

Funding & Ethics Approval

This study was funded by the Janeway Children's Hospital Foundation and has been approved by the provincial Health Research Ethics Board (HREB # 2017.185) of Memorial University of Newfoundland in St. John's, NL, Canada.

Population Cohort

Our infant cohort was developed from the NLSCY cycles 1 – 5. The NLSCY was a longitudinal cohort study conducted biennially between 1994 to 2009 by Statistics Canada and gathered information on Canadian children and youths from infancy to adulthood. It contains information

on demographics, social economic status, lifestyle, and living conditions about the person most knowledgeable about the child (PMK), spouse, and children in each household. Each child was linked via a unique individual identifier across all cycles. Cycle 1 of the NLSCY included 16 903 respondents aged 0 – 11 from all ten provinces in Canada and was conducted in 1994 – 1995. Our study cohort included 4696 children aged 0 or 1 in cycle 1. The number of children (follow-up rate) in subsequent cycles were: 3651 children at age 2-3 in cycle 2 (78%), 3549 children at age 4-5 in cycle 3 (76%), 3186 children at age 6-7 in cycle 4 (68%), and 3093 children at age 8-9 in cycle 5 (66%).

Asthma Phenotypes

The following questions in each cycle determined the childhood asthma phenotypes (early and late) for: Ever-asthma - "Has [the child] ever had asthma that was diagnosed by a health professional?", and Current-asthma - "Has [the child] had an asthma attack in the past 12 months?" A child was deemed to have early childhood asthma if the *earliest* cycle in which a positive response to either the ever-asthma or current-asthma question was in NLSCY cycles 1 or 2 (age 0-3). A child was deemed to have late childhood asthma if the *earliest* cycle in which a positive response to either the ever-asthma or current-asthma question was in NLSCY cycles 3, 4 or 5 (age 4-9). A sensitivity analysis was performed wherein early childhood asthma was defined as the *earliest* cycle with a positive response to either asthma question being NLSCY cycles 1, 2 or 3 (age 0-5) and late childhood asthma was defined as the *earliest* cycle with a positive response to either asthma question being NLSCY cycles 4 or 5 (age 6-9).

Primary Exposure – NO_2 Exposure

NO_2 exposure was modelled using data from the NAPS program. This program was established in 1970 by the Canadian government to monitor long-term air quality across Canada, and currently consists of 286 nationwide monitoring stations (Canada, n.d.). These stations are located in each province and territory and provide 24 hour continuous air pollutant monitoring (Canada, n.d.). Pollutants monitored include carbon monoxide, nitrogen dioxide, and fine particulate matter, among others. This study focused on NO_2, which is the most widely used proxy for TRAP exposure (Canada, n.d.). Hourly NO_2 exposure data, measured in parts per billion (ppb) was aggregated into annual average NO_2 exposure for each monitoring station. This was used to model NO_2 exposure from the years 1993 - 2003 at the forward sortation area (FSA, a geographical region with identical first three postal code digits for each household) level via a land use regression model (LUR). Annual average NO_2 levels are commonly used in epidemiological studies and is also used by World Health Organization (WHO) guidelines for ambient air pollution levels (Achakulwisut et al., 2019; Gehring et al., 2010; World Health Organization, 2005). Land use characteristics at the FSA level were from the Canadian Census. As the census is carried out quinquennially, FSA characteristics for years with no census data were modelled using linear regression, which were then linked to the NAPS dataset. Further information describing data processing of census data can be found in Appendix A. The method of modelling predicted NO_2 exposure is described in Appendix B. After average NO_2 exposure was calculated for each year and FSA, individual early and late TRAP exposure levels were calculated based on timing of asthma onset. Among those diagnosed with an asthma phenotype, the average NO_2 exposure was calculated using the NO_2 exposure of the cycles prior to and including the cycle in which asthma was diagnosed. Average

NO_2 exposure was based on the NO_2 exposure across all 5 cycles for those not diagnosed with a given asthma phenotype.

Other Risk Factors

Other child and parental factors were considered in this study. These include: 1) child risk factors: age of child, sex of child, child's body mass index (BMI) in NLSCY cycle 1, whether the child was breastfed, breast feeding duration, 2) parental risk factors: PMK smoking, spousal smoking, maternal history of asthma, age of mother at birth, age of father at birth, and smoking during pregnancy, and 3) household factors: highest education level attained for either PMK or spouse, and household socioeconomic score (HSES), which was derived by Statistics Canada from five factors: level of education of the PMK, level of education of the spouse/partner, prestige of the PMK's occupation, prestige of the occupation of the spouse/partner, and household income.

Statistical Analysis

The main exposure variable, the average NO_2 exposure (ppb) was categorized into exposure levels based on exposure quartiles. Means and standard deviations at each exposure level were calculated. For continuous variables, mean with standard deviation (SD) and 25^{th}, 50^{th} (median) and 75^{th} percentiles were also calculated. For categorical variables, counts and frequency were calculated. Sampling weights were applied in all statistical analyses. Significant risk factors in the univariate and multivariate analysis were identified by logistic regression using proc surveylogistic. This survey specific procedure accounts for complex survey design issues such as stratification and clustering by allowing for sampling weights and 1000 bootstrap weights in calculating of unbiased estimates and standard errors. Purposeful selection was used to build the final model. In this method, a univariate logistic regression model was fitted for each independent variable. An

interim multivariate model was then fitted, including all statistically significant independent variables at the p = 0.20 level in the univariate regression models. The final model included both statistically significant and clinically important variables. Variables which were not significant in either the early asthma onset nor late asthma onset models but which were deemed clinically significant were the PMK's current smoking status and the age of the child. Association between predictors and outcome risk were presented as odd ratios (OR) with 95% confidence intervals (95% CI). Data analysis was performed using SAS 9.4.

