Effect of Pesticides on Lung Function in the Canadian General Population

by

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Abstract

Although pesticide exposures have been associated with respiratory problems in humans, particularly in occupational settings, little is known about the effect of pesticides on lung function in the general population. The objective of this thesis is to characterize the association between pesticide exposure and lung function among the Canadian general population using data from the Canadian Health Measure Survey (CHMS), a nation-wide cross-sectional health survey.

In this thesis, the effect of organophosphate insecticides, pyrethroid insecticides, the organochlorine pesticide DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane], and the phenoxy herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) on lung function was investigated using human biomonitoring data of pesticide exposures and spirometric measures of lung function among CHMS-Cycle 1 participants aged 6 to 79 years, a representative sample of the Canadian general population. In addition, dietary predictors of exposures to organophosphate (OP) and pyrethroid (PYR) insecticides were also investigated. Multiple linear regression was used to examine the association between pesticide exposures and lung function after controlling potential confounders, including demographic factors, socioeconomic factors, lifestyle factors and environmental factors.

The main findings in this thesis were:

1) Urinary detections of the organophosphate and pyrethroid insecticide metabolites were highly prevalent among the CHMS participants, with dietary consumption of fruit and vegetables being a significant predictor of exposures to OP insecticides and dietary consumption of vegetables, and pulses/nuts for exposures to PYR insecticides;

2) Among adult participants aged 20-79 years, one unit increase in log transformed urinary concentration (nmol/g creatinine) of total dialkyl phosphates (ΣDAP, metabolites of OP

insecticides excreted in urine) was associated with a 32.6 mL reduction in forced vital capacity (FVC, p=0.014), a 32.6 mL reduction in forced expiratory volume in 1 second (FEV_1, p=0.02), a 0.3% reduction in FEV_1/FVC ratio (p=0.36) and a 53.1 mL/s reduction in forced expiratory flow between 25% and 75% of FVC ($FEF_{25\%-75\%}$, p=0.081);

3) One unit increase in log transformed urinary concentration (nmol/g creatinine) of total pyrethorid insecticide metabolites (ΣPYR) was associated with a 17.4 mL reduction in FEV_1 (p=0.045) among participants aged 6-11 years, and a 37.1 mL reduction in FVC (p=0.05) among participants aged 12-19 years;

4) Among adult participants aged 20-79 years, individuals with detectable *p,p'*-DDT had significantly lower mean FVC (difference=310.7mL; p=0.003) and FEV_1 (difference=231.8mL; p=0.015) than those without, and every 100 units (ng/g lipid) increase in the concentrations of *p,p'*-DDE [1,1-bis-(4-chlorophenyl)-2,2-dichloroethene], a metabolite of insecticide DDT, was associated with an 18.8 mL decrease in FVC (p-value=0.002) and an 11.8 mL decrease in FEV_1 (p-value=0.013); and

5) No significant association was observed between urinary concentrations of herbicide 2,4-D and lung function among the CHMS-Cycle 1 participants.

In conclusion, results of this thesis demonstrated a prevalent detection of pesticide metabolites in urine and confirmed the dietary sources of pesticide exposures among the Canadian general population. Urinary concentrations of OP metabolites were significantly associated with impaired lung function in the adult population. The potential adverse effect of pyrethroid insecticides on lung function was mainly significant among children and adolescents. Although it has been banned for more than thirty years in Canada, the environmentally persistent pesticide DDT was associated with decremented lung function among the Canadian adult

population. Findings of this thesis provide the first population-based evidence of the adverse effect of pesticides on lung function in the Canadian general population.

Preface

This thesis is an original work by Ming Ye. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name "Effect of environmental chemicals on respiratory diseases and lung function in Canadian general populations: a cross-sectional study using data from CHMS Cycles 1 and 2", No. Pro00045536, March 24, 2014

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In addition, a version of Chapter 3 and a version of Chapter 4 of this thesis have been submitted for publication as M. Ye, J. Beach, J.W. Martin, and A. Senthilselvan "Association between Dietary Factors and Urinary Concentrations of Organophosphate and Pyrethroid Metabolites in a Canadian General Population", and M. Ye, J. Beach, J.W. Martin, and A. Senthilselvan "Relationship between Urinary Dialkyl Phosphate Concentration and Lung Function in Adolescents and Adults: Results from the Canadian Health Measures Survey", respectively.

