# CHILDHOOD ASTHMA AND ALLERGIES IN BIRTH COHORT STUDIES: TOOLS FOR ENVIRONMENTAL EXPOSURE ASSESSMENT.

by

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## Abstract

Pediatric asthma and allergies represent global health problems causing substantial disability. Epidemiological research has established a link between air pollution and exacerbation of asthma. However, the role of air pollutants in relation to atopy and on the development of asthma is unclear.

This thesis examines the relationship between traffic-related air pollution and the development of atopy and asthma using two complementary Canadian birth cohorts where the impact of different exposure assessment approaches on observed associations was evaluated.

Hopanes in house dust, collected in the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort study, were evaluated as markers of indoor infiltrated traffic-related air pollution by measuring their correlation with geographic predictors of outdoor concentrations of nitrogen dioxide. This correlation was dependent on the inclusion of behavioral characteristics, hindering the utility of measuring hopanes in settled dust for exposure assessment.

As an alternative approach to assess exposures in CHILD, city-specific land use regression models, questionnaires and home assessments were used to model personal exposure, including accounting for indoor/outdoor infiltration and time-activity patterns, in relation to early atopy. Spatio-temporally adjusted exposure in the first year of life was positively associated with sensitization to common food or inhalant allergens at age 1 (Odds ratio [95% confidence interval] per interquartile increase in nitrogen dioxide = 1.16 [1.00 – 1.41]).

Because atopy is often a precursor for allergic asthma, 10 years of longitudinal data from the Border Air Quality Study population-based birth cohort were used to evaluate the role of air pollution on asthma development. An interquartile range increase in nitrogen dioxide, adjusted for temporal and spatial variability, increased incident asthma among preschool (age 0-5) children by 9% (95% confidence

interval: 4 – 13%). Surrounding residential greenness mitigated this effect. In further analysis, the course of asthma was found to follow three trajectories: transient asthma, early-, and late-infancy chronic asthma, the latter two being significantly associated with fine particulate matter and nitrogen dioxide.

This dissertation highlights the importance of integrating temporal and spatial variation in traffic-related air pollution exposure assessment and clarifies the role of early exposures on atopy and asthma initiation.

## Preface

This doctoral dissertation summarizes the objectives, methods and results of an overall research plan that was developed under the guidance of my thesis committee following the presentation and acceptance of an initial research proposal. Four research chapters (Chapters 2-5) were written as separate manuscripts intended for publication in peer-reviewed journals. These manuscripts were reviewed by numerous co-authors given that this research was made possible by a multi-university and government agencies collaboration, as well as by my doctoral committee members. For each of the chapters 2 to 5, I developed the study design, conducted all analyses, interpreted the results and prepared the manuscript. I revised manuscripts according to the feedback provided by the committee members as detailed below.

This research was approved by the University of British Columbia Behavioural Research Ethics Board (certificate number H04-80161 for the Border Air Quality Study (BAQS) cohort; project title: Establishment of a childhood respiratory disease cohort using BCLHD/Analysis of birth outcomes in the Greater Vancouver Regional District using the BC Perinatal Database; certificate number: H11-03231 for the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort; project title: Canadian Healthy Infant Longitudinal Study; as well as by the Clinical Research Ethics Board (certificate number: H07-03120 for the hopanes study within 'CHILD').

A version of Chapter 2 has been published [Sbihi H, Brook JR, Allen RW, Curran JH, Dell S, Mandhane P, Scott JA, Sears MR, Subbarao P, Takaro TK, Turvey SE, Wheeler AJ, Brauer M. (2013). A new exposure metric for traffic-related air in settled indoor house dust. *Environmental Health 12(1)*]. HS formulated the research question, gathered spatial data and extracted questionnaire data, conducted the statistical analysis and led the writing of the manuscript. MB guided the study design, provided spatial data for Vancouver, gave critical input in data conditioning for the analysis and revised the manuscript for its intellectual content. RA provided the spatial data for Edmonton and Winnipeg and guided the data

harmonizing across different spatial databases. JB gathered the ambient air samples from the national air monitoring stations and oversaw the chemical analysis of hopanes samples in the Environmental Canada Laboratory. JC performed a preliminary analysis examining personal hopanes in dust and air samples. JB, JS, TK, RA, and MB were part of the Exposure Working Group for the CHILD study where all questionnaires used in this study were designed. This group, along with the site leaders and CHILD PI oversaw the collection and initial processing of CHILD dust samples. ST, PM, PS are site leaders for the CHILD study, they are responsible for the coordination and training of research technicians and data collection. MS is the Principal Investigator of the CHILD study and has revised the manuscript drafts. SD is the Principal Investigator of the Toronto Child Health Evaluation Questionnaire (TCHEQ) study and revised the manuscript. AW has led the Windsor Ontario Exposure Assessment Study (WOEAS), participated in preparing the spatial data for Windsor, and has given critical input to the manuscript drafts.

A version of Chapter 3 has been published [H.Sbihi,, R. Allen, A Becker, J Brook, J. Scott, P Mandhane, M.Sears, P. Subbarao, T. Takaro, S.Turvey, M. Brauer. 2015. Perinatal exposure to traffic-related air pollution and development of atopy in a Canadian birth cohort study. Environmental Health Perspectives. DOI:10.1289/ehp.1408700]. HS and MS formulated the research question. HS defined the specific objectives and analytical approaches to conduct the study. HS gathered environmental researcher-collected and governmental exposure data, wrote the manuscript and subsequent revisions. AB, ST, PM and PJ were site leaders for CHILD cities and oversaw the biological samples collection as well as the administration of questionnaires. TT and JB are leaders of the Environmental Working Group and have designed and facilitated access to the time-activity patterns questionnaire data. RA has provided the infiltration model and insight into using it in the context of a Canadian study. MS is the principal investigator of CHILD and has revised the manuscript drafts.

A version of Chapter 4 has been submitted for publication. HS, MB, MK defined the research theme. LT prepared administrative health data linkages provided by Population Data BC. HS and LT designed analytical and statistical methods. HS analyzed the data, interpreted the results and wrote the paper. All

authors discussed analyses, interpretation and presentation. All authors have contributed to and approved the manuscript. Perry Hystad is acknowledged for assigning greenness exposures to study participants.

A version of Chapter 5 has been submitted for publication. HS and MB conceptualized the research objectives and formulated the research questions. HS designed the analytical approach, prepared and conducted the analysis. HS wrote the manuscript and included edits provided by MB and MK. MK provided feedback on revisions of the manuscript.

Disclaimer for Chapters 4 and 5: “All inferences, opinions, and conclusions drawn in this research are those of the authors, and do not reflect the opinions or policies of the Data Steward(s).”

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## List of Abbreviations

BAQS: Border Air Quality Study

CO: Carbon monoxide

CHILD: Canadian Healthy Infant Longitudinal Development

Der p : *Dermatophagoides pteronyssinus*

Der f: *Dermatophagoides farinae*

$F_{inf}$: Infiltration efficiency

GIS: Geographic Information System

GVRD: Greater Vancouver Regional District

IDW: Inverse-Distance Weighting

IgE: Immunoglobulin E

LUR: Land Use Regression

NDVI: Normalized Difference Vegetation Index

NO: Nitrogen monoxide

$NO_2$: Nitrogen Dioxide

$O_3$: Ozone

$PM_{10}$: Particulate matter with an aerodynamic diameter <10um

$PM_{2.5}$: Particulate matter with an aerodynamic diameter <2.5um

$SO_2$: Sulfur dioxide

TRAP: Traffic-Related Air Pollution

TCHEQ: Toronto Child Health Evaluation Questionnaire

WOEAS: Windsor Ontario Exposure Assessment Study

## Acknowledgments

It is with extreme pleasure that I write this section where I have the opportunity to express my gratitude to all who have made this journey possible and often enjoyable.

First and foremost, Dr. Michael Brauer, my supervisor, has not only been a very supportive, challenging, and insightful supervisor but also a mentor who taught me by example to teach with respect, collaborate with integrity, and conduct research with rigor. Members of my committee, Drs. Mieke Koehoorn and Ryan Allen, provided me with great support and a lot of "food for thought" following our one-on-one meetings throughout the PhD. I am also grateful for the opportunities that Dr. Allen had offered outside of the PhD research work that helped complete my learning experience: reviewing journal manuscripts, giving seminars, and discussing ideas that shaped the trajectory of my research. Overall, I believe I have had the best supervisory team with immense intellectual generosity, thank you!

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I would like to thank the University of British Columbia, Killam Trusts, Canadian Institutes of Health Research, and the province of British Columbia BC Clean Air Research for their generous financial support.

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# Chapter 1: Introduction

## 1.1 Background and significance

### 1.1.1 Asthma and atopy in children

Respiratory and allergic disorders in children are a serious health issue due to their high prevalence worldwide and negative impact on the quality of life during childhood and later throughout adulthood (Bousquet et al. 2005; McColley and Morty 2012).

Traditionally, asthma has been viewed as an allergic disease as demonstrated in several studies where atopy strongly predicts its development (Custovic et al. 2013; Simpson et al. 2010). However, children can also develop asthma without being atopic. Atopy, the condition referring to allergic manifestations following exposure to a trigger allergen, is also an important health issue and can share common etiology with asthma. In light of the substantial geographic variations, both for prevalence rates and trends (Asher et al. 2006; Eder et al. 2006; Pearce et al. 2007), these conditions are likely multifaceted and include not only genetic factors but also environmental and lifestyle factors (Nicolaou et al. 2005; Subbarao et al. 2009). Air pollution constitutes an environmental exposure that has captured particular attention, especially given the results of a child-specific health impact analysis showing health and economic benefits after a reduction in air pollution levels (Wong et al. 2004). In this chapter, the clinical manifestations and characteristics of both asthma and atopy are presented, with an overview of their natural history and epidemiology. The role of known and suspected environmental exposures will be described with a focus on neighborhood exposures: air pollution, especially traffic-related pollutants, and green spaces.

#### 1.1.1.1 Clinical presentation

Asthma is a heterogeneous disease that can be described as a chronic inflammatory disorder of the airways, characterized by intermittent airflow obstruction, shortness of breath and wheeze (Lemanske Jr.

and Busse 2010). The underlying pathophysiological basis of asthma is believed to be inflammatory processes involving cells of the innate and adaptive immune systems. Cytokines, T and B lymphocytes, mast cells, and histamines act together with epithelial cells to cause bronchial hyper-reactivity, a characteristic smooth muscle contraction involving mucous secretion and other changes that lead to airway remodeling if these changes remain unresolved (Maddox and Schwartz 2002). Airway remodeling is thus the irreversible end result of such pathophysiological mechanisms, also referred to as endotypes[1]. Clinically, children often have allergic asthma which is initiated by allergic sensitization. Following this, they may develop allergic rhinitis or other allergic conditions (e.g. eczema) and progress to asthma, a trajectory often referred to as the 'atopic march'. However non-atopic asthma also presents similar clinical manifestations and represents a non-negligible 44% of pediatric asthma in the United States (US) (Arbes et al. 2007), a proportion that has considerable geographic variation (Ronchetti et al. 2009).

Atopic diseases constitute a group of allergic disorders (e.g. allergic asthma, atopic dermatitis, and allergic rhino-conjunctivitis) that are subject to hereditary predisposition, called atopy. Thus atopy is a personal and/or familial propensity to developspecific immunoglobulin E (IgE) antibodies against common allergens. Sensitization is defined as the presence of a specific immune mechanism directed towards a particular antigen and is most often shown by a positive skin-prick test and/or by the presence of specific IgE (sIgE) antibodies. This definition of sensitization does not necessarily imply the presence of allergic symptoms. In fact, the more recent view is that quantification of atopy, either as the level of sIgE and/or the size of skin test wheal diameter, can help identify those at risk of persistent allergic disorders including allergic asthma (Custovic et al. 2013).

[1] Endotype (can also be termed endophenotype): subtype of disease defined by distinct pathophysiological mechanism. A phenotype, which does not point to an underlying pathophysiological process, represents the observable manifestations of each individual's genetics that can change in response to different environmental stimuli (Corren 2013)

While asthma is traditionally categorized into allergic versus non-allergic asthma, there is an increasing recognition that this paradigm has been an oversimplification. Different asthma phenotypes, described by clinical features, are now being clustered by one or several endotypes (Corren 2013; Henderson 2014a; Lötvall et al. 2011). Conversely, a discrete endotype, such as eosinophilic airway inflammation, can be present in several phenotypes including atopic asthma or severe recurrent wheeze whereas neutrophil-predominant airway inflammation was associated with chronic obstructive pulmonary disease in adults and with severe, non-steroid responsive childhood asthma (Henderson 2014a). The identification of all endotypes underpinning the various phenotypes of asthma have yet to be uncovered (Belgrave et al. 2013). Birth cohort studies with long-term follow up can aid in recognizing clinically similar groups of phenotypes or sub-types of asthma.

#### 1.1.1.2 Natural history, epidemiology

Asthma is the most common chronic disease in childhood and a leading cause of emergency department visits, hospitalizations and school absenteeism in children (Bousquet et al. 2005; Ismaila et al. 2013; Masoli et al. 2004). In Canada, based on two national surveys, the prevalence among children aged 0 to 11 years who had been diagnosed with asthma was estimated to be at 11% in the mid-nineties (Millar and Hill 1998); and by 2001 the rate had risen to more than 13%, with a sharper increase among 0- to 5- year olds (Garner and Kohen 2008). Current estimates of childhood asthma prevalence in Canada have shown a plateau (12% to 13%), following an approximate four-fold increase over the past twenty five years (Subbarao et al. 2009). Similar trends have been observed in most developed countries (Eder et al. 2006). Developing countries are likely to follow the increasing trends seen in developed countries where the observed increases are likely related to environmental changes associated with the adoption of a 'modern' lifestyle (Nicolaou et al. 2005).

In the late nineties, global studies of asthma and atopy have shown increases in the prevalence of wheezing, increased in areas where prevalence was initially low (e.g. Asia) (Asher et al. 2006). Prevalence either decreased or remained unchanged in areas where prevalence was already high (Asher et al. 2006). After the 1990s, estimates of temporal trends in the prevalence of asthma in several European and Asian countries were conflicting, possibly because of the greater variation seen in trends in the prevalence of symptoms suggestive of asthma compared to those of diagnosed asthma (Eder et al. 2006). The global variations in asthma prevalence may thus be at least partially related to differences in asthma symptoms definition. Therefore, the main challenge in pediatric asthma has been and still remains to be the characterization of this "heterogeneous condition" (Bisgaard and Bønnelykke 2010). This would in turn imply that childhood asthma does not follow a specific trajectory but that there may be several natural histories to unveil and understand.

Given the evolving and complex nature of asthma, long-term studies are necessary to understand the development of the disease, to identify associations between exposure and the initiation of the disease in order to gain more knowledge about its different phenotypes. One landmark cohort study, the Tucson Children's Respiratory Study (Martinez et al. 1995b) allowed the first segmentation of asthma among children: growing out of symptoms, growing into symptoms, or continuing to have symptoms. Cohort studies have shown that the majority of children belong to the first segment with only 10 to 15% of children being diagnosed with "true" asthma at the time they are at school when 50% of preschool children have wheezing symptoms (Sears et al. 2003). Since the Tucson study, asthma in early infancy and childhood have been further characterized and are now commonly described as transient wheezing, non-atopic wheezing, late-onset wheezing and persistent wheezing.

The assessment of asthma in epidemiologic studies is complex in light of the various trajectories and phenotypes. To date, there is no single instrument to identify asthma with certainty. The "gold standard" is physician-diagnosed asthma, a diagnosis made on the basis of medical history, physical examination,

and assessment of airway obstruction. Studies usually rely on questionnaires where participants either self-report asthma based on symptoms, or report receiving asthma diagnosis from a physician. Atopy is a consistent risk factor for asthma in many epidemiological studies (Custovic et al. 2013). The proportion of asthma attributable to atopy in children has been estimated to be 38%, but there is considerable variation between studies. The multicenter International Study of Asthma and Allergies in Childhood (ISAAC) phase II showed that the population fraction of asthma attributable to atopy differed greatly between countries according to their economic development, being 40.7% in study centres from economically affluent countries and 20.3% in centres from 'non-affluent' countries (Weinmayr et al. 2007).

#### 1.1.1.3 Sensitive Periods of Exposure

Environmental exposures that occur in early-life are the most influential for respiratory and immune systems (Selevan et al. 2000). Newborns have an imbalanced immunity at birth with both an immature innate immune system and an acquired immune system skewed toward Type 2 helper cell (Th2). Adjustments in the T-helper cells and further maturation of immune cells through interaction with environmental factors such as microbial agents are needed for the infant to restore balance among Th-driven responses and to aid in the complete maturation of the innate immune system, which would minimize the risk of Th2-mediated allergic disease (Dietert and Zelikoff 2008). Early observations in farming cohorts where some aspect of farm living in early life (i.e. microbial stimulation) appeared to protect against the development of allergic diseases, and gave support to the role of immune balance efficiency in relation to the development of allergic diseases. This "hygiene hypothesis" stipulates that the altered patterns of exposure to microorganisms resulting from more western lifestyles leads to immune malfunction characterized by the imbalance between Type 1 and Type2 helper T cells [Th1] and [Th2] (Strachan 2000). Any early life environment exposures, including air pollutants, hindering these adjustments towards the restoration of Th-cells balance could be a risk factor for allergic diseases and

asthma. In addition, two main points were summarized in the Workshop to Identify Critical Windows of Exposures for Children's health. The first conclusion was that interference with normal development of the respiratory and immune systems during the intrauterine period and infancy may have lasting consequences. Second to this, it was suggested that immaturity of metabolic pathways hinders the ability of fetuses and young children to clear toxicants effectively (Dietert et al. 2000).

There are many plausible reasons for the vulnerability of young children: they are exposed to higher doses since they breathe more air than adults relative to their body weight and pollutants have more impact on the rapidly developing systems of fetuses and children (Dietert et al. 2000).

The vulnerability of this segment of the population has been consistently reported in the epidemiological literature(Aguilera et al. 2013; Jedrychowski et al. 2005; Morales et al. 2015). For example, second-hand tobacco smoke exposure during early life affects asthma risk (Rosa et al. 2011), while exposure in later life is associated with allergic disorders, asthma exacerbations, but not new asthma onset (Gilliland et al. 2001). While numerous exposures are known to contribute to childhood asthma exacerbations, including ambient air pollution (Tzivian 2011), fewer factors have been well established as risk factors for the development of pediatric asthma, resulting in a long list of other putative risk/protective factors.

### 1.1.2 Risk factors for asthma and allergy

Environmental exposures, especially those encountered during pregnancy as well as in infancy appear to play an important role in the development of pediatric asthma and allergic diseases given the development and continuing maturation of the immune system and the lungs during these periods. Recent studies, mostly birth cohort studies conducted in developed countries, have suggested that exposure to traffic-related outdoor air pollution (TRAP) is related to incident asthma while others have linked common allergens of indoor origin and endotoxins with the development of childhood asthma and wheeze.

The understanding of the contribution made by environmental exposures to asthma and allergies development in children requires the assessment of exposures in all micro-environments where the child develops: in utero, indoors and outdoors. Pregnant women, infants and young children are exposed to a wide range of environmental factors which include those that can be inhaled (indoor and outdoor air), absorbed dermally, ingested, and the microbial milieu (gastro-intestinal flora for example). The main risk factors, excluding host factors, which have been known from long-term studies to be associated with asthma and allergic disorders, are genetics, behavioral (e.g. diet), and environmental (e.g. second-hand tobacco smoke, virus, and bacteria) factors. Following a brief review of these known factors (genetics, second-hand tobacco smoke (SHS), microbial agents), the role of TRAP, a suspected environmental factor for which the epidemiologic evidence is mounting, will be critically reviewed (see sub-section 1.1.3).

#### 1.1.2.1 Genetics

Family and twin studies indicate that genetics play an important role in the study of asthma etiology (Los et al., 2001). Linkage, association studies and genome-wide screening suggest that many genes are involved in the pathogenesis of asthma. It is likely that genetics contributes to the different trajectories of asthma development. However, the dramatic and rapid increase in asthma prevalence in developed countries observed during the second half of the 20$^{th}$ century (Eder et al. 2006) cannot be explained by genetics alone. Short-term changes in the occurrence of asthma are more likely to be influenced by changes in the environment, diet, and lifestyle than by changes in the genetic pool. The role of environmental influences in asthma expression is further supported by natural epidemiological observations within genetically similar populations after the German reunification (Subbarao et al. 2009). Similarly, the clear differences in asthma prevalence in populations with the same ethnic background but differing environmental exposures are convincing arguments that environmental influences cannot be ignored (Wong and Chow 2008). Thus, asthma can be labeled as a genetically complex disease where

multiple genetic variants interact with environmental factors to modify both susceptibility and severity of the disease.

The effects of gene-by-environment interactions in asthma are complex because interactions are affected not only by multiple gene variants and potential exposures, but also by time windows. For instance, in early childhood, genes code for enzymes that detoxify inhaled agents (e.g. glutathione transferase) while *in utero* genes may have a more direct effect via epigenetic mechanisms (e.g. possibility that maternal smoking modifies fetal genetic expression). However, gene-environment interaction studies for asthma have clearly demonstrated interaction effects for some environmental inhalable exposures including endotoxin (Martinez 2007; von Mutius 2009), SHS (Dizier et al. 2007; Wu et al. 2007), and air pollution which encompasses ozone exposure (London 2007; MacIntyre et al. 2014), nitrogen dioxide (MacIntyre et al. 2014; Melén et al. 2008) and particulate matter exposures (Kerkhof et al. 2010). Therefore genetics can be useful to disentangle environmental influences in specific subgroups of the population. Indeed, compared with population-based studies in which the effect of an environmental exposure is compared between groups (exposed versus unexposed), genotyping allows the identification of susceptible individuals that in turn allows for a better estimation of the true magnitude of effect of an environmental exposure for the population at risk. In sum, the role of genetics adds more specificity to the causal relationship under investigation. However, as long as the assessment of environmental exposures lacks accuracy and precision, the ability to understand the effects of gene by environment interactions will be limited.

#### 1.1.2.2 Second Hand Smoke (SHS)

SHS is likely the most important indoor pollutant and exposure of public health relevance. Tobacco smoke contains more than 4000 chemical substances which have known or suspected carcinogenic, mutagenic, toxic or inflammatory properties (California, EPA 1997). For infants and children, exposure to SHS due to maternal smoking during pregnancy is of particular importance. Carlsen and Carlsen provided

a review of the role of SHS and respiratory disorders in infants and young children highlighting the role of genetic polymorphisms (Carlsen and Carlsen 2008). Therefore, it appears that asthma and SHS are impacted by time of exposure, genetic susceptibility and heritability (i.e. history of atopy). Vork and colleagues performed a meta-regression to identify biases and inconsistencies among all the previous reviews and found, based on thirty eight recent studies and previous meta-analyses, that the relative risk of secondhand smoke and ever, current and incident asthma were 1.48 (95% CI 1.32 – 1.65), 1.25 (1.21 – 1-30) and 1.21 (1.03 – 1.36), respectively (Vork et al. 2007).

The weight of evidence is increasingly suggestive of a causal pathway between exposure to SHS mixtures and development of asthma under a set of genetic predispositions.

#### 1.1.2.3 Microbial agents

The list of inhalable indoor factors, reviewed here, that have been linked to asthma development in children includes endotoxins, (1,3)β -D-Glucans (Dales et al. 2006), different indoor aero-allergens such as cockroach allergens (Salam et al. 2004), mouse allergens (Matsui et al. 2006), and house dust mites (Huss et al. 2001; Loan et al. 2003; Peat et al. 1996; Sidenius et al. 2002). The latter exposure is probably the one that has gathered the most attention from researchers in terms of exposure assessment, quantification, analysis and prevention (Loo et al. 2010).

*Damp environments, Endotoxin and Beta-Glucans*

The consistent reporting of several epidemiological findings on the adverse health effects of living in damp homes or those with visible molds has been recently summarize in a meta-analysis by Fisk and colleagues. The associated percent increases in health outcomes (asthma, wheeze, rhinitis) for participants residing in damps homes ranged from 30% to 52% (Fisk et al. 2007). Increased dampness stimulates growth of fungi, bacteria, and other biological agents.

Endotoxin, a component of gram-negative bacteria cell walls, is commonly used as a surrogate for microbial exposure. Other important bio-active components of damp environments are the (1,3)-β-glucans, which are also cell-wall components, but in this case resulting from fungal growth. Both components have inflammatory properties including immune-stimulating characteristics and airways irritation.

In contrast to the findings reported by Fisk and colleagues on visible mold and measured specific mold species, cohort and birth cohort studies showed that early-infancy exposure to (1,3)-ß-D-glucan decreases the risk of developing wheezing symptoms and parental-reported, doctor-diagnosed asthma in young children (Douwes et al. 2006; Iossifova et al. 2007). This finding is in line with the hygiene hypothesis. While this hypothesis involves the role of both environmental and host factors (e.g. via the lack of exposure to infections such as the respiratory syncytial virus early in life), restricting exposures to inhalable factors would postulate that the rise in allergic diseases and asthma is due to increasingly hygienic environments that do not provide sufficient microbial stimuli to allow for the normal maturation of the immune system and development of appropriate immunologic responses. This hypothesis has been extensively debated (Platts-Mills et al. 2001; von Mutius 2001). Since the original theory offered by Strachan (Strachan 2000) mainly holds for allergic asthma but does not characterize observations for non-atopic asthmatics, the hygiene hypothesis has been revisited to include more objective measures of bacterial and fungal exposure. For example, endotoxin has two opposing effects: it protects against atopy but is a risk factor for non-atopic asthma and wheezing. Recently published data tried to improve on our understanding of the impact of microbial exposure on asthma initiation by identifying microorganisms involved in the onset of the disease using two cohorts that relied on complementary techniques, namely the use of single-strand conformation polymorphism (SSCP) analyses and the culture techniques (Ege et al. 2011). These authors found a greater diversity of exposures in farms/rural areas as compared to urban settings; this diversity explained a wide proportion of the inverse relationship to asthma in the study

subjects. Interestingly the diversity did not show associations with atopy, as the latter was inversely associated with the level of gram-negative bacteria exposure. The contrast in findings for asthma and atopy likely indicate that there are different mechanisms by which microbial exposures affect asthma and atopy. This in turns fits the differences in genetic determinants of these two related conditions as shown in the latest Genome-Wide Association Study (GWAS) on asthma and allergies (Moffatt et al. 2010). It has been suspected that (1,3)-ß-D-glucan may have a similar impact on the development of an infant's immune system as an early endotoxin exposure (Iossifova et al. 2007; Schram-Bijkerk et al. 2006).

*House Dust Mites*

Since Sporik's landmark paper in 1990, in which increased exposure to house dust mites (HDM) was proposed as a risk factor for asthma (Sporik et al. 1990), the role of HDM in the development of childhood asthma has been the subject of intensive investigation (see for example (Platts-Mills et al. 2009; von Hertzen and Haahtela 2009)). Nevertheless, recent reviews demonstrate a consensus that early-life exposure to mite-related allergens increases the risk of sensitization, thereby leading to asthma and allergic disease exacerbations (e.g., eczema, rhinitis) among those sensitized (Arshad 2010; Platts-Mills et al. 2007). The question of the role of HDM and/or HDM allergens in the development of asthma remains elusive, although there is mounting evidence for a causative pathway among genetically predisposed subgroups (Bisgaard et al. 2008).

Exposure to house dust mites has likely increased, especially in light of the many recent changes in the human environment that contribute to the increase of "mite load" indoors: increase use of indoor heating and air humidification systems provides optimal environmental condition for mite growth; new "green" detergents free from acaricides does not affect mites; and vacuum cleaners have replaced picking up and beating carpets outside the home leading to re-suspension of the particles carrying the HDM allergens (Heinrich 2010). Despite varying levels across the world, the link between HDM allergens and asthma

development in children has been shown among different populations (general population versus "at risk", varying SES, etc.), which would argue in favor of a causal relationship. Of particular interest is the finding that suggests that the dose-response relationship may not be linear as previously reported (Celedon et al. 2007) but rather bell-shaped. The link between mite allergen and the induction of allergic sensitization has been established on the one hand, and the causal relation between specific allergic sensitization to mites and asthma onset is unequivocal on the other hand. Yet, the direct relation between mite exposure and asthma is still unclear, with both positive association (Celedon et al. 2007; Torrent et al. 2006; Tovey et al. 2008) and no effect reported in birth cohorts assessing exposure at birth (Lau et al. 2005). This would indicate that HDM allergens are associated with certain phenotypes of asthma (e.g. allergic asthma). In the most recent review on this topic, Heinrich also proposes that while no direct evidence for mite exposure and asthma onset can be drawn, "sufficient evidence" can be concluded from an indirect link via the increased risk of sensitization (Heinrich 2010).

### 1.1.3 Air pollution as a risk factor for asthma and allergy

#### 1.1.3.1 Effect of ambient air pollution and respiratory diseases in children

The outdoor environment may impact respiratory health both directly and indirectly by infiltration of outdoor-generated pollutants into indoor environments. For the former association, there is sufficient evidence to suggest that air pollutants, such as ozone and particulate matter, decrease lung function, trigger asthma exacerbation, and increase rates of hospitalization for asthma. This has been observed in various locations, including Toronto, Ontario (Thurston et al. 1997) , Seattle, Washington (Schwartz et al. 1993), and many European cities (Sunyer et al. 1997) .

While studies have demonstrated that exposure to outdoor air pollutants exacerbates pre-existing asthma, the evidence for its effect on the initial development of asthma and allergic disorders remains unclear

(Health Effects Institute 2010; Heinrich and Wichmann 2004). The lack of conclusive evidence is possibly due to poor or inadequate exposure assessment methods. Indeed, assessing long-term exposure to air pollution has always been a significant challenge. The cruder assessment involves estimating pollution at the community level based on fixed-location government monitoring data, thereby assigning each person in the community the same level of exposure. However, within-community differences in air pollution are often as large as or larger than between-community differences. Therefore, people living in the same community may have considerably different exposures and assigning them the same exposure leads to significant misclassification. This type of error in exposure assessment, called Berkson error (Armstrong 1998), reduces the power of a study and implies that a "true" underlying association is less likely to be detected. Furthermore the threat of confounding with this approach can further obscure any associations. Indeed, community differences other than air pollution levels such as socioeconomic status may also confound any potential association between air pollution and childhood asthma and allergic disorders development. Research interest has therefore shifted to assessing within-community differences in exposure. In particular, this interest was directed towards urban within-community air pollution since several studies found that asthma rates are higher in urban areas than in rural areas, suggesting the urban environment may contribute to asthma development (Nicolaou et al. 2005). Another motivating aspect for this accrued interest is that within-city variability may also reflect different pollution sources and pollutant mixtures, often related to traffic. This is somewhat different than the between-city analyses where pollution sources encompass industrial and traffic sources, as well as secondary formation of air pollutants.

An example of a simple approach to examine urban within-community air pollution variation is an assessment based on proximity to emission sources such as major roads and highways. Indeed, proximity to traffic has drawn significant attention. Interest in investigating traffic proximity in relation to asthma development was also motivated by studies of natural experiments such as the East-West German

comparison. Following the reunification of Germany, it was possible to compare genetically similar populations where the main difference of exposure with respect to air pollution was that Western Germany had higher $NO_2$ levels compared to high $SO_2$ levels in Eastern Germany driven by coal combustion (supposedly the main heating source). Since the rates of asthma and atopy in areas of East Germany were found to be much lower than in West Germany, the focus of research has shifted from background air pollution toward pollution from motor vehicles (von Mutius et al. 1994). The literature on pollution from motor vehicles showed that focusing the exposure assessment to the urban pollution portion due to traffic emissions unveiled associations with childhood asthma development that were unseen when estimating general urban/background air pollution. At the ecological level, the ISAAC analysis phase I assessed the urban background concentration of $PM_{10}$ in 55 countries in order to evaluate the role of ambient particulate matter in explaining geographical variation in the prevalence of asthma (Anderson et al. 2010). Anderson and colleagues concluded that urban background $PM_{10}$ has little or no association with childhood asthma. On the other hand, using self-reported truck traffic intensity in the same study did show an association with asthma, giving further weight to shifting the focus to TRAP (Brunekreef et al. 2009). Since these studies, there is growing evidence that air pollutants that are specifically associated with traffic exposure may have a role in incident asthma (Guarnieri and Balmes 2014). A recent review concluded that living close to busy roads is an independent risk factor for the onset of childhood asthma even though it did not reach the level of sufficient evidence (Gowers et al. 2012).

#### 1.1.3.2 Traffic-related air pollution, asthma and allergies: mechanisms, exposure assessment, and epidemiological evidence

The emission of air pollution from motor vehicles affects the quality of the air, especially considering the rapid growth of the motor-vehicle fleet worldwide and despite substantial improvements in emission controls. TRAP is a mixture of particulate matter (PM), volatile organic compounds (VOCs), polycyclic

aromatic hydrocarbons (PAHs), and gases such as nitrogen oxides (NO, $NO_2$) and carbon monoxide (CO). This mixture's composition is a function of the type of vehicle, its operating and maintenance condition, type and quality of fuel, wear of tires and brakes, and engine lubricants. Nitrogen oxides are often used as surrogates for TRAP as data for these air pollutants, associated with a diverse set of health effects, is often routinely collected by governments.

Nitrogen oxides are a group of reactive gases, of which nitrogen dioxide ($NO_2$) and nitric oxide (NO) are most commonly monitored. NO and $NO_2$ are both released from combustion. NO is a primary combustion pollutant that reacts with ozone in the atmosphere to form $NO_2$. Concentrations of both gases are highest near traffic sources. Long-term average $NO_2$ levels in cities worldwide vary from 15-60 $\mu g/m^3$ (WHO 2006). In general, traffic-related pollutants impact ambient air quality on a broad spatial scale ranging from roadside, to urban, to regional background. Based on synthesizing evidence gathered by the panel of Health Effects Institute (HEI) experts, 300 to 500 meters from a major road was identified as the near-source area most impacted by traffic; variations exist depending on meteorology, background pollution, and local factors (Health Effects Institute 2010).

Particulate Matter (PM) refers to a complex and dynamic mixture that can be characterized by mass or number concentrations in specific size categories and/or by chemical composition. Monitoring efforts target particles with an aerodynamic diameter less than 10 micrometers (referred to as $PM_{10}$) because they can be inhaled and deposited into the lungs. A subgroup of $PM_{10}$ is fine particulate matter, or particles with an aerodynamic diameter less than 2.5 micrometers (referred to as $PM_{2.5}$), which are able to penetrate deep into the lungs. For this reason, they have become increasingly monitored. $PM_{10}$ includes coarse PM which, while potentially related to TRAP, might arise from non-combustion sources (e.g. brake and tire wear, suspended road dust into air by wind and traffic) as well as industrial and mobile sources. Primary sources of $PM_{2.5}$ are mainly from combustion. Particulate matter levels can be higher

near emission sources, such as roadways (Health Effects Institute 2010), in particular for black carbon, ultrafine particles and $PM_{10}$ (Karner et al. 2010).

As traffic is currently, and is likely to remain, a major contributor to air pollution in urban settings, understanding its impacts on health is of upmost importance. According to the Health Effects Institute review on TRAP, there is sufficient evidence to infer a causal role for TRAP in the exacerbation of asthma in children (Health Effects Institute 2010) . However, there is also mounting evidence that TRAP may induce asthma onset as discussed below.

***Biological mechanisms and role of TRAP in the incidence of asthma and allergic disorders.***

Experimental studies have identified specific TRAP-induced biological effects. The effect of diesel exhaust particles has received the most attention. It has been shown to enhance allergic inflammation by skewing the immune response towards Th2 activation, therefore increasing the production of IgE. D'Amato and colleagues provide a thorough review of the potential effects of exposure to ozone, $NO_2$, $SO_2$, and inhalable particulate matter on respiratory allergic diseases (D'Amato et al. 2002). These studies, however, argue for the allergic asthma phenotypes. Other mechanisms involving the oxidative stress pathway have also been suggested for the role of TRAP in childhood asthma development. Reactive oxygen species (ROS) and free radicals are generated by traffic related pollutants such as $NO_2$ and PM. Oxidative stress arises when the neutralizing antioxidant defense in the airways, secreted to counteract the effects of ROS, is overwhelmed. In turn, this enhances inflammation, specifically around the respiratory epithelium, and further ROS formation (Bråbäck and Forsberg 2009). In the developing lung, air pollutants may interact with the airway in a way that modifies its structural functions leading thus to asthma (airway remodeling). More recently, epigenetic studies are increasingly supporting the oxidative stress pathway in the association between TRAP and asthma. In particular, following short-term exposure to diesel, changes in the DNA methylation was seen in gene sites involved with inflammation and

oxidative stress responses. This provides a plausibile pathway by which TRAP can impact gene expression and subsequently respiratory system function (Jiang et al. 2014).

The expert panel of the Health Effects Institute found evidence suggesting that TRAP was associated with pediatric asthma onset, a finding further corroborated by recent publications (Clark et al. 2010b; Gehring et al. 2010a; Gruzieva et al. 2013; McConnell et al. 2010). The most relevant studies to assess pediatric asthma onset are birth cohort studies as this design allows the follow up of study participants from birth or earlier during pregnancy, and to characterize their exposures prior to the onset of the disease, establishing the direction of causality. However, assessing the role of TRAP on childhood asthma in epidemiologic studies is fraught with issues related to the case definition. Asthma onset relies on history of symptoms that may indicate not only onset but also exacerbation. Additionally, pediatric asthma diagnosis is challenging and may often confuse wheezing with "true" asthma, since robust diagnosis is often only possible at school age.

Among the first studies to highlight the role of timing of exposure for asthma onset was a case-control study in France in children aged 4 to 14 years. The authors of this study demonstrated an association between traffic density (>30.3 (veh/day)/m) before the age of 3 years and asthma (Zmirou et al. 2004) but no association with lifelong exposure, suggesting that early exposure to traffic related air pollution plays a role in the initiation of asthma.

Adding to the weight of evidence are the generally consistent results from birth cohort studies over different geographical areas spanning Asia, Europe and North America. In Europe, a Dutch cohort (the Prevention and Incidence of Asthma and Mite Allergy [PIAMA]) (Brauer et al. 2002, 2008; Gehring et al. 2010a) and two German cohorts (GINA and LISA) used a similar modeling-based exposure assessment approach to estimate individual estimates at each subject's home address at birth with rigorous control of other covariates related to asthma and allergies. While the German studies suffered from a less robust

case definition, using "obstructive/asthmoid bronchitis" that likely related to the mixed results obtained in these cohorts (Gehring et al. 2002; Morgenstern et al. 2007), the Dutch study examining the children at three time points (2, 4, and 8 years of age) had more consistent results for asthma and allergies. Of particular interest is the follow up study on 4000 Dutch children until age 4 where Brauer *et al.* showed that exposure to higher levels of TRAP was associated with increased risks of doctor diagnosed asthma: 1.3 times increased risk per 0.6 x $10^{-5}$/m increase in level of soot, 1.2 times increased risk per 10 μg/m3 increase in NO2, and 1.2 increased risk per 3.3 μg/m3 increase in PM2.5 (Brauer et al. 2008). The follow up study led by Gehring and colleagues confirmed the association of traffic air pollutants in 8 year old children (Gehring et al. 2010a). A Canadian birth cohort study, also relying on similar modeling method to assess exposure in utero and during the first year of life showed positive associations between early-life TRAP exposures and asthma. Here, the researchers determined that exposure to traffic-derived pollutants in early years, including NO, $NO_2$, CO, and black carbon (BC), were associated with the highest risk estimates compared to other pollutants ($SO_2$, and PMs), although these are not good TRAP proxies (Clark et al. 2010b). In the US, different approaches for exposure assessment to TRAP were used to assess its impact on pediatric asthma incidence. In the Cincinnati birth cohort, residing close to stop-and-go traffic (<100 m) had a 3-fold increased risk of wheeze in the first year of life compared to unexposed infants (Ryan et al. 2005), and increasing modeled levels of traffic soot showed a dose-response relationship with infant wheezing (Ryan et al. 2007). Both studies did not have robust case ascertainment as the identified phenotypes were outcome measures that often resolve subsequently at school age.

However, the classification of the evidence for the role of TRAP on incidence of asthma by the Health Effects Institute expert panel was deemed as suggestive only. Indeed, even when including various phenotypes of asthma, some cohort studies did not find any association between TRAP and childhood asthma. For instance, a cohort of school children in Oslo, Norway where individual exposures to TRAP pollutants ($NO_2$, $PM_{10}$ and $PM_{2.5}$) in early life and during lifetime were calculated using an atmospheric

dispersion model with contribution from busy roads showed no association with asthma onset (Oftedal et al. 2009). Similarly, negative reports from German cohorts showed no relation between exposure to air pollutants and new onset of asthma (GINI/LISA) (Gehring et al. 2002). Therefore, results to date are diverging. It should be noted that the most consistent evidence of an association of TRAP with childhood asthma incidence involved epidemiologic research where the exposure assessment of TRAP included both traffic-specific pollutants and traffic density measures. It is also possible that the overall lack of agreement relates to the confounding effect of genetics and gene-environment interactions. In fact, a 2009 review of the impact of TRAP on respiratory health called for genetic association studies to clarify how polymorphisms may increase susceptibility to traffic exhaust (Bråbäck and Forsberg 2009). However, for such studies to detect an interaction, a rigorous and accurate exposure assessment of TRAP is crucial.

While qualitative critical reviews have suggested that the weight of evidence may indicate possible causal associations between childhood asthma and TRAP (Bråbäck and Forsberg 2009; Health Effects Institute 2010), meta-analyses have demonstrated diverging results. Anderson et al., recently conducted a meta-analyses of the epidemiological evidence published up until 2010 and reported an adverse effect of outdoor air pollution on asthma incidence but no association with lifetime period prevalence of asthma (Anderson et al. 2013a, 2013b). Another meta-analysis showed adverse effects of traffic air pollution on pediatric asthma (Gasana et al. 2012). Although the results of these meta-analyses appear contradictory, a possible explanation could be that the increased risk in incidence reflects the unveiling of the exacerbation of subclinical phenotypes of asthma (Gowers et al. 2012) which highlights the heterogeneity of the disease as well as the need to examine asthma incidence over consecutive time spans. Given that the early life period seems to be a critical time window in the pathogenesis of asthma and allergic diseases, there is a gap for studies following children over time from early life, to preschool and later on into childhood. Epidemiologic results were also inconsistent in studies examining asthma onset in school age participants. For example, positive associations in relation to TRAP exposure have been reported in

the Children's Health Study in California of incident asthma in school age children (Gauderman et al. 2005; Jerrett et al. 2008; McConnell et al. 2010), but no evidence of an association between air pollution and lifetime asthma prevalence in children followed up until age 10 was reported in meta-analysis of birth cohorts from the European Study of Cohorts for Air Pollution Effects initiative (Mölter et al. 2014b). Concomitantly, the discrepancies in results between within-community studies that tend to show positive associations[2] (Gruzieva et al. 2013), and between-communities studies that showed null effects (Anderson et al. 2013b; Mölter et al. 2014b) could also be related to a finer-scale exposure assessment that captures individuals living in close proximity to heavy traffic (MacIntyre et al. 2014). These gaps need to be informed.

***Critical review of exposure assessment methods for TRAP***

In determining the role of TRAP in the incidence of asthma and allergic disorders, various exposure assessment approaches have been employed to estimate individual exposures. They vary in terms of complexity, spatial, and temporal resolution. The simplest approach to assign TRAP exposure to individuals in a study has been either the use of a single piece of information to either derive indicator variables (i.e. proximity measures) or to interpolate information. The former approach uses proximity to major roads as an exposure surrogate for the assessment of temporal and spatial distributions of ambient pollution related to motor vehicles, which has been used extensively in air pollution studies (e.g. distance to major roads (Brunekreef et al. 1997)). Similar to the idea of using proximity to roadways as a surrogate for TRAP exposure, traffic intensity has also been used for assigning traffic pollutants in population-based studies (Gauderman et al. 2005; Salam et al. 2004). Proximity and traffic intensity can provide reasonable approximation because (a) pollutants decay studies have shown that $NO_2$, benzene and PAHs

[2] It should be noted that meta-analyses showing positive association(Anderson et al. 2013a; Gasana et al. 2012) also focused on within-community exposures

are mostly captured between 300m and 500m from roadways (Beckerman et al. 2008), and (b) people tend to live closer to roadways as shown in an investigation of two large North American cities (Los Angeles and Toronto) (Health Effects Institute 2010) and also more recently in a report showing that 10 million Canadians (32% of the population) live within 100m of a major road or 500 m of a highway (Brauer et al. 2012). An important advantage of proximity measures is the ease of interpretation when used in epidemiologic analyses, which helps with knowledge translation and research dissemination for policy-makers. However, traffic intensity and proximity measures are still crude estimates of exposure as they do not account for wind patterns or land topography and do not incorporate measured values of pollutants.