Results

Population characteristics can be found in Table 1. 770 776 participants with sampling weights were analyzed in the overall cohort, with 49% being female and 51% being male. 50% were age 0 and 50% were age 1 in cycle 1. The incidence of early onset asthma over NLSCY cycles 1 – 5 was 9%. Incidence of late onset asthma over NLSCY cycles 1 – 5 was also 9%. 31% of children lived in a house where the PMK smoked, while 24% of children were born to mothers who smoked during pregnancy. 17% of children had mothers who consumed alcohol while pregnant and 31% of children were breastfed. 6% of children were born to mothers with a history of asthma.

Mean ages of the mother and father at childbirth was 28.8 years (Standard Deviation: 5.0) and 32.0 years (SD: 5.3) respectively. Mean HSES was -0.09 (SD: 0.79), and mean BMI at birth was 20.0 kg/m^2 (SD: 7.5). Average breastfeeding duration was 4.32 months (SD: 1.9) Mean pollutant exposures for NO_2 was 13.3 ppb (SD: 5.1). Means for each quartile of NO_2 exposure ranged from 6.31 ppb (SD: 1.54) in the first quartile to 17.87 ppb (SD: 3.77) in the fourth quartile and can be found in Table 1.

Results from univariate analysis of both early and late childhood asthma are in Table 2. Significant risk factors for early childhood asthma onset were male sex (OR: 1.84, 95% CI: 1.29, 2.63), children aged 1 in cycle 1 (OR: 1.42, 95% CI: 1.01, 2.00), living with a PMK who smoked (OR: 1.46, 95% CI: 1.02, 2.09), being born to a mother with asthma (OR: 3.54, 95% CI: 2.10, 5.97), and being born to mothers who smoked during pregnancy (OR: 1.54, 95% CI: 1.04, 2.29). Both an increase in the mother's age at childbirth (OR: 0.93, 95% CI: 0.93, 1.00, p = 0.035) and an increase in HSES (OR: 0.73, 95% CI: 0.58, 0.91) were protective for early childhood asthma risk. Male sex was the only significant risk factor for late childhood asthma onset (OR: 1.46, 95% CI: 1.06, 2.04). NO_2 was not associated with late-asthma onset in univariate analysis. However, high NO_2 exposure (Q3 OR: 2.21, 95% CI: 1.37, 3.51, Q4 OR: 2.08, 95% CI: 1.27, 3.40) was associated with early asthma onset when compared to the lowest quartile of NO_2 exposure.

Multivariate analysis results can be found in Table 3. Onset of early childhood asthma (age 0-3) was associated with NO_2 exposure (Type 3 p-value: 0.003) after controlling for other risk factors. Higher levels of NO_2 exposure were more strongly associated with early childhood asthma (Q3 OR: 2.11, 95% CI: 1.29, 3.44, Q4 OR: 2.16, 95% CI: 1.27, 3.68) compared to the lowest level of NO_2 exposure (Q1). No association was observed with risk of late childhood asthma onset. In our sensitivity analysis, wherein early asthma was diagnosed as asthma onset between the age of 0-5, higher NO_2 exposure was again associated with early childhood asthma (Q3 OR: 1.83, 95% CI: 1.19, 2.82, Q4 OR: 1.87, 95% CI: 1.17, 2.98). There was no association between NO_2 exposure and late childhood asthma onset (age 6-9) in the sensitivity analysis. Other significant factors for onset of early childhood asthma in multivariate analysis include mothers having asthma, child sex, and HSES.

Discussion

Our results show a significant association between NO_2 exposure and early childhood asthma onset (age 0-3); however, there was no association between NO_2 and late childhood asthma onset (age 4-9). Results remained consistent in the sensitivity analysis, wherein early childhood asthma onset was expanded from age 0-3 to age 0-5: NO_2 exposure was associated with early childhood asthma but not late childhood asthma. Specifically, compared to the lowest quartile of NO_2 exposure (mean: 6.31 ppb), children in the third and fourth quartile of NO_2 exposure (mean: 11.83 ppb and 17.9 ppb respectively), were significantly associated with childhood asthma in both regular and sensitivity analyses. These levels of NO_2 exposure are lower than those suggested by WHO guidelines.

Previous studies have found the association between childhood asthma and TRAP is affected by the timing of assessment of asthma. One birth cohort study containing 68 195 children found that NO_2 exposure was associated with asthma onset before the age of 1, but not after age 3 (Sbihi et al., 2017). Another study containing 3840 children found NO_2 exposure was associated with asthma onset in children before age 2, but not in children after this period (Rancière et al., 2017). Our results agree with a meta-analysis of 41 studies across North America, Europe and Asia conducted by Khreis et al. focusing on the association between TRAP and asthma in children aged 0-18 years (Khreis et al., 2017). Among these 41 studies, a meta-analysis of 20 studies measuring NO_2 exposure found NO_2 was associated with childhood asthma onset (OR: 1.05, 95% CI: 1.02, 1.07) (Khreis et al., 2017). As the symptoms and prognosis of childhood asthma can change with the age of diagnosis, an age-specific meta-analysis was also conducted as by Khreis et al. The results suggest a stronger association between NO_2 and asthma in the 7 studies focused on children younger than age 6 years (OR: 1.08, 95% CI: 1.04,1.12) compared to the 14 studies focused on

children older than 6 years (OR: 1.03, 95% CI: 1.00, 1.06) (Khreis et al., 2017). This age-specific analysis focuses on prevalence of childhood asthma, however, and does not consider timing of asthma onset. The results from our study also agree with our previous systematic review focusing on the association between TRAP and childhood asthma phenotypes (Lau et al., 2018). This review included 7 studies defining the timing of asthma onset and suggests that TRAP is associated with asthma phenotypes beginning before age 3 but may not be associated with asthma phenotypes which begin afterwards (Lau et al., 2018).