M. Ye was responsible for study design and data analyses as well as manuscript composition for the above studies. A. Senthilselvan provided guidance to the data analyses,

manuscript preparation and revision. J. Beach and J.W. Martin contributed to the manuscript preparation and revision.

Dedication

This dissertation is dedicated to my family and friends.

Special gratitude to my loving wife, Yuanyuan Qiu, who always believes in me, encourages me and supports me throughout my PhD program. I also thank my son William (Qiushi) Ye and my daughter Kathy (Qiutong) Ye for all the happiness they brought to me.

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List of Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
ACCESS	A Case Control Etiologic Study of Sarcoidosis
AchE	Acetylcholine esterase
AHS	Agricultural Health Study
APC	Antigen-presenting cell
AR	Androgen receptors
ATP	Adenosine triphosphate
ATS	American Thoracic Society
B.C.	Before the birth of Christ
BMI	Body mass index
BTPS	Body temperature, barometric pressure and water saturation
BuChE	Butrylcholinesterase
CD	Cluster of differentiation
CDC	Center for Disease Control and Prevention
CHMS	Canadian Health Measures Survey
CI	Confidence interval
CMA	Census Metropolitan Area
CNS	Central neural system
CoA	Coenzyme A
COPD	Chronic obstructive pulmonary disease
CPAFLA	Canadian Physical Activity, Fitness and Lifestyle Approach

CPES	Children's Pesticide Exposure Study
CYP450	Cytochrome P450
DAP	Dialkyl phosphate
DBCA	3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid
DCCA	3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid
DDA	2,2-bis(4-chlorophenyl)acetic acid
DDD	1,1-dichloro-2,2-bis(p-chlorophenyl)ethane
DDE	1,1-bis-(4-chlorophenyl)-2,2-dichloroethene
DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
DEAP	Diethyl alkylphosphates
DEDTP	Diethyl dithiophosphate
DEE	Daily Energy Expenditure
DEP	Diethyl phosphate
DETP	Diethyl thiophosphate
DHT	Dehydrogen testosterone
DMAP	Dimethyl alkylphophates
DMDTP	Dimethyl dithiophosphate
DMP	Dimethyl phosphate
DMTP	Dimethyl thiophosphate
ED	Emergency Department
EDC	Endocrine-disrupting compound
EFSA	European Food Safety Authority
ENNS	Étude nationale nutrition santé

EPA	Environmental Protection Agency
EPTC	S-ethyl-dipropylthiocarbamate
ER	Estrogen receptors
ERK	Extracellular-signal-regulated kinase
EU	European Union
FEF	Forced expiratory flow between
FEV_1	Forced expiratory volume in 1 second
FFHHSP	Farm Family Health and Hazard Surveillance Program
FTE	Full time equivalent
FVC	Forced vital capacity
GABA	Gamma-aminobutyric acid
GC-MS	Gas chromatography–mass spectrometry
GR	Glutathione reductase
GSH	Glutathione
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
IFN	Interferon
IIA	Irritant-induced asthma
IL	Interleukin
INSPQ	Institut national de santé publique du Québec
IQR	Inter quartile ranges
JNK	c-Jun N-terminal kinases
LOD	Limit of detection

MAPK	Mitogen-activated protein kinase
MCPA	2-methyl-4-chlorophenoxyacetic acid
MEC	Mobile Examination Center
MMAD	Hygroscopicity and mass-mediated aerodynamic diameter
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHANES	National Health And Nutrition Examination Survey
NHL	Non-Hodgkin's lymphoma
NTE	Neuropathy target enzyme
OC	Organochlorines
ODTS	Organic dust toxic syndrome
OP	Organophosphate
OPIDN	Organophosphate-induced delayed neuropathy
OR	Odds ratio
PBA	Phenoxybenzoic acids
PCP	Pentachlorophenol
PEFR	Peak expiratory flow rate
PM	Particulate matters
PNS	Peripheral neural system
PON1	Phosphotriesterase paraoxonase 1
POP	Persistent organic pollutant
PPE	Personal protective equipment