Another approach is to assign exposure by using surrogate measurements such as $NO_2$ from regulatory air quality monitoring networks. Such monitoring networks have the advantage of capturing temporal trends in pollution concentrations, but do not have good spatial density to capture the smaller scale variations (i.e. within city). Moreover, this approach may introduce exposure misclassification in epidemiologic analyses for pollutants that are spatially heterogeneous, which is the case for TRAP. Geographic Information System-based (GIS) spatial interpolation approaches and statistical techniques have been used to obtain a more precise spatial interpolation of ambient monitoring data (e.g. Kriging approaches) providing further refinement of the proximity metrics. However, these approaches require dense monitoring networks and good input data. Even so, they may still not adequately capture the smaller-scale spatial variations such as those occurring near roadways. Since governmental monitoring air quality stations routinely collect $NO_2$, a large portion of the studies using spatial interpolation have assessed TRAP using $NO_2$ as a preferred marker for TRAP. Other advantages of this marker include its inexpensive measurement by passive samplers, and its correlation with other exposure surrogates such as distance to major road or other pollutant markers such as “soot” (also referred to as black carbon). In the Netherlands, the mean outdoor concentration of $NO_2$ measured at twelve schools was positively

correlated with total traffic density on the nearest highway ($r = 0.68$, $p<0.05$), and negatively correlated with the natural logarithm of the distance from highway ($r = -0.83$, $p<0.01$). Black smoke was positively correlated with $NO_2$ ($r = 0.74$, $p<0.01$), and with truck traffic ($r=0.67$, $p<0.05$) but not with cars, total traffic, or with the natural logarithm of the distance from highway (Roorda-Knape et al. 1999).

A limitation of relying on pollutant markers from monitoring networks already in place is that these networks are set up for regulatory purposes. They are not designed to capture hotspots or possible within-city variation for which TRAP is an important source, but rather to reflect ambient levels. Since evidence showed that small-scale variability is often as great or even greater than intercity variation for many air pollutants (Briggs 2005; Miller et al. 2007), exposure assessment techniques with poor spatial resolution increase the potential for misclassification because they provide background levels rather than true variability in a given area. Characterizing small area variations in air pollutant concentrations is of utmost importance in epidemiological studies, especially for large studies conducted within a single urban area. This need prompted the use of models capturing spatial variations in air pollution studies.

Two general approaches can be distinguished for the air pollution models: (1) dispersion models, which attempt to simulate the physical (and to some extent chemical) processes involved in transport; and (2) statistical models, which largely ignore the intervening processes, but represent, by empirically defined equations, the relationship between the source and concentrations at the receptor. The latter class of models includes GIS-based models and represents a technique with good spatial resolution that has been used widely in Europe and North America. GIS-based models are also termed land use regression (LUR) because they are based on empirically-derived regression equations linking land use to measured air pollutant concentrations at a set of monitoring sites. In contrast, dispersion models have been used for many years in air quality management but only recently in applied exposure assessment to provide improved spatial exposure estimates (Marshall et al. 2008a). The main limitation of using dispersion models is that all the relevant input parameters need to be included in the model without necessarily

gaining a significant benefit in terms of spatial resolution. In a comparison of exposure assessment approaches, urban-scale variations were captured by interpolated background air pollution data collected at monitoring stations and a chemical transport model; whereas neighborhood-scale variations were captured by LUR models (Marshall et al. 2008a). In the same vein, European evaluation studies have indicated that LUR models generally perform as well as or better than dispersion models for $NO_2$ estimates (Briggs et al. 2000; Hoek et al. 2008). Another study reported that LUR outperformed proximity models, kriging, and dispersion models for predicting $PM_{10}$ (Gulliver et al. 2011). Hence, for environmental epidemiologic studies where effect size is typically small, the ability of LUR models to capture high spatial variability of urban air pollution make it a preferred methodological approach for TRAP exposure assessment.

LUR modeling generally requires a minimum of 40 to 80 monitoring sites, depending on the size and complexity of the study area (Hoek et al. 2008). Other predictors are usually selected to represent traffic-related emissions (e.g. road length, traffic flows) in the surrounding area, and the effect of local topography (e.g. elevation). The predictor variables included in the model vary widely between studies and are usually obtained via geographical information systems (Hoek et al. 2008). In North American studies, LUR has typically achieved predictions for $NO_2$ in the range of $R^2 = 0.56$ to 0.79 (Health Effects Institute 2010).

Geographic data collected to build LUR models vary by site, as there is no consensus as to which predictor variables must be included. However, numerous studies have demonstrated an association between GIS-derived information related to exposure and respiratory health effects where there was at least one variable related to road use (either traffic, type or distance) (Hoek et al. 2008; Patel and Miller 2009; Venn et al. 2001). Study areas where there was variation in elevation tended to include a variable related to elevation (Ryan and LeMasters 2007). The variable selection depends therefore on the

availability of the data and on the potential sources of pollutant variability in the study area (e.g. shipping emissions, waste burning etc.)

A major limitation of LUR models resides in that they have been mostly used to predict $NO_2$ for logistics reasons. As mentioned earlier, passive samplers for $NO_2$ (e.g. Ogawa sampler) are inexpensive compared with other methods to collect traffic pollutants tracers. Alternate approaches such as mobile monitoring that may be applied to additional pollutants have also been utilized. For example, Larson and colleagues conducted mobile monitoring to collect data for a LUR model predicting black carbon. In general, LUR models suffer from a lack of specificity for TRAP.

Given that the relevant timing of exposure for the development of childhood asthma and allergic diseases is yet to be identified, the ability to assign exposures for precise time windows needs to be improved. This is especially critical as LUR models tend to lack temporal resolution. LUR models are developed based on a large number of measurement locations collected in different seasons usually within a year to predict exposures for studies that examine the effect of chronic TRAP exposures (> 1year). These models have traditionally assumed that there is no change in the relative ranking of the exposure of study participants over time. This assumption is only valid if concentrations of pollutants change systematically in all sections of the study area, which is not always the case, leading in turn to misclassification of the study population, especially in the context of large cohort studies spanning long time periods. Few studies have examined how to improve or combine LUR to model temporal variation in air pollution. Su and colleagues proposed a method where LUR included a source area component to predict hourly $NO_2$ concentrations (Su et al. 2008). They showed that this hybrid model is less data demanding than dispersion models, as it requires only meteorological data on wind speed, wind direction and cloud/insolation. Mölter et al. compared three methods to forecast LUR exposure: (1) apply a temporal trend derived from a local background monitor to the concentrations predicted by the LUR model; (2) change the values of the predictor variables to reflect different time periods; and (3) recalibrate existing

LUR model, which implies that the regression analysis is re-run with updated values of the dependent variable to reflect different time periods (Mölter et al. 2010). Their results conclude that LUR can provide a relatively good temporal resolution for long-term studies provided that recalibration and adjustment are based on current measured data. More recently, the temporal stability (forward and backward) for the LUR model for the greater Vancouver geographic region was examined and found to provide reliable estimates over a period of seven years measurements (Wang et al. 2013). It was shown that when extended in time, the 2003 LUR model explained 60% variation in 2010 measurements, more than it did in 2003 measurements (59%). Since concentrations and their variability decreased overtime, this allows for a reasonable predictive power in forecasting because it consisted of interpolating within the range of measured exposures whereas backcasting involved extrapolating outside the range of measured values.

Limitations associated with LUR estimates of TRAP include: (1) the fact that no study participant stays at a fixed position throughout the study period – although this is true for all methods except measurement –, and (2) the issue of transferability of models over large geographical areas. In large long-term cohort studies, the possibility to transport a model based on one area to another study area needs to be evaluated given the changes in land use characteristics, population densities, and traffic patterns over time. According to the review by Hoek *et al.* (Hoek et al. 2008) LUR methods can benefit from the inclusion of both a spatial and temporal component. Recent studies have investigated the transferability of LUR models for use in wider geographical areas. However, both Poplawski et al. (Poplawski et al. 2009) and Allen et al. (Allen et al. 2011) showed that even for cities with similar characteristics (Seattle, Vancouver, Victoria and Winnipeg, Edmonton, respectively) it was better to create individual local area LUR models than to have the same LUR model for all areas. At the study area level, people are mobile in the course of the day whereas LUR models predict exposures at one location (usually the home of participants), resulting in misclassification for more mobile study subjects. Time-activity patterns can affect personal exposure. For instance, exposures to pollutants in the transport microenvironment are often highly

elevated compared to other microenvironments (Kaur and Nieuwenhuijsen 2009). Data from US travel surveys indicate that in 2001 commuters/adults and children spent an average of 81 and 48 minutes per day in vehicles respectively (Hu 2005), where their exposure to TRAP is likely high. Relative to ambient concentrations measured at monitoring sites, vehicle commuters may receive as much as half the daily exposure in this microenvironment (Gulliver and Briggs 2005; Health Effects Institute 2010). There is a need to adjust LUR model by incorporating mobility information, otherwise the predicted exposure will be inaccurate and may even result in misclassification (Setton et al. 2010). An understanding of factors that influence personal exposures is necessary for a better understanding of the health risks posed by air pollutants in different microenvironments (Kaur and Nieuwenhuijsen 2009). Various approaches have been explored to incorporate mobility into exposure assessments. Through the use of personal sampling, Nethery et al. demonstrated that exposure estimates derived from LUR models were improved when mobility information was taken into consideration (Nethery et al. 2008b). In the case of birth cohort studies, such an approach would be expensive and infeasible. However, questionnaires on time activity patterns can shed light on the amount of time infants spend commuting and in daycare/preschool. Regression models can be improved by incorporating adjustment factors for the portion not predicted by LUR models.

Most of the reviewed literature on assessing exposure to TRAP is concerned with modeling or measuring outdoor pollutants that readily penetrate indoors. Quantifying the PM infiltration efficiency ($F_{inf}$) in residences can help characterize indoor concentrations and reduce exposure misclassification as there are differences in infiltration between homes and over time (Allen et al. 2006). Misclassification of exposure can affect associations either away or closer to the null because of the different sources of variability associated with infiltration: (1) Studies have shown that $F_{inf}$ can vary from 2 to 10-fold between houses that have the same ambient exposure levels (Allen et al. 2006; Clark et al. 2010a; Hystad et al. 2009) and; (2) $F_{inf}$ can also vary within homes due to seasonal changes. For example, on the Canadian west coast,

infiltration varied from 0.49 to 0.72 (Hystad et al. 2009), whereas infiltration in homes in North Carolina varied from 0.50 to 0.63 in heating versus non-heating seasons (Wallace and Williams 2005). In an effort to circumvent the need to assess infiltration, which is deemed impractical in large studies, Hystad and colleagues demonstrated that property assessment data can be useful to model and estimate infiltration based on the value of the structure of a house, also known as improved value. They showed that homes with structure value below the 50$^{th}$ percentile had 15% higher infiltration than homes above the median. Unfortunately, the level of detail on property assessments varies geographically (often by municipality). Clark et al. showed in another property assessment database, for example, that you could not separate the home value into structure and land value (Clark et al. 2010a; Hystad et al. 2009) even within a relatively defined study area.

Finally, TRAP is not solely composed of PM, as outlined previously. This lack of a specific TRAP tracer poses a major challenge for epidemiologic studies of respiratory health effects of air pollution emitted from vehicles.

#### 1.1.3.3 Chemical tracers of traffic-related air pollution in house dust

Despite the variations that occur in dust sampling, house dust measurements have formed the backbone of epidemiological studies of multiple indoor inhalant exposures and asthma. Indeed house dust presents the advantage of providing one matrix for the evaluation of multiple agents related to childhood asthma (e.g. allergens, endotoxins) which is a reasonable proxy for time-integrated exposure (Lioy et al. 2002). While the accumulation of house dust depends on several factors (e.g. infiltration efficiency, pollutant source, cleaning practices, sampling surface), dust concentrations and loadings of pollutants show less variation over time than do indoor air concentrations. Therefore, dust sampling is a particularly useful tool in studies of chronic exposures (Egeghy et al. 2004). The quantification of a TRAP tracer in house dust, as a marker for indoors inhalable hazards and infiltrated pollutants from outdoor origins (e.g. PAHs from vehicles exhaust), would represent a useful and integrated exposure assessment tool. Particulate matter

infiltration is the key determinant of indoor concentrations of ambient particles, yet PM is not a specific measure of traffic pollution exposure. A good tracer of TRAP would be a chemical for which the major source is vehicle emissions and that can be measured accurately and at low levels for reasonable cost.

One such group of tracers may be the hopanes, a class of organic compounds with 27 to 35 carbon atoms in naphthenic structure (Simoneit 1985). Hopanes are are present in engine oil lubricants, but are not found in gasoline and diesel fuel because they are in the higher boiling fraction of petroleum (Pakbin et al. 2009). Hopanes are thus tracers of primary vehicular aerosols (Delfino et al. 2010), particularly on account of their relative stability and non-volatile nature in the atmosphere (Turlington et al. 2010). Hopanes and steranes can be used to distinguish diesel and gasoline engine emissions from other combustion sources and track personal exposure (Sheesley et al. 2009). These stable species can serve as unique tracers in determining the contribution of diesel and gasoline vehicles to the total concentrations measured in ambient air (Rogge et al. 1993), when sources of fuel (e.g. gas station) and coal combustion are not in the vicinity.

In a recent review of organic markers in fine PM, Lin et al. showed that hopanes are commonly used in receptor model source apportionment studies with the three most abundant hopanes being 17α(H),21β(H),29-norhopane, 17α(H),21β(H)-hopane, and 22,29,30-trisnorneohopane (Lin et al. 2010). Still, the potential for indoor dust as a tool for assessing exposure to outdoor-generated pollution has not been extensively evaluated. According to Turlington and colleagues, there are concerns regarding quality control for hopanes (Turlington et al. 2010). These authors showed that compared to PAHs that have been extensively studied in a variety of matrices (e.g. wipes, vacuumed dust, and urine) with numerous validated methods, there are few choices for hopanes analytical standards. Future research aimed at identifying traffic-pollution constituents likely to have few indoor sources, with the capacity to discriminate near-source traffic from background traffic levels, is needed for epidemiologic studies attempting to link traffic to adverse health outcomes (HEI, 2010). In addition, the possibility to collect

such a tracer in house dust offers the possibility to have a time-integrated measure of exposure while capturing the proportion of outdoor TRAP that infiltrates indoors.

### 1.1.4 Greenness, asthma and allergies

The field of research around nature and health has experienced considerable expansion over the past few decades in light of growing worldwide urbanization and related lifestyle changes (Hartig et al. 2014). These factors affect not only air pollution, but also the possibilities of human contact with green spaces, predominantly viewed as beneficial for human health and well-being (Hartig et al. 2014; Lee and Maheswaran 2011). These benefits may act via different pathways, including physical activity (Roemmich et al. 2006), social cohesion, stress reduction and air quality improvement (Figure 1.1). On the other hand, despite the benefits of green spaces, they also pose risks related to human allergic reactions to the airborne plant pollen released during pollination. This coincides with findings which indicate that people living in urban areas are 20% more likely to suffer airborne pollen allergies than people living in rural areas (D'Amato et al. 2007). This situation has been explained by the uniformity of green spaces and the interaction of pollen with air pollutants that potentiate the sensitization response (Cariñanos and Casares-Porcel 2011). In Canada, higher rates of asthma-related hospitalization were shown in relation to an increased load of aero-allergens that can be emitted by certain types of trees suited for urbanized areas (Dales et al. 2004).

The risk posed by pollen allergy is amplified in the context of climate change. Climate change has important effects on ecosystems, notably on the potential load of aeroallergens present in the environment. There is now considerable evidence showing that climate change alters the timing, distribution, quality and quantity of allergenic plants and their associated aeroallergens (Beggs 2004; D'Amato et al. 2002, 2010; D'Amato and Cecchi 2008; Shea et al. 2008) which consequently increases the risk of allergies and asthma severity.

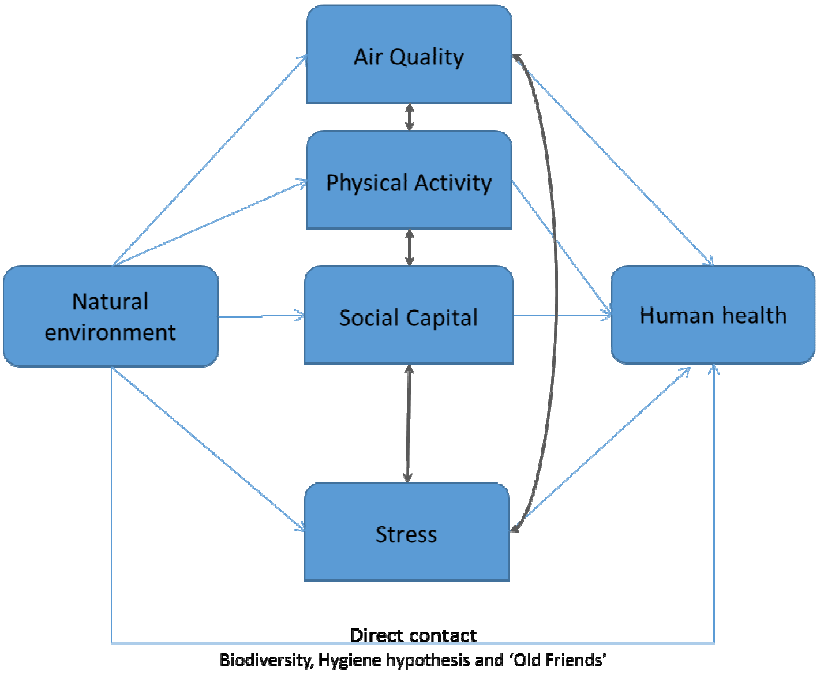


**Figure 1.1: Pathways linking natural spaces to human health (adapted from (Hartig et al. 2014) ). Putative causal pathways in blue, effect modifiers in brown (personal characteristics (e.g. age, gender) and other intrinsic effect modifiers are not depicted for clarity).**

With respect to pediatric asthma and allergies, the epidemiologic evidence on the effect of surrounding greenness remains conflicting. Recent studies have provided support to the negative effect of green space exposures via increased allergic load. For instance, in a birth cohort study conducted in New York, asthma at age 7 was significantly increased in those residing in streets with higher tree canopy coverage (Lovasi et al. 2013). Opposite to these results, a recent review on the role of natural spaces (e.g., parks) in children's health concluded that reduced access to open spaces is related to higher relative prevalence of asthma and allergic conditions in children (McCurdy et al. 2010). This review of evidence is consistent with studies in which exposure to green spaces reduced the risk of asthma (Sherriff et al. 2009) via increased physical activity (Roemmich et al. 2006) or via reduced air pollution exposure (Dadvand et al. 2014; Lovasi et al. 2008). The biodiversity mechanisms (Haahtela et al. 2013) and the revised version of the hygiene hypothesis (Rook 2013) might explain the beneficial role for green spaces with respect to

childhood asthma development whereby the reduction in the human-nature interface hinders immunoregulatory mechanisms. Overall, longitudinal follow up studies examining the effect of green spaces on childhood asthma and allergic diseases development are scarce and needed to verify these proposed mechanisms.

### 1.1.5 Summary of major gaps identified

In view of the above critical review of the literature, most studies examining the role of TRAP on childhood respiratory health have used measurements from a few central ambient monitors and/or some measure of traffic as indicators of exposure, disregarding spatial variability and/or factors influencing personal exposure-ambient concentration. There is a need to investigate novel measurement approaches that would be specific to TRAP and/or modeling methods that account for spatial and temporal variation as well as outdoor/indoor infiltration and time-location patterns.

On the other hand, asthma and allergic disease development in relation to TRAP research need prospective longitudinal follow-up where phenotypes of asthma and allergic diseases are rigorously characterized and environmental exposures are estimated as early as conception, throughout pregnancy into later childhood. Such follow-up can help clarify critical period of disease incidence both for asthma and for atopy, and define whether various trajectories in the disease development occur following exposure to environmental exposures that co-vary and may interact such as air pollution and green spaces. The latter exposure has been scarcely examined in longitudinal birth cohort.

## 1.2 Data sources

The various investigations reported in this dissertation leveraged two existing Canadian birth cohort studies. A description of the study populations, outcome measures and exposure assessment methods are described below.

### 1.2.1 Canadian healthy infant longitudinal development (CHILD) study

#### 1.2.1.1 Study population and outcome data

The Canadian Health Infant Longitudinal Development (CHILD) study is a large (n=3500 mother/infant pairs) prospective birth cohort that was designed to determine the role of environmental factors and their interactions with genetic and host factors in the development of allergy and asthma in children. Pregnant mothers from four Canadian cities (Vancouver, Edmonton, Winnipeg, and Toronto) were recruited between 2008 and 2012. Surveys of environmental exposures using questionnaires and home assessments were complemented by extensive environmental and biologic sampling (Moraes et al. 2015; Takaro et al. 2015).

Inclusion and exclusion criteria for mothers and infants are shown in Table 1.1. Efforts were made to recruit from a representative general population to allow identification of environmental risks and novel genes by avoiding the selection of an at-risk population. Infants born prematurely or with respiratory distress syndrome were excluded as these conditions can independently increase the development of wheeze, asthma and allergic respiratory disorders. Similarly, families who turned to *in vitro* fertilization were excluded since potential environmental effects and epigenetic changes in the embryo developing in vitro could not be assessed. Finally, children who spent less than 80% of their time in the principal residential address were excluded because of the inability to model their exposures (these do not include children spending the equivalent of a work day in daycares). Participation of the father was encouraged but not mandatory for recruitment. All participants provided informed consent at the time of enrolment.

**Table 1.1: Inclusion and exclusion criteria for participants' recruitment in the CHILD study**

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| Pregnant women aged >18 years (>19 years in Vancouver). | Children born with major congenital abnormalities or respiratory distress syndrome (RDS). |

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| Residential proximity (<50 Km) to participating delivery hospital. | Expectation of moving away from a recruitment center within 1 year of recruitment. |
| Ability to read, write and speak English. | Children of multiple births. |
| Willing to donate cord blood. | Children resulting from *in vitro* fertilization. |
| Planning to deliver at a designated recruitment center participating hospital. | Children who will not spend at least 80% of nights in the index home. |
| Infants born at or after 35 weeks. | |

To enable the examination of potential cause-effect relationships relevant to the development of pediatric allergy and asthma, the CHILD study is defined by detailed phenotyping, based on clinical data and objective measurements of atopy and lung function, together with assessment of a diversity of pre- and post-natal environmental exposures and biobanking of multiple biological and environmental samples including blood, breast milk, urine, stool, nasal swabs and house dust for subsequent assays of chemical and biological agents.

All CHILD participants followed the same visit schedule (Table 1.2), except for some tests and biological samples that were city-specific. In particular, families recruited in Toronto had an extensive set of early novel lung development characterization examinations (Pulmonary Function Tests at 3, 12 and 18 months of age). Similarly, only Vancouver mothers were administered a particular psychological scale (the Life Stress Interview) at 36 weeks of pregnancy and when the child was one year old. Finally, only Winnipeg children provided venous blood at the three year clinic visit.

**Table 1.2: CHILD study schedule from recruitment to age 5**

| | Visit | 18 Week | 36 Week | BIRTH | 3 Month home | 6 Month | 1 Year Clinic Visit | 1.5 Years | 2 Years | 2.5 Years | 3 Years Clinic | 4 Years | 5 Years Clinic |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Questionnaires** | Mother Profile/Residential history | √ | | | √ | | √ | | √ | | √ | √ | √ |
| | Mother Health | √ | | | | | | | | | | | √ |
| | Mother Nutrition | √ | | | | | | | | | | | √ |
| | Mother Medications | √ | | | | | | | | | | | |
| | Mother Psychosocial | √ | √ | | | | | | | | | | √ |
| | Parenting stress | | | | | | √ | | √ | | √ | √ | √ |
| | Father Health | √ | | | | | | | | | | | |
| | Socio-economic Status (SES) | √ | | | | | | | | | | | |
| | Child Delivery Chart | | | √ | | | | | | | | | |
| | Child Health | | | | √ | | | | | | | | |
| | Child Nutrition | | | | √ | | | | | | | | |
| | Child Medications | | | | √ | | | | | | | | |
| | Child Clinical Assessment | | | | | | | | | | | | |
| | Home Environment Survey | √ | | | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| | Home Assessment done by RA | | | | √ | | | | | | | | |
| **Tests** | Mother Skin Prick Test | | | | | | √ | | | | | | |
| | Mother Spirometry | | | | | | √ | | | | | | |
| | Father Skin Prick Test | √ | | | | | | | | | | | |
| | Father Spirometry | √ | | | | | | | | | | | |
| | Child Skin Prick Test | | | | | | √ | | | | √ | | √ |
| | Child eNO | | | | | | | | | | | | √ |
| | Child Pulmonary Function Test | | | | | | | | | | | | √ |
| **Samples** | Cord Blood | | | √ | | | | | | | | | |
| | Mother Breast Milk | | | | √ | | | | | | | | |
| | Mother Venous Blood | √ | | | | | √ | | | | | | |
| | Parent Buccal Swab | | | | | | √ | | | | | | |
| | Father Venous Blood | √ | | | | | | | | | | | |
| | Chid Venous Blood | | | | | | √ | | | | | | |
| | Child Nasal Swab | | | | √ | | √ | | | | | | √ |
| | Chidl Buccal Swab | | | | √ | | √ | | | | | | |
| | Child Urine | | | | √ | | √ | | | | | | √ |
| | Child Meconium/Stool | | | | √ | | √ | | | | | | |
| | Home Dust Collection | | | | √ | | | | | | | | |

The analysis in this dissertation took advantage of the availability of skin prick tests administered to the mother/child dyad one year after birth to examine early infancy risk of sensitization with regard to air pollution. During the clinical assessment where the skin allergy test was performed, trained pediatric nurse or research assistant visited each participant.

The skin allergy testing (Appendix A), performed on the arm or the back, used standardized ALK (Horsholm, Denmark) inhalant aeroallergens (tree, grass and weed pollens, cat, dog, *Dermatophagoides pteronyssinus*, *D. farinae*, cockroach, *Alternaria*, *Cladosporium*, *Aspergillus*, and *Penicillium*) and common food allergens (cow's milk, egg white, soybean and peanut). Individual weal sizes for allergens or positive (histamine 0.1%) and negative (glycerin) controls were determined by the mean of the longest diameter and its perpendicular. After adjusting for any response to the negative control, atopy is characterized dichotomously, defined by 1 or more positive skin tests with mean weal diameter ≥2 mm above the negative control response.

#### 1.2.1.2 Environmental factors and covariates

The methodology describing environmental assessments (Table 2) was reported in detail elsewhere (Takaro et al. 2015). In brief, indoor environmental exposures were repeatedly assessed by house and content questionnaires from pregnancy to age 5 years including details of indoor behaviors. For the purpose of this work, tools were administered during pregnancy up to 1-year *post partum*. When the child was around 3 months of age, a detailed walk-through assessment (Appendix B) with dust sampling for multiple allergens and chemical pollutants, including hopanes (Chapter 2), was conducted, along with global positioning system (GPS) tracking that was aimed to complement the self-reported residential history addresses used to identify the index home of each participant. Time activity patterns of the child and parents were tracked by means of questionnaires and included time spent in transit, and mode of

transport. Second-hand tobacco smoke exposure was assessed by repeated questionnaires after enrollment (i.e. during pregnancy, after birth (around 3 months), and when the child was one year of age).

Along with the assessment of environmental risk factors that were delineated in Section 1.1.2, other working groups within CHILD developed questionnaires to assess known asthma risk factors, namely diet, psycho-social environment characteristics, and birth characteristics.

**Table 1.3: Principal exposure assessment methods used in the CHILD Study to characterize the physical environment during pregnancy and infancy periods**

| Method / Exposure example | Questionnaires | Physical home assessment | Home dust samples | GIS model |
|---|---|---|---|---|
| Common allergens (includes pets and pests) | √ | √ | √[3] | |
| Secondhand smoke | √ | √ | | |
| Dampness | √ | √ | | |
| Mold | √ | √ | √[3] | |
| Traffic Related Air Pollution | √ | | √ | √ |

### 1.2.1.3 Traffic air pollutants

LUR models of nitrogen dioxide have been developed for several Canadian cities, including Windsor (Wheeler et al. 2008), Montreal (Gilbert et al. 2005), Edmonton and Winnipeg (Allen et al. 2011), Vancouver where models were developed for different TRAP tracers (Henderson et al. 2007; Larson et al. 2009), and Toronto (Jerrett et al. 2007). CHILD cities have a specific LUR model for $NO_2$ (Table 1.4) which is commonly used as a TRAP marker (Chapter 3) and for which model development methodology was virtually identical for Vancouver, Winnipeg and Edmonton.

[3] Allergens and Mold in house-settled dust were not examined as part of this dissertation. They are merely indicated here to demonstrate the breath of physical exposure assessment methods in CHILD.

**Table 1.4: LUR models for nitrogen dioxide (variables[4] , estimated annual means for all participants and standard deviation (sd)).**

| City | Land Use Regression Model | $R^2$ | Mean [5] (sd) ($\mu g/m^3$) | LUR error (sd)[6] |
|---|---|---|---|---|
| Edmonton(Allen et al. 2011) | $NO_2$ = 16.60 + 0.02(IND_1500) + 0.30(RD1_1000) + 12.01(RD2_50) + 4.66(RD.100) + 0.06(WTR.1000) + 0.58(Distance City Center) | 0.81 | 22.2 (9.1) | 0 (3.0) |
| Winnipeg(Allen et al. 2011) | $NO_2$= 4.11 + 0.007(IND.2000) + 0.33(IND_200)+ 7.14(HM_50) + 5.64(RD.75)+ 0.09(POP_2500) + 0.18(Y) | 0.77 | 8.6 (5.0) | 0 (2.6) |
| Vancouver(Henderson et al. 2007) | $NO_2$= 42.6 + 10.5(RD1_100) + 0.274(RD1_1000) + 4.24(RD2_200) + 0.074(POP_2500) + 0.116(COM) – 0.02(ELEV) – 0.591(X) | 0.76 | 21.2 (8.9) | 0 (5.2) |
| Toronto(Jerrett et al. 2007) | Log($NO_2$) = 8 + 0.18(RD1_200) + 0.6(RD2_50) + 0.0016(IND.750) + $8.3*10^{-5}$ (DC2000) - $8*10^{-6}$(X) + 0.13(D_WIND.1500) + 0.001(TRAF.500) | 0.67 | 27.1 (8.9) | 1.9 (4)<br>0 (0.1)<br>1.4 (3.1) |

The second metric that was examined is hopanes in settled house dust. Details on the methodology and protocol adopted for the collection and analysis of hopanes are found in Chapter 2, which is dedicated to the examination of the utility of this class of chemical as a TRAP tracer. Standard operating procedures are annexed in the appendix B.

[4] All the buffer zones radii for the derived land use variables are indicated in suffix with the radius length in parenthesis. IND: industrial; WTR: Water; RES: Residential; COM: commercial; RD1: Highway; RD2: Major Road; RD: all roads; HM: Highway or Major Road; ELEV: Elevation; POP: population density; DC: density of dwellings; TRAF: Traffic counts; D_WIND: downwind of major expressways; Y: Latitude; X : longitude.
[5] The temporally adjusted annual concentrations and standard errors in $\mu g/m^3$are presented for each city.
[6] For all cities except Toronto, we show the leave-out one error estimate with the standard deviation around the estimate. For Toronto, we show the results (absolute and relative difference) of three cross-validations using 70% randomly selected sites to predict the remaining 30%

### 1.2.2 Border air quality study (BAQS)

Part of the research described in this thesis leveraged the existing Border Air Quality Study (BAQS). BAQS includes a series of research projects aimed at evaluating the health impacts of air pollution in the Georgia Basin / Puget Sound Airshed, located in Southwestern British Columbia (BC), Canada.

#### 1.2.2.1 Study population and area

The work presented here focuses on the study area encompassing the municipalities of the Greater Vancouver Regional District (GVRD) with the addition of the neighbouring municipalities of Abbotsford, Chilliwack, and Mission, which are located in the Fraser Valley (Figure 1.2). The addition of the neighboring municipalities to the GVRD study area is subsequently referred to as GVRD plus. Recent predictions for the GVRD municipalities suggest a population of 4 million inhabitants by 2040 (Greater Vancouver Regional District), that is likely to result in urban sprawl, increased transportation and energy consumption. This will likely impact the region's air quality since the industrial and transportation emissions (motor vehicles and marine transport) are important sources of air pollution year-round.

The birth cohort consisting of all children born in the study area during the 1999 to 2002 period comprised a total of 68,326 children who were successfully linked to administrative health databases and geospatial exposures beginning at conception. The study population is described in more details below (sections: 1.2.2.2 to 1.2.2.4).

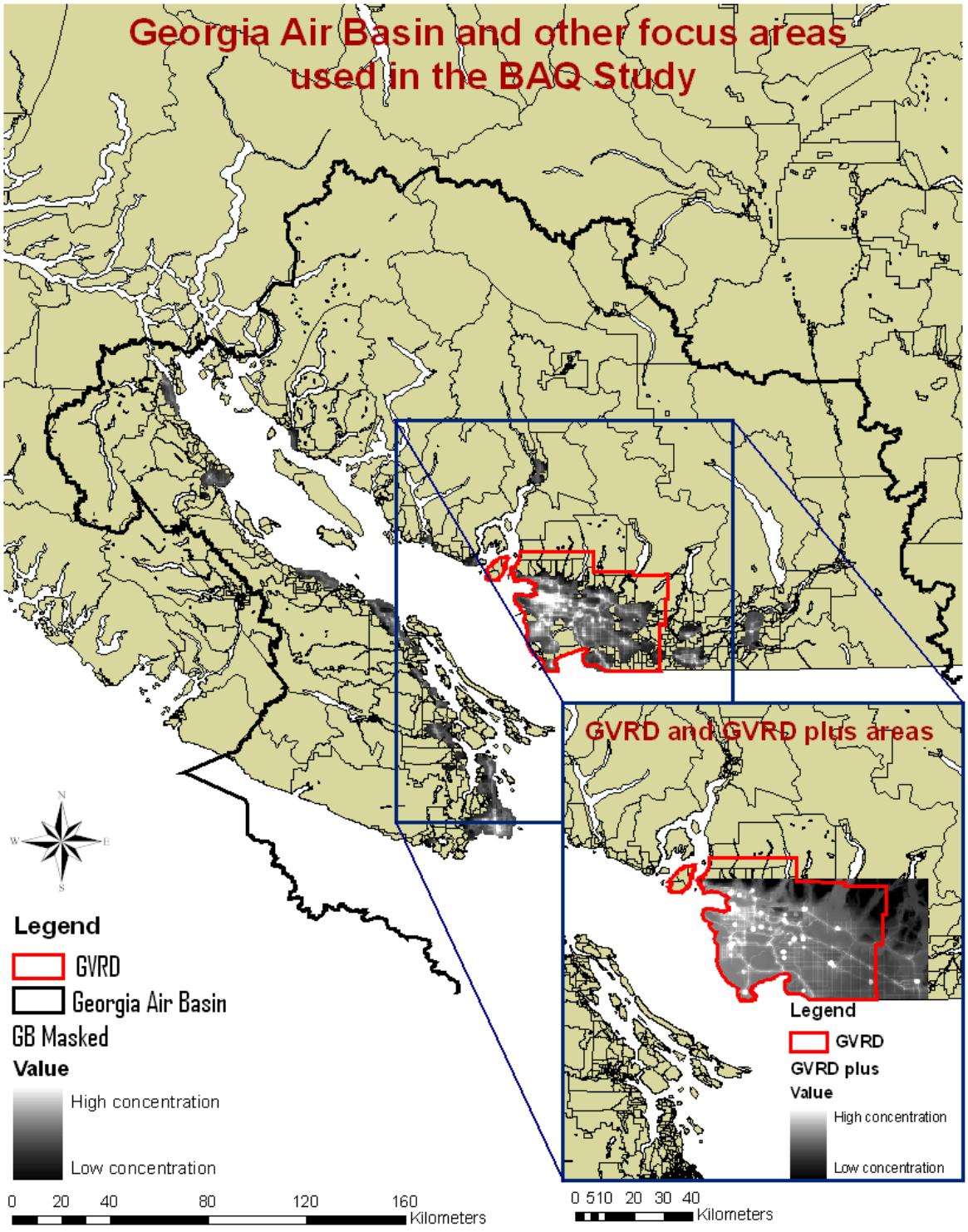


**Figure 1.2: Study area of GVRD plus within the Border Air Quality Study**

#### 1.2.2.2 Defining asthma using administrative databases

Administered by Population Data BC (https://www.popdata.bc.ca), various population-based and health records databases in British Columbia are linked, including Medical Services Plan records (British Columbia Ministry of Health 2009b), hospital separations records (British Columbia Ministry of Health 2009a), births and deaths records through Vital Statistics (British Columbia (BC) Vital Statistics Agency 2009a, 2009b), as well as the BC Perinatal Data Registry (Perinatal Services BC 2009) and Census data (Statistics Canada 2001).

The Medical Services Plan records include information on every outpatient physician visit and medical procedure billed to the provincial medical plan. Enrolment in the provincial plan is mandatory for residents of the province (BC Ministry of Health 2007) and therefore it captures data at a population level.

Physician billing claims to the medical plan require an ICD (International Classification of Diseases) code indicating the primary reason for the encounter as well as administrative details, including physician, date, location, and patient's address. Personal Health Numbers that are unique to individuals can be used to link this data to other provincial databases, including the BC Perinatal Database Registry. This database collects detailed information on every birth in the province, including maternal and birth characteristics. These linked data sources were used to identify and follow the study population, create a residential history file, assess asthma diagnosis via physician visits and hospitalizations throughout the follow up period, and assess risk factors. All data were linked based on personal health number, except for census data linked on census dissemination area of residential location.

Administrative data provide consistently high rates of follow-up that are not always achievable with primary data collection. Therefore, this approach for epidemiological research offers not only an efficient and economical means to examine disease incidence and trends over long periods but also a population-

based record of health information that is of a scale virtually unfeasible in birth cohorts and that circumvents the inherent selection bias in survey-based research reliant on self-reported health outcomes.

Several studies in Canada have evaluated the use of administrative records to identify asthma cases for health research. The relative accuracy of asthma coding in this context was demonstrated in multiple Canadian provinces including Quebec (Blais et al. 2006; Labrèche et al. 2008), Manitoba (Lix et al. 2008), and Ontario (To T et al. 2007; To et al. 2006) where administrative data used for case ascertainment were compared to either physicians' charts or patients self-reports. In Quebec, the investigators verified administrative asthma diagnoses against chart recorded asthma diagnoses in individuals aged 16-80 and demonstrated that the provincial databases were a valid measure of asthma via physician's billing. In Manitoba, Lix and colleagues showed that the case definition algorithm affects the validity of findings compared with self-reports. For children aged 12-18, specificity remained high for all algorithms (92-97%) but sensitivity varied significantly (from 16-87%) depending on the number of years of data examined. The sensitivity increased with the number of years of data available for analysis. To et al. examined asthma diagnoses in the Ontario billing databases for children aged 0-18 years. They found that the diagnosis entered by the primary care physician in a patient's chart was very reliably recorded in the administrative database, with 99% overall agreement (To et al. 2006). The recorded diagnoses were also validated by experts blinded to physician's recorded diagnosis against chart review. These charts agreed well with the administrative data (overall sensitivity was found to be 91% and specificity 83%). The sensitivity was found to be lower (75%) for young children (aged 0-2), but rose to 96% for the 3-5 year old group. Specificity remained consistent at or above 83%. All together, these studies indicate that administrative databases, especially if available over several years, are a sensitive and specific measure of physician-diagnosed asthma.

#### 1.2.2.3 Exposure estimates: air pollution

Air pollution exposure was estimated using proximity to roadways, interpolated measurements of regulatory monitoring data, and LUR modeling, (Brauer et al. 2008; Henderson et al. 2007; Larson et al. 2007).

Exposures were assigned at the level of 6-digit postal codes for residential address. This corresponds to one edge of a block in urban areas, but is larger where population density is lower.

The regulatory monitoring network consists of daily measurements at 24 monitors for $O_3$, 22 for NO and $NO_2$, 14 for $SO_2$, 19 for CO and $PM_{10}$. Exposures were assigned according to inverse distance weighting (IDW), in which the daily values at the three closest monitors within 50 km were weighted by their inverse distance (1/d) to the postal code of interest.

Annual average air pollution LUR models were also developed for the region using measurement data from targeted intensive sampling campaigns. Modeling was used to develop high-resolution (10 meters) maps of NO, $NO_2$, $PM_{2.5}$ and black carbon using GIS data on road density, population density, elevation, and type of land use.

LUR models have improved spatial resolution as compared with the monitoring network approaches, but lower temporal resolution due to sampling data from a single year. For each LUR model, the corresponding monitoring network data were used to temporally adjust LUR estimates (Times Series Forecasting System, version 9; SAS Institute Inc., Cary, NC, USA). Then, these were adjusted to estimate monthly average concentrations (Brauer et al. 2008; Henderson et al. 2007).

For proximity to roadway measures, residential postal codes were defined as being within 50 m or 150 m from highways and major roads (DMTI ArcView street file dataset for BC, Canmap Streetfiles, v2006.3, 2006).

For all exposure metrics, an average exposure was calculated for gestational life up until birth. These exposure estimates were weighted by the time spent at each residence to incorporate individual residential histories derived from BC Ministry of Health data.

#### 1.2.2.4 Exposure estimates: surrounding greenness

Satellite-derived Normalized Difference Vegetation Index (NDVI), a biomass density indicator, was used to assess a continuous measure of greenness across the Metropolitan area of Vancouver, BC. NDVI data ranges from -1 (water) to +1 (dense vegetation), with values close to zero indicating barren areas of rock, sand or snow based on land surface reflectance of visible (red) and near infrared parts of spectrum(Weier and Herring 2000). To assign greenness measures to study postal codes, all cloud free images from Landsat Enhanced Thematic Mapper Plus (ETM+) of the Vancouver region were downloaded from 1999-2002. Average greenness values were extracted for 100 m areas around residential postal codes and yearly greenness values calculated. The small 100 m area was used to assign residential greenness exposure as these distances have been used in prior studies (Dadvand et al. 2012a, 2012b), and are at a similar spatial scale to within-city air pollution variation.

## 1.3 Objectives

Fifty-four percent of the world's population lives in urban areas, a proportion that is projected to rise to 70% by 2050 (Talukder et al. 2015). Despite technological advances in terms of vehicles emissions and the decline in air pollution levels seen in Canadian cities such as Vancouver (Brauer et al. 2012; Brook et al. 2014), the health burden caused by the transport demands is likely to pose serious public health challenges above and beyond today's concerns. Given the ubiquitous nature of traffic-related air pollution and the fact that a "safe concentration" has yet to be identified, there is a clear need for better risk characterization in order to put proper control and mitigation strategies in place. Such risk

characterization is dependent upon several aspects including the ability to assess personal exposures, both accurately and precisely.

This work was motivated by the need to extend/improve existing methods of exposure assessment to TRAP that would account for modifiable factors (e.g. time-activity patterns, potential indoor sources of TRAP markers) in order to reduce exposure misclassification and strengthen the epidemiological evidence on the role of outdoor and indoor air pollutants in pediatric allergic diseases and asthma development, and in particular the role of TRAP.

The overall goals of this research were twofold: first to improve exposure assessment of air pollution for large birth cohort studies of childhood respiratory disease, and secondly to determine the impact of TRAP the development of asthma and allergies both in early years and later in childhood. A specific objective related to the first goal was to describe the methodology and provide descriptive information on TRAP exposures in the Canadian Health Infant Longitudinal Development study (CHILD).

The proposed dissertation is articulated around the following research objectives that stem from the identified gaps in the literature reviewed above and that help answer this dissertation's main objectives:

1- To examine associations between hopanes concentration profiles in outdoor air and hopanes in house dust, and between GIS predictors and $NO_2$ ambient exposures at CHILD participating homes where dust samples were collected (Chapter 2).
2- To develop and apply methods for assessing traffic-related air pollution exposure in a multi-center birth cohort study which account for spatial and temporal variation as well as time-activity patterns and home infiltration efficiency (Chapter 3).
3- To evaluate whether traffic-related air pollution exposure is associated with allergic responses to common inhalant and food allergens (Chapter 3) by applying the exposure estimates in Objective 2 above in the analysis of atopy development in infants at age 1.

4- To examine associations between traffic-related air pollution and the development of asthma among children followed from birth to ten years of age in a population-based birth cohort study and investigate when these associations (if any) with new onset of asthma occur during preschool (0-5 years old) and school age years ( ≥ 6 years old) (Chapter 4).

5- To evaluate the effect of surrounding residential greenness on new asthma onset in children and its potential role in modifying the TRAP-asthma incidence relationship (Chapter 4).

6- To examine asthma trajectories over ten years of follow up using a data-driven approach to clarify time of asthma onset and persistence in relation to neighborhood air pollution and greenness exposures (Chapter 5).

## 1.4 Dissertation structure

The layout of this thesis conforms to the manuscript-based thesis guidelines of the University of British Columbia. Chapters 2 and 3 have been published in a peer reviewed journal, and Chapters 4 and 5 have been submitted for publication.

Chapter 2 is a cross-sectional between-communities investigation of the utility of hopanes as a traffic-related air pollution marker that incorporates the infiltration of ambient pollution inside the homes. Chapter 3 is a longitudinal study in which the potential effects of traffic-related air pollution exposure on allergic health outcomes are explored in the Canadian birth cohort, CHILD. Complementing its previous chapter, chapter 4 relies on an unselected population as it longitudinally examines the influence of traffic-related air pollution in a population-based study (BAQS) where all children are followed up for 10 years. Chapter 5 extends the previous one by identifying group memberships in the same population based on their disease status and recurrence, then examining the influence of traffic-related air pollution on the identified trajectories. Finally, the conclusions and implications of this dissertation, as well as its strengths and limitations, are discussed in Chapter 6 where recommendations for future research are provided.