The postnatal period of childhood from age 0-3 is crucial for lung development, and exposure to TRAP during this period can significantly affect their development. Studies of exposure to TRAP pollutants in children have found NO_2 exposure was associated with increased serum levels of interleukin-6 (IL-6), an inflammatory cytokine and tumor necrosis factor alpha (TNF-α) (Gruzieva et al., 2017; Klümper et al., 2015). TRAP exposure during prenatal, postnatal and early childhood stages of development are also associated with epigenetic changes such as DNA methylation to many genes which may cause lung sensitization to allergens (Rider & Carlsten, 2019) (Fu et al., 2012; Prunicki et al., 2018). In turn, methylation of these genes have been associated with asthma risk (Prunicki et al., 2018). Many population genetics studies also support the association between TRAP exposure and childhood asthma. Studies have found that variants of genes such as Glutathione S-transferase P and Toll-like receptor (TLR) 4 can modify asthma risk in children exposed to NO_2 exposure (Kerkhof et al., 2010; MacIntyre et al., 2014; Vawda et al., 2014). Our study adds to this evidence and suggests the biological effects of NO_2 exposure to the respiratory system may be influenced by the age of the child.

WHO guidelines suggest annual mean exposure to ambient NO_2 should not exceed 40 $\mu g/m^3$ or 21 ppb, and the 2020 CAAQS guidelines suggest annual ambient NO_2 of 17 ppb. (Achakulwisut

et al., 2019; Environment and Climate Change Canada, 2016; World Health Organization, 2005). Many of the studies which have found an association between childhood asthma and NO_2 exposure have NO_2 levels well within range of established guidelines. For instance, the area studied in one of the previously mentioned studies which found a significant association between childhood asthma and NO_2 contained a median NO_2 exposure of 33.3 $\mu g/m^3$ (~17.7 ppb). Our study, however, suggests that children exposed to annual NO_2 concentrations of 12 ppb are susceptible to early-onset asthma. This may have implications for young children, despite ambient NO_2 declining significantly in Canada and NO_2 levels in Canadian urban areas being below both WHO and CAAQS guidelines (Environment and Climate Change Canada, 2016). NO_2 in Canadian urban areas in 2016 was as high as 12 ppb, within the range our study found a significant association with early-onset asthma (Environment and Climate Change Canada, 2016). Furthermore, many international cities contain annual NO_2 levels above WHO guidelines, suggesting a risk for early-onset asthma in children (Environment Canada, 2014). These findings suggest that such guidelines may need to be revisited to better protect young children susceptible to NO_2, although such findings need to be replicated in other studies.

Although NO_2 exposure was the primary exposure variable, our multivariate model also found that maternal history of asthma, child sex, and low HSES were significant risk factors for early childhood asthma onset. Maternal history of asthma has previously been associated with childhood asthma (Thomsen, 2015). However, rather than a single gene being responsible for childhood asthma, childhood asthma has been associated in mutations with several different genes (Thomsen, 2015). Existing studies have also demonstrated a link between male sex and childhood asthma in pre-pubescent children (Naeem & Silveyra, 2019). This may be due to female newborns generally having more developed lungs than male newborns and therefore are less likely to have reduced

lung function after birth and in early childhood (Postma, 2007). Existing literature also suggests there are sex differences in the effect of TRAP on childhood asthma onset, although it is unclear whether male or female children are more strongly affected (Clougherty, 2010; Hsu et al., 2015). Finally, it has been suggested that low HSES may be linked to chronic stress and inflammation response, which are implicated in childhood asthma onset (Kozyrskyj et al., 2010). The effect of TRAP on childhood asthma onset may be greater in children of low HSES (Neidell, 2004).

This study has several limitations. NO_2 exposure was not measured at the individual level and the exposure at each FSA was estimated using land use regression (LUR) based on data from monitoring sites across Canada and census information. However, this method has been widely used to estimate air pollution in large population studies. This study used the NO_2 exposure at each FSA, rather than individually, which poses a risk of ecological fallacy. The ecological fallacy is when inferences are erroneously made about individuals based on group data (Schwartz, 1994). However, we believe differences in NO_2 exposure within an FSA to be minimal. Additionally, information used was based on self-report questionnaires, which are subject to nonresponse bias and self-reporting bias. However, the NLSCY is a longitudinal survey designed and carried out by Statistics Canada. The sample is representative and has been used in many large population studies of health-related issues in Canadian children.

In summary, we have shown that exposure to NO_2 at levels well below national and international guidelines in early childhood is associated with early onset of childhood asthma; however, there is no association with late asthma onset. The results suggest that asthma susceptibility to NO_2 exposure may vary with the childhood developmental stage, and that young children may be susceptible to NO_2 exposure at levels well below national and international guidelines. Our study emphasizes the importance of considering the timing of childhood asthma onset in future studies

and confirms the increased risk of early onset of childhood asthma associated even with relatively low NO_2 exposure levels.

Data Availability

This study uses data from the National Air Pollution Surveillance (NAPS) Program, National Longitudinal Survey of Children and Youths (NLSCY), and the Canadian Census. Data from the NAPS Program used to support the findings of this study is publicly available and can be found at http://maps-cartes.ec.gc.ca/rnspa-naps/data.aspx. Data from the NLSCY and Canadian Census used to support the findings of this study is available at the Statistics Canada Research Data Centre.

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Disclosure

The datasets used are products of Statistics Canada and Environment Canada. The analysis and results presented in this study are those of the authors and not of Statistics Canada nor Environment Canada.