PYR	Pyrethroid
RADS	Reactive airways dysfunction syndrome
RBC	Red blood cell
ROS	Reactive oxygen species
RR	Relative risk
SE	Standard errors
SENSOR	Sentinel Event Notification System for Occupational Risks
SMR	Standardized mortality rate
SOD	Superoxide dismutase
TCP	3,5,6-trichloro-2-pyridinol
Th	T-helper
TRP	Transient receptor potential
UK	United Kingdom
US	United States
WC	Workers' compensation
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Statement of the Problem

Pesticides, including herbicides, insecticides, fungicides, termiticides and rodenticides, are groups of chemicals widely used in occupational settings and residential areas to control pests and prevent pests-induced diseases [1, 2]. Human exposures to pesticides are typically from either the workplace or the environment. For general populations, pesticide exposures are mainly from residential use of pesticides, and/or from pesticide-contaminated food, water or air [2, 3]. According to the US Environmental Protection Agency (EPA), approximately 5 billion pounds of pesticide are used annually worldwide, and only approximately 1% actually reaches target pests [4], suggesting a high possibility for humans to be exposed to pesticides through the environment.

The toxic nature of pesticides poses potential hazards to human health. Respiratory symptoms, such as cough, wheeze and airway inflammation, are commonly observed among people who have been exposed to pesticides [5, 6]. In addition, pesticide exposures have also been linked to the risk of developing respiratory diseases, such as asthma [7-14], COPD [15-19] and lung cancer [20-28]. Ye et al recently published a detailed review on the effect of occupational pesticide exposures on respiratory health [29].

Since Hutchinson first described the possibility of measuring lung function by spirometry in 1846 [30], spirometric results, such as forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), FEV_1/FVC ratio, and the forced expiratory flow between 25% and 75% of forced vital capacity ($FEF_{25\%–75\%}$) have been widely used in clinical diagnosis, therapeutic

assessment, respiratory health surveillance and epidemiological research [31-33]. Lung function increases with age until early adulthood and then declines [34]. According to the Canadian Health Measures Survey (CHMS 2007-2009), adults aged 20-29 years had the highest mean values in lung function parameters FEV_1 and FVC. Lung function also varies with other demographic factors, including sex, ethnicity, weight and height [34, 35]. On average, male subjects often have larger FVC and FEV_1, but slightly smaller FEV_1/FVC than females, and subjects with Caucasian ethnicity often have larger FVC and FEV_1 than subjects with other ethnicity [34, 35]. The distribution of lung function parameters by age and sex was summarized in Table 1-1 [35]. Environmental factors, including tobacco smoking [36] and ambient air pollutants [37] and socioeconomic factors, including education and income [38], are also related to variations in lung function.

Most of epidemiological evidence characterizing the relationship between pesticide exposures and lung function are mainly from occupational settings [29]. For example, a cross-sectional study showed that pesticide-processing workers had significant lower FVC, FEV_1 and $FEF_{25\%–75\%}$ compared to general populations [39]. Another study of farmers in Ethiopia showed that there was a significant reduction in FEV_1 and FVC among pesticide sprayers compared to non-sprayers [40]. A similar study among pesticide sprayers in Spain showed that pesticide exposures, including carbamates, organochlorine insecticide, bipyridil herbicide and fungicide, were associated with significant reduction in lung function FEV_1 and $FEF_{25\%-75\%}$ [41]. In agriculture workers from Sri Lanka and India, acute and/or chronic exposure to cholinesterase-inhibiting insecticides, including organophosphate and carbamate insecticides, was assocated with a significant reduction in lung function FEV_1 and FVC [15, 42]. Salameh *et al*. also found that lung function FEV_1, $FEF_{25\%-75\%}$, and FEV_1/FVC ratio were significantly lower among

pesticide factory workers than controls in a study conducted in Lebanon [43]. An occupational asthma case related to chronic exposure to captafol fungicide also showed a substantial and persistent decrease in lung function FEV_1 [44]. Moreover, in a study conducted among agricultural workers in Colorado and Nebraska, self-reported insecticide or herbicide use was significantly associated with the greater endotoxin-induced reduction in lung function FEV_1 [45].