## Chapter 2: Assessing hopanes as an exposure assessment tool for TRAP exposure[7]

### 2.1 Introduction

The burden of air pollution from traffic on morbidity is also well documented with a variety of negative respiratory and cardiovascular effects (Brauer et al. 2002; Gan et al. 2010) as well as adverse pregnancy outcomes (Brauer et al. 2008) and lung cancer (Beelen et al. 2008; Vineis et al. 2006). According to a recent comprehensive review (HEI, 2010), there is sufficient evidence to infer a causal role for TRAP in the exacerbation of asthma in children and suggestive evidence that it may play a role in the onset of asthma in children (Health Effects Institute 2010).

A number of pollutants (e.g. CO, NOX, and PM components) that are routinely measured at fixed regulatory monitoring sites can be used to represent exposure to TRAP. However, regulatory monitoring data cannot capture the fine-scale spatial pollutant gradients associated with vehicle emissions. Most of the recent epidemiological studies assessing TRAP have improved on such earlier exposure assessment approaches by using more elaborate methods with higher spatial resolution to provide individual-level exposure estimates. These methods generally estimate different surrogates of the traffic mixture (e.g. $NO_2$, black carbon) derived from dispersion or land use regression (LUR) models (Hoek et al. 2008). Despite the advances in TRAP exposure assessment, none of the surrogate pollutants measured or modeled are specific to vehicle emissions.

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[7] A version of this manuscript has been published: Hind Sbihi, Jeffrey R Brook, Ryan W Allen, Jason H Curran, Sharon Dell, Piush Mandhane, James A Scott, Malcolm R Sears, Padmaja Subbarao, Timothy K Takaro, Stuart E Turvey, Amanda J Wheeler and Michael Brauer. 2013. A new exposure metric for traffic-related air pollution? An analysis of determinants of hopanes in settled indoor house dust. *Environmental Health* 2013, **12**:48 doi:10.1186/1476-069X-12-48

In addition to the lack of specificity, all these methods characterize ambient levels and do not consider indoor infiltration. Since individuals in North America spend an average of 87 per cent of their time indoors (Klepeis et al. 2001), (Leech et al. 2002) and many outdoor pollutants readily penetrate indoors, a significant proportion of total exposure to outdoor-generated pollutants occurs indoors. For example, quantifying the PM infiltration efficiency (Finf) in residences can help characterize indoor concentrations and reduce exposure misclassification (Allen et al. 2006) since Finf can vary 2 to 10- fold between houses that have the same ambient concentration levels (Allen et al. 2006; Clark et al. 2010a; Hystad et al. 2009).

Unfortunately, methods for estimating Finf in residences require indoor and outdoor pollution sampling, which makes estimating Finf in large epidemiological studies virtually impossible. To overcome this challenge, prediction models of Finf have been developed (Clark et al. 2010a; Hystad et al. 2009). While they have shown promise, these models have generally been developed for individual cities using relatively small sample sizes and therefore may not be transferable to other locations.

Hence, current approaches to assess individual TRAP exposures (LUR, dispersion model, geostatistical methods) have two consistent limitations: (1) TRAP surrogates are based on non-specific pollutant measures, (2) modeled estimates are capturing concentrations outside the home while most exposure occurs indoors.

Settled house dust is a sink and repository for particle-bound material and semi-volatile organic compounds. Despite the variations that occur in dust sampling, this type of sampling has formed the backbone of epidemiological studies of multiple biological agents (Arrandale et al. 2010). Indeed, house dust presents the advantage of providing one matrix for the evaluation of multiple agents which is a reasonable proxy for time-integrated exposure (Lioy et al. 2002). While the accumulation of house dust depends on several factors (e.g. infiltration efficiency, pollutant source, cleaning practices, sampling surface), dust concentrations and loadings of pollutants show less variation over time than do indoor air

concentrations, therefore, dust sampling is a particularly useful tool in studies of chronic exposures (Egeghy et al. 2004). Using house dust as a marker for indoor inhalable hazards and infiltrated pollutants of outdoor origin (e.g. polycyclic aromatic hydrocarbons (PAHs) from vehicles exhaust) would represent a useful and integrated exposure assessment tool. A good tracer of TRAP in dust would be a chemical: 1) for which the major source is vehicle emissions, 2) for which emissions are correlated with other motor vehicles constituents, 3) that can be measured at low levels for reasonable cost, and 4) that can be measured with accuracy and stability.

One such group of tracers may be the hopanes, a class of organic compounds with 27 to 35 carbon atoms in a naphthenic structure (Simoneit 1985). Hopanes are not found in gasoline and diesel fuel because they are in the higher boiling fraction of petroleum, but are present in engine oil lubricants (Pakbin et al. 2009). Hopanes are tracers of primary vehicular exhaust aerosols (Delfino et al. 2010), particularly on account of their relative stability and non-volatile nature in the atmosphere (Turlington et al. 2010). Schauer et al. showed that hopanes and steranes could be used to distinguish diesel and gasoline engine emissions from other combustion sources (Schauer 2003). These stable species can serve as unique tracers in determining the contribution of diesel and gasoline vehicles to the total particulate matter concentrations measured in outdoor air (Brauer et al. 2002; Rogge et al. 1993). In a recent review of organic markers in fine PM, Lin et al. showed that hopane monomers are commonly used in receptor model source apportionment studies with the three most abundant species being 17α(H),21β(H),29-norhopane, 17α(H),21β(H)-hopane, and 22,29,30-trisnorneohopane ( Lin et al. 2010). The potential for assessing exposure to outdoor-generated pollution in indoor dust has not been evaluated. Measurement of hopanes in house dust offers the possibility to have a time-integrated measure of exposure while capturing variability in the infiltration of TRAP, circumventing the need to indirectly account for infiltration. Therefore we evaluated the association between hopane concentrations in house dust samples to outdoor TRAP surrogates and hopane concentration profiles in outdoor air.

## 2.2 Methods

Our overall goals were to determine (1) whether the hopane mixture in house dust had similar composition as that in outdoor air and (2) the relationship between hopanes in settled house dust with predictors of TRAP spatial variability. We utilized indoor dust measurements from five Canadian cities (Vancouver, Edmonton, Winnipeg, Toronto and Windsor), in order to ensure sufficient variability in hopane levels. Specifically, we conducted a city-level analysis where both city-specific and harmonized LUR variables across all cities were examined. These analyses also included covariates identified in housing characteristics surveys that were administered in the different studies used for this investigation.

### 2.2.1 Population

Samples were collected in three separate studies, briefly described here, in which house dust was collected from inside homes of study participants:

- (1) the Canadian Healthy Infant Longitudinal Development (CHILD) study is a prospective longitudinal, population based birth-cohort study that has enrolled 3650 families from Vancouver, Edmonton, Winnipeg, and Toronto between 2009 and 2012. Homes that (i) underwent home assessment when the child was at an age of 3-months; (ii) completed the questionnaires on environmental factors, and (iii) had dust samples with sufficient dust mass for the analysis of several agents (endotoxins, β-glucans, and hopanes) were selected while ensuring balanced sample representation from the four CHILD cities. Thus, 120 homes analyzed for the suite of hopane monomers by December 2010 were included in this study ;
- (2) the Toronto Child Health Evaluation Questionnaire (TCHEQ) with 1,500 subjects from a nested case-control study were randomly selected from a larger survey of 5,619 students who completed a screening survey for respiratory disease (Dell et al. 2010). Within this nested study, a sub-sample of 50 homes were inspected in 2006/2007 and underwent measurement of

indoor/outdoor concentrations of traffic related pollutants. From these, only 24 homes, included in this study, with sufficient dust mass for the analysis of allergens (Der *p*, Der *f*, Ergosterol and Glucans) were also analyzed for hopanes (Loo et al. 2010);

- (3) during 2005/2006, Health Canada and the University of Windsor conducted a personal exposure study in Windsor (Wheeler et al. 2011) (the Windsor Ontario Exposure Assessment Study, WOEAS), in which in which 48 households were randomly recruited from the larger Windsor Children's Respiratory Health Study (Dales et al. 2009), and where preference was given to spatially distributed households across Windsor. From these households, all homes with sufficient house dust mass were selected (n=27) to examine the hopanes levels in house dust.

### 2.2.2 Hopanes

#### 2.2.2.1 Dust Samples: collection and analysis

House dust samples were collected from the living rooms in all the homes included in the study. Participants were asked not to vacuum during the week prior to the home visit. Sampling was conducted by trained technicians who were instructed to measure the sampling area, note the type of surface and collect a pre-determined amount of dust.

The WOEAS and TCHEQ sampling protocols were similar as technicians vacuumed a 4m2 section of floor for a period of 4 minutes or until at least one gram of dust was collected and used high volume devices. In WOEAS, settled dust was collected using the High Volume Surface Sampling System (HVS3) vacuum (Wheeler et al. 2011), while TCHEQ used the Shop-Vac vacuum (Model: QAM70, 7.0 Amps), another high volume device, equipped with Dust Sampling Socks (X-Cell 100, Midwest Filtration, Cincinnati, OH, USA). In CHILD, house dust samples were collected using a standardized consumer model vacuum cleaner (Sanitaire, model S3686) fitted with a dust collection device designed especially

for the CHILD study with the goal of increasing the collection efficiency without having to vacuum the entire area. This modified collector included slots for two nylon filter thimbles, thereby doubling the filtration surface and was constructed from machined aluminum, and outfitted with Teflon wheels to prevent marring of flooring, and to maintain the collection slot at a fixed distance from the test. The sample was taken from a 2 m2 area by making seven passes of the nozzle over adjacent swaths of flooring. Fresh weights of dust samples were taken, after which they were sieved to 105 µm and this fraction reweighed. These sieved fractions were aliquoted and frozen at –80° C pending further analysis.

Hence, all dust samples were sieved into <150µm size fractions for analysis. The sieved fractions were aliquoted and frozen at -80°C pending further analysis at the Environment Canada laboratory. Extraction in an isooctane solution was conducted with an ASE 200 (Accelerated Solvent Extractor) followed by solvent reduction using a Zymark TurboVap. Recovery standards were added to the dust/solvent matrix before extraction and blow down. A suite of organic compounds were quantified by tandem Gas Chromatography-Mass Spectrometry, including eleven hopane monomers. The final dust-related metric for each of the individual hopanes and the sum of all 11 monomers was expressed as the concentrations per gram of sieved dust (ng/mg), thereby correcting for differences in the total amount of dust collected in each sample.

#### 2.2.2.2 Outdoor hopane measurements

The composition of hopane mixtures, expressed as the abundance of the highest concentration monomer (17α(H), 21β(H)-Hopane) relative to the sum of the concentrations of all 11 measured monomers, was compared between available $PM_{2.5}$ outdoor air samples in Vancouver, Edmonton, Toronto and Windsor with house dust samples for the same cities. In all cities, one 24-hr ambient $PM_{2.5}$ sample was collected at Environment Canada national monitoring network sites (Dabek-Zlotorzynska et al. 2011) within the

same week in the months of January, April, July and October 2010 . The same suite of hopane monomers available in the dust samples were quantified at the same Environment Canada Laboratory by thermal desorption gas chromatography mass spectrometry (Graham et al. 2010) from punches of archived pre-fired quartz filters. Dust and air samples were matched by city and season.

### 2.2.3 Geographic predictor variables

Harmonized geographic data were derived to allow for pooled analysis of all dust hopane measurements from all five cities where samples were collected. We generated 30 variables in 5 categories that are often used in development of LUR models for TRAP (Dijkema et al. 2011; Hoek et al. 2008). Subcategories were generated to characterize the street network, land use, and population density within circular buffer sizes where the radius was set to represent close, medium and large geographical areas around each home where the house dust sampling was conducted (Table 2.1). Highways and major roads were defined by standard road classification categories (DMTI Spatial Inc., Markham, Ontario), with categories 1 (expressway), 2 (principal highway), and 3 (secondary highway) all considered highways (RD1), and category 4 as major roads (RD2). We also examined land use, elevation relative to sea level and the distance to the nearest features within the street network.

All variables in each category were derived from a single spatial dataset in vector format. Input files for the Road Length and Land Use, were taken from the 2006 DMTI Spatial (Markham, Ontario) data files. Population Density categories were generated from the 2006 census distributed by Statistics Canada and converted into point files at the block level. Digital Elevation Data was obtained from GeoBase in raster format at the municipal level. All input files were manipulated in ArcGIS 10.1 (ESRI, Redlands, CA) to produce variable layers in raster format at 10m resolution, except for the digital elevation model where the finest available resolution was 30m. From the latter data, relative elevation was defined as the mean centered city-specific elevation.

**Table 2.1: Harmonized GIS variables across TCHEQ, WOAES, and CHILD cities**

| Category (Number of variables) | Description | Sub-Category | Buffer radii (m) | Source/Type |
|---|---|---|---|---|
| Road Length (8) | Total length of two road types | RD1 (Highways)<br>RD2 (Major Roads) | 50, 100, 500, 1000 | DMTI Road Network (Polyline) |
| Land use (12) | Total area of different land use types (ha) | COMM (commercial)<br>OPEN<br>PARK<br>INDUS (industrial) | 100, 500,1000 | DMTI spatial data (polygon) |
| Distance to nearest feature (6) | Distance to nearest road type (m) | Dist_RD1<br>Dist_RD2 | | DMTI Road Network (Polyline) |
| | Distance to nearest land use type (m) | Dist_Comm<br>Dist_Open<br>Dist_Park<br>Dist_Indus | | DMTI spatial data (polygon) |
| Population density (3) | Density of the population (persons/hectare) | POPDENS | 100, 1000, 2500 | Block level census data (point file) |
| Geographic position | Elevation (m) | ELEV | | Geobase DEM (raster) |

We also extracted city-specific variables that had previously been extracted and used in the development of LUR models for NO2 in each of the cities (Allen et al. 2011; Henderson et al. 2007; Jerrett et al. 2007; Wheeler et al. 2008) (Table 2.2). Since these LUR variables had been used to explain variability in outdoor NO2 in these cities we therefore expected that they would explain variability in dust hopanes concentrations.

**Table 2.2: City-specific GIS variables and buffer sizes extracted from LUR model surfaces.**

| City/ Cohort | Road metrics (m) | Traffic density | Other |
|---|---|---|---|
| Edmonton CHILD | Length of all roads (100,250, 500, 1000m) | | Distance to city center (m)<br>Water area |
| Winnipeg CHILD | | | Y coordinate centered at city center. |
| Vancouver CHILD | Length of Truck roads (100,250, 750, 1000m) | Automobile density<br>Truck density (100, 250, 750, 1000m) | |
| Toronto TCHEQ | Distance to nearest expressway<br><br>Length of expressways (100,500, 1000, 2000m) | | |
| Windsor WOEAS | Distance to nearest local road<br>Distance to nearest class 1, 2 collector<br>Distance to nearest class 1, 2 arterial<br>Distance to nearest highway<br>Distance to nearest scenic drive | | Distance to Ambassador bridge (m)<br>Distance to Windsor/Detroit Tunnel (m) |

### 2.2.4 Questionnaires

We also included data from questionnaires delivered in each of the indoor measurement studies on housing characteristics and lifestyle factors, which may be related to indoor hopane variability and/or infiltration (Table 2.3).

**Table 2.3: Descriptive summary of questions found (as shown with a check mark) in the questionnaires delivered during home visits, recoded for analysis in the pooled investigation of hopanes in dust and land use determinants of traffic pollution.**

| Question type | CHILD | TCHEQ | WOEAS |
|---|---|---|---|
| **Emissions sources within 100m of the home** | √ | × | × |
| Factory | 2% | | |
| Gas Station | 11.3% | | |
| Parking | 15.6% | | |
| Construction Site | 23.5% | | |
| **Shoe removal** | √ | × | × |
| Yes | 94% | | |
| No | 6% | | |
| **Type of Floor** | √ | √ | √ |
| mixed | 4% | | 4% |
| smooth | 21% | 83% | 36% |
| carpets | 75% | 17% | 60% |
| **Cleaning frequency** | √ | √ | √ |
| Rarely | 14% | 4% | |
| Moderately | 80% | 71% | 56% |
| Frequently | 6% | 25% | 44% |
| **Window usage/type** | √ | √ | √ |
| Usually open/sheer | 15% | 37.5% | 33.3% |
| Covered with blinds/curtains | 42.5% | 14.8% | 63% |
| Sealed | 34% | | |
| Opened daytime/ closed night | | 14.8% | 3.7% |
| Other | 2% | 14.8% | 0% |
| **Garages** | √ | √ | √ |
| Yes | 46% | 17% | 52% |
| No | 54% | 83% | 48% |
| **Type of house** | √ | √ | √ |
| single | 64% | 100% | 100% |
| multifamily | 36% | | |
| **Air Conditioning** | √ | √ | √ |
| Yes | 40% | 100% | 81% |
| No | 60% | | 11% |
| **Frequency of AC use** | √ | √ | √ |
| Frequently | 21% | 42% | 15% |
| Sometimes | 19.5% | 46% | |
| Don't know | 59.5% | 12% | 7% |
| Never | 0 | | 78% |

For homes that were part of the CHILD study, home information was gathered from both a questionnaire completed by the parents and the home inspection conducted by research technicians. For homes that were part of TCHEQ, a large amount of housing characteristics data were also available from a questionnaire that was administered at study baseline (633 questions). Finally, from the WOEAS homes for which information on a wide range of housing characteristics and time-activity patterns was collected twice, we used the baseline questionnaire. The questionnaires included questions that were unique to each cohort as well as other questions common across all studies (see Table 2.3), which were recoded to generate a set of harmonized data. Harmonized variables included data related to the season (defined using heating degree day) based on the date when samples were collected, the type of floor (recoded as smooth for hard wood, vinyl and other smooth surfaces, carpets for rugs and carpets, and mixed for samples collected from both smooth and carpet surfaces), the type of household (single or multifamily), the presence or absence of a garage, the type of garage (attached or detached), the presence of air conditioning (central or in a wall or portable unit), the frequency of use of air conditioning (recoded as never, sometimes and frequently), the cleaning frequency (recoded as rarely, sometimes and frequently), and the usage of windows (recoded and grouped from different questions in the CHILD questionnaire) coded into 5 categories: usually open/sheer; covered with blinds or curtains; sealed; open daytime/covered nighttime; other.

## 2.3 Statistical analysis

We first analyzed the association between the mixture of hopanes in outdoor air and indoor dust by comparing the relative abundance of the most abundant monomer. Specifically, we calculated the ratio of the concentration of 17α(H), 21β(H)-Hopane to the total concentration of the eleven monomers. We then compared this relative abundance between the outdoor air and indoor dust samples in each city, and examined this association after accounting for temperature and evaluating multicollinearity between predictors (assessed by the variance inflation factor) in linear regression models .

After examining the distribution of hopane concentrations in a pooled analysis of dust samples from all cities (hereafter "pooled analysis") and separately within each city ("city-specific analysis"), we applied a log transformation to the total hopane concentration (i.e. sum of the 11 monomers) distribution across all cities and within each city. Only hopane concentrations in the family room were considered in the analysis since homes from WOEAS and TCHEQ studies did not provide samples from the bedroom and the bedroom samples in CHILD included a mixture of floor and bed samples. Prior to examining the association between total hopane concentrations and GIS variables in the pooled analysis, we fit a varying intercept model to assess the between- and within-city variability. Both in the pooled and city-specific analyses, questions on lifestyle factors and housing characteristics were examined in bivariate analysis as potential confounders or effect modifiers for the hopane – geographic predictor relationships.

The same model building approach described by Henderson *et al.* (Henderson et al. 2007) to generate physically meaningful predictive models was adopted and consisted of the following steps: (1) Rank all variables by the absolute strength of their correlation with the hopane concentration; (2) Within each sub-category (e.g. all buffer sizes for highway lengths), keep only the highest-ranking; (3) to avoid collinearity examine the correlations between all GIS predictors retained from step 2 as well as questionnaire variables using 0.6 as a cut-off value ; (4)enter all remaining variables into a stepwise linear regression; (5) remove from the available pool any variables that have insignificant t-statistics and variables that show a direction of effect opposite of *a priori* hypotheses. Steps 4 and 5 were repeated until a parsimonious final model was obtained.

The study methodology was reviewed and approved by both the University of British Columbia Behavioral Research Ethics Board (ethics certificate number: H11-03231) and the Clinical Research Ethics Board (H07-03120).

## 2.4 Results

### 2.4.1 Outdoor vs. indoor hopane concentrations comparison

All samples were above the GC/MS limit of detection (LOD). In all cities the monomer 17α(H), 21β(H)-Hopane was consistently detected and showed the highest abundance in the suite of analyzed compounds, therefore the comparison of outdoor and indoor hopane ratios was performed using this monomer relative to the sum of all monomers. The sampling from the air monitoring stations was conducted in 2010 at fixed time points which result in concentrations from air samples with a discrete distribution (Figure 2.1) compared with the house dust samples which were collected throughout the year. In air, the range of the 17α(H), 21β(H)-Hopane relative abundance (0.2 to 0.4) generally corresponded to the same relative abundance in house dust. The correlation of the 17α(H), 21β(H)-Hopane relative abundance in outdoor air and house dust was moderately strong, yet significant ($r=0.48$, $p<0.05$).

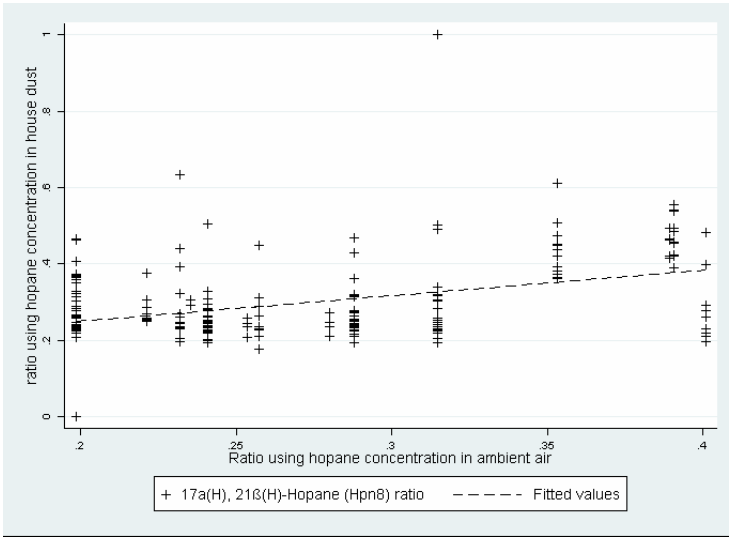


**Figure 2.1: Association between outdoor air and house dust hopanes major monomer ((17α(H), 21β(H)-Hopane) relative abundance.**

After excluding an outlier sample (see in Figure 2.1 data point near zero where the ratio in 17α(H), 21β(H)-Hopane was depleted due to a very low concentration in all monomers), we also examined the relation between the outdoor and the indoor relative abundance in linear regression accounting for the effect of season, and found a stronger statistically significant relationship (slope=0.92, t=6.1) compared to the association without adjustment for season (slope=0.72, t=5.9). The correlation was still significant when the outlier was included. The effect of season was stronger during the spring and summer ($r>0.5$) than during the fall and winter ($r<0.5$).

### 2.4.2 Pooled results

Hopane levels in individual homes varied from a low of 0.4 ng/mg of dust in a Toronto home to a high of 41.8 ng/mg of dust in a Vancouver sample, after excluding an outlier in Toronto from the CHILD study where the concentration was 160.3 ng/mg (more than 29 times higher than the median); for this home we examined the land use characteristics, the road network, and potential outdoor sources as indicated in the questionnaire and did not find any difference that would explain such a high concentration. Analyses were thus run with and without this sample (Table 2.4).

Windsor had the lowest overall indoor hopane levels with a mean level of 5.8 ng/mg (GM= 5.1 ng/mg, GSD =1.8) while the sample of homes in Vancouver showed the highest mean concentration of 9.3ng/mg (GM= 6 ng/mg, GSD= 2.9) when the high (Toronto) outlier was excluded (if the Toronto outlier was retained, then Toronto was ranked first with an AM=9.9ng/mg).

After fitting a null random effect model, the intraclass correlation of 0.007 indicated that city clustering would not contribute to explaining the variability in total hopane concentrations. We therefore built a model without using a city-specific random intercept in the regression analysis and all samples were treated as independent.

From the harmonized questions, only relative elevation and heating degree days at the time of dust collection showed a statistically significant relationship with hopane concentrations. Distance to highway (DIST_RD1) had a statistically significant association with hopane concentrations, but its direction of effect was opposite to what we expected and was therefore excluded from the model. The final model with relative elevation and heating degree days as predictors explained only 6% of the total variability in the hopanes concentration. Including distance to highway did not appreciably improve the amount of explained variability (adjusted $R^2$ =0.08). Excluding the high outlier home in Toronto led to a model with the addition of the presence of an AC unit in the home along with the same predictors as above, but with less overall variability explained (adjusted $R^2$= 0.04).

### 2.4.3 City-specific modeling results

Given the availability of LUR models for predicting $NO_2$ in each study area, we extracted the $NO_2$ concentration at the geocoded participants' home addresses and examined the correlation of hopane concentrations in house dust with city-specific LUR $NO_2$ estimates in each city. Results (Table 2.5) indicated no statistically significant associations except in Windsor ($r=0.44$, $p<0.05$) and Edmonton ($r=0.58$, $p<0.05$).

Leveraging the availability of city-specific LUR models, we further examined separately for each city the association between hopane concentrations and the variables that were used both in the city-specific LUR models describing the $NO_2$ levels (Table 2.2) and those that we generated for the pooled analysis (Table 2.1). The amount of variability explained in each city varied from 10% in Vancouver to 80% in Edmonton (Table 2.4).

**Table 2.4: City-specific determinants of hopane concentrations in house-settled dust.**

| City | Final model with regression coefficients | Partial $R^2$ | Model Adj.$R^2$ |
|---|---|---|---|
| Edmonton | log (hopanes) = 3 – 0.13 cleaning frequency<br>- 1.5 Smooth Flooring<br>-0.15 Air Conditioning | 0.78<br>0.78<br>0.35 | 0.80 |
| Toronto | Log (hopanes) = 2.69 -1.03 Smooth Flooring<br>-0.008 Elevation<br>+ 0.88 Attached Garage -0.66 Detached Garage | 0.29<br>0.13<br>0.13%* | 0.45 |
| Windsor** | log (hopanes) = 5.6 + 0.5 elevation<br>+ 0.17 RD1_100 | 0.36<br>0.13 | 0.39 |
| Winnipeg | log (hopanes) = 1.45 – 0.057Heating degree days<br>– 1.33 multifamily house | 0.17<br>0.16 | 0.33 |
| Vancouver | log (hopanes) = 1.9 – 0.09 Heating Degree Days<br>– 0.07 Shoe removal | 0.09<br>0.07 | 0.10 |

* The 'detached garage' variable has three categories: No garage; Attached garage; Detached garage.

** In Windsor, elevation and distance to the Ambassador Bridge were strongly and significantly correlated. An alternative model with Distance to Ambassador Bridge yielded similar results, yet with smaller $R^2$

Overall, in each city the determinants of indoor dust hopanes were predominantly related to home-specific factors (cleaning, use of AC, shoe removal) and meteorology, except for Windsor where the final model included the length of major roads in a 100m buffer (Table 2.3). In Toronto, the spatial variability provided by the TCHEQ samples was very limited as all homes were within a restricted geographic area within the city. Hence, an additional sub-analysis was run for Toronto with only the CHILD homes

included. This model (not shown) did retain GIS variables (open area within 1000m buffer and elevation) as well as variables related to other possible sources of hopane emissions (garage type, presence of a construction site within 100m) and finally home-specific factors (i.e. the type of floor surface) and explained 86% of the overall variability in indoor dust hopanes. After excluding the house with the outlier concentration value, however, the final model, with an $R^2 = 0.3$, had exactly the same predictors as those shown in table 2.3 where samples from both the TCHEQ and CHILD study homes in Toronto were included.

While the association of hopanes indoors in relation to GIS variables typically used as surrogates for TRAP was only modeled for samples collected in living rooms, Table 2.5 shows the concentration in each city by room type and the number of homes (from the CHILD study) where two rooms were sampled. In CHILD participating households dust samples from the living room as well as a second composite sample from subject child's mattress and adjacent flooring. The ranking by decile showed that the hopanes concentration in the living rooms was significantly greater than that found in the bedrooms.

**Table 2.5: Geometric Mean (GM) and Geometric Standard Deviation (GSD) of total hopanes concentrations in the study population, by room, by homes and correlation with city-specific modeled $NO_2$**

| City | Homes N (number of homes) | Hopanes concentration (ng/mg) | | | | | | Pearson correlation |
|---|---|---|---|---|---|---|---|---|
| | | Bedroom | | Family Room | | Average | | Between $NO_2$ and family room |
| | | n | GM(GSD) | n | GM(GSD) | N | GM (GSD) | r (p-value) |
| Winnipeg (CHILD) | 26 | 23 | 4.9 (2.1) | 21 | 5.8 (2.1) | 40 | 5.3 (2.3) | 0.04 (n.s.) |
| Edmonton (CHILD) | 15 | 12 | 4.7 (2.7) | 14 | 4.1 (2.0) | 26 | 4.5 (2.3) | **0.58 (0.03)** |
| Vancouver (CHILD) | 65 | 56 | 7.4 (2.2) | 54 | 6 (2.9) | 90 | 6.7 (2.6) | -0.12 (n.s.) |
| Toronto (CHILD) | 14 | 13 | 5.9 (1.9) | 12 | 7.7 (2.9) | 22 | 6.6(2.3) | 0.02 (n.s.) |
| Windsor (WOAES) | 27 | NA | | 27 | 5.1 (1.8) | NA | | **0.44(0.02)** |
| Toronto (TCHEQ) | 24 | NA | | 24 | 4 (2.5) | NA | | 0.18 (n.s.) |

n: total number of samples; n.s.: not statistically significant association.

## 2.5 Discussion

Assessing indoor levels of TRAP through the collection and analysis of settled house dust is a new area of study and has the potential to reduce exposure misclassification and increase specificity. In this investigation, we compared measured dust hopanes with ambient air hopane measurements and with GIS-derived land use variables for each city. The availability of contemporaneous cohort studies (CHILD, TCHEQ and WOEAS) offered a unique opportunity to gather a sample of 171 homes where dust was collected using similar protocols in 151 living rooms and where hopanes were analyzed by tandem GC/MS at the same laboratory using a standardized protocol. This sample was representative of different settings ranging from highly urban locations such as Toronto to smaller and less urban cities such as Winnipeg, while also including major transit hubs such as Windsor where an important Canadian-American truck crossing exists. Furthermore, all the cities had previously developed LUR models which reasonably predicted traffic related $NO_2$ spatial variability (from 66% in Vancouver to 81% in Edmonton(Allen et al. 2011; Henderson et al. 2007; Wheeler et al. 2008).

We demonstrated that hopanes can be consistently detected in house dust samples regardless of the type of city and the dust collection location. In addition, after controlling for heating degree days and its impact on infiltration, the major hopane monomer relative abundance in house dust and outdoor air samples were modestly but significantly correlated ($r = 0.48$), suggesting similar hopane sources in the two samples, but there remains substantial unexplained variability in indoor levels. This comparison had relatively good external validity given that the ambient monitoring sites were located to capture urban background concentrations rather than hot spots and since samples were collected in and matched for all seasons. This correlation was stronger in the summer compared to the winter, suggesting an impact of infiltration as windows are more likely to be opened on warmer days. Since hopanes in house dust

accumulate over relatively longer periods of time compared with hopanes in air samples and may have undergone many changes and cycles in temperature, it is likely that the seasonal effect shown in the literature (Olson et al. 2008; Olson and McDow 2009; Rutter et al. 2011) may not hold in this context. In addition, dust sampling, which often is a readily available matrix for sampling multiple agents in epidemiological studies, including hopanes as demonstrated in this study, does not represent similar constraints (e.g. logistics) as those imposed by particle infiltration measurements.

Examining associations between hopanes concentrations and geographic predictors in a pooled analysis indicated that only a small degree of variability in hopanes concentrations in dust was explained by the final model. Further, in this analysis, higher levels of road variables were linked to lower levels of hopanes. Despite the advantages of pooling data from different cohorts, this effort was hindered by the absence of consistency in the supplementary data collected via questionnaires since each study used its own set of questions. While we inspected each question and the research technicians' notes for each sample of house dust collected in order to generate harmonized variables that could affect the hopanes concentration in house dust, recoding variables may have resulted in a loss of specificity.

Unlike the pooled analysis, the city-specific analysis provided more insight into the utility of hopanes as possible markers for TRAP as a moderate to large amount of variability in the total hopane concentration in house dust was explained in each model. This analysis, however, was hindered by the lack of consistency between cities in terms of main predictors of indoor hopane concentrations.

We examined potential modifiers that could alter the relationship between LUR variables and hopanes in dust for each city separately. In addition to geographic surrogates of TRAP, all cities had at least one predictor of hopanes concentration related to the indoor environment or home construction. Thus, the variability in settled dust hopanes concentrations appears to be a function of a mix of parameters that are not exclusively related to traffic emissions.

For example, indoor hopanes in settled dust may also result from coarse PM being tracked indoors. A recent analysis of indoor PAHs indicated the potential importance of this pathway even after adjusting for carpeting, frequency of vacuuming and indoor burning (Mahler et al. 2010). We examined the association in the city-specific analysis for all CHILD participating homes between shoe removal habits and hopane concentrations. We found that only Vancouver samples were correlated with shoe removal habits in the expected direction. Collection of supplementary field data remains a crucial component for assessing the utility of hopanes in house dust since tracked dust seems to contribute to hopanes concentration in house dust. This information was only available in the CHILD homes, and could therefore not be assessed in the pooled analysis.

In our investigation we made a critical assumption that hopanes have few sources beyond engine oil lubricants as we were not able to find information on indoor hopanes sources in the literature. Since hopanes are widespread in recent and ancient sediments, they are constituents of all mineral oil or petroleum-based lubricants and it is therefore possible that unaccounted for indoor sources were present.

House dust remains an attractive metric for exposure assessment because it offers a matrix for multiple indoor contaminants, both biological and chemical and both indoor and outdoor in origin, and can be stored for long time periods, thus providing the opportunity to examine additional research questions when necessary. The utility of hopanes in house dust as an indicator of infiltrated TRAP is limited in the absence of better understanding of its deposition and stability in house dust. House dust is heterogeneous matrix with a complex history in each home as it accumulates contributions from multiple sources including not only fresh emissions of combustion-related particles but also road dust which also contains hopanes. The mode of accumulation also contributes to the variability of vacuum dust. Several factors that may vary among study participants can affect the concentrations of hopanes: cleaning practices and sampling surfaces (carpeted vs. non-carpeted) play a role in the amount of chemicals that deposit inside

the homes as shown in the city-specific analysis. In addition, the metric of exposure for hopanes still lacks consensus as hopanes can be measured in terms of loading of chemicals (concentration normalized by surface area sampled) or expressed as the more traditional approach of normalized concentration to mass of dust collected. Differences in the choice of metric would relate mostly to cleaning practices, which we have tried to account for in our investigation. Future investigations of other species, such as PAHs, on their own or in combination with hopanes, may offer additional insight into the utility of settled house dust as a surrogate for TRAP exposure.

In our study, we compiled the information about presence and frequency of use of air conditioning as this has been shown to be an important predictor of PM infiltration(Allen et al. 2012a), but we found limited explanatory power in both pooled and city-specific analysis.

PM infiltration varies with particle size, with a maximum infiltration efficiency for diameters of approximately 0.2-0.3 μm (Sarnat et al. 2006), while the size distribution of hopanes ranges between 0.7 and 3.3 μm (Huang et al. 2012) which would imply that hopanes infiltration efficiency may be low and might therefore explain variability in the outdoor/indoor correlation (Olson et al. 2008). We could expect that in presence of higher concentration level of hopanes in ambient air (i.e. better ability to detect hopane monomers), the analysis of relative abundance in ambient and indoor hopanes would have shown less unexplained variability.

Overall, the results indicate the indoor dust hopanes concentrations depend on both outdoor TRAP concentrations and a variety of home-specific variables such as cleaning, floor type, and presence of AC. This conclusion is supported by an additional analysis where we examined the relative variation explained by LUR $NO_2$ compared to home-specific factors and also found that in some cities the correlation between hopanes and LUR $NO_2$ is only revealed when accounting for variation due to such home-specific factors.

We examined the utility of measurements of hopanes in house dust as exposure indicators for infiltrated, time-integrated, traffic-related pollutants. When combined with behavioral factors retrieved from questionnaires, and geographic determinants, hopanes in house dust may have the potential to be used as surrogates for infiltrated TRAP. Further characterization of the determinants of hopanes in house dust may result in an improved exposure measure for epidemiologic studies to more precisely analyze relationships between TRAP and chronic health effects.

# Chapter 3: Early-life exposure to traffic-related air pollution and development of atopy in the CHILD birth cohort study[8]

## 3.1 Introduction

The incidence of allergic diseases has increased sharply, especially for people living in urban areas, which raises an important public health concern given the trend to urbanization worldwide (D'Amato et al. 2010). While associations between exposure to air pollution derived from traffic emissions and allergic exacerbations have been demonstrated, the potential role of traffic related air pollution (TRAP) in the onset of allergic diseases is uncertain (Heinrich and Wichmann 2004).

Several epidemiological studies have reported associations between atopy or other allergic phenotypes and exposure to some TRAP exposure surrogates, including nitrogen dioxide ($NO_2$) (Gruzieva et al. 2012; Janssen et al. 2003; Brauer et al. 2007; Morgenstern et al. 2008). However, a number of other studies have not observed these positive associations (Gehring et al. 2010; Oftedal et al. 2007). Differences in TRAP exposure assessment approaches are one possible explanation for these divergent findings.

Despite the importance of early life exposures in the development of allergy, information on the effect of air pollution exposure, particularly during pregnancy, on allergic responses early in life has rarely been assessed (Aguilera et al. 2013; Esplugues et al. 2011; Mortimer et al. 2008) and birth cohort studies addressing this relationship are rare (Bråbäck and Forsberg 2009). Exposure assessment methods have evolved from self-reported measures (e.g. proximity) (Janssen et al. 2003) to atmospheric dispersion (Oftedal et al. 2007) and land use regression (LUR) models capturing within-city air pollution variations (Brauer et al. 2007b; Gehring et al. 2010b). Most LUR models consider annual average concentrations

[8] A version of this manuscript has been published: Hind Sbihi, Ryan W. Allen, Allan Becker, Jeffrey R. Brook, Piush Mandhane, James A. Scott, Malcolm R. Sears, Padmaja Subbarao, Tim K. Takaro, Stuart E. Turvey, and Michael Brauer. 2015. Perinatal Exposure to Traffic-Related Air Pollution and Atopy at 1 Year of Age in a Multi-Center Canadian Birth Cohort Study. *Environmental Health Perspectives* 2015, DOI:10.1289/ehp.1408700

which may over or under-estimate personal exposures as traffic markers such as $NO_2$ are highly variable in time and space (Mölter et al. 2010). Recently the temporal specificity of LUR models has been improved by applying forward (Morgenstern et al. 2008) or backward (Brauer et al. 2007b; Gehring et al. 2010b) time trends from fixed site monitoring stations to the LUR predictions. However, given the potential importance of specific short-duration periods of development, refined approaches to capture fine-scale seasonal variability are needed (Mortimer et al. 2008). Exposure assessment is further complicated by the mobility of study participants, a consideration seldom addressed and rarely measured in large cohort studies (Arrandale et al. 2010), yet one with considerable influence on personal exposures (Nethery et al. 2008a). The inclusion of exposure in other locations where children spend a significant amount of time, such as daycare facilities, is likely to increase the precision of the exposure estimates. Finally, all prior studies have used ambient concentrations at a given location to estimate exposure, although most time is usually spent indoors, especially in the early years of life (Leech et al. 2002). Considering that indoor infiltration for some pollutants can vary significantly between residences (Clark et al. 2010) substantial exposure misclassification may be present in prior studies.

To address these limitations we examined the association between exposure to TRAP and the development of atopy in a population-based multi-center birth cohort, the Canadian Healthy Infant Longitudinal Development (CHILD) study. We estimated exposure to $NO_2$ as surrogate for TRAP during pregnancy and the first year of life while accounting for both spatial and temporal variability in ambient concentrations. In stratified analyses we evaluated the influence of time-location patterns, daycare attendance, and modeled estimates of infiltration.

## 3.2 Methods

### 3.2.1 Study population

CHILD is a prospective longitudinal national birth-cohort that recruited over 3,600 families between 2008 and 2012 in four Canadian cities (Vancouver (2.31 Million inhabitants), Edmonton (1.16 M), Winnipeg (0.73 M), and Toronto (5.58 M)) (http://www.canadianchildstudy.ca) (Takaro et al., 2015). Participants had to be pregnant, 18 years or older (19 in Vancouver), reside in reasonable proximity to a recruitment center (originally set about 50km distance from the study center), communicate in English, provide informed consent, intend to give birth at one of the recruitment centers, and able to provide valid personal contact information as well as two alternate contact individuals. Eligible infants had to be born at ≥ 35½ weeks gestation with weight ≥2500 grams. Infants were excluded under any of the following criteria: conception via in-vitro fertilization, product of a multiple gestation pregnancy, major congenital abnormalities, spending less than 80% of nights in the principal residential home. Recruitment ideally occurred at or soon after the routine 18 week ultrasound examination, but a significant proportion was recruited in later pregnancy (34% between 24 and 30 weeks, and 31% after 30 weeks of gestation). Each study center (universities and hospitals) obtained ethics approval from their governing health and ethics board and the CHILD study was reviewed and approved by the Hamilton Integrated Ethics Board (certificate number: 07-2929).

The start of follow up was defined as the date of conception based on the expected and actual dates of delivery. End of follow up was defined as the earliest of October 15$^{th}$ 2013 or when the participating child was assessed with skin allergy testing at approximately one year of age.

### 3.2.2 Skin prick tests

To determine individual allergen sensitization, epicutaneous skin tests were administered to each infant at approximately 1 year of age for the six inhalant (*Alternaria alternata*, Cat Hair, Dog Epithelium, House

Dust Mites (*Dermatophagoides pteronyssinus* and *D. farinae*), German cockroach) and four food (whole cow's milk, egg white, soybean, peanut) allergens. Histamine 1mg/mL and Glycerin were used as positive and negative controls. To define maternal atopy, each mother was tested with a panel of allergens: *Alternaria alternata*, Cat Hair, Dog Epithelium, House Dust Mites, German cockroach, *Cladosporium sp*, *Penicillium* mixed, *Aspergillus fumigatus*, Midwest trees, grass mix, weeds, mixed ragweed and peanut. Atopic status was determined using a positive response to any allergen. We defined two clusters of atopic responses in the children – inhalant allergy and food allergy.

Participants reporting use of antihistamines during the seven days prior to the date of skin prick test were excluded. The wheal responses were measured at 10 (histamine) and 15 (allergen) minutes. We averaged the maximum diameter and its orthogonal, and defined a positive response as a wheal diameter ≥2mm greater than the response to the negative control. We included all participants with a positive response to histamine and no response to glycerin, or those with one or more positive responses (≥2mm) to any allergen, even if there was a weak or no response to histamine. Participants with a positive response to one or more allergens but also a response to the negative control were included with adjustment for the negative control (subtraction of the mean wheal diameter of the negative control from each positive test wheal diameter). In some cases specific tests were omitted (e.g. some families declined infant peanut testing) and these specific allergens responses were recorded as missing but all other data from that participant were included. Participants with no response to histamine and no response to any allergen were excluded, as were participants with dermatographism, when the response to the negative control was as large as any other response.

### 3.2.3 Air pollution exposure assessment

City-specific LUR models were developed to estimate $NO_2$ concentrations and used to assign exposures at the residential locations of all participants. The models are described in detail elsewhere and were

developed at different time points between 2003 and 2008 (Allen et al. 2011; Henderson et al. 2007; Jerrett et al. 2007). Models were developed using road and land use data provided by Desktop Mapping Technologies Inc. (DMTI http://www.dmtispatial.com), as well as additional city-specific data sources (e.g. traffic counts from the city of Toronto).

While individual models differed between locations, the variables used for model building had relatively similar grouping categories (Table 1.4): (1) land use; (2) road and traffic; (3) population; (4) physical geography; and (5) meteorology. All statistical models were consistently developed using the methodology adopted in the initial Vancouver model (Henderson et al. 2007). These models allow cost-effective, relatively precise individual predictions of TRAP at the participants' residence.

Individual estimates of exposure were extracted for each geo-referenced residence reported by participants from address at conception to the address where the participating child resided at age one. Exposure to $NO_2$ was assessed in two case scenarios. In the first scenario, we used the traditional approach of using only the address at the time of birth. Secondly, for those reporting multiple addresses we considered their exposure at every address during the study period and computed their time weighted average exposure. All exposures were subsequently temporally adjusted based on local fixed-site ambient monitoring data on a bi-weekly basis (see equation 1 for one 2-week interval) over the entire pregnancy and first year of life by multiplying the LUR estimate by the ratio of the contemporaneous average concentrations measured at all fixed-site governmental monitoring stations (Vancouver: N=12, Toronto: N=7, Edmonton: N=4, and Winnipeg N=2) to the annual average at the same stations for the year the model was developed.

$$\text{adj}^{9}.\ NO_2 = NO_{2(LUR)}\ (\text{2-week}\ NO_{2(monitor)}/\text{yearly}\ NO_{2(monitor)}) \quad [1];$$

[9] adj.$NO_2$ refers to temporally adjusted individual estimate of nitrogen dioxide.