Table 5 Baseline (cycle 1) descriptive characteristics of study population (n = 770 776)

Variable (Continuous)	**Mean (SD)**
Breast Feeding Duration (months)	4.32 (1.86)
Biological Mother Age at Birth (years)	28.81 (5.02)
Biological Father Age at Birth (years)	32.03 (5.31)
BMI at Birth (kg/m^2)	20.04 (7.51)
House Socioeconomic Score	-0.09 (0.79)
NO_2 (ppb)	13.34 (5.05)
NO_2 Quartile 1 (ppb)	6.31 (1.54)
NO_2 Quartile 2 (ppb)	9.45 (0.73)
NO_2 Quartile 3 (ppb)	11.83 (0.73)
NO_2 Quartile 4 (ppb)	17.87 (3.77)

Variable (Categorical)	**Status**	**Count (%)**
Early Asthma Status	Yes	72 622 (9%)
	No	698 154 (91%)
Late Asthma Status	Yes	66 592 (9%)
	No	704 184 (91%)
Sex of Child	Female	375 030 (49%)
	Male	395 746 (51%)
Age of Child in Cycle 1	0	381 636 (50%)
	1	389 140 (50%)
Breast Feeding	Yes	416 082 (69%)
	No	183 746 (31%)
Alcohol Consumption during Pregnancy	Yes	118 932 (17%)
	No	561 283 (83%)
Smoking during Pregnancy	Yes	161 176 (24%)
	No	519 784 (76%)
PMK Smoker Status	Yes	235 980 (31%)
	No	530 135 (69%)
Mother's History of Asthma	Yes	37 267 (5%)
	No	652 530 (95%)

NO_2 = Nitrogen Dioxide. Ppb = Parts per billion. SD = Standard Deviation. PMK = Person Most Knowledgeable about the child

Table 6 Univariate results for early and late childhood asthma

Variable	Status	Early Asthma				Late Asthma			
		OR	95% CI	p-value	Type-3 P-value	OR	95% CI	p-value	Type-3 P-value
Sex of Child	Female	1				1	1.06, 2.04		
	Male	1.84	1.29, 2.63	**0.008**		1.46		**0.023**	
Age of Child in Cycle 1	0	1				1	0.58, 1.13		
	1	1.42	1.01, 2.00	**0.045**		0.81		0.213	
Breast Feeding	Yes	1.20	0.77, 1.86	0.417		0.88	0.59, 1.30	0.510	
	No	1				1			
Alcohol Consumption during Pregnancy	Yes	0.62	0.38, 0.99	**0.045**		0.91	0.51, 1.46	0.684	
	No	1				1			
Smoking during Pregnancy	Yes	1.54	1.04, 2.29	**0.033**		1.24	0.81, 1.89	0.327	
	No	1				1			
PMK Smoker Status	Yes	1.46	1.02, 2.09	**0.040**		0.94	0.79, 1.13	0.358	
	No	1				1			
Mother's History of Asthma	Yes	3.54	2.10, 5.97	**<0.001**		0.905	0.49, 1.67	0.750	
	No	1				1			
NO_2 (ppb)	Quartile 1	1			**0.002**	1			0.638
	Quartile 2	1.31	0.81, 2.14	0.272		1.13	0.75, 1.72	0.558	
	Quartile 3	2.21	1.37, 3.51	**0.008**		1.07	0.67, 1.69	0.784	
	Quartile 4	2.08	1.27, 3.40	**0.004**		1.31	0.86, 1.89	0.208	
Breast Feeding Duration (months)		0.93	0.81, 1.07	0.294		1.05	0.95, 1.17	0.360	
Biological Mother Age at Birth (years)		0.96	0.93, 1.00	**0.035**		1.02	0.98, 1.05	0.789	
Biological Father Age at Birth (years)		0.98	0.95, 1.02	**0.292**		1.01	0.97, 1.04	0.789	
BMI at Birth		1.01	0.99, 1.03	0.477		1.00	0.98, 1.03	0.909	
House Socioeconomic Score		0.73	0.58, 0.91	**0.006**		0.94	0.79, 1.13	0.520	

PMK = Person most knowledgeable about the child. NO_2 = Nitrogen Dioxide. Ppb = Parts per billion. BMI = Body Mass Index. 95% CI = 95% Confidence Interval

Table 7 Multivariate results for early and late childhood asthma

Variable	Status	Early Asthma (age 0 – 3)				Late Asthma (age 4 – 9)			
		OR	95% CI	p-value	Type-3 P-value	OR	95% CI	p-value	Type-3 P-value
NO_2 (ppb)	Quartile 1	1			**0.003**	1			0.486
	Quartile 2	1.17	0.71, 1.94	0.555		1.17	0.75, 1.82	0.481	
	Quartile 3	2.11	1.29, 3.44	**0.003**		1.22	0.76, 1.97	0.416	
	Quartile 4	2.16	1.27, 3.68	**0.005**		1.43	0.91, 2.26	0.121	
Sensitivity analysis for asthma phenotypes using modified age cut points									
		Early Asthma (age 0 – 5)				Late Asthma (Age 6 – 9)			
		OR	95% CI	p-value	Type-3 P-value	OR	95% CI	p-value	Type-3 P-value
NO_2 (ppb)	Quartile 1	1			**0.018**	1			0.317
	Quartile 2	1.31	0.85, 2.02	0.222		0.95	0.57, 1.58	0.845	
	Quartile 3	1.83	1.19, 2.82	**0.006**		1.12	0.65, 1.94	0.681	
	Quartile 4	1.87	1.17, 2.98	**0.009**		1.51	0.92, 2.49	0.105	

The multivariate analysis included the following confounders: household socioeconomic status score (HSES), sex of child, smoking status of the PMK, and mother's history of asthma. Ppb = parts per billion. 95% CI = 95% confidence interval.

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Chapter 5: Discussion and Conclusion

Summary of Systematic Review

The first study was a PRISMA systematic review which assessed the current knowledge on the relationship between TRAP and childhood asthma phenotypes based on longitudinal wheezing patterns. Seven studies focusing on this relationship were included, but the number of studies including each of the common longitudinal asthma or wheezing phenotypes was limited. Multiple studies suggested that various pollutants, such as $PM_{2.5}$ and NO_2 are associated with transient or persistent childhood asthma or wheezing phenotypes, but the majority of studies suggest there is no association between TRAP and late-onset childhood asthma. All included studies were found to be of sufficient quality according to the CASP checklist, although there were common limitations such as potential for recall bias. No meta-analysis was performed due to the limited number of studies per pollutant and asthma phenotype.