Although these studies have suggested the adverse effect of pesticide on lung function, few studies have been conducted to assess the association between pesticide exposures and lung function in the general population [46]. The goal of this study is to characterize the effect of pesticides on lung function among the general populations by an epidemiological approach.

Since 2007, Statistics Canada, in partnerships with Health Canada and the Public Health Agency of Canada, has been conducting the Canadian Health Measures Survey (CHMS), a nation-wide population-based cross-sectional survey, to collect the baseline health information of Canadians [47]. In CHMS-Cycle 1 (2007-2009), 5,604 individuals responded to the questionnaire and subsequently performed physical and medical measurements [47]. The 5,604 participants of the CHMS-Cycle1, comprised participants from New Brunswick (411), Quebec (1545), Ontario (2,092), Alberta (766) and British Columbia (790), and were considered representative of 96.3% of the Canadian population [47].

To address "data gaps and limitations in existing health information", CHMS-Cycle 1 conducted "direct measures", including measurements of lung function by spirometry and pesticide metabolite concentrations in urine or blood samples using a human biomonitoring approach [47]. In this study, concentrations of pesticides and pesticide metabolites in urine or blood samples and their associations with lung function were examined among the Canadian general populations using data from the Canadian Health Measures Survey (CHMS-Cycle 1).

1.2 Study Objectives

The primary objective of this study was to investigate the effect of pesticides on lung function in the Canadian general population. The specific objectives of this study were: (i) to examine dietary factors as a predictor of pesticide exposures; (ii) to examine the effect of organophosphate insecticides on lung function; (iii) to examine the effect of pyrethroid insecticides on lung function; (iv) to examine the effect of an environmentally persistent pesticide DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] on lung function; and (v) to explore the potential effect of herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) on lung function, in the Canadian general population.

1.3 Thesis Submitted for Partial Fulfillment of PhD

This thesis consists of a comprehensive literature review on pesticide exposures and their effects on respiratory health in occupational settings and general populations (Chapter 2). It is followed by five studies (Chapter 3, 4, 5, 6 and 7) designed to address each of the specific objectives.

Chapter 2 consists of a literture review of what is currently known about pesticide exposures and respiratory health. In Section 2.2 and 2.3, pesticide classification, pesticide exposures, pesticide transformation and toxicity of pesticides, are summarized. In Section 2.4, epidemiological studies on the association between occupational pesticide exposures and respiratory health, including respiratory symptoms, lung function, asthma, COPD and lung cancer, are reviewed. In Section 2.5, epidemiological studies of the relationships between pesticide exposures and respiratory health in general populations are reviewed. In addition, views on the potential issues in epidemiological research were presented in Section 2.5. A version of

Chapter 2 has been published in the *International Journal of Environmental Research and Public Health* [29].

In Chapter 3, results of the first study are presented. In this chapter, the associations of dietary factors and urinary concentrations of pesticide metabolites were examined among the Canadian general population using data from 5,604 CHMS-Cycle 1 participants. A version of Chapter 3 has been submitted and is currently under review for publication.

In Chapter 4, results of the second study are presented. In this chapter, the associations between urinary concentrations of dialkyl phosphate (DAP) metabolites and lung function were investigated using data from 4,446 CHMS-Cycle 1 participants aged 12-79 years. A version of Chapter 4 has been submitted for publication and is currently under review.

In Chapter 5, results of the third study are presented. In this chapter, the associations between urinary concentrations of pyrethroid insecticide metabolites and lung function were investigated among children, adolescent and adult subjects using data from 5,436 CHMS-Cycle 1 participants.

In Chapter 6, results of the fourth study are presented. In this chapter, the associations between plasma DDT levels and lung function were investigated among 1,696 adult participants of the CHMS-Cycle 1. A version of Chapter 6 has been been published in the *Environmental Health Perspectives* [48].

In Chapter 7, results of the fifth study are presented. In this chapter, the associations between urinary concentrations of 2,4-D and lung function were investigated among 5,604 CHMS-Cycle 1 participants.