Since the LUR models did not in all cases cover the full areas where participants resided with the exception of Vancouver, it was necessary to impute exposures for those outside of the LUR model domain. We restricted the locations where exposures were imputed to those residences within 50 km from the municipal center, defined as the location of the city hall. Participants with home addresses located outside of a 50km buffer from the city hall were not assigned TRAP exposure. To reduce discontinuities in exposure estimates on the periphery of the study area, we first defined core city limits within the original LUR surface where the models were applied directly to estimate exposures. For all homes outside these core city limits and within the 50km buffer, the minimum value of the LUR surface in the core city area was assigned to homes within 100m of highway (defined by standard road classification categories (DMTI Spatial Inc., Markham, Ontario), with categories 1 (expressway), 2 (principal highway), and 3 (secondary highway)) and the minimum value of the original LUR surface was assigned to homes located farther than 100m away from a highway. Finally, participants with a TRAP concentration estimated for at least 75% of the time window of interest (i.e. full pregnancy or first year of life) were assigned the mean exposure of the corresponding time window.

#### 3.2.3.1 Time-activity patterns and daycare attendance

The CHILD home environmental questionnaires were used to gather information on the micro-environments within and outside the home of each participating child since birth. From the 3, 6, and 12 month home questionnaires we derived: (a) the average time spent away from home (i.e. time weighted average of hours per day spent outside the house based on typical weekday and weekend time-activity patterns) that was divided into two strata by the city-specific median time away from the house, (b) whether the participating child ever attended a daycare or other indoor location for at least 7hrs per week or 1 hr. per day regularly at any time point during the first year of life. Using these estimates we evaluated the association between TRAP exposure and atopy in stratified analyses comparing children ever/never

attending daycare and children spending more time or less time than the city-specific median outside the home.

#### 3.2.3.2 Infiltration

To assess the potential impact of ventilation, we adapted a model previously developed (Allen et al. 2012a) to predict fine particle ($PM_{2.5}$) infiltration in 6 US cities as part of the MESA Air study. The model included information on residence characteristics and behaviors related to infiltration, including presence/use of air conditioning (AC), outdoor air temperature, window opening, and use/type of heating. As the CHILD and MESA-Air questionnaires differed, we mapped the MESA-Air variables onto those collected in CHILD to predict infiltration (Table 3.1). For behaviors that varied seasonally, participants were asked about typical behavior in mid-summer and mid-winter. Season was defined by the Environment Canada daily temperature data (http://climate.weather.gc.ca/), collected from the same monitors from 2008 to 2013, where the warm season was defined as every two-week period with a median temperature above 18°C, similar to the MESA Air study. We predicted home-specific seasonal infiltration efficiencies and classified homes based on the city-specific $80^{th}$ percentile of infiltration efficiency for each season, where participating houses above this threshold were classified as "leaky". As our exposure of interest was $NO_2$, we used modeled particle infiltration as a surrogate for home ventilation and infiltration of TRAP in general, and assessed effect modification by infiltration in stratified analyses.

**Table 3.1: Particle Infiltration ($F_{inf}$) variables; mapping questions used in the MESA-Air (Allen et al. 2012) and CHILD cohorts.**

| Season | MESA-Air Variable | CHILD Variable | Partial $R^2$ in the MESA-Air model |
|---|---|---|---|
| Warm (≥18° C) | Central AC used > ½ time in past July | Central AC used regularly in the summer [a] | 0.560 |
| | Windows open ≥ ½ time in past summer | Windows open ≥ 1hr more than 2 times/week in mid-summer [a] | 0.080 |
| | Central AC used > ½ time in past July and 2-week avg. outdoor temperature > $23^0$C | Central AC used regularly in the summer [a] and 2-week government monitors average [b] | 0.051 |
| | Central AC used a few days in past July | Central AC use occasionally in the summer [a] | 0.013 |
| | 2-week avg. outdoor temperature > $23^0$C | 2-week avg. outdoor temperature > $23^0$C [b] | 0.000 |
| Cold (<18° C) | 2-week avg. outdoor temperature ($^0$C) | 2-week avg. outdoor temperature [b] | 0.222 |
| | Home has forced air heat | Home has furnace [c] | 0.166 |
| | Windows open ≥ ½ time in past summer | Windows open ≥ 1hr more than 2 times/week in mid-summer [c] | 0.069 |

a:Home environmental questionnaire; b: Environment Canada (http://climate.weather.gc.ca/), c: Home inspection;

## 3.3 Questionnaires and home inspections

Covariates relating to (1) indoor and outdoor environments, (2) socioeconomic and parental risk factors, and (3) nutrition were derived from self-reported questionnaires, or from inspections by trained technicians (for timetable of assessments, see Table 1.2). Using standardized protocols, technicians carried out a home assessment when infants were ~3-4 months old and gathered information about residential characteristics and activity patterns of the occupants. Secondhand smoke exposure (SHS),

presence of leaks, mold, pets, insects and other pests were assessed by questionnaires at four time points (at time of enrollment, and at 3, 6, and 12 months of age).

Three measures of SES were examined using the father and mother's education status and the household income reported at enrolment.

Information on maternal history of asthma, smoking (before/during pregnancy), secondhand smoke exposure during pregnancy and prior live births was obtained at enrolment by questionnaire. Additionally, birth chart data provided information on type of delivery (vaginal *vs.* Cesarean-section), length of gestation, and child's sex.

Information on breastfeeding/feeding practices was provided by the mother in postnatal questionnaires (approximately at 3, 6 and 12 months). Breastfed participants were defined as having been breastfed when the mother answered positively at any time point. Children with formula, soy and cow's milk intake were similarly derived. Variables relating to the introduction of solid foods were derived using any positive answer to the same question across all three questionnaires asking mothers to "indicate which foods you currently feed your child". Timing of food introduction was not considered in this investigation.

## 3.4 Statistical analysis

Prior to investigating the association between TRAP and atopy, we assessed bivariate associations between sensitization at one year and all covariates mentioned above from the environmental (furry pets, SHS, leaks, mold, pests), parental (parity, maternal asthma, maternal atopic status, maternal smoking status during pregnancy, type of delivery), socio-economic (income, maternal and paternal education) and diet risk factors (breastfeeding, formula, soymilk, and introduction of different solid foods) for each of the three outcomes of interest (sensitization to any allergens, to any inhalant allergens and to any food allergens) as well as sex and presence of an attached garage, to test for potential confounding. In these analyses, we first examined covariates for each time point separately. Subsequently, never/ever variables were generated whereby for any positive response to a risk factor, the participant was categorized as

having this risk factor. Further bivariate analyses, evaluating the associations with each outcome separately (sensitization to any allergens, to any inhalant allergen, and to any food allergen), were carried out with the latter never/ever variables. Covariates that were significant predictors ($p<0.05$) of each of the three outcomes of interest from the bivariate analyses were considered in models of $NO_2$ and atopy for each time window. We performed a manual stepwise backward multiple variable logistic regression with city as random intercept to obtain a parsimonious model with significantly ($p<0.05$) associated predictors of each outcome.

In the pregnancy time period, the model for inhalant allergens included presence of an attached garage and mold, whereas the food allergens model included maternal atopy, presence of furry pets, and household income. In the first year of life, inhalant models controlled for presence of furry pets and any consumption of nuts, while the food allergen model controlled for maternal atopy, presence of furry pets, and any consumption of eggs, processed cereals, and peanuts. For sensitization to any allergen, the significant predictors were similar to those obtained for the food allergen model in the first year analysis. During pregnancy, the model for this outcome included maternal atopy and presence of furry pets.

The main analysis, which included a random study center intercept to adjust for the clustering within city, focused on predicted outdoor $NO_2$ exposure at the home address(es) only, while we assessed time spent away from home, daycare attendance and infiltration in sensitivity analyses. Additional analyses for effect modification by parity, sex and maternal atopy were carried out. Predictors for these analyses were the same as those used in the main analysis. Effect estimates are presented for a 10 $\mu g/m^3$ increase in $NO_2$, approximately representing the overall standard deviation.

## 3.5 Results

### 3.5.1 Population characteristics and atopy

Atopy data were examined for 2482 children who had been assessed at age one by October 15th, 2013. Five children were excluded from the analysis due to non-interpretable skin prick test results (n=2), antihistamine medication taken prior to skin testing (n=1), or test postponement (n=2). Of the 2477 participants, 400 infants were sensitized to at least one of the 10 tested allergens (Table 3.2).

**Table 3.2: Atopic outcomes (inhalant, food and any allergies) by city among 2477 CHILD participants with valid skin prick test at age 1**

| City (N, % sensitized) | Inhalant sensitization n(%)[a] | Food sensitization n(%)[a] | Any sensitization n(%)[a] |
|---|---|---|---|
| Vancouver (575, 23% ) | 55 (42) | 92 (30) | 132 (33) |
| Edmonton (641, 17%) | 28 (21) | 85 (28) | 108 (27) |
| Winnipeg (680, 9%) | 13 (10) | 50 (16) | 60 (15) |
| Toronto (581, 17%) | 36 (27) | 82 (27) | 100 (25) |
| Total (2477, 16%) | 132 (5) | 309 (12) | 400 (16) |

[a]Data are shown as n, number of sensitized children and (%)proportion within each atopy outcome examined.

Across all cities, 309 (12.5%) infants were sensitized to any food allergen while 132 (5.3%) had a positive response to any inhalant allergens. Vancouver had the largest proportion of atopic children (23.5%) followed by Toronto and Edmonton (both 17%), and only 9% in Winnipeg (Table 3.2). As expected for infants, prevalence of individual allergens was low (Table 3.3), precluding the investigation of associations between $NO_2$ and individual allergens. However, in preliminary bivariate analysis, dog (OR = 1.20; 95% CI: 1.01 – 1.21), *Der p* (OR = 1.14, 95% CI: 1.07 – 1.27) and peanut sensitization (OR = 1.07; 95% CI: 1.03 – 1.11) were individually associated with temporally adjusted exposure at the birth address.

**Table 3.3: Proportion of positive responses to individual allergen skin prick tests by CHILD city (in (%))**

| City | Alt | *Der p* | *Der f* | Cat | Dog | Blatt | Peanut | Milk | Eggs | Soy |
|---|---|---|---|---|---|---|---|---|---|---|
| Vancouver (N = 575) | 1.9 | 1.4 | 3.0 | 3.1 | 1.0 | 1.2 | 6.1 | 8.5 | 4.0 | 1.7 |
| Edmonton (N = 641) | 1.1 | 0.5 | 0.9 | 1.4 | 0.3 | 0.3 | 6.2 | 9.2 | 2.3 | 1.1 |
| Winnipeg (N = 680) | 0.1 | 0.1 | 0.1 | 1.2 | 0.1 | 0.4 | 3.2 | 5.3 | 0.4 | 0.1 |
| Toronto (N = 581) | 1.2 | 1.4 | 0.7 | 2.2 | 2.2 | 1.0 | 6.5 | 9.1 | 1.7 | 2.1 |

Only participants with non-missing exposure and health data (pregnancy: n=2123; first year: n=2173) as well as completed questionnaire information about environmental, parental, socio-economic, and nutritional risk factors were considered for analysis. Pregnancy and first year of life subsets used for analysis and the full cohort with sensitization results (n=2477) were virtually identical in the distribution of predictors and disease prevalence (data not shown). We also examined the effect of the exposure imputation, which was performed to include participants residing outside of the LUR surfaces. Rates of allergic sensitization were not statistically different between children assigned LUR estimates (16%) and those who had imputed concentrations (13.5%). Covariates considered for the analysis between TRAP and atopy risk were similarly distributed in the sample with imputed concentrations and that with LUR derived exposure estimates (data not shown).

Most mothers were highly educated (93% completed at least some university or college education or more), nonsmokers during pregnancy (97%), non-asthmatics (77%) yet with a high prevalence of sensitization to at least one of the ten allergens tested (61%) and had a vaginal delivery (65%). About half of mothers had previous live births (47%). The majority of participating families (70%) belonged to higher SES based on household income ≥ $80,000/year (Table 3.4)

**Table 3.4: Cohort characteristics[b] among 2477 children at one year of age with valid skin allergy tests, and crude Odds Ratio (OR) for sensitization to any allergens with 95% Confidence Interval (95% CI)**

| | N (%) | Non-atopic | Atopic | OR | 95% CI |
|---|---|---|---|---|---|
| **Personal/Maternal covariates** | | | | | |
| Sex | | | | | |
| Male | 1282, (52) | 1067, (51) | 215, (54) | 0.94 | (0.76, 1.18) |
| Female | 1195, (48) | 1010, (49) | 185, (46) | | |
| Maternal atopy status[c] | | | | | |
| Yes | 1509, (61) | 1227, (59) | 282, (71) | 1.68 | (1.33, 2.12) |
| No | 966, (39) | 848, (41) | 118, (30) | | |
| Missing | 2 | 0 | 2 | | |
| Maternal asthma status | | | | | |
| Yes | 522, (23) | 435 (23) | 87, (23) | 1.09 | (0.83, 1.42) |
| No | 1771, (77) | 1485 (77) | 286, (77) | | |
| Missing | 184 | 157 | 27 | | |
| Maternal smoking in pregnancy | | | | | |
| Yes | 74, (3) | 66, (3) | 8, (2) | 0.74 | (0.35, 1.57) |
| No | 2219, (97) | 1854, (97) | 365, (98) | | |
| Missing | 184 | 157 | 27 | | |
| Maternal smoking ≥ 1 year | | | | | |
| Yes | 617, (27) | 527, (27) | 90, (24) | 0.85 | (0.65, 1.10) |
| No | 1674, (73) | 1391, (73) | 283, (76) | | |
| Missing | 186 | 159 | 27 | | |
| Parity | | | | | |
| Has previous births | 1075, (47) | 914, (48) | 161, (43) | 0.85 | (0.68, 1.07) |
| No previous births | 1219, (53) | 1007, (52) | 212, (57) | | |
| Missing | 183 | 156 | 27 | | |
| Delivery mode | | | | | |
| Vaginal | 1481, (65) | 1244, (75) | 237, (73) | 1.05 | (0.79, 1.37) |
| C-section | 501, (22) | 413, (25) | 88, (27) | | |
| Missing | 495 | 420 | 75 | | |
| **Socio-economic covariates** | | | | | |
| Maternal education | | | | | |
| High School | 166, (7) | 144, (7) | 22, (6) | ref | |
| College or University | 1686, (74) | 1415, (74) | 271, (73) | 0.99 | (0.61, 1.59) |
| Postgraduate Education | 439, (19) | 362, (19) | 77, (21) | 0.98 | (0.58, 1.67) |
| missing | 186 | 156 | 30 | | |
| Household income | | | | | |
| < 40K | 155, (7) | 132, (8) | 23, (7) | ref | |
| 40-80K | 490, (24) | 417, (24) | 73, (22) | 0.96 | (0.58, 1.61) |
| 80 - 150K | 928, (45) | 777, (44) | 151, (46) | 0.95 | (0.59, 1.55) |
| > 150K | 511, (25) | 431, (25) | 80, (24) | 0.83 | (0.49, 1.40) |
| Missing | 393 | 320 | 73 | | |
| **Environmental[d] covariates** | | | | | |
| Furry Pets | | | | | |
| Yes | 1134, (65) | 977, (54) | 157, (44) | 0.72 | (0.58, 0.90) |
| No | 1048, (22) | 846, (46) | 202, (56) | | |
| Missing | 295 | 254 | 41 | | |
| Garage | | | | | |
| yes | 751, (65) | 623, (41) | 128, (45) | 1.35 | (1.03, 1.79) |
| no | 1070, (22) | 912, (59) | 158, (55) | | |
| Missing | 656 | 542 | 114 | | |

| | | N (%) | Non-atopic | Atopic | OR | 95% CI |
|---|---|---|---|---|---|---|
| **Personal/Maternal covariates** | | | | | | |
| **Introduced food in first year[e]** | | | | | | |
| Dairy products | | | | | | |
| | Yes | 1944, (95) | 1639, (96) | 305, (92) | 0.51 | (0.32, 0.79) |
| | No | 102, (5) | 75, (4) | 27, (8) | | |
| | Missing | 431 | 363, | 68 | | |
| Processed cereals | | | | | | |
| | Yes | 1661, (83) | 1413, (85) | 248, (76) | 0.58 | (0.44, 0.76) |
| | No | 334, (17) | 256, (15) | 78, (24) | | |
| | Missing | 482 | 408 | 74 | | |
| Eggs | | | | | | |
| | Yes | 1742, (85) | 1478, (86) | 264, (80) | 0.54 | (0.40, 0.73) |
| | No | 303, (15) | 235, (14) | 68, (20) | | |
| | Missing | 432 | 364 | 68 | | |
| Nuts | | | | | | |
| | Yes | 625, (31) | 1180, (69) | 240, (72) | 0.72 | (0.55, 0.93) |
| | No | 1420, (69) | 533, (31) | 92, (28) | | |
| | Missing | 432 | 364 | 68 | | |
| Peanuts | | | | | | |
| | Yes | 941, (46) | 816, (48) | 125, (38) | 0.63 | (0.49, 0.79) |
| | No | 1102, (54) | 896, (52) | 206, (62) | | |
| | Missing | 432 | 365 | 69 | | |

[b]Data are shown as n (%). The percentages are calculated using the number of observations with known values as the denominator. [c]Positive skin prick test response to any of the allergens tested.
[d]Environmental covariates are based on any self-reported positive response during pregnancy and at 3, 6, or 12 months. [e]Information on feeding practices are based on any self-reported positive response at 3, 6, or 12 months.

Compared to atopic participants, non-atopic children were more likely in their first year to consume dairy products, eggs, nuts, peanuts, grains and processed cereals, to reside in a home with pets , and less likely to have a garage attached to their home (Table 3.4). Children of atopic mothers were more likely to be atopic than children of non-atopic mothers (crude OR = 1.68; 95% CI: 1.33 – 2.12).

### 3.5.2 Exposure levels and association with atopy development

Exposure estimates were unavailable for 12% of the 2477 children with skin prick test data, as 173 participants had homes located more than 50km from the city centre and 131 did not provide their residential history since enrolment.

Among the 2173 participants with complete residential histories who resided within the 50km buffer, 252 homes fell outside of each city core limits and were assigned imputed $NO_2$ concentrations. Mean exposure levels differed significantly by city; ranging from 28 $\mu g/m^3$ in Toronto to 9.9 $\mu g/m^3$ in Winnipeg (Table 1.3). After applying the bi-weekly temporal adjustment and accounting for residential mobility (83% did not change their address), estimated exposures were lower across all cities due to decreasing ambient concentrations between the development of the LUR model and the date of birth (Brook et al. 2014), with a greater decline for older LUR surfaces. The difference between these estimates is not likely due to addresses change, but mostly to the temporal adjustment from original LUR models to the time of the present of investigation. When examining the pairwise differences of spatiotemporal biweekly means by time window, we found significant differences ($p<0.05$) between exposure during pregnancy and the first year of life, unlike the non-significant differences obtained with estimates not accounting for residential mobility ($p=0.3$).

Compared to TRAP exposure estimated at the birth address with no temporal adjustment, $NO_2$ estimates that incorporated temporal variability in ambient concentrations increased the magnitude of the effect estimates for the first year of life analysis (any allergens: aOR = 1.10; 95% CI: 0.96 – 1.34), yet without reaching statistical significance (Table 3.5). Further, estimates of effect generally increased when temporally adjusted models further accounted for mobility (Figure 3.1B). However the increased spatial resolution also led to larger confidence intervals around the atopy risk estimates.

**Table 3.5: Adjusted[10] Odds Ratios (aOR) for risk of atopy per 10 µg/m$^3$ increase in $NO_2$ exposures during pregnancy and the first year of life.**

| | All Allergens | | Food Allergens | | Inhalant Allergens | |
|---|---|---|---|---|---|---|
| | aOR | 95% CI | aOR | 95% CI | aOR | 95% CI |
| Pregnancy | | | | | | |
| Exposure at birth address | 1.06 | 0.95 – 1.28 | 1.05 | 0.87 – 1.34 | 1.05 | 0.77 – 1.40 |
| Temporal adjustment(birth address) | 1.01 | 0.81 – 1.16 | 0.99 | 0.73 – 1.10 | 1.16 | 0.86 – 1.61 |
| Spatio-temporal adjustment | 1.02 | 0.86 – 1.22 | 1.00 | 0.77 – 1.61 | 1.18 | 0.77 – 1.61 |
| First Year | | | | | | |
| Exposure at birth address | 1.05 | 0.95 – 1.28 | 1.08 | 0.95 – 1.28 | 1.16 | 0.91 – 1.40 |
| Temporal adjustment(birth address) | 1.10 | 0.96 - 1.34 | 1.15 | 0.95 – 1.40 | 1.22 | 0.92 - 1.62 |
| Spatio-temporal adjustment | 1.16 | 1.00- 1.41 | 1.17 | 0.95 – 1.47 | 1.28 | 0.93 - 1. |

During the first year of life, $NO_2$ was associated with sensitization to any allergen tested at one year of age (aOR = 1.16; 95% CI: 1.00 – 1.41) when considering temporally adjusted exposures that also accounted for residential mobility. When examining each group of allergens separately, we also found positive, but non-significant associations (aOR = 1.17; 95% CI: 0.95 – 1.47 for inhalant allergies and aOR = 1.27; 95% CI: 0.93 – 1.76 for food allergies) (Figure 3.1B).

[10] Model covariates for each outcome/time window pair are as follows:
Any allergies and $NO_2$ during pregnancy: mother's atopic status, presence of furry pets.
Any allergies and $NO_2$ during first year: mother's atopic status, presence of furry pets, consumption of eggs, consumption of processed cereals, and consumption of peanuts
Food allergies and $NO_2$ during pregnancy: mother's atopic status, presence of furry pets, and household income
Food allergies and $NO_2$ during first year: mother's atopic status, presence of furry pets, consumption of eggs, consumption of processed cereals, and consumption of peanuts
Inhalant allergies and $NO_2$ during pregnancy: presence of an attached garage, presence of mold.
Inhalant allergies and $NO_2$ during first year: presence of furry pets and consumption of nuts.

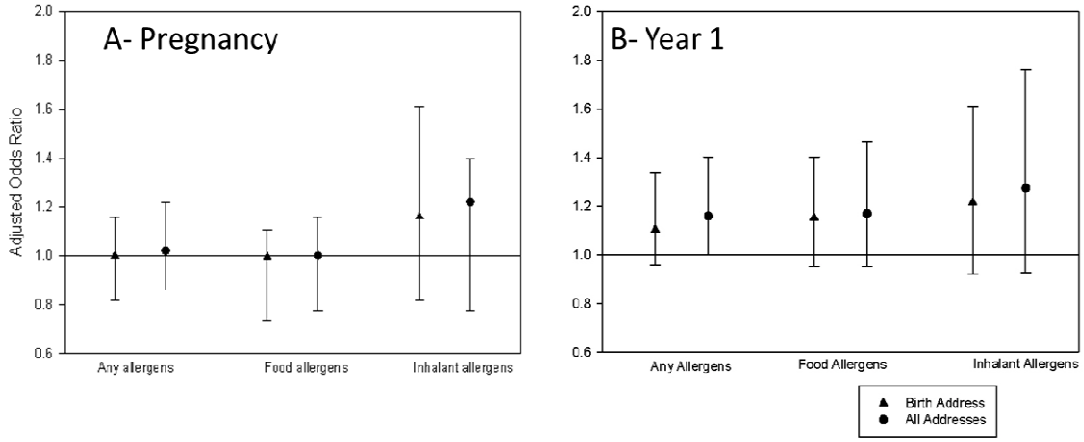


**Figure 3.1: Adjusted Odds Ratio for risk of atopy per 10 µg/m3 increase in $NO_2$ exposures (triangle) temporally adjusted at birth address, (circle) temporally adjusted and accounting for residential mobility. (A) during pregnancy: inhalant allergens model controlled for presence of an attached garage and mold (n=1836); food allergens model controlled for mother's atopic status, presence of furry pets, household income (n=1913); any allergens model (n=2123) controlled for mother's atopic status and presence of furry pets and (B) during the first year of life: inhalant model (n=2058) controlled for presence of furry pets and any consumption of nuts since birth; food allergen analysis (n=2002)adjusted for mother's atopic status, presence of furry pets, and any consumption of eggs, processed cereals, and peanuts); any allergen analysis (n=2173) adjusted for mother's atopic status, presence of furry pets, consumption of eggs, processed cereals and peanuts**

In contrast, during pregnancy (Figure 3.1A), effect estimates were null for sensitization to any allergens (aOR = 1.02; 95% CI: 0.86 – 1.22) and for sensitization to food allergens (aOR = 1.00; 95% CI: 0.77 – 1.61). In this time window, the association between exposure and inhalant allergens atopy was non-significant (aOR = 1.18; 95% CI: 0.77 – 1.61).

Analyses of the effect of greater or lesser time spent away from the home indicated improved precision in estimates among children spending more time at the home, and identified a potential source of exposure misclassification (Figure 3.2A). Participants (n= 976) who spent more time away from the home (median: 3.3 hours per day for all cities) generally had slightly smaller effect estimates with larger confidence intervals (any allergens: aOR = 1.16; 95% CI: 0.85 – 1.53) than those spending less time (n=1026) away from their home addresses (aOR =1.22; 95%CI: 1.00 – 1.47). This association was likely driven by the sensitization to inhalant allergens (children spending ≤ city-specific median time away from home aOR

=1.61; 95% CI: 1.15; 2.19 vs. those spending more than the city-specific median time away from their homes aOR =1.10; 95% CI: 0.69, 1.68).

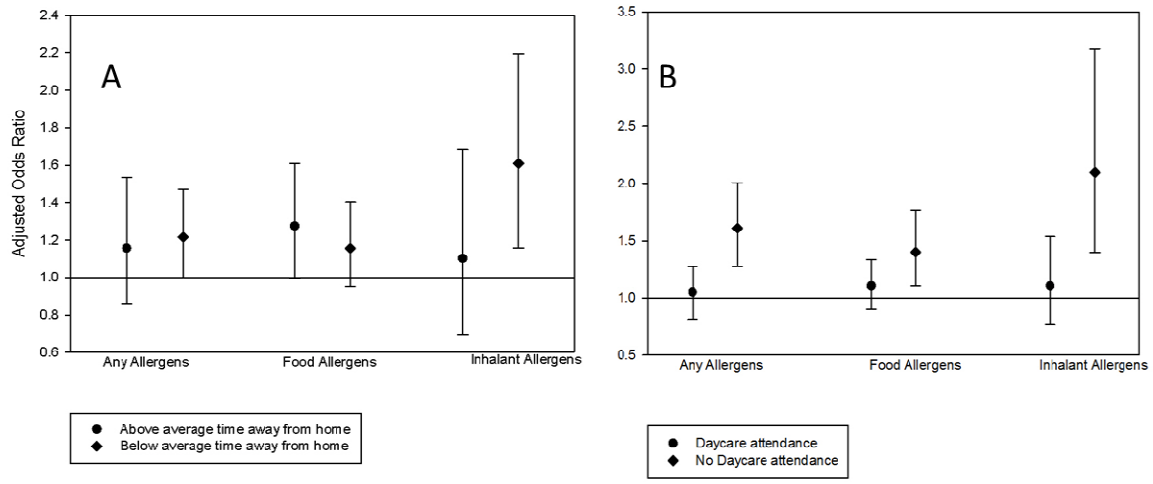


**Figure 3.2: Adjusted OR of atopy per 10µg/m3 $NO_2$ increase during the first year stratified by: (A) time-activity patterns (defined by the city-specific median hours per day based on the three questionnaires submitted after birth around 3,6, and 12 months) among children spending more time (n=976) and those spending less time (n=1026) away from the home; and (B) daycare facilities attendance among daycare attendees (n=765) and children never attending daycare (n=1236).**

Stratifying the cohort by daycare attendance (35% attended daycare) also suggested a source of exposure misclassification, as non-daycare attendees (n= 1236) had 61% increased odds of developing atopy (aOR = 1.61; 95% CI: 1.28 – 2.01) whereas the risk was smaller and not significant in children who did attend daycare (n=765; aOR = 1.05; 95% CI: 0.81 – 1.28) (Figure 3.2B). The sensitization to inhalant allergens showed the largest association with $NO_2$ exposure (aOR = 2.1; 95% CI: 1.40 – 3.17 for children never attending daycare *vs.* aOR = 1.10; 95% CI: 0.77 – 1.54 for daycare attendees). Given these results, we investigated whether exposures other than $NO_2$ (e.g. contact with other children) may play a role in the sensitization onset. Thus, we ran a stratified analysis by presence of siblings which showed that participants who were in households with other siblings had lower odds of developing sensitization to any

allergens (n = 874, aOR = 1.16; 95% CI = 0.91 – 1.54) following exposure to TRAP than those with no siblings (n= 1085, aOR = 1.28; 95% CI = 1.0 – 1.54) (Figure 3.3).

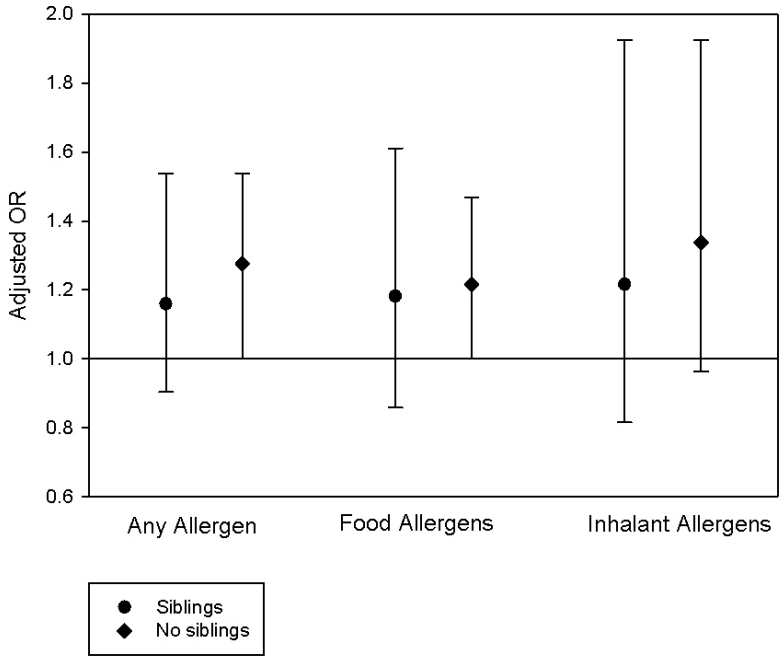


**Figure 3.3: Adjusted OR of risk of atopy in CHILD families for a 10 µg/m$^3$ increase in $NO_2$ during the first year of life, stratified by presence of siblings in participants families (group with no siblings, n= 1085; group with sibling, n= 874). All models are adjusted for the same covariates as those used in the main analysis (Figure 1B)**

For a 10 µg/m$^3$ increase in $NO_2$ exposure, the odds of sensitization to any allergen for children living in homes with greater ventilation (n= 687) was slightly higher (aOR = 1.22; 95% CI: 0.91 – 1.61) than for children living in tighter homes (n= 824, aOR = 1.10; 95% CI: 0.82 – 1.47) during the heating season, but not in the warm season (Figure 3.4).

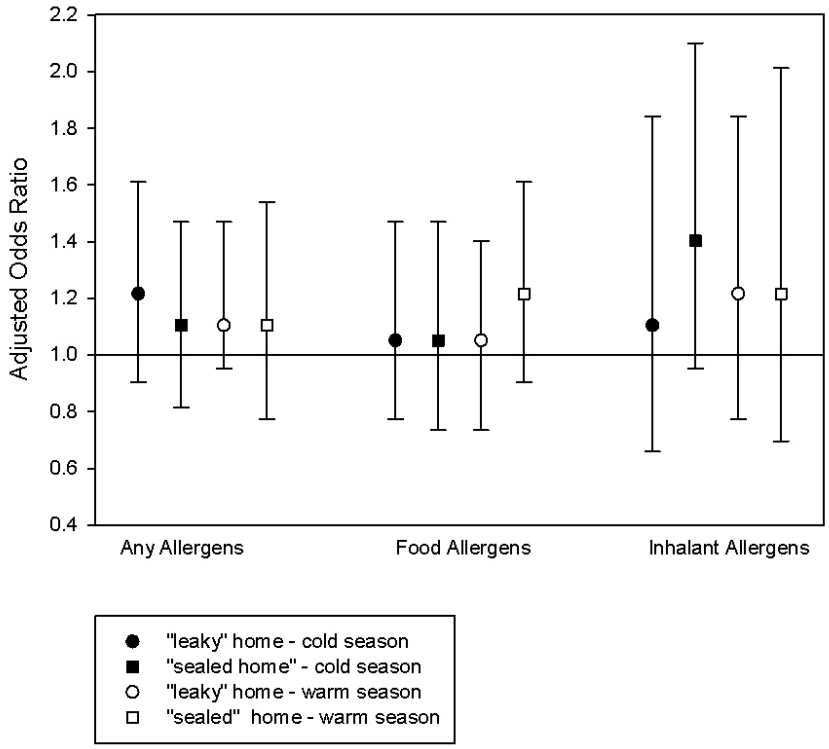


**Figure 3.4: Adjusted Odds Ratio of risk of atopy for 10 µg/m$^3$ increase in $NO_2$ during the first year of life stratified by season (defined using weekly average of 18°C as cutoff to define cold and warm) and by home PM infiltration status (defined based on city-specific 80$^{th}$ percentile predicted household PM infiltration efficiency; "leaky" homes: n= 687; "sealed" homes: n=824). All models are adjusted for the same covariates as in the main analysis (Figure 3.1B)**

## 3.6 Discussion

In this prospective multi-centre birth cohort study, exposure to $NO_2$ during the first year of life, but not during pregnancy, was positively associated with atopy at age one year. To our knowledge, this is the first birth cohort study where atopy in relation to traffic-related air pollution was determined in the first year of life (Brauer et al. 2007b; Gruzieva et al. 2012; Nordling et al. 2008). Positive associations between $NO_2$ and specific sensitization to common food, but not inhalant allergens were observed in a subgroup of 700 Dutch children from the PIAMA cohort at age 4 (Brauer et al. 2007). In the Swedish BAMSE cohort, exposure during the first year of life was associated with an increased risk of only pollen sensitization at age 4 (no association with food allergens) (Gruzieva et al. 2012).

The ability to refine individual estimates of exposure to TRAP by incorporating temporal changes in air pollution concentrations and in participants' residential mobility led to larger effect estimates; however the improvement in precision of these effects was negligible and not consistently improved across all three outcomes. It is important to note that the exposure assessment was derived from modeled estimates rather than measurements, increasing error propagation in the estimates used for evaluating the association with atopy outcomes. However, the correlation between the exposures during the entire pregnancy and the first year of life decreased with more specific exposure measures suggesting that refined exposure assessment enables improved differentiation between exposure periods. This finding is supported by a study showing that temporally updated (based on air dispersion model data) LUR models provide accurate exposure estimates (Mölter et al. 2010).

In light of the recently published European meta-analysis of air pollution with allergic sensitization (Gruzieva et al. 2014), we explored potential effect modification by sex or maternal atopy. Similar to their results, no effect modification by sex was found. However maternal atopy showed borderline significant effects for exposure during pregnancy and smaller magnitude of effect compared with the main model in the first year (aOR for Maternal atopy $^{\times}NO_2$ interaction term = 1.04; 95% CI: 0.98 – 1.10 during pregnancy and aOR = 1.04; 95% CI: 1.00 – 1.10 during the first year).

We demonstrated stronger associations between TRAP and atopy in our stratified analyses when daycare attendance and individual time-activity patterns were considered. In particular for exposures during the first year of life, when inhalant allergen sensitization was considered separately, participants for whom exposure misclassification was less likely (i.e. those spending more than the city median time at home, and those who did not attend daycare) had stronger associations. In a small subsample of participants providing daycare addresses (n=235), exposures were not significantly different in homes and in daycares (data not shown), making it unlikely that lower exposures outside the homes would explain reduced effects amongst those attending daycare. While the observed differences in these sub-analyses could also

be due to more variability suggestive of a classical exposure error, we explored the possibility that the differences observed were related to exposure other than TRAP. Odds ratios of sensitization to any allergens were lower for participants spending more time in the home or not attending daycare compared to daycare attendees or those spending more time away from the home for the same rate of TRAP increase, suggesting that this latter group might be exposed to other exogenous protective exposure such as presence of other children. The additional stratified analysis by presence of siblings seemed to support the argument that exposure to other children is likely to play a protective role in the development of atopy.

Along with refined exposure assessment modeling, major strengths of our study are the prospective design from early in pregnancy and the objective definition of sensitization. Comparisons between the few birth cohorts examining early life exposures to traffic pollution are complicated by the various definitions of atopy or allergic sensitization, most often assessed by self-reported symptoms (Bråbäck and Forsberg 2009) which can lead to misclassification of outcomes. In the present study, atopic status was based on objective skin prick tests using a common protocol for all participants. Gathering questionnaire and home inspection data enabled us to collect extensive individual data on known and suspected risk factors about indoor and outdoor environments, and parental health status, as well as detailed dietary information that are seldom acquired in large cohort studies as early as in this investigation. However, the number of missing covariates is a limitation as sample sizes for individual analyses were substantially reduced.

Despite the advantage of multiple questionnaires and detailed home assessments, the use of self-reported information on environmental risk factors, which may be biased by parental health status, is a concern. Although the cohort was unselected and the prevalence of parental allergy and current asthma similar to that in the Canadian population, there is a bias towards higher SES compared with the general population, as is often the case with birth cohorts. Further, while this is one of few analyses of TRAP to examine the

role of infiltration, our assessment was limited by the use of a model for particle infiltration developed for cities in the US (Allen et al. 2012) to classify infiltration of TRAP in Canadian homes. We mapped variables in the MESA-Air cohort questionnaires to the most similar questions available in CHILD; however it is likely that we introduced some error in recoding the CHILD variables, and thus in developing infiltration estimates which are already difficult to model based on actual measurements. In addition, the model was developed in US cities spanning a wider north-south geographical area, and consequently developed for a hotter climate, which led to a temperature threshold variable (23°C) that might differ from the cut-off obtained using Canadian data. In the case of the cold season infiltration models, we found the expected differences between homes with participants in the "leaky" homes showing stronger associations between TRAP exposure and sensitization to any allergens only. Lack of sufficient power precluded the identification of differences in this analysis. Future studies should consider an infiltration measurement sub-study to develop a study-specific model.

Children at one year of age developed more sensitization to food (12.5%) than inhalant allergens (5.5%) similar to findings in the European birth cohorts in which participants were older and showed higher prevalence rates of sensitization (BAMSE with 16% and 15% (Gruzieva et al 2012) and PIAMA with 23.9% and 8.5% (Gehring et al. 2010b) for food and inhalant allergens respectively). However, we observed that exposures during first year of life may contribute differently to the potential load of sensitization. In conclusion, this study demonstrates that in cities with low-levels of ambient traffic-related air pollution, incorporating different tools (GIS, monitoring data, questionnaires, and home environmental assessment) to account for temporal variation, residential history, and time-location patterns in the estimation of individual-level exposures can help clarify the association between early life exposure to traffic-related air pollution and the development of allergic sensitization to common inhalant and food allergens.

# Chapter 4: Asthma development in relation to air pollution and greenness in the BAQS birth cohort: a follow up from birth to age 10.

## 4.1 Introduction

Among pediatric respiratory diseases, asthma is the most common pathology and represents a heavy burden of illness and costs (Ismaila et al. 2013). It is recognized that both environmental and genetic factors play a role in its development (Subbarao et al. 2009). Among the environmental factors, air pollution has attracted interest because of its link to asthma severity, its ubiquitous nature and the possibility of preventive strategies via exposure reduction (Guarnieri and Balmes 2014).

Several qualitative critical reviews have suggested causal associations (Bråbäck and Forsberg 2009; Health Effects Institute 2010) between air pollution, traffic-related air pollution, and incident childhood asthma, although recent meta-analyses of the epidemiological evidence show diverging results. An adverse effect of outdoor air pollution on asthma incidence, but no effect with lifetime period prevalence of asthma, was reported in meta-analyses of studies published up until 2010 (Anderson et al. 2013a, 2013b). Examining air pollution from traffic sources, an American meta-analysis showed adverse effects on childhood asthma (Gasana et al. 2012) while no evidence of an association was found among children followed up to age 10 in a meta-analysis of birth cohorts from the European Study of Cohorts for Air Pollution Effects (ESCAPE) initiative (Mölter et al. 2014b). While the results of these meta-analyses appear contradictory, a possible explanation could be that the increased risk in incidence reflects the unveiling of the presentation of subclinical phenotypes of asthma (Gowers et al. 2012) which highlights the need to examine asthma incidence over different age periods. The current study is able to build on the previous literature by examining the risk of asthma onset among two different age groups (preschool and

school-aged) using data from a 10-year follow-up of over 68,000 children as part of the Border Air Quality Study (BAQS) in British Columbia, Canada.

Traffic not only produces air pollutants, it is also likely to affect urban form via reduced access to green spaces. Green spaces have been linked to positive health outcomes (Lee and Maheswaran 2011), to higher physical activity (Roemmich et al. 2006) which is itself associated with decreased asthma development (Sherriff et al. 2009), and to reduced air pollution provided by trees, thereby protecting respiratory health (Lovasi et al. 2008). A recent review on the role of natural spaces in children's health concluded that reduced access to open spaces is related to higher relative prevalence of asthma and allergic conditions (McCurdy et al. 2010). However, a birth cohort study conducted in New York, reported that asthma at age 7 was significantly increased in those residing in streets with higher tree canopy coverage (Lovasi et al. 2013). Overall, longitudinal follow up studies examining the effect of green spaces on asthma development is scarce and inconsistent. The current investigation will also examine the role of greenness in the development of this disease.

## 4.2 Methods

### 4.2.1 Cohort description

Using linked administrative data sets originating from the British Columbia (BC) Ministry of Health, the BC Vital Statistics Agency, and BC Perinatal Data Registry, the birth cohort comprised all 1999 to 2002 births in the greater Vancouver metropolitan region (Brauer et al. 2008). The study protocol was approved by University of British Columbia Research Ethics Board (#H04-80161).

Children were followed for 10 years providing they continued to reside in the Metropolitan region of Vancouver based on the Ministry of Health registry data and their mothers also had to be registered as continually residing in the study region during their complete pregnancy. Members were lost to follow-up if they moved outside of the study region or had a gap of over half-a year in residency. From the 73, 387

births to mothers with complete information on residential history during pregnancy, children were excluded if they died during follow up (n=101) or were a multiple birth (n=988). The analysis cohort comprised 68,320 singletons with complete covariate information. .

### 4.2.2 Confounders

Birth date, sex, birth weight and gestational length of each child were accessed via Vital Statistics data; and maternal parity, maternal age, intention to breastfeed and maternal smoking during pregnancy were obtained from the BC Perinatal Data Registry. Since, no individual-level data were available for other socio-economic factors, income (quintiles) and maternal level of education (quartiles) were assigned from census data at the Census Dissemination Areas (DA), a resolution which approximates the neighborhood-level with target populations of 400–700 persons

### 4.2.3 Incident asthma case definition

Records of the utilization of all billed outpatient and inpatient medical services from 1999 to 2009 were obtained from the British Columbia Ministry of Health (http://www.popdata.bc.ca/data). Asthma diagnoses were identified from physician billing records and hospital discharge records for codes 493 and J45 from the 9$^{th}$ and 10$^{th}$ revision of ICD, respectively. Using a validated case definition of asthma (Prosser et al. 2008), children with a minimum of two primary care physician diagnoses in a rolling 12-month period or a minimum of one hospital admission over the study period were identified as asthma cases.

### 4.2.4 Exposure assessment

All spatially-derived exposure variables as well as residential greenness exposure were assigned to corresponding residential histories throughout pregnancy since all residential postal codes were recorded at each contact with the health care system. An average exposure was calculated by the time spent at each postal code and successfully assigned to 65,254 study subjects with complete covariate information.

#### 4.2.4.1 Air pollution exposure assessment

Exposure to air pollution for each cohort member was assigned at their six-digit postal code(s), which corresponds to one block-face in urban areas, by three different approaches (described in detail in Brauer *et al.*, 2008) namely, land use regression models, interpolation metrics of regulatory monitoring stations operated by the BC Ministry of Environment and Metro Vancouver, and proximity measures.

Land use regression models provided high spatial resolution (10 m precision), individual estimates of exposures to traffic-related air pollutants, including black carbon (BC), fine particulate matter (< 2.5 µm, $PM_{2.5}$), nitrogen dioxide ($NO_2$), and nitric oxide (NO) (Brauer et al. 2008; Henderson et al. 2007). Since the temporal stability of LUR surfaces showed reasonable backward predictions (Wang et al. 2013), each LUR model, developed after the 1999 – 2002 cohort inception period, was temporally adjusted based on daily air monitoring measurements to estimate monthly average concentrations that were assigned to individual subjects' recorded home address postal code at birth (For detailed description, see (Brauer et al. 2008)) .

Data on $NO_2$, NO, BC, $PM_{10}$, carbon monoxide (CO) and sulfur dioxide ($SO_2$) from monitoring stations were used to compute an inverse-distance weighted (IDW) average of the three closest monitors within 50 km providing high temporal resolution (daily) estimates assigned to participants residential postal codes (Marshall et al. 2008b).

Finally, road proximity for home postal codes of all cohort members was calculated using a geographic database (DMTI ArcView street file data set for BC, Canmap Streetfiles version 2006.3; DMTI Spatial Markham, Ontario, Canada). Home postal codes were categorized as within 50m or 150m of a primary highway, within 50 or 150m of a major road, or within 150m of a primary highway and within 50m of a major road.