Summary of Longitudinal Infant Cohort Study

Our second study was a longitudinal infant cohort study which assessed the relationship between NO_2 (a proxy for TRAP) and early and late-onset childhood asthma phenotypes. This study used data from a Statistics Canada survey on children and youths and from a nationwide air pollution monitoring program. 770 776 children (after including funnel and bootstrap weights), were included in this study as they aged from 0 to 9 years old. NO_2 was associated with early-onset (0 – 3 years old) childhood asthma (p-value: 0.003), although significant differences were observed only when the 3rd quartile (Q3 OR: 2.11, 95% CI: 1.29, 3.44), and 4th quartile (Q4 OR: 2.16, 95% CI: 1.27, 3.68) were compared to the 1st or lowest quartile of NO_2 exposure. There was no association between NO_2 and late-onset (4 – 9 years old) childhood asthma, and these results

remained consistent when the definitions of early-onset and late-onset childhood asthma were modified in the sensitivity analysis. Other significant risk factors for early-onset asthma were sex of child, maternal history of asthma, and HSES.

Findings in Context of the Literature

As discussed in Chapter 4, previous studies have found the timing of TRAP exposure may play a role in subsequent asthma onset. [1, 2]. Our results also agree with a meta-analysis of 41 studies from multiple countries conducted by Khreis et al. focusing on the association between TRAP and asthma in children aged 0-18 years (Khreis et al., 2017). Among these 41 studies, a meta-analysis of 20 studies measuring NO_2 exposure found NO_2 was associated with childhood asthma onset (OR: 1.05, 95% CI: 1.02, 1.07) (Khreis et al., 2017). Furthermore, the age specific meta-analysis from this study suggested a stronger association between NO_2 and asthma in children younger than age 6 years (OR: 1.08, 95% CI: 1.04,1.12) compared to children older than 6 years (OR: 1.03, 95% CI: 1.00, 1.06) (Khreis et al., 2017). However, this age-specific analysis focuses on prevalence of childhood asthma and does not consider timing of asthma onset.

Previous studies have also looked the development of childhood asthma and TRAP in Canadian children. A study using administrative data from 65 000 children in British Columbia, Canada found that an interquartile increase of NO_2 was associated with a 51% increase in early-onset chronic asthma risk compared to no asthma (adjusted weighted relative risk [RR]: 1.51, 95% CI: 1.22–1.89). NO_2 exposure was also associated with late-onset chronic asthma in this study; however, there was only a 26% increase in risk when compared to no asthma (adjusted weighted RR: 1.25; 95% CI: 1.07–1.46) (Sbihi et al., 2017). A separate study involved 1286 children from Toronto, Canada who were followed from birth for a mean 17 years. This study found that

exposure to NO_2 before birth was more strongly associated with asthma onset before age 4 (Hazard ratio [HR]: 1.19, 95% CI: 1.04–1.36 when compared to asthma onset after age 4 1.19 (HR: 1.19, 95% CI: 0.96–1.46) (To et al., 2019). These studies are consistent with our results, which suggest that TRAP exposure in early childhood can affect the timing of asthma onset.

Strengths and Limitations – Systematic Review

Many studies and SRMAs have previously investigated the association between TRAP and childhood asthma. However, we believe our systematic review is the first attempt to systematically evaluate the available literature on the effect of TRAP exposure with the development of childhood asthma or wheezing phenotypes. The search strategy encompassed multiple terms for TRAP to capture as many studies as possible and we believe our modified definitions of the asthma phenotypes strike a balance between maintaining a clear definition for each phenotype and including as many studies as possible. Our systematic review followed PRISMA guidelines and utilized the CASP checklist to assess study quality, both of which ensured the transparency of our systematic review.

Our systematic review does contain several limitations, which have been previously described (Chapter 2) and will be summarized here. Briefly, the first limitation is the difficulty in drawing any firm conclusions due to the low number of eligible studies. This limitation was exacerbated due to our systematic review assessing each pollutant separately, further reducing the number of studies per pollutant. A second limitation is the heterogeneity in phenotype definitions and in study follow-up length. Depending on the phenotypic definition used, a child's phenotypic classification may differ between studies. Finally, the majority of studies used a self-report questionnaire to measure asthma or wheezing outcomes in children. While this is typical of many similar studies, this may lead to recall bias in the results. Recall bias can be due to due to several factors, such as

the length of the recall period (often a year or more), disease characteristics (such as acute or chronic) or study duration (Althubaiti, 2016). Not accounting for recall bias can lead to either underestimation or overestimation of the true effect size (Althubaiti, 2016). Methods to address recall bias include shortening the recall period, using memory aids, or adjusting for potential recall errors in the statistical analysis (Althubaiti, 2016).

Strengths and Limitations – Longitudinal Infant Cohort Study

The foremost strength of our study is the sample size of our cohort, which was made possible due to Statistics Canada performing the data collection. After using the appropriate weights, our cohort assessed 770 776 children from the initial longitudinal cohort, with a follow-up which remained at 66% nine years after Cycle 1. Additionally, by using NO_2 data from monitoring sites nationwide, we are able to predict NO_2 exposure for children from a diverse range of backgrounds and regions across Canada.

The limitations of this study have been discussed (Chapter 4). NO_2 exposure was not estimated at the individual level, but at the FSA level. Individual level exposures in large cohorts can be estimated if detailed individual (such as residential address) and geographical (such as residential or industrial land use) information is used (Ostro et al., 2010; Pope et al., 2002). However, this was unfeasible for our study given the confidentiality requirements of Statistics Canada. Assessing pollutant exposure in a larger area is common in large population studies, however, and we believe differences in NO_2 exposure within an FSA are minimal. Additionally, information used was based on self-report questionnaires, which are subject to nonresponse bias and self-reporting bias.

Future Research

Although the results of this thesis contribute to the knowledge surrounding the association between TRAP and childhood asthma phenotypes, there remain many areas for future research. One future direction is to study the effect of TRAP on other phenotypes. We did not study the effect of TRAP on transient or persistent childhood asthma phenotypes due to the inconsistent definitions of these phenotypes in the current literature. Defining these additional phenotypes and studying their association with TRAP would generate additional knowledge regarding this topic. A second future direction would be to study the effect of TRAP on asthma phenotypes which are not based on longitudinal wheezing patterns or endotypes, wherein specific biological mechanisms explain the phenotypic properties which are observed. For instance, childhood asthma is often divided into Type-2 high asthma, which is caused by eosinophilic inflammation (seen in inflammation markers such as IL-25 or IL-33), and Type-2 low asthma (seen in inflammation markers such as IL-8), which is linked to neutrophilic inflammation ((Licari et al., 2018). However, this would require a detailed dataset which includes not only self-reported data on asthma symptoms, but biological data regarding the asthma phenotype or endotype. A third direction for future research would be to study the association of TRAP and asthma phenotypes in a more recent cohort. Many lifestyle and demographic factors have likely changed in the 26 years since the NLSCY longitudinal infant cohort was followed starting from 1994 – 1995. Pollution levels have generally decreased within Canada and North America, and there is now a greater awareness for both asthma and the harmful effects of TRAP. Unfortunately, the NLSCY was discontinued in 2009 and any such studies would have to incorporate a different longitudinal birth cohort.

Conclusion

The studies which we conducted suggest that the effect of TRAP exposure and childhood asthma in children is affected by the age of the child. We have added to the evidence which suggests asthma risk factors may affect individual children differently. Many studies focusing on asthma simply focus on diagnosing asthma broadly, without considering phenotypic differences. However, the many different phenotypes of asthma also exhibit differences in risk factors, biological mechanisms, diagnosis, prognosis and treatment. It therefore may be important to consider differences in asthma phenotypes when providing care and conducting research.

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Appendices

Appendix A – Letter of Ethics Approval from the Health Research Ethics Board

Ethics Office
Suite 200, Eastern Trust Building
95 Bonaventure Avenue
St. John's, NL
A1B 2X5

September 05, 2017

Faculty of Medicine
Discipline of Epidemiology

Dear Mr. Lau:

Researcher Portal File # 20180693
Reference # 2017.185

RE: "Determinants of Childhood Asthma Phenotypes"

Your application received a delegated review by a sub-committee of the Health Research Ethics Board (HREB). ***Full approval*** of this research study is granted for one year effective **September 5, 2017**.

This is your ethics approval only. Organizational approval may also be required. It is your responsibility to seek the necessary organizational approval from the Regional Health Authority (RHA) or other organization as appropriate. You can refer to the HREA website for further guidance on organizational approvals.

This is to confirm that the HREB reviewed and approved or acknowledged the following documents (as indicated):

- Application, approved
- List of variables, approved
- Budget, approved
- Funding letter, acknowledged
- Approval letter from SSHRC and Statistics Canada, acknowledged

MARK THE DATE

This approval will lapse on September 5, 2018. It is your responsibility to ensure that the Ethics Renewal form is submitted prior to the renewal date; you may not receive a reminder. The Ethics Renewal form can be found on the Researcher Portal as an Event form.

If you do not return the completed Ethics Renewal form prior to date of renewal:

- ***You will no longer have ethics approval***
- *You will be required to stop research activity immediately*
- *You may not be permitted to restart the study until you reapply for and receive approval to undertake the study again*
- *Lapse in ethics approval* ***may result in interruption or termination of funding***

You are solely responsible for providing a copy of this letter, along with your approved HREB application form; **to Research Grant and Contract Services** should your research depend on funding administered through that office.

Modifications of the protocol/consent are not permitted without prior approval from the HREB. **Implementing changes without HREB approval may result in your ethics approval being revoked, meaning your research must stop**. Request for modification to the protocol/consent must be outlined on an amendment form (available on the Researcher Portal website as an Event form) and submitted to the HREB for review.

The HREB operates according to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2), the Health Research Ethics Authority Act (HREA Act) and applicable laws and regulations.

You are responsible for the ethical conduct of this research, notwithstanding the approval of the HREB.

We wish you every success with your study.

Sincerely,

Patricia Grainger

Ms. Patricia Grainger (Chair, Non-Clinical Trials Health Research Ethics Board)
Dr. Joy Maddigan (Vice-Chair, Non-Clinical Trials Health Research Ethics Board)

Appendix B – Processing of Census Data

As data collection for the Canadian Census is performed once every five years, a linear regression was performed using census data from 1996, 2001, and 2006 to predict the census characteristics for each year in which there was no census data collected. For each census variable except the total population in each FSA, the number of people in each FSA for that variable was divided by the total number of predicted people in the FSA in that year to arrive the proportion of people in each FSA for which the variable is relevant to. All variables were calculated using census weights provided by Statistics Canada.

Included census variables at the FSA level were: total number of people, percent of total people who take a vehicle to work, proportion of people aged 0-17, proportion of people aged 18-64, proportion of people whose marital status is together, , proportion of non-immigrants, proportion of people attending school, proportion of non-low-income people, proportion of people who didn't work in past year, weeks worked in the past year (divided into 4 categories: 0-13, 14-26, 27-39, 40-52 weeks) distance to work (divided into quartiles), census family income (divided into quartiles).

Appendix C – Land Use Regression Method for Modelling NO_2 Exposure

Pollution exposure was estimated via the following method. Hourly NAPS NO_2 exposure data was used to calculate the mean daily NO_2 exposure at each monitoring station for each year from 1993-2003. A land use regression (LUR) was used to predict the mean daily NO_2 exposure for each year from 1993-2003 for every FSA in Canada . This was performed by using the annual mean daily NO_2 exposure data taken from NAPS combined with the census variables at the FSA level (described in Appendix A) for each year. Predicted NO_2 exposures in a given FSA and year which were below zero were replaced using a linear regression containing the remaining predicted NO_2 variables and year as the predictor. As data collection for the NLSCY occurs biennially, the mean daily NO_2 exposure for each NLSCY cycle was calculated by using the predicted mean daily NO_2 exposure for the year preceding data collection and the year in which data collection occurred.