#### 4.2.4.2 Greenness

Satellite-derived Normalized Difference Vegetation Index (NDVI) provided a continuous measure of greenness across the study region. NDVI ranges from -1 to 1 (with the minimum value indicating water and higher values indicating more greenness) based on land surface reflectance of visible (red: 0.4 – 0.7μm) and near-infrared (0.7 – 1.1 μm) parts of spectrum(Weier and Herring 2000). Landsat 7 Enhanced Thematic Mapper Plus (ETM+) cloud free images downloaded from 1999 to 2002 were used to assign greenness measures to study postal codes. Average yearly greenness values were calculated for 100 m areas around residential postal codes during pregnancy (Hystad et al. 2014). These buffer areas are at a similar spatial scale to within-city air pollution variation.

### 4.2.5 Analytical methods

Nested case-control design using incidence density sampling within the study cohort was adopted for analytic efficiency. Each asthma case was randomly matched to five controls by sex and birth month and year. Nested conditional logistic regression models included breastfeeding status at the time of discharge, parity, maternal education (in area-level quartiles), household income (in area-level quintiles), gestational length and birth weight (both as continuous variables). The risk of incident asthma was examined in relation to air pollution and greenness in two time windows: between birth and up to the child's $6^{th}$ birthday (denoted preschool group thereafter) and from age 6 until the end of follow up (school age group). The preschool group mirrored the initial analysis of a subsample of this cohort (Clark et al. 2010b), while the school age group provided new insight into the effect of air pollutants and greenness on asthma incidence later in childhood. In addition, greenness and air pollutants were analyzed in joint exposure models.

The effects of low birth weight (<2500g) and gestational duration ($\geq$37 weeks; 30 - 37 weeks; < 30 weeks) on asthma incidence were also examined in stratified analyses. Similarly, a series of stratified analysis were conducted to investigate potential effect modification by sex, parity, and socio-economic

factors (neighborhood-level household income < 40$^{th}$ percentile), maternal post-secondary education < median neighborhood level of maternal education, and tertiles of maternal age at birth).

## 4.3 Results

A total of 6,948 and 1,711 children met the case definition of asthma onset in the preschool and school age groups respectively. Descriptive statistics by confounder data, stratified by case status are summarized in Table 4.1. Mean age at time of diagnosis (sd) was 2.64 (1.41) and 7.00 (1.21) years in the preschool and school age groups.

**Table 4.1: Case and control characteristics (n (%) for categorical covariates; mean (standard deviation) for continuous covariates) for children meeting the case definition, matched controls by time window.**

| | | Preschool age | | School age | |
|---|---|---|---|---|---|
| | | **Control (34621)** | **Cases (6948)** | **Control (8577)** | **Cases (1711)** |
| **Sex** | Female | 13143 (38) | 2646 (38) | 3480 (41) | 690 (40) |
| | Male | 21478 (62) | 4302 (62) | 5097 (59) | 1021 (60) |
| **Newborn Feeding at discharge** | | | | | |
| | No breastfeeding | 2430 (7) | 545 (8) | 626 (7) | 141 (8) |
| | Breastfeeding | 32028 (93) | 6358 (92) | 7911 (93) | 1561 (92) |
| | Unknown | 163 (0) | 45 (0) | 40 (0) | 9 (1) |
| **Maternal post-secondary education quartiles** | | | | | |
| | 1 (lowest) | 7670 (22) | 1882 (27) | 1959 (22) | 459 (27) |
| | 2 | 7973 (23) | 1622 (23) | 1996 (23) | 411 (23) |
| | 3 | 9407 (27) | 1781 (26) | 2270 (27) | 421 (26) |
| | 4 | 9571 (28) | 1663 (24) | 2352 (28) | 420 (24) |
| **Area-based household income quintiles** | | | | | |
| | 1 (poorest) | 7468 (22) | 1693 (24) | 1833 (21) | 392 (23) |
| | 2 | 7719 (22) | 1697 (24) | 1905 (22) | 425 (25) |
| | 3 | 7178 (21) | 1460 (21) | 1893 (21) | 377 (22) |
| | 4 | 6799 (20) | 1185 (17) | 1629 (19) | 301 (18) |
| | 5 | 5457 (16) | 913 (13) | 1317 (16) | 216 (13) |
| **Parity** | 0 | 15504 (45) | 3332(52) | 3879 (45) | 892 (48) |
| | 1+ | 19117 (55) | 3616 (48) | 4698 (55) | 819 (52) |
| **Gestation length (weeks)** | | 39.4 (1.15) | 38.9 (1.94) | 39.1 (1.68) | 39.0 (1.8) |
| **Maternal age (years)** | | 31.5 (5.06) | 31.2 (5.09) | 31.5 (5.12) | 30.8 (5.3) |
| **Birth Weight (g)** | | 3510 (458) | 3362 (567) | 3437 (527) | 3385 (538) |

Significant differences were observed for all risk factors in Table 4.1 between cases and controls for both the preschool and the school age groups. Asthmatic children were more likely to have been born to a younger mother, from a lower socio-economic stratum (as indicated by maternal educational attainment and household income), and less likely to have been breastfed. Also, the gestation period and birth weight were smaller among cases compared with their matched-controls.

All risk factors except for sex were similar when comparing matched controls from the entire pool of children who did not meet the case definition (i.e. all potential non-asthmatic controls), indicating non-biased selection of control subjects as shown in Table 4.2

**Table 4.2 : Cohort characteristics (n (%) for categorical covariates; mean (standard deviation) for continuous covariates) for control children and all non-asthmatics by cohort age groups**

| | **Preschool group** | | **School group** | |
|---|---|---|---|---|
| | **Controls (34621)** | **No asthma (58306)** | **Controls (8577)** | **No asthma (63543)** |
| **Sex** | | | | |
| Female | 13143(38) | 31591(50) | 3480(41) | 30901(49) |
| Male | 21478(62) | 33663(50) | 5097(59) | 32642(51) |
| **Newborn Feeding** | | | | |
| No breastfeeding | 2430(7) | 4632(7) | 626(7) | 4491(7) |
| Breastfeeding | 32028(93) | 60296(93) | 7911(93) | 58735(93) |
| Unknown | 163(0) | 326(0) | 40(0) | 317(0) |
| **Female post-secondary education quartiles (1=lowest)** | | | | |
| 1 | 7670(22) | 14441(23) | 1959(22) | 13982(22) |
| 2 | 7973(23) | 14776(23) | 1996(23) | 14365(23) |
| 3 | 9407(27) | 17792(26) | 2270(27) | 17371(27) |
| 4 | 9571(28) | 18245(27) | 2352(28) | 17825(28) |

| | Preschool group | | School group | |
|---|---|---|---|---|
| | **Controls (34621)** | **No asthma (58306)** | **Controls (8577)** | **No asthma (63543)** |
| **Area-based household income quintiles (1=poorest)** | | | | |
| 1 | 7468(22) | 13950(21) | 1833(21) | 13558(21) |
| 2 | 7719(22) | 14446(22) | 1905(22) | 14021(22) |
| 3 | 7178(21) | 17792(21) | 1893(21) | 13344(22) |
| 4 | 6799(20) | 12654(20) | 1629(19) | 12353(19) |
| 5 | 5457(16) | 10413(16) | 1317(16) | 10267(15) |
| **Parity** | | | | |
| 0 | 15504(45) | 29602(45) | 3879(45) | 28710(45) |
| 1 | 19117(55) | 35652(55) | 4698(55) | 34833(55) |
| **Gestation (weeks)** | 39.4 (1.15) | 39.1 (1.66) | 39.1 (1.68) | 39.1 (1.69) |
| **Maternal age (years)** | 31.5 (5.06) | 31.4 (5.07) | 31.5 (5.12) | 31.4 (5.06) |
| **Birth Weight (g)** | 3510 (458) | 3447 (533) | 3437 (527) | 3431 (533) |

Table 4.3 summarizes the distribution of all spatially-derived exposure variables and the residential greenness metric for each nested case-control cohort. The levels of air pollutants in metropolitan Vancouver were relatively low, compared to other major Canadian cities and international urban areas with populations of over 1 million (Government of Canada 2007) , while the average greenness was high compared to other cities in Europe and the US with similar or larger size (Dzhambov et al. 2014) .

**Table 4.3: Mean exposure levels, standard deviation (SD), and Interquartile Range (IQR) among cases and controls for carbon monoxide (CO), nitrogen monoxide (NO), nitrogen dioxide ($NO_2$), ozone ($O_3$), sulfur dioxide ($SO_2$), particulate matter ($PM_{10}$, $PM_{2.5}$), and black carbon (BC) derived using inverse-distance weighting (IDW) and/or land-use regression (LUR) models and for greenness using Normalized Difference Vegetation Index (NDVI).**

| | | Preschool (0-5 years) | | | School (> 6 years) | | |
|---|---|---|---|---|---|---|---|
| | Pollutants_ metric | Mean | IQR | SD | Mean | IQR | SD |
| Cases | CO_IDW | 628.7 | 165.0 | 119.6 | 628.2 | 160.4 | 120.6 |
| Controls | ($\mu g/m^3$) | 626.7 | 163.9 | 119.7 | 636.7 | 165.7 | 121.9 |
| Cases | $NO_2$_IDW[11] | 33.7 | 9.7 | 6.8 | 33.4 | 9.4 | 6.8 |
| Controls | ($\mu g/m^3$) | 33.3 | 10.0 | 7.0 | 33.7 | 10.1 | 7.0 |
| Cases | NO_IDW | 23.0 | 15.6 | 10.9 | 22.5 | 15.1 | 11.0 |
| Controls | ($\mu g/m^3$) | 22.7 | 15.4 | 10.9 | 23.0 | 16.0 | 11.1 |
| Cases | $O_3$_IDW[10] | 27.7 | 8.6 | 5.8 | 27.9 | 8.7 | 6.0 |
| Controls | ($\mu g/m^3$) | 27.9 | 8.5 | 5.8 | 28.3 | 8.6 | 5.9 |
| Cases | $PM_{10}$_IDW | 12.5 | 1.3 | 1.0 | 12.5 | 1.4 | 1.1 |
| Controls | ($\mu g/m^3$) | 12.5 | 1.3 | 1.0 | 12.5 | 1.3 | 1.1 |
| Cases | $SO_2$_IDW | 5.7 | 3.2 | 2.4 | 5.5 | 2.8 | 2.4 |
| Controls | ($\mu g/m^3$) | 5.6 | 3.1 | 2.4 | 5.7 | 3.3 | 2.5 |
| Cases | $PM_{2.5}$_LUR | 4.09 | 1.4 | 1.6 | 4.1 | 1.4 | 1.6 |
| Controls | ($\mu g/m^3$) | 4.06 | 1.5 | 1.7 | 4.0 | 1.5 | 1.7 |
| Cases | $NO_2$_LUR | 33.6 | 9.3 | 8.8 | 33.2 | 9.1 | 8.6 |
| Controls | ($\mu g/m^3$) | 33.6 | 9.0 | 9.1 | 33.5 | 9.5 | 8.9 |
| Cases | NO_LUR | 31.3 | 14.5 | 13.4 | 31.4 | 13.7 | 13.2 |
| Controls | ($\mu g/m^3$) | 31.3 | 14.6 | 13.8 | 31.4 | 14.2 | 13.6 |
| Cases | BC_LUR | 1.6 | 1.2 | 1.2 | 1.6 | 1.1 | 1.2 |
| Controls | ($10^{-5}$/m) | 1.6 | 1.1 | 1.2 | 1.6 | 1.1 | 1.2 |
| Cases | Greenness_ | 0.23 | 0.12 | 0.09 | 0.23 | 0.11 | 0.09 |
| Controls | NDVI[10] | 0.23 | 0.12 | 0.09 | 0.24 | 0.11 | 0.09 |

Air pollutants were positively correlated (except for ozone which was negatively correlated with other air pollutants) as reported previously (Clark et al. 2010b) and greenness was only moderately correlated with air pollution ($r \leq 0.4$). The land use derived pollutants showed differences compared to the IDW derived pollutants both for children with incident asthma captured during preschool and school age periods, in particular for NO where the interpolation method was significantly lower (23 µg/m3 *vs.* 31 µg /m3 for

[11] Significant paired t-test comparison ($p<0.05$)

IDW and LUR respectively). The assigned exposure estimates were significantly lower among controls compared to their matched cases for $NO_2$, Ozone, and greenness.

### 4.3.1 Asthma in preschool years

For children with a diagnosis of asthma established in preschool years, all interpolation-based exposures were positively associated with an increased risk in adjusted models, except for ozone. No elevated odds ratios were found when using land use regression estimates (Figure 4.1A). Proximity to highway and major roads increased the risk of new asthma onset by 25% and 5% respectively (Table 4.4). Greenness at birth was protective as asthma risk decreased by 4% for each 0.11 NDVI increase in a 100m buffer around the residential home address at birth (Table 4.4).

Consistent results for preschool children were seen for exposures categorized in quartiles (See Table 4.5 for exposure levels by quartiles). An increased risk was associated with increasing quartiles of exposure for all IDW exposures but ozone (Figure 4.1A), whereas no consistent trends were seen for quartiles of exposure from land use regression derived estimates. The associations with ozone were negatively correlated with asthma incidence due to the negative correlation of ozone with traffic-related air pollutants.

**Table 4.4: Adjusted[12] OR (aOR) for new asthma onset relative to an interquartile (IQR) increase in air pollutants (carbon monoxide (CO), nitrogen monoxide (NO), nitrogen dioxide (NO2), ozone (O3), sulfur dioxide (SO2), particulate matter (PM10, PM2.5)) derived using LUR and IDW, to road proximity, to surrounding greenness, and to joint greenness and air pollutants exposures.**

| | | Preschool | | School | |
|---|---|---|---|---|---|
| **Single Pollutants** | Unit | IQR | aOR (95%CI) | IQR | aOR (95%CI) |
| **NO_IDW** | $\mu g/m^3$ | 15.5 | 1.06 (1.01 - 1.11) | 15.5 | 0.93 (0.85 - 1.02) |
| **NO_LUR** | $\mu g/m^3$ | 14.4 | 1.00 (0.97 - 1.03) | 14.4 | 1.00 (0.94 - 1.06) |
| **$NO_2$_IDW** | $\mu g/m^3$ | 9.9 | 1.09 (1.04 - 1.13) | 9.6 | 0.95 (0.88 - 1.03) |
| **$NO_2$_LUR** | $\mu g/m^3$ | 9.5 | 0.99 (0.96 - 1.02) | 9.3 | 0.95 (0.90 - 1.01) |
| **CO_IDW** | $\mu g/m^3$ | 163.9 | 1.05 (1.01 - 1.10) | 164.6 | 0.90 (0.83 - 0.98) |
| **$O_3$_IDW** | $\mu g/m^3$ | 8.5 | 0.92 (0.87 - 0.97) | 8.5 | 1.18 (1.07 - 1.31) |
| **$PM_{10}$_IDW** | $\mu g/m^3$ | 1.3 | 1.12 (1.05 - 1.19) | 1.36 | 1.09 (0.96 - 1.24) |
| **$PM_{2.5}$_LUR** | $\mu g/m^3$ | 1.45 | 0.99 (0.97 - 1.01) | 1.46 | 1.01 (0.97 - 1.06) |
| **Black carbon_LUR** | $10^{-5}/m$ | 1.13 | 1.01 (0.99 - 1.04) | 1.13 | 0.99 (0.95 - 1.05) |
| **$SO_2$_IDW** | $\mu g/m^3$ | 3.1 | 1.05 (1.01 - 1.09) | 3.1 | 0.89 (0.83 - 0.96) |
| **hwy50**[13] | m | | 1.25 (1.04 - 1.49) | | 0.81 (0.55 - 1.19) |
| **mjr150**[12] | m | | 1.03 (0.98 - 1.09) | | 1.04 (0.92 - 1.16) |
| **h150m50**[12] | m | | 1.00 (0.94 - 1.08) | | 1.06 (0.92 - 1.21) |
| **Greenness_NDVI** | | 0.11 | 0.96 (0.93 - 0.99) | 0.1 | 0.99 (0.93 - 1.07) |
| **Joint Models** | | | Preschool | | School |
| **NO_IDW** | $\mu g/m^3$ | 15.5 | 1.05 (1.00 - 1.10) | 15.5 | 0.92 (0.84 - 1.01) |
| **NO_LUR** | $\mu g/m^3$ | 14.4 | 0.99 (0.95 - 1.02) | 14.4 | 1.00 (0.93 - 1.07) |
| **$NO_2$_IDW** | $\mu g/m^3$ | 9.9 | 1.08 (1.03 - 1.13) | 9.6 | 0.94 (0.87 - 1.03) |
| **$NO_2$_LUR** | $\mu g/m^3$ | 9.5 | 0.97 (0.94 - 1.01) | 9.3 | 0.94 (0.88 - 1.00) |

[12] Models were adjusted for newborn breastfeeding status at the time of discharge, parity, maternal education (in area-level quartiles), household income (in area-level quintiles), gestational length and birth weight.
[13] Road metrics: hwy50: within 50m of highway; mjr150: within 150m of major road; h150m50: within 150m of highway or 50m of major road

| | | Preschool | | School | |
|---|---|---|---|---|---|
| **CO_IDW** | $\mu g/m^3$ | 163.9 | 1.04 (0.99 - 1.09) | 164.6 | 0.88 (0.8 - 0.96) |
| **$O_3$_IDW** | $\mu g/m^3$ | 8.5 | 0.93 (0.88 - 0.98) | 8.5 | 1.22 (1.09 - 1.36) |
| **$PM_{10}$_IDW** | $\mu g/m^3$ | 1.3 | 0.98 (0.96 - 1.01) | 1.36 | 1.09 (0.96 - 1.25) |
| **$PM_{2.5}$_LUR** | $\mu g/m^3$ | 1.45 | 0.98 (0.96 - 1.01) | 1.46 | 1.01 (0.96 - 1.07) |
| **Black carbon_LUR** | $10^{-5}/m$ | 1.13 | 1.01 (0.98 - 1.03) | 1.13 | 0.99 (0.94 - 1.05) |
| **$SO_2$_IDW** | $\mu g/m^3$ | 3.1 | 1.04 (1.00 - 1.08) | 3.10 | 0.88 (0.81 - 0.95) |
| **hwy50*** | m | | 1.21 (1.01 - 1.45) | | 0.80 (0.54 - 1.19) |
| **mjr150*** | m | | 1.02 (0.96 - 1.08) | | 1.04 (0.92 - 1.17) |
| **h150m50*** | m | | 0.99 (0.93 - 1.07) | | 1.06 (0.92 - 1.22) |

For both continuous and categorical exposures (see Table 4.5 for exposure levels by quartile range), all adjusted analyses were robust to the inclusion of the risk factors that were considered (breastfeeding, parity, maternal age, income quintile, maternal education level quartile, birth weight and gestational length) with odds ratio showing similar direction and magnitude of effects to that observed in the crude analyses.

**Table 4.5: Exposure levels (N, mean) by quartile in the preschool age analysis among cases and controls**

| | | **NO_IDW** | | **$NO_2$_IDW** | | **CO_IDW** | | **$PM_{10}$_IDW** | | **$SO_2$_IDW** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Rank** | N | mean | N | mean | N | mean | N | mean | N | mean |
| **Controls** | **0** | 8177 | 10.7 | 7254 | 23.7 | 7881 | 479.2 | 8439 | 11.1 | 7007 | 3.3 |
| | **1** | 8444 | 16.6 | 9278 | 30.3 | 8904 | 570.3 | 9041 | 12.2 | 9291 | 4.2 |
| | **2** | 9151 | 24.3 | 9164 | 35.4 | 8932 | 653.1 | 8730 | 12.8 | 9323 | 5.4 |
| | **3** | 8845 | 38.1 | 8924 | 42.2 | 8894 | 787.3 | 8410 | 13.8 | 8999 | 9.2 |
| **Cases** | **0** | 1578 | 10.9 | 1302 | 23.8 | 1529 | 481.3 | 1680 | 11.1 | 1386 | 3.3 |
| | **1** | 1702 | 16.6 | 1845 | 30.4 | 1835 | 569.9 | 1717 | 12.2 | 1843 | 4.1 |
| | **2** | 1845 | 24.3 | 1913 | 35.3 | 1762 | 652.6 | 1807 | 12.8 | 1831 | 5.4 |
| | **3** | 1821 | 38.1 | 1888 | 42.2 | 1821 | 788.8 | 1744 | 13.8 | 1888 | 9.1 |

| | | NO_LUR | | $NO_2$_LUR | | Black Carbon | | $PM_{2.5}$_LUR | |
|---|---|---|---|---|---|---|---|---|---|
| | **Rank** | N | mean | N | mean | N | mean | N | mean |
| **Controls** | **0** | 8157 | 18.2 | 7573 | 24.3 | 8397 | 0.7 | 7028 | 4.6 |
| | **1** | 8689 | 24.9 | 8925 | 29.0 | 8739 | 0.9 | 7728 | 5.1 |
| | **2** | 8910 | 31.6 | 9193 | 33.9 | 8916 | 1.4 | 7628 | 5.6 |
| | **3** | 8865 | 49.3 | 8930 | 45.7 | 8569 | 3.5 | 7547 | 6.4 |
| **Cases** | **0** | 1591 | 18.3 | 1424 | 24.4 | 1654 | 0.7 | 1393 | 4.6 |
| | **1** | 1791 | 24.9 | 1810 | 29.0 | 1743 | 0.9 | 1541 | 5.1 |
| | **2** | 1773 | 31.6 | 1873 | 33.9 | 1761 | 1.4 | 1571 | 5.6 |
| | **3** | 1793 | 49.1 | 1841 | 45.0 | 1790 | 3.5 | 1497 | 6.5 |

### 4.3.2 Asthma in school age years

Children where incident asthma was only evident from age 6 onwards showed no consistent risk of developing asthma in relation to air pollutants exposures (Figure 4.1B). Most of the associations seen in early years were no longer evident, with the exception of exposure to $PM_{10}$ that exhibited some consistency between the two time periods. The effect of ozone switched from having a protective effect during preschool to increasing the risk of developing asthma after 6 years of age (1.18 (1.06 – 1.31). No associations were found with regard to greenness exposure.

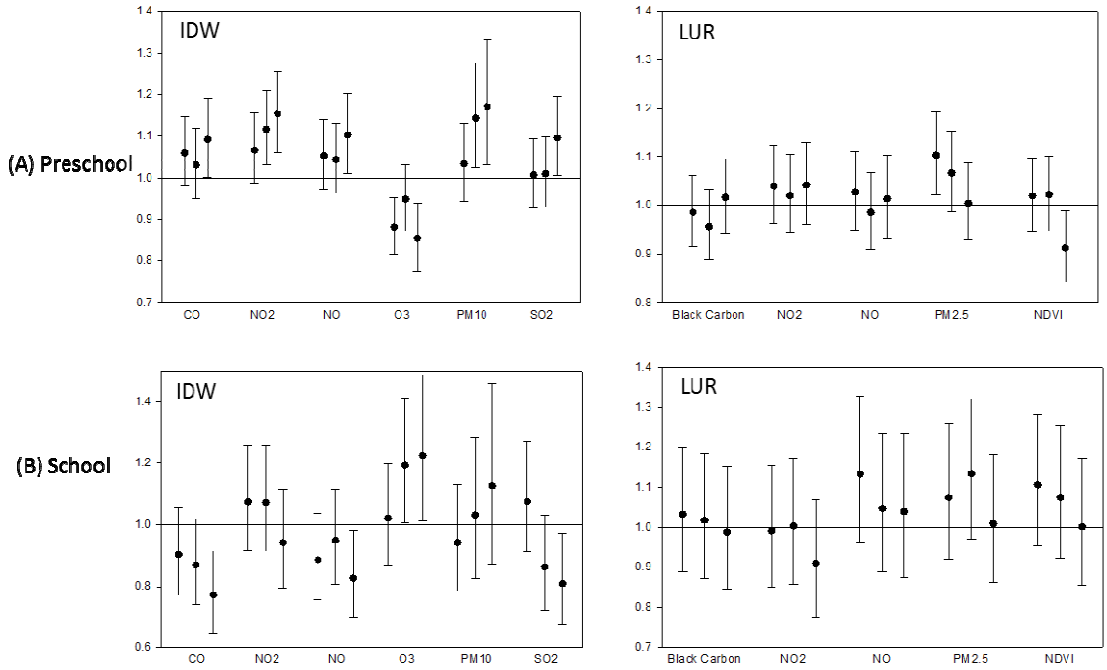


**Figure 4.1: Adjusted Odds Ratio of incident asthma in children meeting the case definition in the (A) preschool and (B) school groups relative to exposure quartiles of air pollutants (carbon monoxide (CO), nitrogen monoxide (NO), nitrogen dioxide ($NO_2$), ozone (O3), sulfur dioxide ($SO_2$), particulate matter (PM10, $PM_{2.5}$)) derived by interpolation (IDW) and by land use regression (LUR) ; and relative to measured greenness (NDVI).**

### 4.3.3 Joint models

Given the moderately low correlation between air pollutants and surrounding greenness, joint exposure models of air pollutants with the greenness index were examined. Controlling for surrounding greenness only impacted the effect of $PM_{10}$ on asthma risk in preschool children. The association between $PM_{10}$ and asthma incidence disappeared from 1.12 (1.05 - 1.19) to 0.98 (0.96 - 1.01). For all other air pollutants and road proximity metrics, the direction and magnitude of effects on new onset asthma in young children as well as later in life did not change. On the other hand, the protective effect of surrounding greenness on asthma risk was increased after adjustment for air pollutants (Table 4.6).

**Table 4.6: Effects of surrounding greenness on incident asthma risk, adjusted for air pollutants in preschool and school children.**

| | Preschool [14] OR (95% CI) | School OR (95% CI) |
|---|---|---|
| **Greenness only** | **0.96 (0.93 - 0.99)** | **0.99 (0.93 – 1.07)** |
| + NO_IDW | 0.83 (0.60 - 1.16) | 0.77 (0.40 - 1.51) |
| + NO_LUR | 0.69 (0.49 - 0.97) | 0.96 (0.48 - 1.91) |
| + $NO_2$_IDW | 0.93 (0.67 - 1.3) | 0.81 (0.41 - 1.59) |
| + $NO_2$_LUR | 0.66 (0.47 - 0.92) | 0.75 (0.38 - 1.48) |
| + CO_IDW | 0.82 (0.59 - 1.14) | 0.68 (0.35 - 1.33) |
| + $O_3$_IDW | 0.88 (0.63 - 1.23) | 0.61 (0.31 - 1.19) |
| + $PM_{10}$_IDW | 0.80 (0.59 - 1.1) | 1.05 (0.56 - 1.97) |
| + $PM_{2.5}$_LUR | 0.68 (0.49 - 0.94) | 1.04 (0.54 - 2) |
| + Black carbon_LUR | 0.76 (0.55 - 1.05) | 0.95 (0.5 - 1.8) |
| + $SO_2$_IDW | 0.83 (0.59 - 1.15) | 0.65 (0.33 - 1.27) |
| + h150m50 | 0.70 (0.5 - 0.97) | 1.08 (0.55 - 2.09) |
| + hwy150 | 0.71 (0.51 - 0.98) | 0.99 (0.51 - 1.91) |
| + mjr150 | 0.72 (0.52 - 1) | 1.07 (0.55 - 2.1) |

### 4.3.4 Sensitivity analyses

Stratified regression analyses were conducted only for the preschool period since this accounted for 70% of all identified cases and stratified models for the school age period did not converge.

Among children who met the case definition during the preschool period, 535 (6.5% of all incident asthma cases) had between 30 and 37 weeks of gestational length and only 83 (1%) had less than 30

[14]All models are adjusted for the following risk factors: maternal age at delivery, birth weight, gestational length, neighborhood-level household income, neighborhood-level maternal education, parity, newborn feeding at discharge

weeks, while 442 (6%) were below 2500 g at birth. Gestational length categories were grouped into a small gestational age category (<37 weeks) to ensure model convergence.

Suggestive evidence of effect modification by birth weight but not gestational age was demonstrated in stratified analyses(Figure 4.2). All the increased risks observed for children with birth weight less than 2500 g, for IDW-derived air pollutant exposures were near double that seen in children with birth weight more than 2500 g. Furthermore, stratifying by birth weight indicated consistent effects using land use regressions exposure estimates. NDVI in low birth weight children showed a consistent protective effect with near 50% reduction in the odds of new asthma onset.

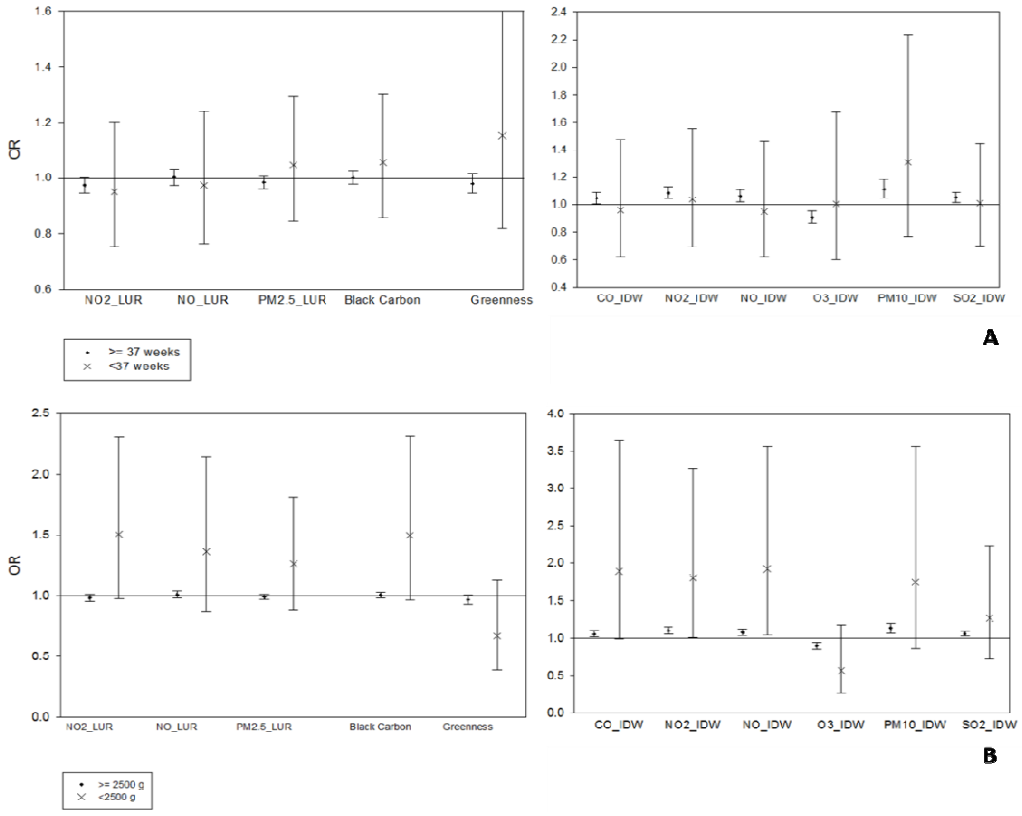


**Figure 4.2: Effect modification of greenness in 100m buffer and air pollution estimates derived using inverse distance weighting (IDW) and/or land use regression (LUR)models on new asthma onset among preschool age children by (A) gestational length (<37 weeks) and (B) birth weight (<2500g). All models are adjusted for maternal age at delivery, neighborhood-level household income, neighborhood-level maternal education, parity, and breastfeeding.**

Stratified regressions by sex revealed that girls were consistently at increased odds of new asthma compared with boys for the same air pollutants identified in the main analysis and that they were more protected from the odds of new asthma onset with respect to the surrounding greenness compared to boys for whom the protective effect disappeared (Figure 4.4). Stratification by parity showed similar but less pronounced results: all exposures that had an effect on odds of asthma in the main analysis, except $PM_{10}$, showed a heightened impact for children without siblings compared to those with siblings.

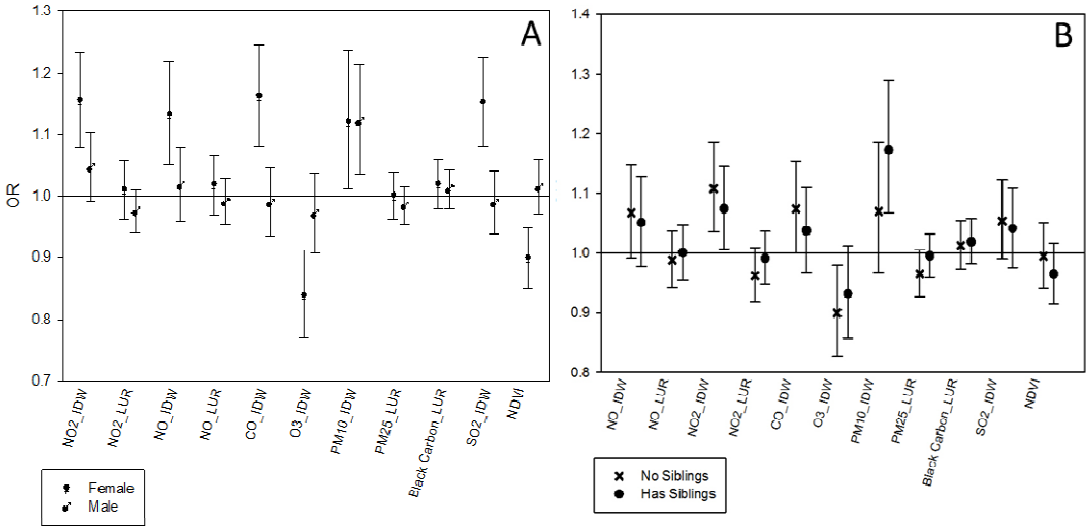


**Figure 4.3: Risk of asthma incidence in the preschool group (n=6948), stratified by (A) sex and by (B) parity in relation to exposure to inverse distance weighting (IDW)- and land use regression (LUR)-derived air pollutants and surrounding greenness (NDVI) in 100m buffer. All models are adjusted for birth weight, gestation period, maternal age at delivery, neighborhood-level household income, neighborhood-level maternal education, parity, and breastfeeding**

Lower socio-economic status (based on neighborhood income and education measures) children demonstrated high odds of asthma associated with interpolated air pollutants exposures (Figure 4.5). Stratification by maternal age at birth showed less consistent results: relative to interpolated air pollutants, both the oldest and youngest mothers were likely to have children at higher odds of new asthma development, while LUR estimates showed some trend with age

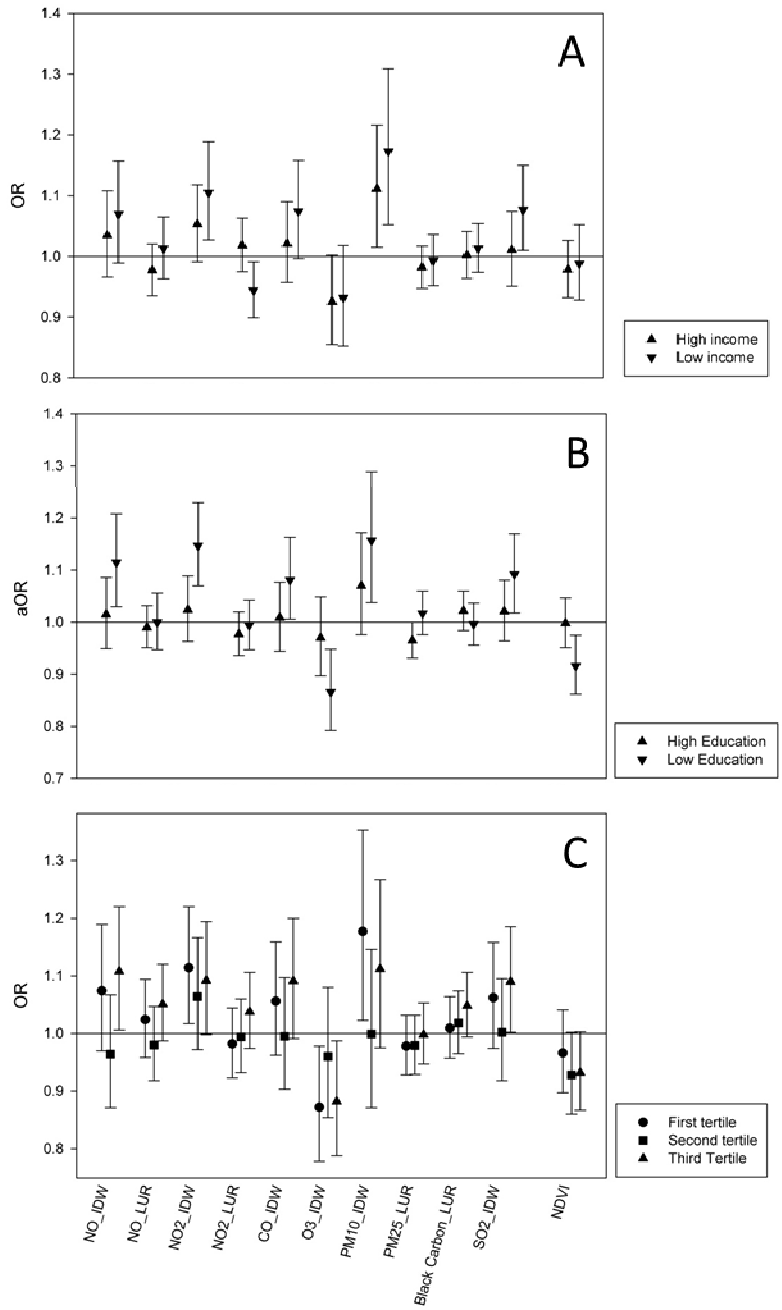


**Figure 4.4: Stratified asthma risk analysis by three indicators of Socio-economic status (A: Household Income; B: Maternal Education status; C: Maternal age at birth) in relation to air pollutants and surrounding greenness in preschool age children**

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## 4.4 Discussion

With 65,254 children followed up for 10 years, in one of the largest birth cohort studies to examine within-community air pollution contrasts, we found 25% increased odds of asthma for children living near highways and 5% to 10% increase for an interquartile increase in NOx and CO, pointing to traffic pollutant exposure in the etiology of asthma. Similar to an earlier analysis of a subset of this cohort (Clark et al. 2010b), we also found positive associations with $PM_{10}$ and $SO_2$ exposure (Clark et al. 2010b). The school age analysis revealed that exposures to air pollution generally did not affect asthma onset when the child was older than 6 years.

Surrounding greenness elicited a robust protective effect in the preschool age analysis. This finding may follow a hypothesis that biodiversity loss( Rook 2013) and subsequent reduction in exposure to microbial diversity, reflected of less interaction with natural spaces, leads to immune dysfunction, inflammation and asthma (Haahtela et al. 2013). Greenness was not protective for asthma onset during school age years. This could be related to the mediating effect of physical activity. While this pathway is not likely to pertain to children between birth and age 5, it may come to play during school age years (Dadvand et al. 2014). Data on time activity patterns were not available, preventing exploration of such mechanisms.

Correlations between air pollution and greenness levels were moderate, allowing the investigation of joint effects models on asthma incidence. During preschool years, the air pollution effects were largely unchanged after adjustment for greenness, whereas the protective

effect of greenness was strengthened after adjustment for air pollutants. This finding provides further support to the biodiversity hypothesis in that tolerance mechanisms are less impaired when interaction between human and environmental microbiotas are enhanced (Haahtela et al. 2013).

The increased risk of asthma incidence in early years was only evident with exposures estimated by interpolation methods (IDW). Although effect estimates based on exposures estimated by LUR models had smaller confidence intervals than those based on IDW, they showed no increased risk. There are several possible explanations for this discrepancy. First, these metrics capture different spatial scales (Marshall et al., 2008). IDW may better represent secondary pollutants such as $NO_2$ that display less spatial heterogeneity compared with primary pollutants (e.g. black carbon). Second, it is possible that LUR models, developed using 2003 measurements, may result in an over-smoothed surface for this cohort as exposure estimates were extrapolated backwards during a period time where concentration levels were decreasing Finally, these differences between monitor-based and LUR model exposure estimates suggest some independence of risks between regional and local traffic-related air pollution (Brauer et al, 2008), however the positive associations observed with roadway proximity metrics would suggest otherwise..

The most recent birth cohort studies with similar follow-up periods show mixed results with regard to the role of air pollution. They include the Swedish cohort (BAMSE) where positive associations between air pollution and asthma after follow up until age 12 years were found (Gruzieva et al. 2013), whereas the British birth cohort found no association between $NO_2$ nor $PM_{10}$ and asthma in children who were

followed up until age 11 years (Mölter et al. 2014a). The number of identified asthma cases in BAMSE at increasing ages did not follow the decreasing pattern found in our study.

The Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) cohort study offers an interesting comparison (Gehring et al. 2010a). As in our study, positive associations between $NO_2$ and asthma at age 2 and 4 (Brauer et al. 2007a; Gehring et al. 2002) were reported and most incident cases were apparent earlier in the children's life. However, unlike our study, the increased risk of incident asthma in relation to $NO_2$ and $PM_{2.5}$ exposure was still evident for incident asthma at older ages (up to age 8) in the Dutch study, possibly due to higher air pollution levels (Gehring et al. 2010a). In this investigation, the difference observed in the positive association between air pollution and development of asthma between birth up until age 6 and the absence of association later in life is likely to reflect that childhood asthma develops in early years rather than later in childhood. Additional analyses where the association was examined every year from birth to end of follow up confirmed that administrative records capture asthma incidence in the first 3 to 4 years of life leaving a very small pool of participants eligible for incidence analysis and thereby leading to a lack of power to detect any association between early life exposure and late childhood asthma development (data not shown).

Our asthma case definition, although verified in other population-based administrative data analyses (Kozyrskyj et al. 2004; To et al. 2006), relied on billing records for health care encounters and may have captured wheezing illnesses that have varying trajectories over time (Stein and Martinez 2004). Transient wheezing is common in infants and often resolves in childhood (Martinez et al. 1995a) as shown in a population-based Canadian study, where half the children diagnosed with asthma before age 6 went into remission by age 12 (To T et al. 2007). It is possible that the preschool analysis has included children whose symptoms may resolve later, weakening the ability to detect associations during school years. Future studies of asthma trajectories using linked health databases can help elucidate this question.

Administrative data are collected for non-research purposes and lack individual level information on important confounders (e.g., SES measure) and therefore may be subject to residual confounding. However, such linked health records provide unprecedented opportunities to investigate multiple risk factors in a large population. Preschool children weighing less than 2500g at birth were consistently, across all metrics, at increased risk for new asthma onset in relation to air pollution exposure, supporting the idea of greater susceptibility among low birth weight subpopulations (Strickland et al. 2014). However, the protective effect of surrounding greenness among this subgroup was 10-fold higher compared to children weighing more than 2500g at birth, raising the prospect of preventative public health interventions during pregnancy. Similar to the Children's Health Study results (Salam et al. 2004), being born to a nulliparous mother decreased the risk of asthma incidence. This observation may reflect the importance of the *in utero* environment, given evidence of reduced cord blood mononuclear cells proliferative responses with increasing parity (Devereux et al. 2002). We also confirmed previously observed effect modification of SES with air pollution's effects on childhood respiratory morbidity (Gehring et al. 2006). Further, older mothers were are at higher risk of giving birth to children who were impacted by air pollution exposure, a relevant finding as British Columbia has the highest proportion of mothers giving birth over the age of 35 years in Canada (Canadian Institute for Health Information (CIHI) 2011).

In conclusion, air pollution was associated with incident cases of asthma in preschool children, but was not consistently related to initial asthma onset in school age children. For incident asthma during preschool years, surrounding greenness offered some protective effect independent from the observed associations with air pollution.

# Chapter 5: Pediatric asthma patterns and effects of environmental factors.

## 5.1 Introduction

Childhood asthma is a heterogeneous disease that encompasses a wide spectrum of presentations based on clinical characteristics or symptoms such as wheezing, especially in early childhood (Savenije et al. 2011). In order to conduct informative population-based studies, it is crucial to extend the current asthma cohort research beyond capturing incidence of a condition whose specific diagnosis remains elusive, to incorporate more detailed characterization of the different presentations of this disease.

The Tucson Children's Respiratory Study (TCRS) was among the first studies to identify phenotypic groups for asthma based on clinical observations of wheezing (Martinez et al. 1995b): (1) non-wheezers, (2) transient wheezers (at least one wheezing symptom before age 3, no wheezing by age 6), (3) late onset wheezers (no wheeze until age 3, but wheezing by age 6) and, (4) persistent wheeze (wheezed in the first 3 years of life, and persistent wheezing at age 6). Subsequent studies have used latent class analysis to classify phenotypes of wheezing, complementing the initial categorization offered by the TCRS and contributing to clinical diagnosis of asthma (Henderson et al. 2008; Savenije et al. 2011; Spycher et al. 2008). The use of data-driven techniques - including but not limited to latent class modeling- to explore whether clusters exist within a population has been limited to a few studies of generally small sample size (Savenije et al. 2011) with the notable exception of one nationally representative birth cohort study of 11,652 British children (Panico et al. 2014), which relied on parental report of wheeze and atopic symptom (eczema and hayfever) episodes to classify four distinct trajectories. Since the use of self-reported conditions or symptoms is fraught with threats of bias, and given the logistical and financial burden of direct testing of children in large population-based studies, the use of administrative health records offers a unique opportunity to identify asthma trajectories, and to distinguish chronic and transient

conditions, thus capturing more specificity in relation to asthma phenotypes. Furthermore, with enhanced classification of asthma trajectories, we sought to conduct a novel evaluation of the role of environmental exposures on the different asthma groupings.

In a population-based birth cohort study (the Border Air Quality Study (BAQS)) of over 65,000 children residing in Metropolitan Vancouver, British Columbia (BC), associations between air pollutants and asthma incidence were reported in children between birth and age 5, as well as an independent protective effect of surrounding residential green spaces, but not in the small proportion of cases whose disease developed between ages 6 and 10 (Chapter 4). It is plausible that the two age groupings that were examined represent different phenotypes, as identified in the TCRS groupings, with different risk. To further understand the role of air pollution and surrounding green spaces in childhood asthma etiology, the current study will examine annual asthma prevalence rates in the BAQS cohort to identify whether children follow different asthma trajectories that could not be captured in the prior analysis, and to understand how air pollution and greenness may affect the likelihood of belonging to the identified trajectories.

Group-based trajectory modeling is a specialized form of finite mixture modeling. The method is designed to identify groups of individuals following similarly developmental trajectories (Nagin 2005; Nagin and Odgers 2010). Unlike, hierarchical and latent class modeling, group-based trajectory modeling does not require parameters to be continuously distributed throughout the population according to the multivariate normal distribution. This approach assumes that there may be clusters of distinct developmental trajectories that may reflect unique etiologies. This offers the ability to model distinct subgroups of the population, allowing for the classification of children who belong to distinct trajectories with group-specific time-related parameters and facilitating the exploration of common risk factors and environmental exposures that may be associated with each asthma trajectory.

## 5.2 Methods

### 5.2.1 Cohort description

Linked administrative data, accessible via the pan-provincial data services platform of Population Data BC (https:/www.popdata.bc.ca) were used to establish a birth cohort that comprised all single births from 1999 to 2002 to mothers with verified residential history in the metropolitan area of Vancouver, British Columbia, Canada throughout their pregnancy (Brauer et al. 2008). This cohort included all residents with a primary address in BC as registration with the universal health insurance program is mandatory for those residing in the province for at least 3 months. Children born to these identified mothers were followed for 10 years (1999-2009) or until lost to follow-up, whichever occurred first. Loss to follow up occurred if they moved outside of study region, or when a gap in health registry exceeded 183 days. From the 73, 387 births to mothers with complete information on residential history during pregnancy, 988 multiple births were excluded, 101 children died during study follow up, and 4103 had missing covariate information leaving 68,195 singletons available for analysis.

Population Data BC provides access to the Ministry of Health medical services and hospitalization data (British Columbia Ministry of Health 2009b) which inform the asthma definition, as well as vital statistics birth and deaths data (British Columbia (BC) Vital Statistics Agency 2009a, 2009b) which include birth date, sex, and First Nations status, obtained from the British Columbia Vital Statistics Agency. Population Data BC also links the preceding data with data from the BC Perinatal Data Registry (Perinatal Services BC 2009) which provides information on intention to breastfeed, maternal age, parity and maternal smoking during pregnancy. Since no individual-level data were available for socio-economic factors, census data on income and maternal level of education were assigned to children based on their neighbourhood of residence at birth at the Census Dissemination Areas (DA) level (Statistics Canada 2001), a unit representing a target population of 400 to 700 persons. The study protocol was approved by the Institutional Review Board of The University of British Columbia (#H04-80161).

### 5.2.2 Asthma case definition

Asthma diagnoses were identified from physician billing and hospital discharge records (International Classification of Diseases [ICD]-9th revision: 493; ICD-10: J45) for the1999-2009 period. Using a validated case definition of asthma (Prosser et al. 2008), children with a minimum of two primary care physician diagnoses in a rolling 12-month period or a minimum of one hospital admission were identified as asthma cases each year from birth until end of follow up, providing an annual asthma prevalence.

### 5.2.3 Exposure assessment

Residential air pollution exposure during pregnancy was assessed using high resolution land use regression (LUR) air pollution models developed for the Vancouver metropolitan area for nitrogen dioxide (NO2), fine particulate matter ($PM_{2.5}$), and black carbon (BC). The creation and evaluation of the models has been described previously (Brauer et al. 2008; Henderson et al. 2007; Larson et al. 2009) as has their application to the epidemiologic analysis of childhood respiratory disease (Clark et al. 2010b; Karr et al. 2009; MacIntyre et al. 2011, and Chapter 4)

Measurements of $NO_2$, NO, BC, $PM_{2.5}$, $PM_{10}$, carbon monoxide (CO) and sulfur dioxide ($SO_2$) collected from regulatory air quality monitoring network stations were used to compute inverse-distance weighted (IDW) averages of the three closest monitors within 50 km. This provided daily estimates assigned to participants residential postal codes, then aggregated over the pregnancy period (Marshall et al. 2008b). A 6-digit residential postal code, in urban setting, refers to an urban block face with a target population of 400 to 700 people.

The Normalized Difference Vegetation Index (NDVI) provided a continuous measure of greenness from -1 to 1 across the study region. Using Landsat 7 Enhanced Thematic Mapper Plus (ETM+) cloud free images downloaded from 1999 to 2002, NDVI derived from land surface reflectance of visible (red: 0.4 –

0.7μm) and near-infrared (0.7 – 1.1 μm) parts of spectrum (Weier and Herring 2000), was calculated for 100m buffers around residential postal codes during pregnancy (Hystad et al. 2014).

### 5.2.4 Analytical methods

The group-based approach utilizes a multinomial modeling strategy. Maximum likelihood is used for the estimation of the model parameters. The fundamental concept of interest is the distribution of asthma outcomes conditional on age (continuous) over time; that is, the distribution of outcome trajectories denoted by $P(Y_i \mid Age_i),$ where the random vector $Y_i$ represents individual *i*'s longitudinal sequence of outcomes (asthma diagnosis) and the vector $Age_i$ represents individual *i*'s age when each of those measurements is recorded. The group-based trajectory model assumes that the population distribution of trajectories arises from a finite mixture of unknown order *J*. The likelihood for each individual *i*, conditional on the number of groups *J*, may be written as

$$P(Y_i \mid Age_i) = \sum_{j=1}^{J} \pi^j \cdot P(Y_i \mid Age_i, j; \beta^j) \quad (1),$$

where $\pi^j$ is the probability of membership in group j, and the conditional distribution of $Y_i$ given membership in j is indexed by the unknown parameter vector βj that determines the shape of the group-specific trajectory. For a given j, conditional independence is assumed for the sequential realizations of the elements of $Y_i$ , $y_{it}$, over the T periods of measurement, written as

$$P(Y_i \mid Age_i, j; \beta^j) = \prod_{t=1}^{T} p(y_{it} \mid age_{it}, j; \beta^j) \quad (2),$$

where p(.) is the distribution of $y_{it}$ conditional on membership in group j and the age of individual i at time t.

The binary logit distribution was used to to specify p(.) for the analysis of longitudinal data on a dichotomous outcome variable, here whether or not the subjects meets the asthma definition at age t.

In order to choose the appropriate number of groups, the model was repeatedly fit with a step-wise increase in the number of groups, starting with two groups fixing the order (i.e. shape) to quadratic for each group, since we assumed that there would be at least one non-asthmatic and one asthmatics group. As there is no single statistical test to determine the correct number of trajectory groups (Nagin 2005), model fit was assessed using Bayesian Information Criteria (BIC) and log likelihood. A number of models with different orders in an interactive two-step process were tested: (1) selection of the number of trajectory groups, and (2) selection of the shape (also designated as order) for each trajectory group, using both statistical and subjective knowledge on asthma natural history.

Following model selection, risk factors for the probability of membership in the asthma trajectory groups, relative to the non-asthma group, were tested using weighted multinomial logistic regression. Weights were calculated as products of trajectory group posterior probabilities. Combining trajectory groups with weighted regression methods can provide risk profiles for individuals most likely to follow certain trajectories over time. To build an overall prediction model, risk factors were selected in separate models using block testing. Block 1 included clinically important factors and known risk factors: sex, parity, First Nations status, birth weight, gestational age at birth, intention to breastfeed, household income, maternal education and smoking. Block 2 considered each of the environmental exposures separately (i.e. unadjusted exposure models). The final model consisted of the selected covariates from Block 1 and only significant environmental exposures from Block 2.

## 5.3 Results

### 5.3.1 Cohort and asthma trajectories description

As the cohort inception included children born between 1999 and 2002, their contribution to years of follow up was examined. Participants were registered for nearly all of the study follow-up, with an average of 8.2 years (Table 5.1).

Descriptive statistics of the overall cohort (n=68195) with complete covariate and exposure assessment data (n= 65254) are summarized and stratified by trajectory group membership in Table 5.2. A total of 10,275 children (15 % of the sample) met the case definition during the course of the follow-up period. The prevalence of asthma cases rose monotonically until children reached age 5 up to 19.8%, at which point it steadily decreased until the end of the 10-year follow down to 5.2%.

**Table 5.1: Summary statistics of number of elapsed years of data coverage by birth year**

| Cohort Birth year | N | Mean | Min | Max |
|---|---|---|---|---|
| **Born in 1999** | 16979 | 9.58 | 0.00 | 10.00 |
| **Born in 2000** | 17801 | 8.63 | 0.00 | 9.00 |
| **Born in 2001** | 17981 | 7.70 | 0.00 | 8.00 |
| **Born in 2002** | 15434 | 6.73 | 0.00 | 7.00 |

The majority of the cohort's subjects were born after 37 weeks of pregnancy from non-smoking mothers (mean age at birth 31 years) who had initiated breastfeeding at time of discharge. Over half of participants (54.2%) were born to mothers with a previous live birth (Table 5.2). Birth weight at delivery was available as a continuous variable (mean = 3410g) but dichotomized as term birth weight less than 2500g for analysis, previously identified as an important modifier of the association between air pollution and asthma development (Sbihi et al, 2015 submitted). Similarly, gestational age at birth (mean: 39.1 weeks) was defined as binary variable using 37 weeks cut-off.

**Table 5.2: Risk factor distribution (frequency counts for categorical variables; mean and standard deviation (sd)) by asthma trajectory groups among 68195 children.**

| Risk factors | N (%) | Non-asthmatics (n=63166) | Transient asthma cases (n=2282) | Late infancy onset chronic asthmatics (n=1828) | Early infancy onset chronic asthmatics (n=919) |
|---|---|---|---|---|---|
| **Maternal Non-smoker** | 63597 (93.3%) | 58843 (93.2%) | 2139 (93.7%) | 1739 (95.1%) | 876 (95.3%) |
| **Native status** | 431 (0.63%) | 402 (0.6%) | 15 (0.7%) | 8 (0.4%) | 6 (0.7%) |
| **Female** | 33052 (48.5%) | 31265 (49.5%) | 779 (34.1%) | 698 (38.2%) | 310 (33.7%) |
| **Nulliparous** | 31309 (45.9%) | 28919 (45.8%) | 946 (41.5%) | 994 (54.4%) | 450 (49%) |
| **High SES** | 38335 (56.2%) | 35778 (56.6) | 1193 (52.3) | 915 (50.1) | 449 (48.9) |
| **High maternal education** | 37591 (55.1%) | 35121 (55.6%) | 1127 (49.4%) | 903 (49.4%) | 440 (47.9%) |
| **Newborn feeding at discharge** | 63015 (92.4%) | 58443 (92.5%) | 2055 (90.1%) | 1691 (92.5%) | 826 (89.9%) |
| **Birth weight ≥2500g** | 65696 (96.3%) | 60947 (92.5%) | 2160 (90.1%) | 1742 (92.5%) | 847 (89.9%) |
| **Gestation ≥37 weeks** | 64555 (94.3%) | 59901 (96.5%) | 2106 (94.7%) | 1712 (95.3%) | 836 (92.2%) |
| **Maternal age at birth (mean (sd))** | 68195 | 31.3 (5.11) | 31.2 (5.09) | 31.0 (5.21) | 31.1 (5.02) |

The model with four different groups and cubic order was chosen as the best fit as it had the lowest BIC value. The four trajectory asthma groups that emerged from the group-based modelling (Figure 5.1) can be summarized as follow:

- *Non-asthma trajectory (NA)*: 88.8% of the study population has a quasi-null probability of meeting the case definition throughout the study follow up time.

- *Late-Infancy Chronic Asthmatics* (LI CA) *trajectory*: 4.1% of the study population have a non-remitting diagnosis throughout the 10 year follow up; in this group children start developing asthma around age 2, with a peak prevalence of asthma when they are 6 years old and maintain their asthmatic status up until end of follow up. This trajectory is akin to growing in to asthma phenotype.
- *Early Infancy Chronic asthmatics* (EI CA) *trajectory*: 1.5% of the population have a similar trajectory to that of the LICA trajectory as children maintain their asthmatic status until the end of follow up but after having met the asthma case definition in early infancy (before age 2) with a peak in prevalence around age 4. This trajectory is akin to continuing to have asthma symptoms.
- *Transient asthma trajectory*: 5.6% of the study participants follow a transient pattern (i.e. grow out of asthma) where the asthma case definition is met in infancy with peak prevalence around age 2. No asthma activity is seen after children turn 6 years of age.

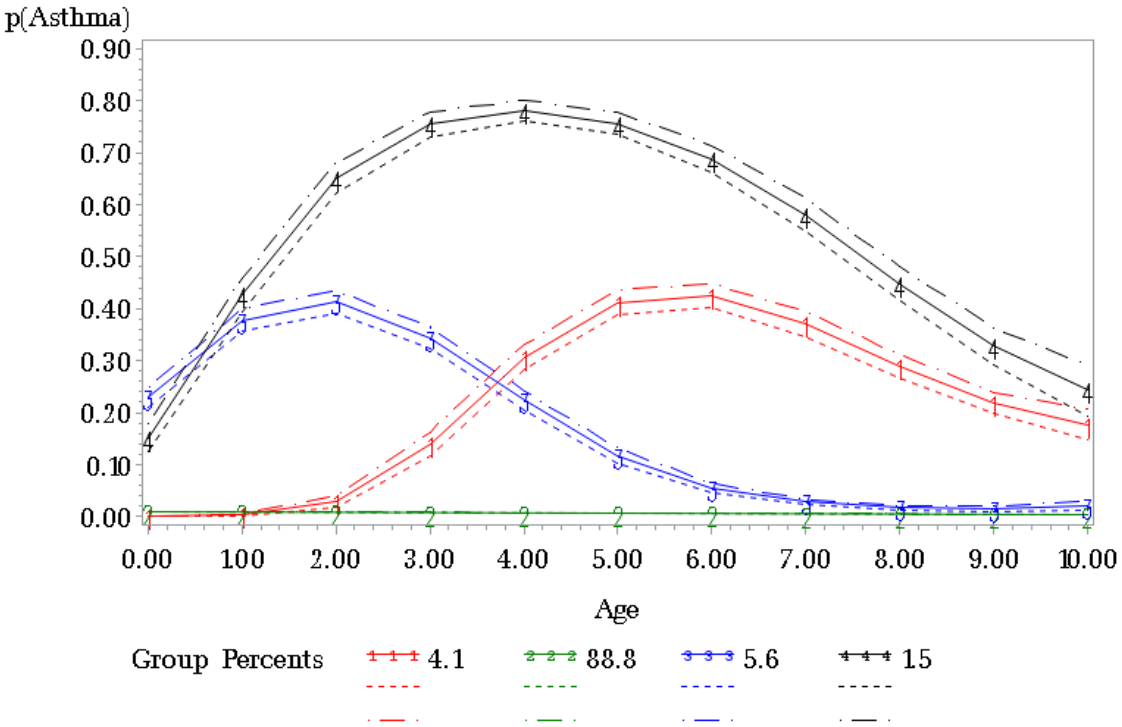


**Figure 5.1: Group memberships and confidence intervals for the posterior distributions of probability of asthma at each time point between birth and age 10 for each of the trajectories in BAQS cohort. Trajectory 1 (in red) represents the late infancy onset chronic asthmatics (LI CA), trajectory 2 (in green) represents the non-asthmatics, trajectory 3 (in blue) represents the transient group, and trajectory 4 (in black) represents the early infancy onset chronic asthmatics (EI CA).**

While only 919 children developed chronic asthma in early infancy, their trajectory was significantly different from that of the late infancy onset chronic asthmatics as indicated by the distinct confidence intervals around the predicted posterior probabilities (Table 5.3). Among children who met the case definition of asthma, transient asthmatics (n=2282) with resolved symptoms by the end of follow up represented the same proportion of the study population as those who were chronic asthmatics regardless of the period of asthma onset (5.6%).

**Table 5.3: Posterior probability for each trajectory at each year of age (Non-Asthmtics; Transient; LICA: Late Infancy Chronic Asthma; EICA: Early Infancy Chronic Asthma) and 95% confidence intervals**

| Year | p(Non asthma) | | p(Transient) | | p(LICA) | | p(EICA) | |
|---|---|---|---|---|---|---|---|---|
| **0** | 0.01 | [0.01 ; 0.01] | 0.23 | [0.21 ; 0.25] | 0.00 | [0 ; 0] | 0.15 | [0.13 ; 0.18] |
| **1** | 0.01 | [0.01 ; 0.01] | 0.38 | [0.36 ; 0.40] | 0.00 | [0 ; 0] | 0.43 | [0.39 ; 0.46] |
| **2** | 0.01 | [0.01 ; 0.01] | 0.41 | [0.39 ; 0.43] | 0.03 | [0.02 ; 0.04] | 0.65 | [0.62 ; 0.68] |
| **3** | 0.01 | [0.01 ; 0.01] | 0.34 | [0.31 ; 0.36] | 0.14 | [0.12 ; 0.16] | 0.75 | [0.73 ; 0.78] |
| **4** | 0.01 | [0.01 ; 0.01] | 0.22 | [0.21 ; 0.24] | 0.31 | [0.28 ; 0.33] | 0.78 | [0.76 ; 0.8] |
| **5** | 0.01 | [0.01 ; 0.01] | 0.12 | [0.10 ; 0.13] | 0.41 | [0.39 ; 0.44] | 0.76 | [0.73 ; 0.78] |
| **6** | 0.01 | [0 ; 0.01] | 0.06 | [0.05 ; 0.06] | 0.42 | [0.4 ; 0.45] | 0.69 | [0.66 ; 0.71] |
| **7** | 0.00 | [0 ; 0.01] | 0.03 | [0.02 ; 0.03] | 0.37 | [0.35 ; 0.39] | 0.58 | [0.55 ; 0.61] |
| **8** | 0.00 | [0 ; 0] | 0.02 | [0.01 ; 0.02] | 0.29 | [0.27 ; 0.31] | 0.45 | [0.42 ; 0.48] |
| **9** | 0.00 | [0 ; 0] | 0.02 | [0.01 ; 0.03] | 0.22 | [0.2 ; 0.24] | 0.33 | [0.29 ; 0.36] |
| **10** | 0.00 | [0 ; 0] | 0.02 | [0.01 ; 0.03] | 0.18 | [0.15 ; 0.21] | 0.24 | [0.19 ; 0.29] |

All risk factors except for First Nations status were significantly different by group membership. Early infancy chronic asthmatic children were less likely to have been breastfed than non-asthmatics (10.1% vs. 7.5% respectively). Children in the non-asthma group were born to families with significantly higher neighborhood SES (household income and maternal educational attainment) compared to all other trajectories (Table 5.2). Both chronic asthma trajectories had more children born to nulliparous mother (54% and 49% for late and early infancy onset) than the transient cases and non-asthmatics (41 % and 46%, respectively). Following ANOVA, term birth weight and gestational period (when examined as continuous variables) showed significant differences across trajectories. These differences were driven by the contrast between non-asthmatics and children belonging to the three trajectories (i.e. Transient, Early Infancy Chronic Asthma (EICA), Late Infancy Chronic Asthma (LICA)). The former group has significantly larger weight and gestation length at birth. Further, within those meeting the case definition, the two chronic asthma group children had significantly smaller weight and gestation length at delivery compared to children belonging to the transient asthma group. Mothers of chronic asthmatics were significantly younger (mean (in years) (sd): 31.1 (5.1) and 31.0 (5.2) for early and late infancy onset respectively) than those of children who did not meet the asthma case definition (31.3 (5.1)).

The weighted multinomial regression relative to the risk factors available for analysis (Table 5.4) shows that smoking, sex, parity, household income, maternal education (high educational attainment being greater than median of neighborhood level), breastfeeding and birth weight had significant effects on the odds of membership in all of the three asthma trajectories.

**Table 5.4: Odds Ratio (OR) of adhering to a trajectory group relative to risk factors based on weighted multinomial logistic regression[15]**

| Effect | Category | Group[16] | OR (vs NA group) | 95% Wald CI | p-value |
|---|---|---|---|---|---|
| **Maternal smoking** | Yes vs No | T | 0.86 | 0.71; 1.03 | 0.10 |
| **Maternal smoking** | Yes vs No | LI CA | 0.66 | 0.53; 0.83 | 0.00 |
| **Maternal smoking** | Yes vs No | EI CA | 0.60 | 0.42; 0.84 | 0.00 |
| **Sex** | F vs M | T | 0.52 | 0.47; 0.57 | <.0001 |
| **Sex** | F vs M | LI CA | 0.63 | 0.57; 0.70 | <.0001 |
| **Sex** | F vs M | EI CA | 0.50 | 0.43; 0.58 | <.0001 |
| **Parity** | 1 vs 0 | T | 1.21 | 1.10; 1.33 | <.0001 |
| **Parity** | 1 vs 0 | LI CA | 0.70 | 0.63; 0.78 | <.0001 |
| **Parity** | 1 vs 0 | EI CA | 0.89 | 0.77; 1.04 | 0.15 |
| **Maternal age** | 1st vs 3rd tertile | T | 1.14 | 1.02; 1.28 | 0.05 |
| **Maternal age** | 1st vs 3rd tertile | LI CA | 1.06 | 0.93; 1.20 | 0.11 |
| **Maternal age** | 1st vs 3rd tertile | EI CA | 1.15 | 0.96; 1.37 | 0.26 |
| **Maternal age** | 2nd vs 3rd tertile | T | 1.08 | 0.96; 1.20 | 0.91 |
| **Maternal age** | 2nd vs 3rd tertile | LI CA | 0.94 | 0.82; 1.06 | 0.09 |
| **Maternal age** | 2nd vs 3rd tertiles | EI CA | 1.1 | 0.92; 1.32 | 0.71 |
| **SES** | H vs L | T | 0.91 | 0.83; 1.00 | 0.05 |
| **SES** | H vs L | LI CA | 0.82 | 0.74; 0.91 | 0.00 |

[15] $\chi^2(30) < 0.001$, N= 64264 (3931 observations were deleted due to missing values for the explanatory variables)
[16] T: Transient; LICA: Late Infancy Chronic Asthma; EICA: Early Infancy Chronic Asthma

| Effect | Category | Group[16] | OR (vs NA group) | 95% Wald CI | p-value |
|---|---|---|---|---|---|
| **SES** | H vs L | EI CA | 0.81 | 0.70; 0.94 | 0.01 |
| **Education** | H vs L | T | 0.81 | 0.74; 0.89 | <.0001 |
| **Education** | H vs L | LI CA | 0.81 | 0.73; 0.90 | 0.00 |
| **Education** | H vs L | EI CA | 0.77 | 0.67; 0.91 | 0.00 |
| **Breastfeeding** | No vs Yes | T | 1.24 | 1.06; 1.44 | 0.00 |
| **Breastfeeding** | No vs Yes | LI CA | 0.95 | 0.78; 1.16 | 0.64 |
| **Breastfeeding** | No vs Yes | EI CA | 1.28 | 1.01; 1.63 | 0.04 |
| **Birth weight** | ≥2500 vs <2500 g | T | 0.75 | 0.59; 0.96 | 0.02 |
| **Birth weight** | ≥2500 vs <2500 g | LI CA | 0.79 | 0.59; 1.05 | 0.10 |
| **Birth weight** | ≥2500 vs <2500 g | EI CA | 0.49 | 0.35; 0.68 | <.000 |
| **Gestation** | ≥37 vs <37 weeks | T | 0.75 | 0.61; 0.92 | 0.01 |
| **Gestation** | ≥37 vs <37 weeks | LI CA | 0.91 | 0.71; 1.17 | 0.48 |
| **Gestation** | ≥37 vs <37 weeks | EI CA | 0.84 | 0.61; 1.16 | 0.28 |

### 5.3.2 Impact of environmental factors on asthma trajectories

Exposure metrics were successfully assigned to nearly all cohort members (Table 5.5) with complete covariate information (99.7%) except for IDW-derived ozone and fine particulate matter PM2.5 (95% and 85.6%, respectively).

The study area had low levels of air pollution with median $NO_2$ of 33.3 µg/m$^3$, a median inhalable particulate matter ($PM_{10}$) concentration of 12.5 µg/m$^3$, and a relatively high green space coverage (median NDVI =0.24).

**Table 5.5: Exposure levels of greenness, IDW-derived pollutants and LUR-derived $PM_{2.5}$, by trajectory among CHILD study participants**

| Exposure | Trajectory | N | Median | Mean | SD | Min | Max |
|---|---|---|---|---|---|---|---|
| **Greenness** | No Asthma | 63017 | 0.24 | 0.23 | 0.09 | -0.08 | 0.59 |
| | Transient | 2271 | 0.23 | 0.23 | 0.09 | -0.08 | 0.56 |
| | LI CA | 1822 | 0.24 | 0.23 | 0.09 | -0.05 | 0.56 |
| | EI CA | 919 | 0.24 | 0.23 | 0.08 | -0.04 | 0.49 |
| **Carbon Monoxide** | No Asthma | 62997 | 618.2 | 629.5 | 121.4 | 362.8 | 1062.3 |
| | Transient | 2270 | 610.6 | 629.4 | 123.3 | 381 | 1021.6 |
| | LI CA | 1820 | 613.3 | 626.5 | 117.8 | 384.8 | 1024.3 |
| | EI CA | 919 | 609.0 | 621.2 | 113.8 | 395.9 | 983.2 |
| **Nitrogen Monoxide** ($\mu g/m^3$) | No Asthma | 63000 | 20.8 | 23.0 | 11.1 | 2.8 | 65.2 |
| | Transient | 2271 | 20.4 | 23.0 | 11.3 | 2.9 | 63.2 |
| | LI CA | 1821 | 20.3 | 22.5 | 10.6 | 3.6 | 61.0 |
| | EI CA | 919 | 19.7 | 22.3 | 10.6 | 4.2 | 54.8 |
| **Nitrogen Dioxide** ($\mu g/m^3$) | No Asthma | 63012 | 33.3 | 33.5 | 7.0 | 15.0 | 53.7 |
| | Transient | 2271 | 33.3 | 33.7 | 7.0 | 16.9 | 51.2 |
| | LI CA | 1822 | 33.5 | 33.7 | 6.6 | 17.3 | 52.4 |
| | EI CA | 919 | 33.1 | 33.4 | 6.5 | 16.9 | 51.0 |
| **Ozone** | No Asthma | 60451 | 28.0 | 27.8 | 5.9 | 9.8 | 48.2 |
| | Transient | 2162 | 28.1 | 27.9 | 6.1 | 10.9 | 45.2 |
| | LI CA | 1758 | 28.3 | 27.9 | 6.0 | 11.4 | 46.7 |
| | EI CA | 879 | 28.3 | 27.9 | 5.8 | 11.1 | 43.3 |
| **$SO_2$** | No Asthma | 63012 | 4.7 | 5.7 | 2.5 | 1.9 | 17.8 |
| | Transient | 2271 | 4.7 | 5.7 | 2.5 | 2.1 | 16.1 |
| | LI CA | 1822 | 4.7 | 5.6 | 2.4 | 2.2 | 16.0 |
| | EI CA | 919 | 4.7 | 5.5 | 2.3 | 2.2 | 13.8 |
| **$PM_{2.5}$** | No Asthma | 54160 | 5.4 | 5.5 | 0.7 | 3.2 | 7.6 |
| | Transient | 1952 | 5.4 | 5.5 | 0.7 | 3.8 | 7.2 |
| | LI CA | 1507 | 5.4 | 5.5 | 0.7 | 3.5 | 7.2 |
| | EI CA | 781 | 5.4 | 5.5 | 0.7 | 4.2 | 7.1 |

| Exposure | Trajectory | N | Median | Mean | SD | Min | Max |
|---|---|---|---|---|---|---|---|
| $PM_{2.5}$[17] | No Asthma | 63017 | 4.03 | 4.07 | 1.74 | 0 | 11.3 |
| | Transient | 2271 | 4.06 | 4.07 | 1.58 | 0 | 10.5 |
| | LI CA | 1822 | 4.05 | 4.11 | 1.59 | 0 | 10.5 |
| | EI CA | 919 | 3.97 | 4.08 | 1.68 | 0 | 10.4 |

From all the exposures that were considered, only inverse-distance weighted $NO_2$ and LUR-derived $PM_{2.5}$ showed a significant impact on membership in one of the three asthma trajectories relative to the asymptomatic group; the effect of greenness was only seen for an interquartile increase in exposure. Table 5.6 presents the multinomial logistic unadjusted and adjusted regressions for the relative risk of being in a given trajectory compared to being in non-asthmatic trajectory for air pollution ($NO_2$ and $PM_{2.5}$) and greenness exposures.

For a one quartile increase in exposure during pregnancy, $NO_2$ significantly increased the risk of being in a chronic asthma group relative to non-asthmatics group by 51% and 25% for early and late infancy onset respectively (Adjusted Weighted Relative Risk Ratios (RRR) and 95% Confidence Interval (95% CI): 1.51 (1.22 – 1.89) and 1.25 (1.07 – 1.46) for early and late infancy onset). Relative to the non-asthmatic group, the transient asthma group was affected less by $NO_2$ compared to those with chronic asthma trajectories (RRR (95% CI): 1.08(0.94 – 1.23)). The $NO_2$ exposure gradient showed a decreasing effect in risk (from 51% to 23% for EICA and from 18% to 4% for LICA), yet it remained significantly positive for the early infancy chronic asthma group. In utero exposure to fine particulate matter was significant across all asthma phenotype trajectories as a one quartile increase in $PM_{2.5}$ exposure resulted in a 28%, 21% and 22% increased risk of membership in the early and late-infancy chronic asthma and transient

[17] Since the interpolated fine particulate exposure was assigned to only 85% of the study population, we report the land-use regression estimates of $PM_{2.5}$which covers 98% of the participants with complete covariate information and show the overall exposure for the two metrics

groups compared to the non-asthmatic group, respectively. The effect of $PM_{2.5}$ was most prominent among the transient asthma group as the relative risk remained significantly associated after adjustment for other risk factors.

Residential greenness in a buffer of 100m surrounding the home location was associated with a decreased risk of belonging to a chronic asthma group for the highest exposure category only (RRR (95%CI): 0.88 (0.72 – 1.09) and 0.92(0.80 – 1.07) for early and late infancy chronic asthma, respectively). The likelihood for children to follow a transient asthma trajectory relative to non-asthma group was only observed for an interquartile increase in greenness exposure. Unlike $PM_{2.5}$ and $NO_2$ exposures which were robust to risk factors adjustment, the effect of greenness was less consistent (Table 5.6).

**Table 5.6: Multinomial logistic regressions of unadjusted and adjusted single exposure models: greenness (NDVI),nitrogen dioxide ($NO_2$), and fine particulate matter ($PM_{2.5}$) models by asthma trajectories in BAQS children born between 1999 and 2002.**

| | | | Unadjusted Models | | | Adjusted Models | | |
|---|---|---|---|---|---|---|---|---|
| Exposure | Category | Group[18] | RRR | 95% CI | | RRR | 95% CI | |
| **NDVI** | 3 vs 0 | T | 0.88 | 0.88 | 1.00 | 0.91 | 0.79 | 1.04 |
| | | LI CA | 0.92 | 0.79 | 1.07 | 1.05 | 0.90 | 1.23 |
| | | EI CA | 0.88 | 0.72 | 1.09 | 0.99 | 0.80 | 1.22 |
| | 2 vs 0 | T | 0.98 | 0.87 | 1.11 | 0.99 | 0.87 | 1.13 |
| | | LI CA | 1.2 | 1.04 | 1.38 | 1.28 | 1.11 | 1.48 |
| | | EI CA | 1.08 | 0.89 | 1.31 | 1.12 | 0.92 | 1.36 |
| | 1 vs 0 | T | 1.0 | 0.88 | 1.13 | 0.98 | 0.86 | 1.12 |
| | | LI CA | 1.06 | 0.92 | 1.23 | 1.09 | 0.94 | 1.26 |
| | | EI CA | 0.96 | 0.78 | 1.18 | 0.94 | 0.77 | 1.15 |
| **$NO_2$** | 3 vs 0 | T | 1.13 | 0.98 | 1.29 | 1.11 | 0.96 | 1.27 |
| | | LI CA | 1.11 | 0.95 | 1.3 | 1.04 | 0.88 | 1.22 |
| | | EI CA | 1.21 | 0.96 | 1.51 | 1.23 | 0.98 | 1.55 |
| | 2 vs 0 | T | 1.08 | 0.94 | 1.23 | 1.02 | 0.89 | 1.18 |
| | | LI CA | 1.39 | 1.19 | 1.62 | 1.31 | 1.12 | 1.53 |
| | | EI CA | 1.27 | 1.02 | 1.59 | 1.31 | 1.04 | 1.64 |

[18] T: Transient; LICA: Late Infancy Chronic Asthma ; EICA: Early Infancy Chronic Asthma

| | | | Unadjusted Models | | | Adjusted Models | | |
|---|---|---|---|---|---|---|---|---|
| Exposure | Category | Group[18] | RRR | 95% CI | | RRR | 95% CI | |
| | 1 vs 0 | T | 1.13 | 0.98 | 1.29 | 1.1 | 0.96 | 1.26 |
| | | LI CA | 1.25 | 1.07 | 1.46 | 1.18 | 1.01 | 1.39 |
| | | EI CA | 1.51 | 1.22 | 1.89 | 1.51 | 1.21 | 1.88 |
| | 3 vs 0 | T | 1.10 | 0.96 | 1.27 | 1.07 | 0.93 | 1.23 |
| | | LI CA | 1.03 | 0.88 | 1.21 | 0.95 | 0.81 | 1.12 |
| | | EI CA | 0.94 | 0.76 | 1.16 | 0.85 | 0.69 | 1.06 |
| | 2 vs 0 | T | 1.32 | 1.15 | 1.50 | 1.24 | 1.09 | 1.42 |
| **$PM_{2.5}$** | | LI CA | 1.36 | 1.18 | 1.58 | 1.24 | 1.07 | 1.45 |
| | | EI CA | 1.11 | 0.90 | 1.37 | 0.99 | 0.80 | 1.22 |
| | 1 vs 0 | T | 1.22 | 1.06 | 1.39 | 1.16 | 1.01 | 1.33 |
| | | LI CA | 1.21 | 1.04 | 1.40 | 1.13 | 0.97 | 1.32 |
| | | EI CA | 1.28 | 1.04 | 1.56 | 1.17 | 0.95 | 1.43 |

Except for parity, all covariates had similar direction of effect as that observed in the risk factors multinomial logistic regression with the largest magnitude observed among the early infancy chronic asthma group. These children were more likely to not have been breastfed, and to be born to a multiparous and non-smoking mother. Most notably, the relative risk to develop chronic asthma in early infancy more than doubled if the child's term birth weight was less than 2500g (RRR (95%CI): 2.22 (1.69 – 2.90)).

### 5.3.3 Co-exposures models

Given the low correlation between greenness and air pollutants (Figure 5.2), we examined two co-exposures models where NDVI was entered with $NO_2$ and $PM_{2.5}$ respectively as well as $NO_2$ with $PM_{2.5}$ to examine the relative contribution of these environmental exposures.

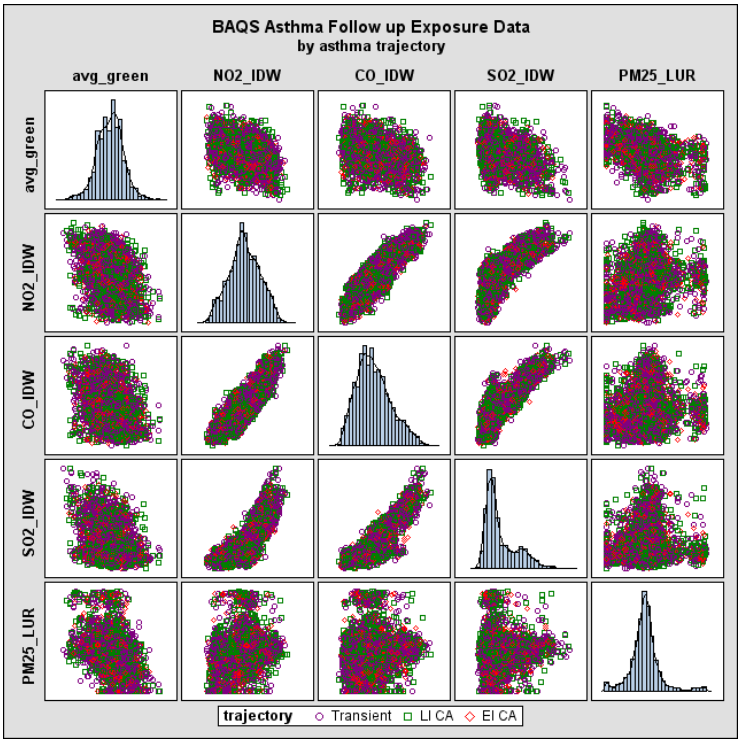


**Figure 5.2: Scatter plot matrix of air pollutants and greenness exposure by asthma trajectory and exposure distributions among children with one of the three asthma trajectories.**

As shown in Table 5.7, $PM_{2.5}$ and $NO_2$ have independent effects on the likelihood to be in a chronic or transient asthma trajectory compared to the non-asthmatic trajectory. Since there were suggestions of protective effects of greenness in relation to the three trajectories, we also investigated the effect of greenness added to air pollutants in stratified analysis by $NO_2$ and $PM_{2.5}$ tertiles. Exposure to greenness was only borderline protective amongst the two chronic asthmatics groups in high levels of air pollution ($2^{nd}$ and $3^{rd}$ tertiles of $NO_2$ levels and $3^{rd}$ tertile of $PM_{2.5}$ levels; data not shown).

**Table 5.7: Co-pollutants multinomial logistic regression of air pollution (IDW-derived $NO_2$ and LUR-derived $PM_{2.5}$), greenness (NDVI), controlling for significant risk factors on the likelihood of belonging to transient , late infancy chronic asthma (LICA), or early infancy chronic asthma (EICA) in BAQS children.**

| Trajectory | Exposure | Category | OR | 95% CI | |
|---|---|---|---|---|---|
| **EI CA** | **$NO_2$** | 1 vs 0 | 1.42 | 1.1 | 1.8 |
| | | 2 vs 0 | 1.20 | 1.0 | 1.5 |
| | | 3 vs 0 | 1.17 | 0.9 | 1.5 |
| | **$PM_{2.5}$** | 1 vs 0 | 1.14 | 0.9 | 1.4 |
| | | 2 vs 0 | 0.98 | 0.8 | 1.2 |
| | | 3 vs 0 | 0.86 | 0.7 | 1.1 |
| **LI CA** | **$NO_2$** | 1 vs 0 | 1.18 | 1.0 | 1.4 |
| | | 2 vs 0 | 1.29 | 1.1 | 1.5 |
| | | 3 vs 0 | 1.00 | 0.8 | 1.2 |
| | **$PM_{2.5}$** | 1 vs 0 | 1.12 | 1.0 | 1.3 |
| | | 2 vs 0 | 1.27 | 1.1 | 1.5 |
| | | 3 vs 0 | 0.97 | 0.8 | 1.1 |
| **Transient** | **$NO_2$** | 1 vs 0 | 1.10 | 1.0 | 1.3 |
| | | 2 vs 0 | 1.04 | 0.9 | 1.2 |
| | | 3 vs 0 | 1.10 | 1.0 | 1.3 |
| | **$PM_{2.5}$** | 1 vs 0 | 1.15 | 1.0 | 1.3 |
| | | 2 vs 0 | 1.22 | 1.1 | 1.4 |
| | | 3 vs 0 | 1.05 | 0.9 | 1.2 |

## 5.4 Discussion

In this work, we report yearly prevalence of asthma in a population-based birth cohort study of 68,195 children followed from birth to age 10 in the Greater Vancouver metropolitan region. Three distinct trajectories of asthma were identified using Group Based modelling. Our model not only distinguished transient from chronic asthmatic children but also allowed for the differentiation within chronic asthmatic children of those who develop asthma in early infancy (EI CA) from those who develop asthma after age 3 years. While most of the previous literature has distinguished between transient and chronic phenotypes

of asthma such as wheezing measured via participants' self-report, the present study offers new and objective insight into the development of asthma in early infancy and provides support, in a large population-based dataset, to a similar classification (early versus late infancy asthma onset) reported in an American birth cohort of 689 children (Chen et al. 2012).

This work was motivated by an earlier investigation in the same population where associations between air pollution prenatal exposures and asthma incidence were found in preschool children (i.e. between birth and 6 years), but not for school age children (between age 6 until end of follow up) (Sbihi *et al.* submitted). One explanation for these previous findings was that most incident cases were captured in early years, leaving a small number of incident cases during the school age period. With the approach adopted here, we quantitatively confirmed this pattern. In particular, among the 2282 transient asthma cases identified in this analysis, only 2% of this group met the case definition after 6 years of age. Given the course of asthma development in the late onset asthmatic children (peak between 4-5 years of age and decline after age 6), the inability to detect associations between air pollution and asthma incidence during school age can be more confidently related to trajectories indicating that development of asthma occurs either in the first three years of age or resolves during school age years

Longitudinal studies have provided evidence for three wheeze phenotypes in childhood (Stein and Martinez 2004): (1) transient wheeze that is associated with lower respiratory tract illnesses in early life that may resolve by 6 years of age; (2) non-atopic wheezing occurring in the first 3 years of life that is associated with lower respiratory tract illnesses, which may persist beyond 6 years into chronic asthma, and is not associated with atopy; and (3) atopic wheezing that starts before 6 years, may persist into adulthood, is strongly associated with atopy, and has a more severe clinical course. We note a striking parallel with the three asthma trajectories unveiled in our objective analysis of administrative health data: the transient group mirrors the transient wheeze phenotype, indicating that there is a significant proportion of children identified through administrative health databases as asthma cases when they are

most likely transient wheezing children. The two chronic asthma trajectories also reflect similarities with the non-atopic and atopic wheezing groups. In the absence of doctor's diagnosis, and without more detailed clinical evaluation or medication utilization as has been used in other cohort studies of the developmental trajectory of childhood asthma(Wu et al. 2012), we can only speculate that early infancy chronic asthma would capture non-atopic asthma while the late infancy chronic asthma would capture the atopic asthma subgroup.

Our study, which encompasses over 65,000 children followed over 10 years, is to the best of our knowledge, the first large population-based study to explore both the trajectories of asthma based on administrative health records and the impact of environmental predictors on different trajectories of childhood asthma. The data-driven approach of Group-Based Trajectory Modeling (GBTM) indicates that nearly all asthma-like phenotypes can be captured in early infancy. In addition, transient, early and late infancy onset of chronic asthma trajectories closely match those found in studies of asthma and wheezing using similar approaches of clustering individuals into distinct class such as latent class analysis (Chen et al. 2012; Granell et al. 2012). We note however that one study reported seven patterns for a similar follow up period by defining two-year asthma period prevalence(Wu et al. 2012), although the ad-hoc definition of period prevalence length which is neither data-driven nor supported by timing of biological mechanisms may be open to criticism.

Another particular strength and novel aspect of this study is in the methodology adopted following trajectory modeling. We have extended previous efforts to advance asthma cohort research through identification of classes of asthma based on individual patterns over time by quantifying the effect of air pollution, greenness and other risk factors on the probability of belonging to the identified classes. Despite its strengths, GBTM is not immune to the same model inference pitfalls (estimates error, validity) as generalized linear models in the context of an observational study (i.e. non randomized data). In order to reduce the possibilities for bias, we used posterior probability as weights in the regression analyses

when comparing the "non-disease" to the diseased (Nagin and Odgers 2010). Combining trajectory groups with weighted regression methods provided risk profiles where elevated exposures to $NO_2$ and $PM_{2.5}$, two markers of traffic-related air pollution, were shown to independently increase the probability of adhering to a chronic pattern of disease, especially for infants (EICA group). Another important gap that was informed relates to the modest propensity of early life green spaces exposure to mitigate the increased risk for infants to grow into a chronic asthma trajectory following high levels of exposure to traffic-related air pollutants such as $NO_2$. Despite the fact that children may grow out of their asthma-like symptoms, this work identified that greenness may provide some preventive measure given the decreased relative risks that were found.

Recent advances in group based trajectory modeling include the ability to follow the developmental course of two distinct but related outcomes. Future studies can benefit from such a dual trajectory model as it provides a rich statistical summary of the developmental linkages between the two outcomes of interest such as asthma and other comorbidities that evolve contemporaneously (e.g. bronchiolitis and asthma) or that evolve over different time periods that may or may not overlap (e.g., asthma during childhood and school achievement in school age year or during adolescence).

In conclusion, trajectory modeling of physician's diagnosed asthma during a 10 year since birth identified three distinct groups with direct assessment of disease status, that closely resemble asthma phenotypes identified in previous studies. This investigation indicated that an equal proportion of children diagnosed at least once during follow-up will not remit and develop chronic asthma; this condition (i.e. chronic asthma, whether onset is in early or late infancy) which is affected by both intrinsic and behavioral risk factors is significantly associated with early life traffic-related air pollution, especially among the group who develop chronic asthma in early infancy.