Appendix D – SAS Output – Early Asthma Univariate Analysis

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
Age_child	4.03	1	1000	0.0449

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
age_child 1 vs 0	4.03	1.008	2.001

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
sex_child	11.38	1	1000	0.0008

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
sex_child M vs F	1.844	1.292	2.632

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
brst_fd_ever	0.66	1	1000	0.4165

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
brst_fd_ever 1 vs 2	1.198	0.774	1.855

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-1.5761	0.3136	-5.03	<.0001
brst_fd_dur	-0.0741	0.0706	-1.05	0.2940

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
brst_fd_dur	0.929	0.808	1.067

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-0.9748	0.4988	-1.95	0.0509
age_mo_bir	-0.0369	0.0175	-2.11	0.0352

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
age_mo_bir	0.964	0.931	0.997

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-1.5572	0.5180	-3.01	0.0027
age_fa_bir	-0.0172	0.0163	-1.05	0.2927

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
age_fa_bir	0.983	0.952	1.015

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
smk_mo_prg	4.55	1	1000	0.0332

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.1395	0.1172	-18.25	<.0001
smk_mo_prg 1	0.4307	0.2020	2.13	0.0332
smk_mo_prg 0	0	.	.	.

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
smk_mo_prg 1 vs 0	1.538	1.035	2.287

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
alc_mo_prg	4.04	1	1000	0.0448

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
alc_mo_prg 1 vs 0	0.615	0.382	0.989

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.2220	0.2172	-10.23	<.0001
BMI_c1	0.00703	0.00989	0.71	0.4770

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
BMI_c1	1.007	0.988	1.027

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
PMK_smk_cur_c1v2	4.27	1	1000	0.0390

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
PMK_smk_cur_c1v2 1 vs 0	1.457	1.019	2.081

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.0121	0.1119	-17.98	<.0001
spo_cig_cur_c1	-0.00025	0.00227	-0.11	0.9141

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
spo_cig_cur	1.000	0.995	1.004

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.1035	0.1433	-14.68	<.0001
hou_smk_cur_c1v2	0.1604	0.1848	0.87	0.3858

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
hou_smk_cur_c1v2	1.174	0.817	1.687

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.0708	0.0954	-21.71	<.0001
house_SES_c1	-0.3195	0.1170	-2.73	0.0064

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
house_SES_c1	0.726	0.577	0.914

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
Edu_hi_c1	4.39	1	1000	0.0044

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.1497	0.1205	-17.84	<.0001
edu_hi_c1 1	0.8443	0.3075	2.75	0.0061
edu_hi_c1 2	-0.3808	0.2559	-1.49	0.1370
edu_hi_c1 3	0.2453	0.2158	1.14	0.2559
edu_hi_c1 4	0	.	.	.

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
edu_hi_c1 1 vs 4	2.326	1.272	4.253
edu_hi_c1 2 vs 4	0.683	0.414	1.129
edu_hi_c1 3 vs 4	1.278	0.837	1.952

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
mo_asthma_ever_c1	22.59	1	1000	<.0001

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
Mo_asthma_ever_c1 1 vs 0	3.543	2.101	5.972

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
NO2_early_all_rank4	4.94	3	1000	0.0021

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.6004	0.1868	-13.92	<.0001
NO2_early_all_rank4 1	0.2726	0.2480	1.10	0.2721
NO2_early_all_rank4 2	0.7913	0.2361	3.35	0.0008
NO2_early_all_rank4 3	0.7312	0.2505	2.92	0.0036
NO2_early_all_rank4 0	0	.	.	.

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
NO2_early_all_rank4 1 vs 0	1.313	0.807	2.137
NO2_early_all_rank4 2 vs 0	2.206	1.388	3.507
NO2_early_all_rank4 3 vs 0	2.078	1.271	3.396

Appendix E – SAS Output – Late Asthma Univariate Analysis

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
age_child	1.55	1	1000	0.2130

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
age_child 1 vs 0	0.807	0.576	1.131

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
sex_child	5.20	1	1000	0.0228

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
sex_child M vs F	1.466	1.055	2.037

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
brst_fd_ever	0.44	1	1000	0.5097

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
brst_fd_ever 1 vs 2	0.875	0.588	1.302

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.2888	0.2382	-9.61	<.0001
brst_fd_dur	0.0487	0.0532	0.92	0.3597

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
brst_fd_dur	1.050	0.946	1.165

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.4701	0.5496	-4.49	<.0001
age_mo_bir	0.0155	0.0190	0.82	0.4134

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
age_mo_bir	1.016	0.979	1.054

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t value	Pr > |t|
Intercept	-2.1774	0.6015	-3.62	0.0003
age_fa_bir	0.00500	0.0187	0.27	0.7890

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
Age_fa_bir	1.005	0.969	1.043

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
smk_mo_prg	0.96	1	1000	0.3266

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
smk_mo_prg 1 vs 0	1.237	0.809	1.891

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
alc_mo_prg	0.17	1	1000	0.6841

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
alc_mo_prg 1 vs 0	0.905	0.561	1.462

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-1.9959	0.2769	-7.21	<.0001
BMI_c1	0.00148	0.0130	0.11	0.9090

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
BMI_c1	1.001	0.976	1.027

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
PMK_smk_cur_c1v2	0.87	1	1000	0.3525

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
PMK_smk_cur_c1v2 1 vs 0	1.188	0.826	1.707

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.0748	0.0983	-21.11	<.0001
PMK_smk_cur_c1v2 1	0.1720	0.1850	0.93	0.3525
PMK_smk_cur_c1v2 0	0	.	.	.