## Chapter 6: Discussion and conclusions

The work presented in this thesis addresses methodological questions for researchers investigating the relationship between early life environmental exposures and the development of asthma and allergies in childhood. These questions are mostly centered on exposure assessment challenges. I investigated a novel measurement option and modeling methods where I adopted pragmatic approaches for exposure assessment in the context of individual-level temporally varying exposure estimates in large cohort populations. The contribution of other neighborhood exposures that may co-vary with air pollution was also examined by the inclusion of surrounding green spaces, and its impact on asthma was quantified. The different contributions summarized here inform questions such as: "What should we measure in air pollution health studies to maximize the precision of exposure estimates?," "How can information gathered in the context of observational birth cohort studies help the estimation of personal exposures?," and "What other co-varying environmental exposures may mitigate the adverse effects of air pollution on pediatric respiratory health?".

A subsequent body of work in this dissertation focused on the complexity of the asthma syndrome that potentially covers multiple discrete entities of the disease. This work concluded by demonstrating the need to advance epidemiologic cohort studies on pediatric asthma and allergies by the examination of incidence of these heterogeneous chronic conditions to the investigation of their patterns and the role of risk factors over time.

## 6.1 Summary and contributions

### 6.1.1 Key findings

The overall main conclusions of this dissertation are:

(1) Early lifel exposure to traffic-related air pollution increases the risk of childhood asthma and atopy in the first years of life. The finding for atopy is novel in its analysis of atopy development in early infancy. The finding for asthma development is in line with a recent pooled analysis of several European and Canadian birth cohorts, the Traffic, Asthma and Genetics Study (TAG), where effects of TRAP were associated with the development of childhood asthma (MacIntyre et al. 2014) as well as with a meta-analysis that demonstrated a significant increased risk for asthma incidence (Anderson et al. 2013a).

(2) The risk for asthma initiation following early life TRAP exposure is diluted over time, most likely due to the heterogeneity of the disease. However, TRAP exposure increases the odds of having an early-life onset chronic asthma course.

(3) Assessing traffic-related air pollution (TRAP) for large birth cohort studies based on modeled surrogate measures of TRAP such as $NO_2$ that are spatio-temporally adjusted is warranted, while the use of hopanes in house dust was found to be of limited utlity.

(4) Surrounding residential greenness may offset the adverse effects of traffic-related air pollution on asthma incidence in the early years of life.

In the following subsections, the contributions related to the above conclusions are outlined.

#### 6.1.1.1 Are hopanes a useful metric for infiltrated traffic-related air pollution?

As detailed in the background review of the literature (Chapter 1), there is a need to identify air pollution exposure metrics that can account for infiltration of air pollutants and integrate over longer time periods than are feasible for air sampling. Hopanes, which have been shown to be good tracers of primary vehicle

exhaust, can be detected in indoor house dust, suggesting possible use as a surrogate for personal exposure to infiltrated traffic-related air pollution.

After comparing measured dust hopanes with ambient air hopanes measurements and with GIS-derived land use variables for each city, the signature profile of ambient hopanes was significantly correlated, albeit modestly, with that found in home-settled dust. A key feature of this particular finding was its external validity, considering that the data, analyzed rigorously using the same protocol in all cities, was gathered in a varied setting ranging from highly urban locations (e.g., Toronto) to smaller cities (e.g., Winnipeg). Indoor measurements of hopanes levels in house dust were not correlated with GIS-derived land use variables unless behavioral and/or home characteristics were considered. This lack of correlation was evident whether the GIS-derived variables were generic land use predictors or variables more targeted to the land use specificities of each city. Examining predictors of LUR models rather than predicted $NO_2$ estimates of LUR is a more precise approach to assess the utility of hopanes in settled dust as indicators of traffic-related air pollution exposure because $NO_2$ estimates have a certain amount of error associated with the proportion of variability unexplained by the LUR models.

Taken together, the use of hopanes as a potential advance over existing traffic-related air pollution exposure assessment approaches was found to be limited, as hopanes only appeared to be useful if the determinants of house-settled dust variability were also characterized. These findings led to examining other approaches, such as modeling (as performed in the subsequent studies of this dissertation) to account for the aspects modifying total personal exposure (indoor/outdoor infiltration, time-activity patterns) that hopanes would have captured if this initial investigation had proved more successful. The subsequent sections highlight key findings when using modeling approaches of personal exposures.

#### 6.1.1.2 Sensitization in infants and traffic-related air pollution

The work undertaken in the context of the CHILD study illustrated the utility of incorporating information from different sources to improve exposure assessment as a means to increase understanding of vulnerable time windows of exposure during which air pollution may impact the development and maturation of the immune system, and thus affect the onset of childhood atopy and, possibly, asthma.

Typically, LUR models are used to assess individual exposures, and studies often rely on the residential birth address for their exposure assessments. Aspects such as the infiltration of outdoor air pollution indoors, residential mobility, and personal time-activity patterns may be important modifiers of personal exposures. Approaches to account for these factors, as well as temporal trends in outdoor pollution concentrations, were developed to estimate TRAP exposures (Chapter 3). These estimates were then applied in the analysis of association with sensitization among 2482 infants who were at least one year of age. Questionnaires, home inspections, governmental air-monitoring measurements, and geographic predictors were used to: account for temporal changes and residential mobility, examine indoor infiltration of TRAP based on a season-specific model, and facilitate stratified analyses based on time activity patterns.

TRAP exposure during the first year of life but not during pregnancy increased the risk for development of atopy for any of the ten allergens that were administered during skin prick tests. The associations were largely driven by sensitization to inhalant allergens rather than food allergens. When residential mobility was not considered, a weaker association was found (aOR = 1.10; 95% CI: (0.96 – 1.34) first year) compared to the odds of developing atopy after adjustment for all addresses where participants resided (1.16 (1.00 – 1.41)). This association was stronger among those for whom the exposure was less susceptible to misclassification, i.e., participants spending more time in the home and participants not attending daycare. However, there may be an alternative explanation other than misclassification. A portion of the difference found in the sensitivity analyses of time-location patterns was likely attributable

to exposures other than TRAP. For example, analysis stratified by presence of siblings indicated that those with older siblings in the household were less likely to develop an allergic reaction to the skin prick tests. This explanation was also likely to hold for the analysis stratified by daycare attendance. Our results regarding indoor PM infiltration were inconclusive, showing an increased risk in homes with higher ventilation only during periods where the weekly average temperature was below 18°C. In an effort to explain the absence of effect modification in the warm season, attention was given to the possibility of an infiltration threshold being surpassed. All the homes, except those in Toronto, experienced significantly greater $F_{inf}$ in the warm compared to the cold period. This could imply that all homes (even those below the city-median infiltration efficiency) are in fact still "leaky". In addition, as the challenge posed by infiltration mostly pertains to its variability over time, the variability in predicted $F_{inf}$ during the warm season was found to be much smaller in all CHILD cities, introducing misclassification error that would not necessarily affect health estimates, but merely modify the outdoor concentrations by a constant. These additional investigations and possible explanations argue in favor of developing Canadian-specific infiltration models.

In this work, temporal adjustment of land use regression estimates at all residential home locations where the mother and child lived for at least 15 days captured associations that otherwise would have remained veiled. Through the use of a more refined methodology, we added new evidence to the existing TRAP and allergies literature. This conclusion is in line with the findings seen in a Dutch birth cohort study, where positive associations were demonstrated among a subset of non-movers (Gehring et al. 2010b) , suggesting that reducing exposure misclassification can help clarify the adverse role of TRAP on allergic diseases. The trajectory of atopy is likely to be heterogeneous (Simpson et al. 2010): longer-term studies can help clarify the role of TRAP in the etiology of allergies. With this perspective, the next step of this dissertation took advantage of a 10-year follow-up in the BAQS birth cohort to investigate the effects of TRAP on the initiation of childhood asthma, as atopy is a known risk factor for IgE-mediated asthma.

#### 6.1.1.3 Childhood asthma and traffic-related air pollution

The follow up over 10 years in BAQS, a population-based birth cohort, extended the initial investigation on pediatric asthma in early years (Clark et al. 2010b) by examining the effects of TRAP at different ages using the LUR modeling approach, among other exposure metrics, and adjusting for temporal and spatial variability (Chapter 3). Starting with the premise that early life exposure is a critical window, issues related to the time-course of developing chronic conditions such as asthma were evaluated. In addition, the role of potentially co-varying environmental exposures (air pollution and surrounding greenness) in asthma initiation was examined.

The investigation of incident asthma showed that air pollution was associated with the odds of asthma in children during the ages between birth and age 5, but not at older ages. These associations with air pollution during the preschool period were partially offset by the independent protective effect of the level of residential greenness surrounding the home location (Chapter 4). In an effort to better understand the course of asthma defined using administrative databases, annual incidence rates were also examined, and confirmed that most new asthma cases occurred in the first three years of life.

To further evaluate the time course of childhood asthma in the BAQS cohort and to assess whether effects were focused on specific time periods, trajectories of asthma phenotypes were determined. Chapter 5 described two trajectories of children with chronic asthma: those who developed the disease starting in early infancy and those who started to show healthcare utilization for asthma treatment and/or diagnosis around age 5 or 6, coinciding with the cut-off year between preschool and school-age time periods that were examined in Chapter 4. The effect of TRAP exposure was most prominent among the chronic asthmatics who developed the disease in early infancy.

### 6.1.2 Synthesis

The results in Chapters 2 and 3 of this dissertation provide different perspectives on the complexity of assessing personal air pollution exposures that occur mostly indoors, while the vast majority of the assessment tools at our disposal estimate ambient concentrations. These two chapters provided a measurement and a modeling approach to address the indoor infiltration of air pollutants.

Assessing indoor levels of TRAP through the collection and analysis of hopanes in settled house dust is a new area of study that has the potential to reduce exposure misclassification and increase specificity of personal exposure by capturing the infiltrated portion of the pollutants related to asthma and allergies. While the utility of hopanes as markers for infiltrated TRAP exposure were inconclusive, Chapter 3 tackled the issue of infiltration by examining whether modeled infiltration modified the association between air pollution and sensitization. This analysis indicated that modeled infiltration was partially useful given the observed modifying effect on the association between TRAP exposure and sensitization only during the winter season when ambient temperature was below 18ºC. However, several limitations should be described here. First, homes were classified according to the city-level 80$^{th}$ percentile of individual homes' particle infiltration efficiency, aggregating individual-level data and thus introducing misclassification error that affects the precision of the estimates. Second, infiltration efficiency was determined using a model calibrated among homes included in an air pollution cohort study from the USA (Allen et al. 2012b). While this model explained 60% of the variance in infiltration efficiency during the summer and winter, the unexplained variability is likely to propagate error in our investigation and, more importantly, it was not validated for Canadian homes, possibly introducing measurement error. The effect of infiltration remains an open question because we have shown that the contribution of ambient TRAP to the indoor mix is likely to modify the total exposure.

The stratified analyses accounting for locations other than the homes, which indicated potential exposure misclassification, corroborated the importance of developing methods for estimating exposure in all

microenvironments (Chapter 3). The likelihood of misclassification when other microenvironments are not considered is supported by results shown in the cohort study in the UK (Mölter et al. 2013). In this study, investigators developed a model to integrate home, school, and journey exposures to assess children's total individual exposure (Mölter et al. 2012a) for an epidemiological analysis of lung function among school age children (Mölter et al. 2013) and demonstrated a high correlation with personal measurements. The degree of misclassification in the presented sensitization analysis was probably non-negligible because infants in their first year of life tend to spend the vast majority of time in their homes, but accounting for residential histories and temporal trends provided reasonable personal exposure estimates.

Moving from a smaller birth cohort with detailed information to a large population-based birth cohort without the same level of detailed individual information, investigations of pediatric asthma outcomes were conducted using temporally adjusted ambient air pollution levels at the residential location from conception to birth (Chapters 4 and 5). Chapter 3 indicated that early life exposures are critical (Clark et al. 2010a) as it demonstrated that with increased temporal and spatial specificity, the impact of exposures within narrower time windows (*in utero* vs. first year *post-partum*) can be pinpointed and their relative contribution in the etiology of allergic sensitizations uncovered.

Although the same study design was adopted in most of the work undertaken (Chapters 3-5), the birth cohort studies that were examined had varying degrees of information regarding potential effect modifiers on the health effects of air pollution. In the BAQS study, a notable finding was the effect modification by term birth weight. The identification of this susceptible subgroup (children weighing less than 2500g at term birth) is of clinical importance and is in line with previous studies reporting the susceptibility of premature children to pediatric lung disease development (Jobe and Tibboel 2014; Strickland et al. 2014). However, in the BAQS study several individual-level confounders were not accessible, limiting the ability to examine in detail other potentially susceptible populations. For instance, children of asthmatic

or atopic parents are more susceptible to TRAP effects on the development of allergic diseases (Morgenstern et al. 2008). Maternal atopic status was a major predictor in the atopy analysis; a confirmation of this finding in a population-based study would have given more weight to this identification of a susceptible group. In the same vein, the BAQS study may have been limited in its ability to investigate how socio-economic variables may affect the impact of air pollution on asthma development in children. Although household income and educational attainment were considered at the neighborhood level, residual confounding still remains. However, recent evidence has shown that area-level SES should also be considered in studies of air pollution and allergies (Gruzieva et al. 2014), which alleviates this threat of confounding.

In contrast, the CHILD study provided very detailed individual information. In particular, the physical characterization data allowed the examination of an identified gap in the literature on assessing personal exposure to air pollution with regard to the development of childhood asthma and allergies. Although the stratification by home ventilation level is novel and may help advance the field of air pollution exposure assessment, the process of stratification reduced the sample size, which, in turn, reduces the ability to detect small-magnitude effects. This limitation also applies to the stratification by daycare attendance and time-activity patterns. In these latter analyses, individual time spent away from the house was averaged across participants by city to stratify the cohort into two groups, introducing a Berkson-like type of misclassification.

Berkson- and classical-type errors constitute the two general classes of measurement error that are unavoidable in exposure assessment. Berkson errors generally introduce no bias in linear – and, to some extent, generalized linear – health effect models because they measure part of the “true” exposure, whereas classical errors measure the true exposure plus varying degrees of noise, thereby introducing some bias away or toward the null (Sheppard et al. 2011). In air pollution cohort studies, the impact of

measurement error is an active area of research because these two types of error come into play: the use of prediction models to estimate exposure will likely reduce variability (smoothing effect, "Berkson-like" error) and the spatial correlation of the exposure model parameter estimates may bias the health effect parameter estimation (spatial misalignment, "classical-like" error) (Dominici and Cefalu 2015).

In addition, the analysis of daycare attendance could have been strengthened by geocoding the addresses of daycares or similar sites where each child spent time outside the home, assigning exposures at these addresses and creating an average total exposure profile. At the time of data acquisition and cleaning, however, the number of missing values hindered this approach, leaving stratification as the next-best way to examine effect modification by exposure at locations other than the home.

Given the lack of specific markers, most of the birth cohort studies of pediatric respiratory outcomes examine inhalable particles, fine particles, or nitrogen dioxide. This dissertation has mostly focused on nitrogen dioxide, for which recent efforts to establish a dose-response formulation were developed based on the prolific set of population health studies in which $NO_2$ is the principal exposure of interest (Favarato et al. 2014). Our results show that variation in $NO_2$ exposure consistently predicts childhood asthma and sensitization, a finding that is aligned with the view that morbidity associated with ambient nitrogen dioxide is not only explained by its associated toxicants, but also with $NO_2$ itself (World Health Organization Regional Office for Europe 2013).

Although Chapters 3 and 4 examine the incidence of two different health outcomes, it is important to keep in mind that: (a) sensitization and asthma can be interrelated and (b) the two studies both used land use regression modeling of $NO_2$ concentrations, a common exposure. These two chapters are complementary in the sense that one examines an unselected population-based birth cohort, the BAQS cohort, while the second utilizes primary data gathered in a non-random birth cohort of participants recruited by the CHILD researchers. In CHILD, considerable efforts were deployed to gather a large amount of data

through questionnaires, biological samples, and home inspections. The inclusion of such data is expected to enhance the precision of individual exposures estimates, despite the potential introduction of additional sources of error. This level of in-depth individual information is not accessible in study designs that rely on linked administrative databases. On the other hand, the BAQS studies (Chapters 4 and 5) offered the opportunity to investigate childhood asthma in a large sample of over 65,000 children, with objectively assessed exposures and diagnoses of asthma based on health care utilization records. Administrative databases such as these offer great promise in epidemiological research because they provide a comprehensive, longitudinal, and population-based record of health information that eliminates issues of self-report, recall, and selection bias inherent in survey-based research.

Collectively, this work demonstrated the importance of early-infancy exposures, a critical time window, and the adverse effects of TRAP on asthma and sensitization incidence, as well as the potential health benefits provided by surrounding green spaces. The associations demonstrated in the presented studies point to the heterogeneity of the asthma syndrome given the dissolution of effects over time, as well as to the complexity of atopy status definition. This heterogeneity begs for a different research framework, such as finite mixture models, to advance our understanding of the role of environmental exposures in the etiology of these chronic respiratory conditions. Our results also confirm the recently reported increased susceptibility of children with parents reporting an allergic disease (Gruzieva et al. 2012; Morgenstern et al. 2008).

### 6.1.3 Strengths and limitations

Birth cohorts offer multiple advantages in the assessment of chronic disease initiation in relation to environmental exposures. In both BAQS and CHILD, participants were prospectively followed from conception onward, providing the best setting for the investigation of the temporal sequence of events and

indicating the direction of causality. The present dissertation does not prove causality, but it adds to the weight of evidence for a causal relationship between TRAP and incident asthma and allergies in young children.

An additional strength of this body of work is the complementarity between the study populations that were leveraged to examine the effect of traffic-related air pollution on asthma and allergies. Each of the two cohort studies, which included outcomes that share some common etiology, provides aspects that are lacking in the other (e.g. size, individual-level characteristics). As a whole, they strengthen the weight of the reported findings.

BAQS is a population-based cohort, which, in addition to its large sample size, offers a sample with good external validity, whereas CHILD may have children with parents of a higher SES than the overall Canadian population. It is therefore unclear to what extent the results reported from CHILD study are generalizable to a low-SES segment of the Canadian population. In addition, other than moving out of the province, the BAQS studies do not suffer from the same threat of selection bias attributable to loss of follow-up that is inherent to a birth cohort study such as CHILD.

#### 6.1.3.1 Assessment of environmental neighborhood exposures

Environmental exposures linked to the development of childhood allergies and asthma occur from conception through the prenatal period and into childhood, with the latest evidence pointing at either the first or second year of life as the critical time window (Arrandale et al. 2010) due to the immaturity of the lungs and immune system. The question of when to measure these exposures is complex, and consequently one of this dissertation's main strengths resides in the utility of the methods that were employed to assign exposure for precise time windows.

Air pollution exposure assessment in the present dissertation was estimated for the early life period using previously developed and validated LUR models. These types of models have been widely used throughout the past two decades of air pollution epidemiological studies without assessing whether they are valid for study periods that are distinct from the time when the models were developed, thereby incorrectly assuming that spatial patterns are stable. In the context of this work, $NO_2$ concentrations have decreased over time in all of the cities examined in this dissertation (Brook et al. 2014). In Vancouver, it has been demonstrated that when $NO_2$ concentrations decrease over time the spatial concentration patterns remain relatively stable (Wang et al. 2013), and thus temporal adjustments using government fixed-site monitoring are appropriate.

LUR models in the present work are a strong tool for generating individual exposure estimates. They provide a high spatial resolution that was augmented by tracking the residential histories throughout pregnancy and during the first year of life, in comparison to previous studies that solely relied on estimates of traffic-related air pollution at the home address at time of birth. Accounting for residential mobility over a time period of increased susceptibility helps attenuate the misclassification that would have occurred if exposure was estimated solely using the birth address. While exposure levels around the early life period were of primary interest, this work would have benefited from examining the relative importance of exposure effects at birth address versus current home address, and confirming earlier findings such as those shown in the BAMSE birth cohort studies (Gruzieva et al. 2012, 2013), which indicate that the early years are a critical time window for asthma and allergies.

Another important strength of the present work is the examination of the effect of exposures that occur away from the home. The inclusion of all of the places in which a young child spends time during the first year of life should reduce exposure misclassification. Among school-age children, McConnell and colleagues showed that TRAP exposure at school and home may contribute to the development of asthma (McConnell et al. 2010), whereas in younger children, TRAP exposures in different locations have been

considered rarely, so the present work fills an important gap. Although exposures were not quantitatively assigned, we showed that time-activity patterns are likely to modify the association between TRAP and the development of allergies in early-life.

An additional strength of the environmental exposure assessments is the adjustment of air pollution for the presence of green spaces in the BAQS cohort (Chapters 4 and 5). These two exposures were only moderately correlated, enabling the examination of co-exposure models and the demonstration that air pollution affects respiratory outcomes independently from the effect of green spaces. Therefore, the assessment of multiple exposures related to urban form (various air pollutants and NDVI-based greenness) is an important contribution of this work. While the Normalized Difference Vegetation Index (NDVI) offers an objective assessment of green spaces, it remains a non-specific measure of the type of greenness surrounding the homes of study participants. Dadvand *et al.* showed that different types of greenness have differential risk and health benefits (Dadvand et al. 2014). The mechanisms of access to green spaces on pediatric respiratory health can be explained by the biodiversity loss hypothesis, increased aero-allergic load, or the reduction in air pollution. Small buffer sizes were used around the home address, decreasing the likelihood of varying degrees of allergenicity of the surrounding greenness. However, the last set of analyses involving greenness at birth, even when measured using NDVI in small buffer areas rather than relying on an estimate of plant species by differing degree of allergenicity, is not a relevant metric for effects later in childhood. Other environmental exposures that may be relevant to this work but were not considered include the effect of climate (e.g., variations in precipitation, temperature, and relative humidity). A major potential indirect effect of climate change on public health is predicted to arise via climate-induced changes in aeroallergens, the primary risk factors for atopy and allergic asthma (D'Amato et al. 2010; Shea et al. 2008).

The investigations in this dissertation relied on modeled $NO_2$, which is a very good marker of within-city variability in TRAP. However, an important limitation arises from the fact that there were no personal

exposure measurements in this research. Relying on LUR models is likely to introduce error because these models explain between 76% of the variability in Vancouver to 81% in Winnipeg, leaving a non-negligible amount of unexplained variability and propagating error in the estimates subsequently used in further regressions for the epidemiological analyses.

Another limitation to note in this dissertation is the generally low level of air pollution. Throughout Canada, and certainly in the cities that were included in the present work, there has been a documented decline in $NO_2$ measured at governmental air monitoring stations (Brook et al. 2014). Such low urban concentrations of $NO_2$ are likely to hinder the ability to detect associations of modeled $NO_2$ estimates with childhood asthma and allergies. However, studies of low-level exposures can be valuable in demonstrating that effects are harmful even at the low levels as shown in the present work, reinforcing the notion that "no safe concentration" has been identified.

#### 6.1.3.2 Outcomes case definition

Throughout the present dissertation, the ascertainment of both outcomes (childhood asthma and atopy) relied on an objective assessment and not on self-reported health status, which suffers from the threat of recall bias. However, there are different limitations related to each outcome that are best discussed separately, as follows.

The assessment of atopy relied on an objective measure of sensitization by administering skin prick tests at each of the CHILD study center clinics. Skin allergy tests avoid the threat of recall bias and provide a reliable measure because the outcome is ascertained following a rigorous and consistent protocol across study sites. Furthermore, the study reported in this dissertation used a wide array of various allergens that capture the two main routes of exposure: ingestion and inhalation. However, criticism around this mode of case definition for atopy includes the lack of specificity when using a binary outcome based on an ad-

hoc cut-off (Custovic et al. 2013). It has been shown that, when defined in this way, such positive allergy skin tests may indicate a positive allergic response without confirming clinical reactivity, where such defined atopic individuals have no evidence of asthma (Custovic et al. 1994). Instead, a continuous measure of wheal size would provide the opportunity to quantify the magnitude of the effect that air pollution has on allergic sensitization and to define "true" atopy in young children, which, when diagnosed among those with a history of wheezing, indicates a high risk of developing persistent asthma (Simpson et al. 2010; Sly et al. 2008).

The definition of childhood asthma was based on administrative health records, which provides the advantage of avoiding recall bias. Asthma is a heterogeneous disease whose diagnosis is complex, even more so in the early years where individual wheezing episodes can be diagnosed wrongly as the development of asthma. Adding to this complexity is the fact that all asthma-related outcomes reported in this dissertation relied on physicians' billing records for a visit related to asthma symptoms or diagnosis, rather than on a clinical record or an asthma-related medication prescription. Other issues include the validity of asthma case definitions using administrative data. A physician visit may be coded as asthma for suspected asthma in the absence of further testing for unusual symptoms that appear asthma-like, or in the absence of clear diagnostic criteria. However, these limitations are alleviated by the positive reports of multiple studies examining the ability of administrative data to identify asthma in Canada using visit or billing data collected as part of provincial universal healthcare system databases (Kozyrskyj et al. 2004; Prosser et al. 2008; To T et al. 2007). Furthermore, Chapter 5 confirms that linked administrative databases from mandatory healthcare insurance programs are suited for moving from incidence analyses to longer-term investigations of chronic episodic conditions. The limitation of defining asthma at one point in time, as performed in Chapter 4, is further confirmed by other Canadian studies of asthma recurrence in childhood. In Ontario, a similar administrative database was used to identify children who were diagnosed with asthma prior to the age of six and to track their asthma diagnoses until the age of 11.

In this study, asthma diagnosis was based on the same criteria as those used in the current dissertation. Children who continued to have asthma diagnoses past the age of six were considered to be persistent asthma cases. By age 12, half of the children were in remission. This finding highlights the advantage of using longitudinal and linked health databases to identify distinct long-term asthma groups for population-based studies.

## 6.2 Recommendations for future research

Given the asthma "epidemic" and the fact that the impact of exposure to many indoor and outdoor pollutants remains unresolved, the issue of an integrated assessment of environmental influences is of high importance because it may identify at-risk subpopulations (Gowers et al. 2012) as well as modifiable factors for which policy-makers can take appropriate measures.

The findings of this dissertation point to sources of exposure misclassification that may be present when relying on outdoor pollutant concentrations. These include infiltration and other exposures encountered in diverse microenvironments. Personal monitoring studies have shown relatively poor agreement with outdoor concentrations (Mölter et al. 2012a) and, more importantly, epidemiological studies using personal monitors have been unable to replicate associations (Mölter et al. 2013) found with outdoor pollutant estimates (Krämer et al. 2000; Pattenden et al. 2006). Could the associations between air pollution and pediatric respiratory outcomes found in previous studies based on outdoor concentrations be type I errors? Or were these outdoor concentrations a proxy for other environmental effects (e.g., moving from single to co-exposures related to built environment models)?

Conflicting findings in past studies of air pollution epidemiology may have been due not only to exposure misclassification but also to poor outcome definitions and the lack of identification of susceptible subgroups. Were associations found in large population-based studies capturing a heterogeneous population encompassing several susceptible subgroups? A possible means of addressing this point in the

era of “big data” is by complementing incidence studies with the examination of the trajectory of this chronic heterogeneous outcome to encompass several endotypes of asthma. Two lines of research, detailed in the following sections, can be envisioned arising from these questions: 1) an exposure assessment research agenda and 2) cohort research with a focus on asthma endotype characterization.

### 6.2.1 Future directions for exposure assessment research

Modeling indoor $NO_2$ across micro-environments where a child typically spends known amounts of time has been shown to correlate well with personal measurements and to be implementable without prohibitive financial costs and overburdening participants in birth cohort studies (Mölter et al. 2012b). The next feasible step could be reconstructing a total exposure profile by time-averaging exposures in each micro-environment (Gulliver and Briggs 2005), especially because children do not display great variability in their time-activity patterns (Adgate et al. 2004). This approach holds promise to reduce exposure misclassification for cohort studies such as CHILD, especially in the presence of low levels of air pollution that require additional precision.

With regard to exposure modeling, capturing infiltrated exposure can either be modeled based on particulate matter, or modeled using simplified chemical mass balance models, such as the INDAIR model used in the MAAS cohort study (Mölter et al. 2013). There are ongoing efforts to model infiltration efficiencies using easily obtainable predictors (Allen et al. 2012a) because infiltration may be an important source of exposure heterogeneity in epidemiologic studies of exposure to pollutants of outdoor origin. Similar models should be considered for use in Canadian settings, especially considering the efforts deployed in characterizing the physical environment in the CHILD study.

Hopanes can be found in different media, including house dust or window wipes and we explored their potential use in house dust. However, house dust as an exposure matrix has limitations related to the mode of accumulation. Several factors that may vary among study participants can affect the

concentrations of hopanes: cleaning practices and sampling surfaces (carpeted versus non-carpeted) play a significant role in the amount of chemicals that deposit inside the home. In addition, the metric of exposure for hopanes still lacks consensus, because hopanes can be measured in terms of the loading of chemicals (concentration normalized by surface area sampled) or expressed by the more traditional approach of normalized concentration to mass dust collected. The need to improve our understanding of the composition and deposition mechanisms in house dust is warranted. Work that was undertaken by Whitehead et al. with respect to PAHs in house dust should help guide future research (Whitehead et al. 2011, 2012, 2013). For example, it was shown that repeated measurements of house dust within study participants' homes can improve the precision of PAH exposure estimates and limit attenuation bias. Investigation of hopanes using window wipes is another avenue for exploratory research, which may show less heterogeneity than house-settled dust.

LUR models provide high spatial resolution for exposure estimates of within-city variability, which has been shown to vary as much as that between cities. However, in light of urbanization trends and subsequent urban sprawl, methods are needed to expand the domain of LUR surfaces to include peri-urban/semi-rural areas. Hybrid models, which take advantage of the ability of dispersion modeling (Molter et al. 2010) or satellite data (Mao et al. 2012) to cover large geographical areas, represent a promising area of research.

In the case of population-based birth cohort studies such as BAQS, exposure assessment improvement can also be obtained through linkage with records from ministries of education. Cross-walk linkage between personal health identifiers and personal education numbers could therefore allow for the geocoding of schools and the estimation of exposure levels at these locations where children spend a significant amount of time. Time-weighted averages based on the home and school micro-environments would help shed more light on asthma analyses conducted on school-age participants. In addition, GIS tools can be used to approximate journey exposures, as shown by Gulliver and Briggs (Gulliver and

Briggs 2005). While this future research agenda will have limitations due to the coexistence of public and private school systems, it may shed more light on the disease etiology by reducing exposure misclassification for the school-age period.

### 6.2.2 Future directions for asthma and allergies research

As mentioned previously, prospective birth cohorts are the most powerful study design to assess the long-term effects of air pollution exposure on children's respiratory health, taking into account that certain limitations as outlined above (see section 6.1.3) are addressed. In CHILD, the definition of childhood atopy should be defined as a continuous outcome to avoid a heuristic, albeit often-used, 2mm cut-off. The follow-up over time of allergy tests co-examined with other allergic phenotypes (e.g., eczema) can be used to predict different endotypes of asthma and to help understand the relationship between atopy and asthma. The lack of understanding of this relationship is related to the absence of a universally accepted definition of asthma. It is increasingly recognized that asthma actually represents several different diseases presenting with similar symptoms. Although sharing similar phenotypes, these distinct endotypes arise via unique and separate pathophysiological mechanisms that are likely to be associated with different environmental exposures and genetic markers (Lötvall et al. 2011). The most desirable aspect of endotyping asthma and atopy is that such endotypes would be expected to be more stable than their observable phenotypic features (e.g., wheezing for asthma or sensitization for atopy) (Anderson 2008; Custovic et al. 2013; Henderson 2014b). A more precise case definition that would result from this new paradigm of diagnosis would reduce the dilution of a true association with environmental exposures of interest. There has been a shift in the analysis of observed or collected symptom data in recent cohort research, mainly relying on new statistical approaches including latent class analysis (Henderson 2014b) with clusters replicated across different cohorts (Savenije et al. 2011), and on the unsupervised clustering

approach (PCA) to determine groups of phenotypes and define specific asthma endotypes in an unbiased way (Custovic et al. 2013; Lazic et al. 2013; Simpson et al. 2010). An improvement on the unsupervised approach to generate asthma classifications is adding the dimension of time to take into account longitudinal phenotype changes that may not be stable over time. We explored the use of Group Based Trajectory Modeling (GBTM) due to the fact that this tool offers promising research directions. For example, while our work examined time-invariant factors, GBTM can also incorporate time-varying covariates as well as multiple outcomes (Nagin and Odgers 2010), thus offering the possibility of examining co-morbidities (e.g., obesity) or behavioral outcomes (e.g., school absenteeism) that either overlap with or precede the development of asthma.

From a translational research perspective, such an approach can help inform the design of endotype-specific treatments. From a public health intervention standpoint, the ability to conduct informative population-based studies is dependent upon identifying different, longer-term asthma endotypes, such as persistent allergic asthma, that may be amenable to public health intervention via air pollution reduction measures or urban planning and design initiatives. Gilliland describes an additional facet to the exposure reduction paradigm by demonstrating that treatment of asthma phenotypes may be improved by the induction of enzymatic antioxidant defenses to pollutant insults to the immune system via dietary means, especially for individuals with high-risk genetic variants of key antioxidant enzymes (Gilliland 2009).

The identification of a potentially protective role of greenness in this work is novel and important to note. Greenness may mitigate the effects of air pollution by improving air quality (Escobedo et al. 2011). Several other mechanisms by which the protective effect of green spaces may offset the risks of asthma and allergies, as outlined in the subsection 6.1.3.1, need to be explored further. Of particular interest is the finding by Mitchell and Popham (Mitchell and Popham 2008) that those exposed to the greenest environments also have the lowest levels of health inequality. Subsequent studies confirmed that the beneficial effects of green spaces on pregnancy outcomes seem to be more prominent among segments of

the population with low socio-economic status (Dadvand et al. 2012a). Because pregnancy outcomes have an impact on the newborn's immune system, such studies need to be extended to pediatric outcomes. Physical environments that promote good health, such as green spaces, might be important to reduce socioeconomic health inequalities and therefore reduce the susceptibility of the lower-SES groups to the adverse effects of air pollution on respiratory health. Future studies of the effects of air pollution on pediatric chronic respiratory diseases would greatly benefit from the inclusion of other attributes of the built environment (e.g., green spaces, "blue" spaces, walkability) in order to inform policy-making decisions.

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# Appendices[19]

## Appendix A Allergy skin prick testing

### A.1 Performing the allergy skin prick test

#### A.1.1 Allergy skin prick supplies

- Allergens (ALK Abello)
- Duotip II Dipwell Trays (Lincoln Diagnostics)
- Duotips II (Lincoln Diagnostics)
- Bic Ultra Round Stic Grip pen for circling wheals
- 3M Scotch® Tear-by-hand™ Tape 105-NA, 48 mm x 16 mm
- Antiseptic Isopropyl Alcohol Pad
- Tissues
- Epipen
- Stop watch or timer
- Allergy Skin Test Record Forms – Skin Test Worksheet

#### A.1.2 Quality control of allergens

Some allergens may not expire for up to 3 years. The variability of allergen effectiveness at room, fridge and Dipwell temperature is unknown. For this reason we will replace each antigen yearly at all centers. The central site will order the same lot numbers for all study centers on a yearly basis and keep logs to track lot numbers and expiry dates

[19] CHILD Standard Operating Procedure from © CHILD Version date May 15 2010; accessed from health diary resources, adapted with permission from Principal Investigator.

### A.1.3 Timeline and checklist

| | DAD | MOM | CHILD | | | |
|---|---|---|---|---|---|---|
| **Allergen (ALK Item #)** | **18 wk** | **1 year** | **1 year** | **2 year (PFT)** | **3 year** | **5 year** |
| 1. Histamine | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 2. Glycerin Control | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 3. Alternaria *alternate* | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 4. Cladosporium | ✓ | ✓ | | | ✓ | ✓ |
| 5. Penicillium Mixed | ✓ | ✓ | | | ✓ | ✓ |
| 6. Aspergillus *fumigatus* | ✓ | ✓ | | | ✓ | ✓ |
| 7. Cat Hair, Standardized | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 8. Dog Epithelium | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 9. D. Pteronyssinus | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 10. D. Farinae | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 11. Cockroach, German | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 12. Trees Midwest | ✓ | ✓ | | | ✓ | ✓ |
| 13. Grass Mix | ✓ | ✓ | | ✓ | ✓ | ✓ |
| 14. Weeds | ✓ | ✓ | | | ✓ | ✓ |
| 15. Ragweed Mixed | ✓ | ✓ | | ✓ | ✓ | ✓ |
| 16. Peanut | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 17. Milk, Whole Cow's | | | ✓ | ✓ | ✓ | ✓ |
| 18. Egg White | | | ✓ | ✓ | ✓ | ✓ |
| 19. Soybean | | | ✓ | ✓ | ✓ | ✓ |

| **Child-Dipwell Tray 2: Labels at 1 year** | | | | | |
|---|---|---|---|---|---|
| 9. D. Pteronyssinus | 8. Dog Epithelium | 7. Cat Hair, Standardized | 3.Alternaria alternata | 2.Glycerin Control | 1. |
| 19. Soybean | 18. Egg White | 17. Milk, Whole Cow's | 16. Peanut | 11. Cockroach, | 10. D. Farinae |

### A.1.4 Skin testing procedure

Directions for using the Dipwell Tray II: The tray consists of 40 clear plastic wells that hold test solutions and Duotip-Test II devices. The 40 wells are arranged in four rows of ten, with each row identified by letters A through D.

i. Type or print the appropriate extract or control information in the blank boxes provided on the label. Apply labels over designated areas (each label contains 10 rectangular spaces).

ii. Remove sterile wells from their package of 40 and insert 16 wells (2 rows of 8). Use enough pressure when placing wells into the receiving holes to push them downward as far as they will travel.

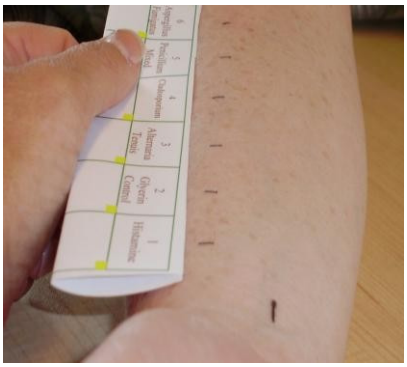
iii. Individual test solutions are placed into individual wells so that each well contains the test solution that corresponds with that test solution's well location shown on the label.

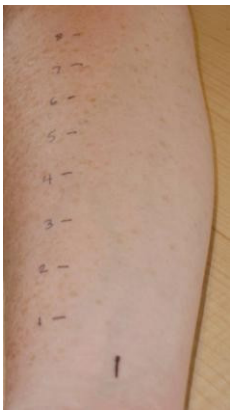
iv. Dispense 0.25 ml of each skin test solution into its properly identified and numbered well, using the dropper in the 10 mL vial. The dropper must be inserted down into the well. Each well must contain enough volume to allow complete submersion of the Duotip II (at least 4 drops of solution from the vial dropper).

v. Place clean duotips in the wells and place the lid securely over duotips/ wells and keep refrigerated when not in use. Remove tray with wells 15 minutes before skin testing and keep at room temperature.

### A.1.4.1 Procedure for skin prick test

1. Explain procedure to child's mother or father as appropriate. Provide a Skin Prick Test Result Informational Handout (Appendix A.2 Figure 2)
2. Ascertain whether the child has taken medications or herbal supplements in the last 7 days. Give medication/supplement prompt list (Appendix A.2 Supplementary Material 2) as a prompt and fill out the medication/supplement skin test form (Appendix A.2 Figure 3).
3. Seat the child in the parent's lap if possible and place their forearm in a supine position on a flat surface.
4. Clean and dry inner aspect of child's forearms with an alcohol swab (70% isopropyl alcohol).
5. Make small dots with pen on the radial aspect of the forearm, in rows corresponding to the numbers of allergens that will be used. Include the controls in the total number. Use a folded photocopied allergy skin test sheet to space the dots by placing the dot directly beside the numbers.

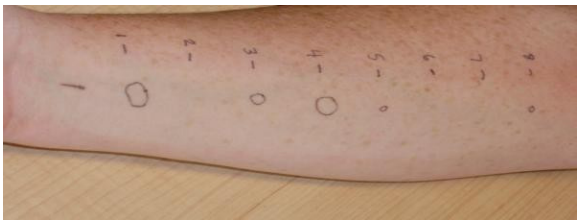




6. Identify the allergens by corresponding allergen numbers on the radial aspect of the

appropriate dot so that the labelling will not be included in the results.

7. Place one sterile Duotip II in each allergen well. Follow the order on the skin test form
8. At a site 2 cm medial (ulnar) to the pen mark, using a sterile Duotip for each allergen, hold device so its shaft forms a 45 degree angle with the skin plane. Prick **with one point** and lift skin simultaneously. Do not prick twice.
9. Discard Duotip in the appropriate sharps container.
10. Blot (DO NOT WIPE) skin dry with tissue making sure not to cross contaminate allergens.
11. Instruct child not to scratch the area and to remain in the waiting area until tests are read.
12. Ten minutes after the first (Histamine) prick, use a Bic pen to outline the wheal of the Histamine. A wheal response, also called a welt, is a firm elevated swelling of the skin. Use your fingers to feel the swelling or bump to help identify a wheal response. After 20 minutes, outline wheal responses for the allergens. Wheal tracings must be closed without any gaps. Do not record the flare. If there is no wheal, do not make any marks. Use cross-lighting if needed to identify the edges of the wheals.



13. Using transparent adhesive tape, cut a piece large enough from the roll to cover the histamine outline, press gently, and remove.
14. Apply tape to the skin test form.
15. Repeat for all other allergy wheals that are outlined.
16. All allergy sheets are bar coded and numbered specifically for each case. Tape must adhere completely flat (ideally without any creases and without hair or other material attached).

Wheal tracings cannot cross the allergen box or touch the yellow box on the skin test form. (See Appendix A.2 Figure 5, for an example of a correctly completed form).

17. If there is hair attached to the tape that you cannot readily remove:
    a. Stick the tape to a BLANK piece of paper.
    b. Place another piece of tape, sticky side up, and trace the outline on the sticky side of the new piece of tape.
    c. Transfer the new piece of tape with copied outline to the skin test worksheet.
18. Wipe the arm with an alcohol swab to remove ink and to help decrease itch.

Allergy Skin Testing for the child at 1 year should not be done in the field. The child's test must be done in the clinic

### A.1.4.2 Taking care of allergens and Epipen instructions

Testing will be to common outdoor allergens, indoor allergens and food allergens. The testing will be carried out by the study personnel listed as per SOP. The local site investigator or his/her physician delegate must be aware of the testing and be available if an adverse event occurs. If the investigator is not available, an alternate contact approved by the investigator must be available.

FALSE POSITIVES

may occur if…

i. the prick is too hard
ii. patient has sensitive skin (i.e. Dematographism)
iii. there is cross contamination between allergens

FALSE NEGATIVES

may occur if…

i. the antigen site is not pricked hard enough or skin is not lifted
ii. antihistamines or medication with antihistamine activity (e.g., Gravol, antidepressants such

as amitryptiline) have not been withheld for the appropriate length of time

CARING FOR ALLERGENS

i. Refrigerate allergens when not in use.
ii. Take the allergens out of the fridge 15 minutes before using and leave at room temperature.
iii. Take great care to return dropper into correct vial to avoid cross contamination.
iv. Discard allergen if the extracts look cloudy.

SAFETY PRECAUTIONS: Always have two Epipens or Twinjects available at the testing site and check the expiry dates regularly.

If systemic symptoms including urticaria, rhinitis, conjunctivitis, angioedema, cough, wheezing, faintness or hypotension occur, give 1 injection of Epipen intramuscularly. Obtain vital signs. Ensure that the physician attends immediately. Repeat the dose of Epipen after 5 or 10 minutes if necessary.

An Epipen Jr is a disposable, prefilled automatic injection device used for allergic emergencies. They contain a single dose of epinephrine which is injected into the outer thigh. It has an expiry date and should be replaced before this date. It should be kept at room temperature. Do not store in refrigerator. For complete instructions see Supplementary material 1: Epipen instructions.

### A.1.4.3 Measurements of Wheals

1. Introduction and Purpose: To measure the taped skin prick reactions to assess individual hypersensitivity to allergens.
2. Scope: This SOP applies to the activities involved in assessing children enrolled in the CHILD Study.
3. Responsibility: The Data Manager at the Winnipeg site will ensure appropriate final analysis of allergy skin test measurements using computer analysis and recognition.
4. Measurement Procedure

a. The allergy forms are bar coded and numbered specifically for each study participant.

b. Skin tests will be initially measured in Hamilton and recorded on the subject's Skin test data form (Figure 4, page 23).

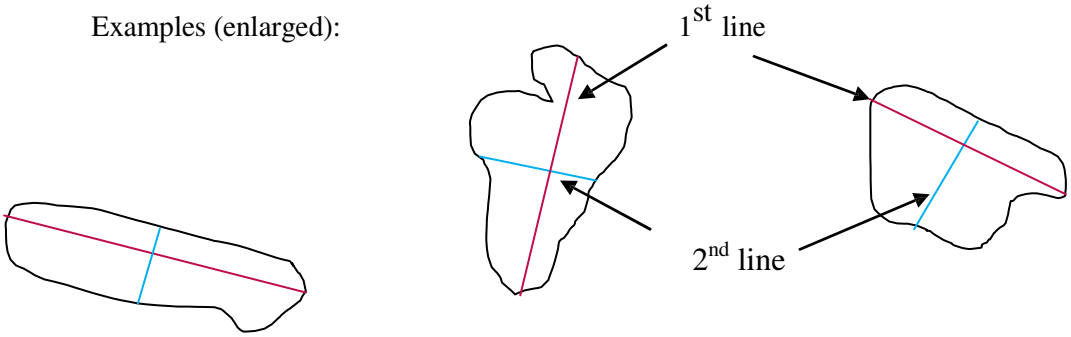
c. Measurements will be taken of the wheal only and be made to the nearest 0.5 mm.

d. The length (1st line, seen below) of the wheal is measured first, followed by the width measured at the midpoint of, and perpendicular to, the length (2nd line, seen below).

e. The length and width will be double entered into HealthDiary.

f. Original forms will then be sent to the Winnipeg Pediatric Allergy Lab, and scanned into the Winnipeg database.

Examples (enlarged):



## A.2 Figures and supplemental materials

### A.2.1 Appointment Letter – Child

Dear Ms. __

Thank you for your willingness to participate in the Canadian Healthy Infant Longitudinal Development (CHILD) Study. We look forward to meeting you at the clinic.

We have arranged an appointment for your child to have a clinical assessment and an allergy skin test and to give a sample of blood. We will see you at

**PLACE**

**DAY**

**DATE**

**TIME**

**If you need to change this appointment, please call the number at the end of the letter**

Please read the following information regarding the allergy and breathing tests.

**What is an allergy skin prick test?**

The test is done to see if you have sensitivities to common substances such as molds, pollens, animal dander, dust mites and some foods to name a few. Before the test, you will be asked about your child's medication history and any antihistamines he/she may have taken. A series of 12 pricks will be made on the child's arm with plastic Duotip devices. Each one carries a drop of an allergen into the superficial layer of the skin. You are asked to wait 20 minutes and then we measure any reactions on your child's arm. A reaction looks very similar to a mosquito bite, with itchiness, redness and swelling of the skin.

**What is involved in the clinical assessment?**

The study (*paediatrician, team, nurse, staff etc* ) will examine your child's skin for any signs of skin redness or inflammation, listen to his/her chest and take a height, weight and head circumference measurement.

**HOW SHOULD I PREPARE MY CHILD FOR THE APPOINTMENT?**

The study staff would also like to collect a stool sample, urine sample and nasal swab.

**For 4 days before the appointment:**

Please do not give your child any anti-histamines (medications such as Hismanol, Benadryl, cough medications, Gravol or motion sickness medication) as these will affect the allergy skin prick test results.

When you come in to the clinic, **bring all your child's medications with you including any over the counter medication,** as treatments may affect the results of these tests in several ways, and so we need to know exactly what your child is taking.

I have attached a map, directions and our contact numbers.

We look forward to seeing you.

Sincerely;

Dr. [site coordinator]

For any change of appointment, call ____

## A.2.2 Informational Skin Test Handout

# Skin Prick Test Results for your child

*What do the results mean?*

CHILD

***What is an allergy skin prick test?***

An allergy skin prick test is the most common allergy test used to provide information about a person's sensitivity to certain substances (allergens). It is used to test for environmental and food allergies. Examples of environmental allergens are grass pollens, trees, and weeds, mold spores, dander from cats, dogs and other furry animals, debris from cockroaches, and house dust mites. Common foods that may cause allergies are milk, soy, eggs, peanuts, tree nuts, fish, shellfish, and wheat.
**The test** includes a "positive" and "negative" control. The positive control will cause a reaction on all people and confirms that the test is being done properly. A reaction is a swelling of the skin that looks like a "bump" referred to as a wheal. Most people do not react to the negative control, however there may be some "redness" due to irritation from the prick itself. After allergens are put on the skin, the wheals are circled with a pen after 15 - 20 minutes and recorded on a test result sheet.

***What does a negative test result mean?***

In most cases, a negative response to allergens in a skin prick test indicates that your child is not sensitive to that allergen. Sometimes children may have a negative test but still be allergic to the substance. This may occur if your child is taking anti-histamines or medications that block the effect of histamine.

***What does a positive test result mean?***

A positive test result means that your child reacted to a specific allergen. Often, but not always, a positive test result means that your child has been exposed to the substance and has sensitivity to it. In general, a stronger response means that your child is more sensitive to that substance. Doctors generally regard wheals over 3 mm in diameter as positive.

Skin tests are generally reliable, however false test results can occur. It is not uncommon for children to have positive responses to one or more substances but not have any problems with those specific substances in everyday life.

The repeatability of allergy testing varies quite a bit. Even the same test performed at different times on a person may give different results.

**Skin prick testing with food allergens is less reliable than with other allergens such as dust mite and pollens.**

***What should I do if my child has a positive skin allergy skin test?***

**It is important to understand that skin prick testing with food allergens may show a positive test (sensitization) in the absence of any true allergy to the food**. If your child has a positive test for any food but regularly eats that food without any obvious problem there is no reason to stop the food. If there is any concern, your child's doctor or allergist can help sort this out. The CHILD study staff would be happy to provide you with a copy of the test result so you can take it to your doctor.

Young children who have food allergies often outgrow their allergy — but not always. For example, most kids who are allergic to milk, eggs, wheat, or soy outgrow their allergies by the time they are 5 years old. Nut allergies are less likely to be outgrown.

If you have any questions, please call the CHILD team at 789-3978

### A.2.3 Child medications and supplements form

**Child Skin Test Meds/Supplements** ID | | | | | Y|2|0| | |M| | |D| |

1. Has your child taken any antihistamines in the last 7 days? ○ Yes ○ No

   1.1 If Yes, specify date and time taken:

   |Y|R|2|0| | |M| | |D| | | | | : | | | (24-hour clock)

2. Has your child taken any prescription or over-the-counter medications in the last 7 days?

   ○ Yes ○ No, go to Q3

   2.1 Name of drug: _____

   2.1a Date and time taken:

   |Y|R|2|0| | |M| | |D| | | | | : | | | (24-hour clock)

   2.2 Name of drug: _____

   2.2a Date and time taken:

   |Y|R|2|0| | |M| | |D| | | | | : | | | (24-hour clock)

   2.3 Name of drug: _____

   2.3a Date and time taken:

   |Y|R|2|0| | |M| | |D| | | | | : | | | (24-hour clock)

3. Has your child taken any of the drugs or herbal supplements listed on the Medications and Supplements Prompt list in the last 7 days?

   ○ Yes ○ No, go to Q4

   3.1 Name of drug: _____

   3.1a Date and time taken:

   |Y|R|2|0| | |M| | |D| | | | | : | | | (24-hour clock)

   3.2 Name of drug: _____

   3.2a Date and time taken:

   |Y|R|2|0| | |M| | |D| | | | | : | | | (24-hour clock)

   3.3 Name of drug: _____

   3.3a Date and time taken:

   |Y|R|2|0| | |M| | |D| | | | | : | | | (24-hour clock)

4. What is TODAY'S DATE? |Y|R|2|0| | |M| | |D| | |

5. Time skin prick test started (from histamine prick): | | | : | | | (24-hour clock)

6. Time skin prick test read: | | | : | | | (24-hour clock)

7. RA performing test: | | |

********** FOR ADMINISTRATIVE USE ONLY **********

SITE OFFICE

Interviewer: | | |

(First number is site-specific; If no interviewer, enter 099)

Date checked: |Y|R|2|0| | |M| | |D| | |

Data checked for completeness by: | | |

### A.2.4 Skin test measurement form

## Child's Skin Prick Test One Year

ID | | | | | Y 2 0 | | M | | D | |

### ALLERGY SKIN PRICK TEST

All the measurements are in millimetres.

| | | | |
|---|---|---|---|
| 1. | Positive control (Histamine): | Wheal width | Wheal length |
| 2. | Negative control (Glycerin): | Wheal width | Wheal length |
| 3. | Alternaria Tenuis: | Wheal width | Wheal length |
| 7. | Cat Hair, Standardized: | Wheal width | Wheal length |
| 8. | Dog Epithelium: | Wheal width | Wheal length |
| 9. | D. Pteronyssinus: | Wheal width | Wheal length |
| 10. | D. Farinae: | Wheal width | Wheal length |
| 11. | Cockroach, German: | Wheal width | Wheal length |
| 16. | Peanut: | Wheal width | Wheal length |
| 17. | Milk, Whole Cow's: | Wheal width | Wheal length |
| 18. | Egg White: | Wheal width | Wheal length |
| 19. | Soybean: | Wheal width | Wheal length |

********** FOR ADMINISTRATIVE USE ONLY **********

SITE OFFICE

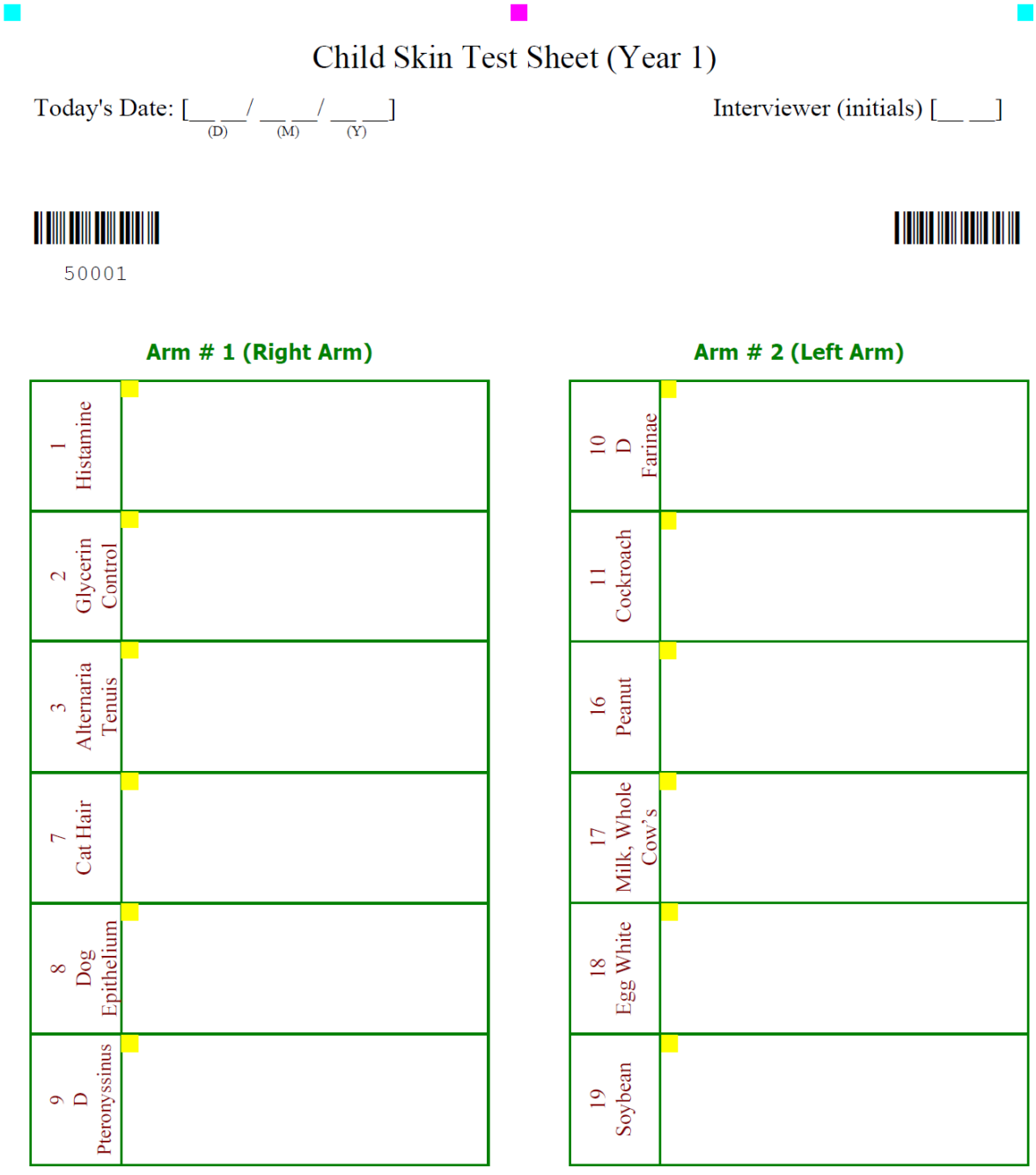
Interviewer | | |

(First number is site-specific; If no interviewer, enter 099)

Data checked for completeness by: | | |

Date checked: Y R 2 0 | | M | | D | |

### A.2.5 Skin test result form



Child Skin Test Sheet (Year 1)

Today's Date: [__ __/ __ __/ __ __] (D) (M) (Y)

Interviewer (initials) [__ __]

50001

**Arm # 1 (Right Arm)**

| | |
|---|---|
| 1 Histamine | |
| 2 Glycerin Control | |
| 3 Alternaria Tenuis | |
| 7 Cat Hair | |
| 8 Dog Epithelium | |
| 9 D Pteronyssinus | |

**Arm # 2 (Left Arm)**

| | |
|---|---|
| 10 D Farinae | |
| 11 Cockroach | |
| 16 Peanut | |
| 17 Milk, Whole Cow's | |
| 18 Egg White | |
| 19 Soybean | |

### A.2.6 Supplementary material 1: Epipen instructions

## DIRECTIONS FOR USE

- **NEVER PUT THUMB, FINGERS, OR HAND OVER BLACK TIP.**
- **DO NOT REMOVE GRAY SAFETY RELEASE UNTIL READY TO USE.**
- **DO NOT USE IF SOLUTION IS DISCOLORED OR RED FLAG APPEARS IN CLEAR WINDOW.**
- **DO NOT PLACE PATIENT INSERT OR ANY OTHER FOREIGN OBJECTS IN CARRIER WITH AUTO-INJECTOR, AS THIS MAY PREVENT YOU FROM REMOVING THE AUTO-INJECTOR FOR USE.**

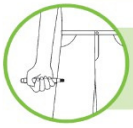

**1.** Unscrew the yellow or green cap off of the EpiPen® or EpiPen® Jr carrying case and remove the EpiPen® or EpiPen® Jr auto-injector from its storage tube.

**2.** Grasp unit with the black tip pointing downward.

**3.** Form fist around the unit (black tip down).

**4.** With your other hand, pull off the gray safety release.

**5.** Hold black tip near outer thigh.



**6.** Swing and **jab firmly** into outer thigh until it clicks so that unit is perpendicular (at a 90° angle) to the thigh. (Auto-injector is designed to work through clothing.)

**7.** Hold **firmly against thigh** for approximately 10 seconds. (The injection is now complete. Window on auto-injector will show red.)

**8.** Remove unit from thigh and massage injection area for 10 seconds.

**9.** Call 911 and seek immediate medical attention.

**10.** Carefully place the used auto-injector (without bending the needle), needle-end first, into the storage tube of the carrying case that provides built-in needle protection after use. Then screw the cap of the storage tube back on completely, and take it with you to the hospital emergency room.

**Note: Most of the liquid (about 85%) stays in the auto-injector and cannot be reused. However, you have received the correct dose of the medication if the red flag appears in window.**

**⚠ WARNING**

- **NEVER** put thumb, fingers, or hand over black tip. The needle comes out of black tip. Accidental injection into hands or feet may result in loss of blood flow to these areas. If this happens, go immediately to the nearest emergency room.
- EpiPen® and EpiPen® Jr should be injected only into the outer thigh (see "Directions for Use").
- Do NOT remove gray safety release until ready to use.

## A.2.7 Supplementary material 2: medications and supplements Prompt List

### Medications and Supplements that affect skin test.

| Brand Name | Generic Name | Use/Conditions | Mean Days Suppressed | Max Days Suppressed | Dose |
|---|---|---|---|---|---|
| **Tricyclic Antidepressants and tranquilizers** | | | | | |
| Norpramin | Desipramine | Depression | 2[7b] | | 25 mg single |
| Tofranil | Imipramine | Depression/ Bed Wetting | >10[8c] | | |
| Sinequan | Doxepin | Depression | 6[7b] | | 25 mg single |
| Zonalon | Doxepin topical | Anti-itch | 11[9c] | | |
| **Histamine$_2$ Antihistamines** | | | | | |
| Zantac | Ranitidine | Ulcers, Stomach Acid | <1[10c] | | 150 mg single |
| **Cysteinyl leukotriene antagonists** | | | | | |
| Singulair | Monteleukast | Asthma (bronchospasm) | 0[11,12] | | 10 mg |
| Accolate | Zafirlukast | Asthma | 0[13] | | 20 mg |
| **Local anesthetic** | | | | | |
| EMLA cream | Lidocaine-Prilocaine | Prevents Needle pain | 0 wheal[14] 150-100%[14] | | 5 g over volar surface of arm 1 hour before test suppression of erythema |

| Brand Name | Generic Name | Use/Conditions | Mean days Suppressed | Max. days Suppressed | Dose |
|---|---|---|---|---|---|
| **First Generation** | | | | | |
| Aller-Chlor | Chlorpheniramine | Allergic Conjunctivitis | 2[1b],3[2c] | 6[1c] | 4 mg 4 times daily |
| C.P.M. | Chlorpheniramine | Allergies | 2[1b],3[2c] | 6[1c] | 4 mg 4 times daily |
| Chlo-Amine | Chlorpheniramine | Allergies | 2[1b],3[2c] | 6[1c] | 4 mg 4 times daily |
| Chlor-Alllergy | Chlorpheniramine | Allergies & Colds | 2[1b],3[2c] | 6[1c] | 4 mg 4 times daily |
| Chlor-Mal | Chlorpheniramine | Allergies & Colds | 2[1b],3[2c] | 6[1c] | 4 mg 4 times daily |
| Chlor-Trimeton | Chlorpheniramine | Allergic Conjunctivitis | 2[1b],3[2c] | 6[1c] | 4 mg 4 times daily |
| Chlorphen | Chlorpheniramine | Colds, Flu & Allergies | 2[1b],3[2c] | 6[1c] | 4 mg 4 times daily |
| Allerhist-1 | Clemastine | Allergies & Colds | 5[3c] | 10[3c] | 1 mg twice daily |
| Contac 12 Hr. Allergy | Clemastine | Allergies & Colds | 5[3c] | 10[3c] | 1 mg twice daily |
| Tavist-1 | Clemastine | Allergies & Colds | 5[3c] | 10[3c] | 1 mg twice daily |
| Periactin | Cyproheptadine | Allergies | 9[4c] | 11[4c] | 8 mg/d |
| Polaramine | Dexchlorpheniramine | Allergic Conjunctivitis | 4[4c] | 4[4c] | 4 mg/d |
| Actifed Sinus Day | Diphenhydramine | Allergies, Flu, Colds | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Aler-Dryl | Diphenhydramine | Itching & Pain | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Benadryl | Diphenhydramine | Allergies & Colds | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Calm-Aid | Diphenhydramine | Sleep | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Compoz Nighttime | Diphenhydramine | Sleep | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Diphedryl | Diphenhydramine | Allergic Conjunctivitis | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Diphen-Allergy | Diphenhydramine | Parkinson Symptoms | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Genahist | Diphenhydramine | Parkinson Symptoms | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Hydramine | Diphenhydramine | Parkinson Symptoms | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Nytol | Diphenhydramine | Sleep | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Scot-Tussin Allergy | Diphenhydramine | Allergies & Colds | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Sominex | Diphenhydramine | Sleep | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Twilite | Diphenhydramine | Sleep | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Tylenol PM | Diphenhydramine | Allergies, Sleep, Pain | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Unisom Sleepgels | Diphenhydramine | Allergies & Sleep | 2[2c] | 5[2c] | 50 mg 4 times daily |
| Atarax | Hydroxyzine | Nervousness | 5[2c] | 8[2c] | 25 mg 4 times daily |
| Rezine | Hydroxyzine | Nervousness | 5[2c] | 8[2c] | 25 mg 4 times daily |
| Phenergan | Promethazine | Nausea & vomiting | 3[2c] | 5[2c] | 25 mg 4 times daily |
| Promethazine | Promethazine | Motion sickness | 3[2c] | 5[2c] | 25 mg 4 times daily |
| Prorex 25 & 50 | Promethazine[89] | Nausea & vomiting | 3[2c] | 5[2c] | 25 mg 4 times daily |
| PBZ & PBZ-SR | Tripelennamine | Allergic Conjunctivitis | 3[2c] | 7[2c] | 50 mg 4 times daily |
| **Second Generation** | | | | | |
| Astelin | Azelastine | Nose allergies | 2[5,6c] | | 1% twice daily |
| Optivar | Azelastine | Eye allergies | 2[5,6c] | | 1% twice daily |
| Zyrtec | Cetirizine | Allergies & Hives | 3[5c] | | 10 mg/d |
| Allegra | Fexofenadine (60mg) | Allergies & Hives | 2[5c] | | 60 mg twice daily |
| Alavert | Loratadine | Allergies & Hives | 7[5c] | | 10 mg/d |
| Claritin | Loratadine | Allergies & Hives | 7[5c] | | 10 mg/d |
| Livostin | Levocabastine Opth | Allergic Conjunctivitis | 0[5c] | | 0.05% twice daily |
| | Levocabastine nasal | | 0[5c] | | 50 micro/sp twice d |

## Appendix B: Home assessment (including dust sampling)

### B.1 Preparation of the visit

#### B.1.1 Goals

The research coordinator will call the mother to set up a 3-month home visit 2 weeks in advance and will follow the phone script template. Ideally, the research coordinator who recruited the family into the study will make the first postnatal telephone call approximately 1-2 months after delivery. The goals are:

- To reconnect with the family.
- To ensure that the family is still eligible (i.e. has not moved, has not refused to further participate, etc.).
- To arrange for a home visit: to discuss possible dates and times that might be feasible for a visit, and to confirm the address.
- To go over home visit package instructions for breast milk collection and stool collection
- To ask the mother's to fill out the Environmental home questionnaire
- To remind the parent about collection of house dust from the bed the child sleeps in and the most often used living area

The participant is reminded about the confidentiality and all measures taken to maintain their information non-identifiable. Two days before the visit, phone to confirm the visit and ask the mother if she has her breast milk container. Remind the mother to start collecting breast milk a day before the visit, and to save a soiled diaper and a wet diaper from the day before the visit.

### B.1.2 Before the home visit

#### B.1.2.1 Home assessment kit protocol

1. Prepare Home Assessment Kit

   Sanitaire Canister Vac: Model S3680

   (Serial#074002221)

   a) Connection Hose

   b) Telescoping Wand

   c) Vacuum Bag

   d) Vacuum Head - depyrogenated

   e) Sterile Tweezers

   f) Sterile scissors

2. Cooler
3. Ice Pack (not included)
4. Tool Bag

   a) Laser Distance Meter

   b) GPS

   c) Flashlight

   d) Tape Measure

   e) Green Painter's Tape

   f) Scissors

   g) Pens (not included)

   h) Sharpie

   i) Extra Batteries

5. Two Clipboards

**"Hognose" © Vacuum Head**

6. Driver's Log Book
7. Dirty Equipment Bag
8. Extra Soiled Diaper Bag
9. Sample Collection Box
    a) Refractometer
    b) 20 mL Syringe
    c) 3 Cotton Pads
    d) Nitrile Gloves
    e) Respirator Mask (3M 8210 N95)
    f) Alcohol Wipes


10. Map book (not included)
11. Samples' Bag
    a) 6 pre-labelled cryovials: See SOP
    b) Extra Breast Milk Bottle (x1): See SOP
    c) Dust Bottles (x4)
    d) 4 stool pre-labelled cryovials: See SOP
    e) Nasal Swab and Media (x1) and 6 pre-labelled cryovials: See SOP
12. Extra Biohazard Bags
13. Postnatal Questionnaire Package

### B.1.2.2 Protocol for cleaning

- Vacuum heads and tweezers before visit:
    - Rinse the dirty vacuum head and tweezers with water for 5 minutes.
    - Remove the Teflon wheels of the vacuum head
    - Wrap both the head (without wheels) and tweezers together in tin foil.

- Put both in the laboratory oven (Fisher Oven ISOTMP STD 2.5 Cubic ft. Cat @ 13 247 625G) and bake at 220°C for 2 hours. Remove from oven and allow to cool.

- Protocol for cleaning vacuum: After use, clean the vacuum, especially the wheels, with an alcohol wipe to prevent dust contamination from house to house.
- Protocol for cleaning refractometer:
    - After use, rinse the refractometer with water.
    - Pat dry with a paper towel.
    - Wipe with an alcohol swab.

### B.1.2.3 HealthDiary

Print out sample collection forms and labels from HealthDiary. These forms will contain pre-printed information including the subject's Identification number (ID), the visit (3MON), the sample type (NASAL, URINE, STOOL, BRSMILK, MUDUST, CBDUST), and a summary of collection instructions (see Appendix B.3 Figure 1, for example of completed Dust Collection Form).

- 6 aliquot labels for urine cyrovials
- 1 labeled for extra breast milk bottle (One is given to mother in advance at the birth visit)
- 1 sample (collection) label for diaper bag, 4 cryrovial (aliquot) labels
- 1 label for nasal sample vial, and 6 labels for cryovials.
- 2 sample labels for the dust collection bottles with thimbles for the Most Used Living Area (MUDUST).
- 2 sample labels for the dust sample collection bottles with thimbles for the child's sleeping area and bed (CBDUST; see Appendix B.2 Figure 2).

Ensure ice packs are frozen for the visit.

Take cooler with ice packs in them to the visit.

### B.1.3 At the home visit

Administer questionnaires and conduct home assessment:

| 3-Months Questionnaires/ Task | HD Code | Version |
|---|---|---|
| Q: Residences to 3 Months | Q160RESIDEN3M | 2009M11D11 |
| Q: Home Environment 3 Months | Q114HENVQ3M | 2009M09D15 |
| Q: Environmental Assessment RA 3 Months | Q117ENVRA3M | 2009M08D07 |
| Q: Mother's Nutrition | | -- |
| Q: Mother's Meds-Birth to 3 Months | Q151MMED3M | 2009M08D31 |
| Q: Mother's Vitamins and Supplements 3 Months | Q154MSUPPL3M | 2009M11D30 |
| Q: Child Health 3 Months | Q127CHLTH3M | 2009M09D15 |
| Q: Child Medications-Birth to 3 Months | Q148CMED3M | 2009M08D31 |
| TCF: Mother Breast Milk 3 Months | BRSTMLK-F55SPECIMEN | 2008M12D15 |
| TCF: Child Nasal 3 Months | Nasal-F37SPECIMEN | 2008M12D15 |
| TCF: Child Urine 3 Months | Urine-F61SPECIMEN | 2008M12D15 |
| TCF: Child Stool 3 Months | Stool-F37SPECIMEN | 2008M12D15 |
| TCF: Dust Child's Bedroom 3 Months | CBDust-F47SPECIMEN | 2008M12D15 |
| TCF: Dust Most-Used Living Space 3 Months | MUDust-F47SPECIMEN | 2008M12D15 |
| TCF: Control Dust 3 Months | Urine QC-F61SPECIMEN | 2008M12D15 |

#### B.1.3.1 Dust samples

- Two thimbles of dust to be collected from the most used living area and two thimbles to be collected from the child's sleeping area (bed and floor). Two dust samples in total will be collected (from three sites): The first sample to be taken is a combined dust sample collected from the bed that baby sleeps (could be mom's bed if the baby sleeps with her) and the floor in the room where baby sleeps. A separate sample will be collected from the most used living area room floor.
- In conjunction with the home inspection, a dust sample will be collected from a 2- metre square area of floor (or greater area if necessary) using the above standardized consumer model

vacuum cleaner fitted with a dust collection device. This area is created by four separate squares of 0.71 m x 0.71 m. A mask may be worn if sensitive to dust. If there is not enough carpet, vacuum available carpet and check thimbles to see if full. If the thimble is full, no more vacuuming is necessary. Record the exact area in HealthDiary. If there is not enough dust, continue vacuuming any other flooring type and record the exact area.

- If the flooring is hardwood or laminate, the research assistant will vacuum as much area as possible with effort made to collect dust from the corners/edges of the room.

| **Carpeted area > 2 square metres total** | **Hardwood Only** | **Area rug < 2 square metres** |
|---|---|---|
| 1. Tape off 4 square areas of 0.71 m x 0.71 m in separate locations. (not in direct sunlight) | 1. Vacuum whole floor room to fill dust thimbles.<br><br>Measure and record the ***exact area*** vacuumed in HealthDiary. | 1. If there is an area rug less than 2 square metres, vacuum rug, check thimble. If there is enough dust, document the ***exact area*** vacuumed.<br><br>2. If there is not enough dust, vacuum hardwood area of the room and document |

First dust sample will be taken from the child's bedding and bedroom floor:

- Don a pair of nitrile gloves. Open the pyrogen-free sampling package. Remove the bag containing the tweezers, and carefully open the bag at the closed end of the tweezers. Do not touch the open end of the tweezers. Carefully remove the clean sampler head. Using the tweezers, place two collection thimbles into the sampler head, taking care to ensure that the parts are touched only by the tweezers. Connect the sampler head to the vacuum. Place the tweezers back into the sterile bag/tinfoil, with the open end of the tweezers going in first, and set them aside in an undisturbed place.
- Plug the vacuum cleaner into the nearest electrical outlet, using the extension cord if necessary. Try not to unplug household items, especially if it is something that requires being reset, such as a clock, microwave, or VCR. If you must unplug something, try to unplug a lamp. Do not

unplug refrigerators or freezers unless you are immediately able to plug it into a power strip. Be careful not to walk or drag the cord or vacuum through the floor area to be vacuumed (floor template area).

- Pull back the bed covers and the top sheet, and vacuum the bottom sheet.
- If the child sleeps in the parents' bed, vacuum the half of the bed usually used by the child. If a pillow is used, vacuum the pillow with the case on (the outside of the case), then remove case and vacuum the pillow or the subsequent cover (for a total 1.5-2 minutes; use timer). If child sleeps in a bassinette with a plastic- covered mattress, vacuum the cover or sheet.
- Using the same vacuum head and collection thimbles, the floor sample will be taken from four 0.71-metre squares that are each at different distances from the door of the room. This method is used when floor is carpeted.
- If there is not enough floor space in the child‟s bedroom, add the room in which the child spends the next most amount of time.
- With tape, mark four 0.71-metre squares for the sample.
- Choose the locations for the four sampling squares in the bedroom as follows:
    - One square on any cleared area where the child is likely to play.
    - Three other squares that are away from direct sunlight, outside the main foot-traffic pattern, and at different distances from door (this will often not be possible).
- Vacuum in one direction in a straight line inside the template, making 10 passes back-and-forth along each line until the area has been completely vacuumed. Using the same sampling filter, move to the next square and sample it in the same way until all four squares have been sampled. Do not include the area directly under the bed or wet areas.
- Holding the vacuum head with the screws facing up, unscrew the plate, remove the thimble using the sterile tweezers, and estimate the mass of dust collected. If the thimble is not full, reassemble

and continue to vacuum in the prescribed way, adding 0.71-metre squares as needed to obtain as much dust as is required. For some hard or very clean floors, you will need to vacuum the entire floor area (e.g. hardwood or laminate flooring). Measure and record the area vacuumed in this circumstance.

- When dust collection is complete, carefully open the labelled storage vial for the bedroom sample without touching the inside of the lid.
- Holding the vacuum head with the screws facing up, unscrew the plate, remove the thimble using the sterile tweezers, and transfer the thimble into the vial. Close the vial and store it in the transport bag.

<u>Second sample from the most used living area in which child plays on the floor</u>

Lay out four 0.71-metre squares with tape. If an area rug is present, use this first to reach the goal of a 2-metre square. Vacuum in the same manner as in the bedroom. Observe the thimbles for dust quantity. Once 2 m2 is vacuumed, hold the vacuum head with the screws facing up, unscrew the plate, remove the thimbles using the sterile tweezers, and transfer the thimbles into a storage vial labelled "most used living area" (MULA).

Leave the room as you found it. After collecting all samples required in a room, reconnect any lamps or other electrical devices that were disconnected. Remake bed as you found it, replacing pillows or stuffed animals in the same location. If the bed was unmade, leave it unmade. Replace any items you moved from the bed or floor surface in order to take the dust samples.

### B.1.3.2 Volume measurements

Use a work sheet to figure out the volume of the home (Figure 3: worksheet example).

Do not measure individual closets

Do not measure every room or hall way.

For basement volumes, if possible foundation length x width x height

### B.1.3.3 Take GPS measurements

1) Record position while standing at the front door. If a signal cannot be found at the front door, attempt to acquire coordinates at a point nearest to the front door on the perimeter of the residence.

2) Turn on the GPS using the 'power' button on the bottom right.

3) Once the 'tracking satellites' screen comes on (takes approx 5 seconds) push the top right button, labeled "page" 4 times. The Menu screen will come up and 'Mark' will be highlighted

4) Push the 'enter' button (bottom left) and the coordinates will be displayed.

5) Record coordinates on questionnaire.


6) Record accuracy measurement

7) Ensuring proper set up

of the GPS The reading

should be shown as:

N__ __ °_____ '__ __.__"

W__ __ __ °__ __' __ __.__" (See picture above)

If the reading is NOT shown as above, please correct format by the following:

8) Go back to menu by pressing the page button, top right.
9) Select "setup" on menu by using top left scroll buttons
10) Press enter , lower left
11) Select units, by using top left scroll buttons. Press enter
12) Position format , select enter

13) Select hddd 0mm"ss.s"
14) Press Page to get back

### B.1.4 After the home visit

- Questionnaires: Check questionnaires and home assessment forms for completeness.
- Aliquot biological samples:
    - Breast milk into 6 pre-labelled cryovials and frozen
    - Nasal samples into 6 pre-labelled cryovials and frozen
    - Stool sample into 4 pre-labelled cryovials and frozen
    - Six aliquoted (in the home ) urine cryovials to be frozen

Freeze all biological samples at -80 degrees.

Fill out HealthDiary forms, including processing and boxing of samples.

- Dust samples

Enter dust sample collection form into HealthDiary.

Leave dust sample at room temperature until shipping every two weeks on a Monday to the Gage Dust Processing Centre in Toronto.

Toronto and Vancouver will ship every other Monday 1st and 3rd week of the month. Edmonton and Winnipeg will ship every othe Monday 2nd and 4th week of the month.

- Ensure all paperwork for dust sample is complete. DO NOT PROCESS THESE SAMPLES by "accepting these under sample processing".
- See full HealthDiary dust sample Standard Operating procedures for collection and shipping of dust.

#### B.1.4.1 Health Concerns and Precautions

For those coordinators and research assistants that may have allergy/asthma concerns with collecting dust, you may require the use of a mask as follows:

A. Educational message

The process of vacuuming a home is very important for the study, BUT it can produce a lot of dust. This dust is in the air and easily breathed for approximately one hour after vacuuming. For this reason, we ask that any individuals in the home at the time of sampling who may have asthma be not present in the same room while such sampling is done. If the research assistant has asthma, they, too, can be irritated by the dust from cleaning. A proper dust mask worn over the mouth and nose can reduce this irritation and make cleaning safer. The mask does not eliminate all dusts, but reduces the dust you breathe by at least 95%. You could still have an asthmatic attack from this amount of dust, but it will be less likely than if you do not use the mask.

B. How to use a mask

Depending upon how dusty the job is, one mask should be usable for 3-5 cleanings. A mask that appears to be dirty should not be used. The mask should fit snugly over the face (beards generally prevent a snug fit). Exhalation should be felt through the coloured button in the front- center of the mask and not around the edges of the mask. The mask should be used during sampling and for about one-half hour afterwards if the technician stays in the rooms that were vacuumed. After use, it should be air-dried in the sun and sealed in a plastic bag between uses.

C. Type of mask used: 3M - #8210 - N95

### B.1.5 Quality control and quality assurance

#### B.1.5.1 Home visit data collection quality assurance/quality control

**A.** Objective

Home assessments performed at age three months (±15 days) for all children born to CHILD Study participants will be monitored for quality control for uniformity in the collection, transport, and storage of environmental data and dust collection.

**B.** Responsabilities of CHILD team

Research Coordinator (RC):

The Research Coordinator will be responsible for overall coordination of visits, QA/QC for data collection in the field, delivery of dust samples to the CHILD envirobank, and checking data collection forms are complete.

The Research Coordinator will provide a monthly report for the national environmental teleconference, and address issues raised by the Research Assistants (RAs) with guidance from their national working group lead as needed. They may request additional resources, such as seeking advice from local health authorities or CMHC consultants, and serve as the liaison with these people.

This standard operating procedure (SOP) applies to those members of the CHILD research team and personnel involved in the home visit. An experienced RA is one that has attended 1 or more training sessions and conducted 25 visits. An experienced RA can train other staff members to conduct a home assessment. A trained RA has attended 1 or more training session and conducted 5 supervised visits, and deemed competent by the experienced site trainer. See Figure 1 for certificate of training criteria

The Research Coordinator will perform the QA/QC in data collection by shadowing the RAs and collecting a separate questionnaire and RA assessment form in 1 home every 50 home assessments.

Data will be double-entered from the paper questionnaire into HealthDiary at the NCC.

Research Assistant (RA)

The RA will prepare for and perform the home assessment under the supervision of the research coordinator. They will be responsible for knowing the protocols as outlined in the manual and assuring that all the data are collected, dust samples are properly stored, equipment is collected, and forms are complete at the end of each visit.

**C.** Other CHILD study quality assurance support

National environmental assessment Leads

The leads will be available as a resource to the team and respond promptly to questions raised by the RC. They will remain in close communication with the site's Principal Investigators (PIs) and national CHILD Executive and National Coordinating Centre. Communication with experts in the environmental field outside of CHILD will be through the national EWG CHILD leads and the Project Managers of the CHILD study. The EWG leads will maintain communication with the research coordinators through monthly coordinating teleconferences set up by the National CHILD Project Managers, and will be responsible for coordinating the uniform collection of environmental data. These duties include recommending any changes to protocols or data collection instruments for review and final approval by the CHILD Executive.

Canada Mortgage and Housing Corporation (CMHC)

Canada mortgage and housing consultants may accompany the RAs and RCs on home visits on occasion as part of the QA/QC process, particularly at the beginning of CHILD. They will participate in the monthly teleconferences.

Other resources

In addition, access is available to local health authorities if issues arise in the assessments that require such attention. CHILD will be enhanced by authorities knowing that we are working in their jurisdiction. Additional expertise will be sought if required from external advisors that can be accessed by the national environmental assessment lead, in collaboration with the CHILD Executive. Each city will have a named Research Coordinator from each city maintain contact with the respective National Environmental Lead.

### B.1.5.2 Protocol for preparation of 'QC Samples of Dust'

*To be performed once **every 12 homes.*** On *average this should work out to be a dust control included in every batch of dust shipped to Gage every two weeks.*

a. The Research Coordinator/Research Assistant will pre-clean and depyrogenate a stainless steel spatula for transferring NIST dust to a standard dust collection bottle.

b. Take the dust collection bottle with a thimble from the supply provided by Gage. This bottle will have a batch ID, but no other labels.

c. Add "create a task" in HD to create the control dust sample.

d. Print a sample ID control dust label using HealthDiary ("CNDust - 7-xxxxxx0-1") and affix to the bottle.

e. Obtain bottle of NIST (standardized control sample) dust from its storage location (room temperature) and bring to a designated lab bench along with the pre-labelled dust collection bottle and pre-cleaned spatula.

f. Open dust collection bottle and place within reach.

g. Open NIST dust bottle and using the pre-cleaned spatula, scoop ~200 mg (size of a quarter) of dust and transfer to the dust collection bottle, dumping the NIST dust into the thimble.

h. Close NIST dust bottle and the dust collection bottle.

i. Place spatula in area where used lab supplies are kept prior to cleaning.

j. Clean working space.

k. Prepare all other supplies needed for home visit.

l. Pack the dust collection bottle with thimble (tightly sealed), now containing NIST dust labelled with "CNDust – 7-xxxxxx-0-1," along with all the other home visit supplies.

m. Bring this bottle to the home(s) visited for that day.

n. Open the NIST dust lid for 30 seconds while preparing the vacuum head and placing the thimbles in the hognose. Close lid

o. When returning to the lab at the end of the day‟s home visits, this CNDust bottle can be placed in a dust box and information recorded in HD. The time of collection is the same as the first collected dust and the area vacuumed will be entered as “0”.

## B.2 Figures and supplemental material

### B.2.1 Figure 1: completed dust collection form (Child's Bedroom)

**CHILD Study** ID 90094

Event Name: 3MONTHS

Sample Full Name: Dust Child's Bedroom

Sample Short Name: CBDUST

Sample ID: 7-018464-0-1

**Study Coordinator**

Sample Collected: (●) Yes ( ) No

Sample Collection Date and Time: Y2009M05D22T : (24 hr clock)

Area Vacuumed: 2 s sq. meters

Notes (e.g. if not collected or protocol deviation):

Floor Surface Type:
- (●) Polished Floor
- ( ) Tight Wood Floor
- ( ) Crack Between Wood
- ( ) Tight Carpet
- ( ) Thick Carpet
- ( ) Throw Rug
- ( ) Other

Sample Collected by: Joanne Duncan

**Instructions**

Before Visit:

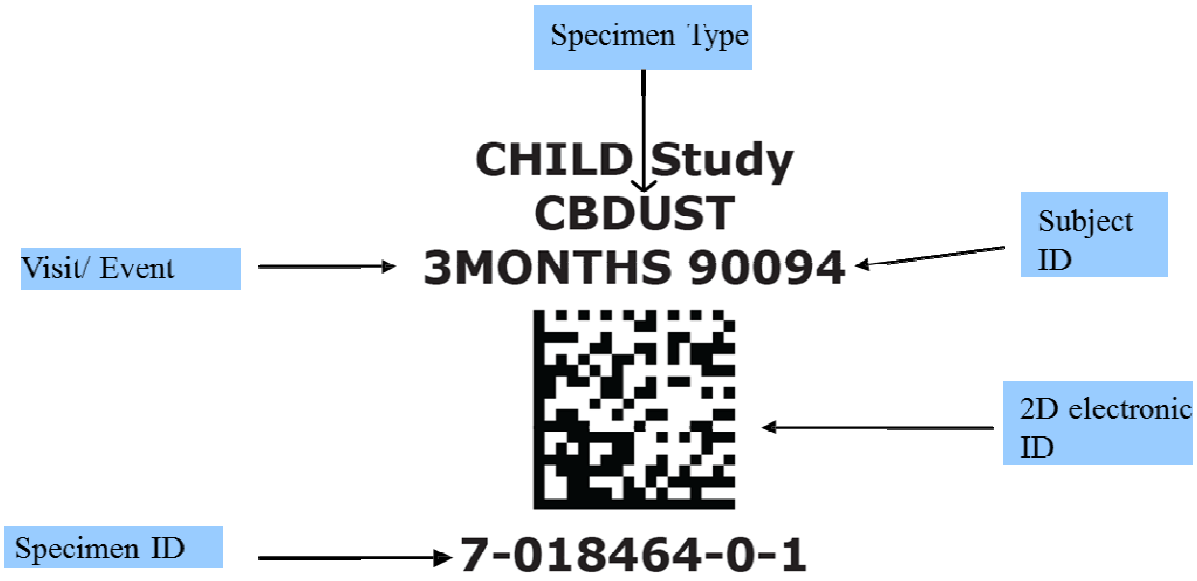
1. Print out dust collection form for the subject.
2. Print out 2 dust sample labels and affix one label to each of two dust collection bottles with thimble inserts.
3. Ensure necessary equipment and supplies are available, including a vacuum, hognose head, wand collection head and nitrile gloves.

During Visit:

1. Don a pair of new nitrile gloves.
2. If the flooring is smooth or low pile carpet, attach the wand dust collector to the vacuum cleaner nozzle, otherwise, attach the clean hognose collector to the vacuum cleaner nozzle.
3. Remove the dust collection bottles labeled "DustCB", open each bottle, and transfer the two thimble into the double thimble collector, reclose each bottle.
4. Carefully vacuum the an area of 2 m. sq. of the single most abundant flooring material, spending approximately 5 minutes using even strokes in a single direction.
5. Switch off the vacuum cleaner and visibly inspect the thimbles to ensure that they are at least half full of dust. If not, vacuum additional floor area in 1 m. sq. increments in the manner described above.
6. Remove the thimbles one by one using the forceps and place them back into the dust bottle.

### B.2.2 Figure 2: HealthDiary specimen collection label

***These labels are 1” by 1” in size



### B.2.3 Figure 3: worksheet example

Family ID:_____
Style of Home_____

**HOME VOLUME WORKSHEET**

M = Main Floors
U = Upper Floors

Area Schematics (Draw rough sketch for each level if needed):

| MAIN AND UPPER FLOORS: | | | | | |
|---|---|---|---|---|---|
| Floor | Area Number | Length (m) L | Width (m) W | Height (m) H | Volume ($m^3$) L x W xH |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| Cathedral Ceiling | Area Number | Length (m) | Width (m) | Height (m) | Volume ($m^3$) (L x W x 2.4) + L x W x [(h-2.4)/2] |
| | | | | | |
| | | | | | |
| | | | | | |
| | Total Volume ($m^3$) | | | | |

| BASEMENT: | | | | | |
|---|---|---|---|---|---|
| Basement Area | Area Number | Length (m) L | Width (m) W | Height (m) H | Volume ($m^3$) L x W xH |
| | | | | | |
| | | | | | |
| | | | | | |
| | Total Volume ($m^3$) | | | | |

Version Form: March 22, 2010