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.0927	0.1049	-19.94	<.0001
hou_smk_cur_c1v2	0.1536	0.1668	0.92	0.3575

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
hou_smk_cur_c1v2	1.166	0.840	1.618

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.0325	0.0836	-24.31	<.0001
house_SES_c1	-0.0582	0.0903	-0.64	0.5197

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
house_SES_c1	0.943	0.790	1.126

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
edu_hi_c1	4.15	1	1000	0.0062

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.0348	0.1077	-18.89	<.0001
edu_hi_c1 1	-0.9319	0.3210	-2.90	0.0038
edu_hi_c1 2	0.4317	0.2661	1.62	0.1050
edu_hi_c1 3	0.0432	0.1919	0.23	0.8218
edu_hi_c1 4	0	.	.	.

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
edu_hi_c1 1 vs 4	0.394	0.210	0.739
edu_hi_c1 2 vs 4	1.540	0.914	2.596
edu_hi_c1 3 vs 4	1.044	0.717	1.522

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
mo_asthma_ever	0.10	1	1000	0.7498

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
mo_asthma_ever_c1 1 vs 0	0.905	0.489	1.674

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
NO2_late_rank4	0.57	3	1000	0.6375

Analysis of Maximum Likelihood Estimates

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-2.1803	0.1525	-14.30	<.0001
NO2_late_rank4 1	0.1241	0.2117	0.59	0.5579
NO2_late_rank4 2	0.0645	0.2359	0.27	0.7844
NO2_late_rank4 3	0.2687	0.2132	1.26	0.2079
NO2_late_rank4 0	0	.	.	.

Odds Ratio Estimates

Effect	Point Estimate	95% Confidence Limits	
NO2_late_rank4 1 vs 0	1.132	0.747	1.715
NO2_late_rank4 2 vs 0	1.067	0.671	1.694
NO2_late_rank4 3 vs 0	1.308	0.861	1.988

Appendix F – SAS Output – Early Asthma Multivariate Analysis

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
house_SES_c1	8.88	1	1000	0.0332
sex_child	12.46	1	1000	0.0004
age_child	1.33	1	1000	0.2500
PMK_smk_cur_c1v2	0.59	1	1000	0.4445
mo_asthma_ever_c1	27.49	1	1000	<.0001
NO2_early_all_rank4	4.72	3	1000	0.0028

The SURVEYLOGISTIC Procedure

Analysis of Maximum Likelihood Estimates

Parameter		Estimate	Standard Error	t value	Pr > |t|
Intercept		-3.3450	0.2733	-12.24	<.0001
House_SES_c1		-0.3863	0.1297	-2.98	0.0030
sex_child	M	0.7033	0.1993	3.53	0.0004
sex_child	F	0	.	.	.
age_child	1	0.2320	0.2016	1.15	0.2500
age_child	0	0	.	.	.
PMK_smk_cur_c1v2	1	0.1590	0.2078	0.76	0.4445
PMK_smk_cur_c1v2	0	0	.	.	.
Mo_asthma_ever_c1	1	1.3547	0.2584	5.24	<.0001
Mo_asthma_ever_c1	0	0	.	.	.
NO2_early_all_rank4	1	0.1532	0.2594	0.59	0.5548
NO2_early_all_rank4	2	0.7460	0.2501	2.98	0.0029
NO2_early_all_rank4	3	0.7693	0.2712	2.84	0.0047
NO2_early_all_rank4	0	0	.	.	.

Odds Ratio Estimates

Effect		Point Estimate	95% Confidence Limits	
house_SES_c1		0.680	0.527	0.876
sex_child	M vs F	2.020	1.367	2.987
age_child	1 vs 0	1.261	0.849	1.873
PMK_smk_cur_c1v2	1 vs 0	1.172	0.780	1.763
mo_asthma_ever_c1	1 vs 0	3.875	2.334	6.435
NO2_early_all_rank4	1 vs 0	1.313	0.807	2.137
NO2_early_all_rank4	2 vs 0	2.206	1.388	3.507
NO2_early_all_rank4	3 vs 0	2.078	1.271	3.396

Appendix G – SAS Output – Late Asthma Multivariate Analysis

Type 3 Analysis of Effects

Effect	F Value	Num DF	Den DF	Pr > F
house_SES_c1	0.01	1	1000	0.9160
sex_child	5.53	1	1000	0.0188
age_child	0.59	1	1000	0.4410
PMK_smk_cur_c1v2	0.33	1	1000	0.5637
mo_asthma_ever_c1	0.05	1	1000	0.8170
NO2_late_rank4	0.81	3	1000	0.4860

The SURVEYLOGISTIC Procedure

Analysis of Maximum Likelihood Estimates

Parameter		Estimate	Standard Error	t value	Pr > |t|
Intercept		-2.4288	0.2222	-10.93	<.0001
House_SES_c1		-0.0118	0.1115	-0.11	0.9160
sex_child	M	0.4143	0.1761	2.35	0.0188
sex_child	F	0	.	.	.
age_child	1	-0.1440	0.1869	-0.77	0.4410
age_child	0	0	.	.	.
PMK_smk_cur_c1v2	1	0.1301	0.2253	0.58	0.5637
PMK_smk_cur_c1v2	0	0	.	.	.
Mo_asthma_ever_c1	1	-0.0730	0.3155	-0.23	0.8170
Mo_asthma_ever_c1	0	0	.	.	.
NO2_late_rank4	1	0.1581	0.2243	0.70	0.4810
NO2_late_rank4	2	0.1995	0.2450	0.81	0.4156
NO2_late_rank4	3	0.3604	0.2322	1.55	0.1210
NO2_late_rank4	0	0	.	.	.

Odds Ratio Estimates

Effect		Point Estimate	95% Confidence Limits	
house_SES_c1		0.988	0.794	1.230
sex_child	M vs F	1.513	1.071	2.138
age_child	1 vs 0	0.866	0.600	1.249
PMK_smk_cur_c1v2	1 vs 0	1.139	0.732	1.772
mo_asthma_ever_c1	1 vs 0	0.930	0.500	1.726
NO2_late_rank4	1 vs 0	1.171	0.754	1.819
NO2_late_rank4	2 vs 0	1.221	0.755	1.974
NO2_late_rank4	3 vs 0	1.434	0.909	2.262