In the final chapter, Chapter 8, general discussion and conclusions are presented. This chapter includes an overview of the thesis research, a summary of the results from five studies,

discussion of the importance of the research, strength and limitations of the studies, conclusions and implications for future research.

Table 1-1. Distribution of lung function parameters by age and sex*

		FVC (L) Mean (95% CI)	FEV_1 (L) Mean (95% CI)	FEV_1/FVC (%) Mean (95% CI)	$FEF_{25\%-75\%}$ (L/s) Mean (95% CI)
			6-11 years		
Total sample		2.20 (2.15-2.25)	1.86 (1.82-1.90)	84.6 (83.9-85.2)	2.01 (1.94-2.08)
Sex					
	Female	2.15 (2.09-2.21)	1.84 (1.79-1.89)	85.6 (84.9-86.2)	2.04 (1.94-2.14)
	Male	2.26 (2.18-2.34)	1.88 (1.81-1.94)	83.5 (82.5-84.5)	1.98 (1.87-2.08)
			12-19 years		
Total sample		4.13 (4.05-4.21)	3.45 (3.40-3.50)	84.0 (83.5-84.5)	3.55 (3.46-3.65)
Sex					
	Female	3.70 (3.65-3.75)	3.15 (3.10-3.20)	85.4 (84.9-86.0)	3.39 (3.27-3.52)
	Male	4.53 (4.38-4.67)	3.73 (3.63-3.84)	82.6 (81.6-83.6)	3.69 (3.53-3.85)
			20-79 years		
Total sample		4.13 (4.05-4.21)	3.19 (3.13-3.24)	77.1 (76.5-77.7)	2.88 (2.80-2.96)
Age group					
	20-29 years	4.71 (4.49-4.93)	3.81 (3.62-3.99)	81.4 (80.3-82.5)	3.69 (3.43-3.96)
	30-79 years	4.00 (3.93-4.07)	3.05 (2.99-3.10)	76.2 (75.6-76.7)	2.70 (2.63-2.76)
Sex					
	Female	3.45 (3.40-3.50)	2.68 (2.64-2.72)	77.6 (76.9-78.3)	2.48 (2.39-2.57)
	Male	4.81 (4.69-4.93)	3.69 (3.59-3.79)	76.6 (75.8-77.5)	3.28 (3.14-3.42)

*Adapted from *Canadian Health Measures Survey: Cycle 1 Data Tables* [35]

CHAPTER 2

PESTICIDE EXPOSURES AND RESPIRATORY HEALTH

[This chapter is an expanded version of the paper, M. Ye, J. Beach, J.W. Martin and A. Senthilselvan, *Occupational Pesticide Exposures and Respiratory Health,* that has been published in *International Journal of Environmental Research and Public Health* 2013 Nov 28;10(12):6442-71.]

2.1 Introduction

Pesticides, including herbicides, insecticides, fungicides, bactericides and rodenticides, are widely used to control pests and pest-induced diseases [1]. Worldwide, approximately five billion pounds of pesticide are consumed annually [2], among which organophosphate (OP) and carbamate insecticides (34%), dithiocarbamate fungicides (18%) and phenoxyl herbicides (12%) are the most commonly used [3]. In many occupational settings, including agriculture, fishery, forestry and food industry, pesticides have been widely used in large quantities [2].

Human exposures to pesticides can occur in either workplace or environmental settings. Occupational exposures to pesticides occur during the production, transportation, preparation and application of pesticides in the workplace [1, 4]. It is also quite common for agricultural workers to experience pesticide exposures even when performing tasks not specifically related to pesticide use [5-7]. Non-occupational or environmental exposure to pesticides is mainly from residential use of pesticides, or from the pesticide-contaminated food, water or air [1, 8].

The toxic properties of pesticides pose a potential hazard to human health. It has been estimated that the incidence rate of pesticide-related illness in the workplace was approximately 1.17 per 100,000 full time equivalent workers (FTEs) [9]. Respiratory symptoms, such as coughing, wheezing and airway inflammation, are commonly observed among people exposed to

pesticides [10, 11]. Epidemiological studies have attempted to investigate the association between pesticide exposures, mainly in agricultural occupations, and respiratory health, including chronic respiratory diseases, such asthma, chronic obstructive pulmonary disease (COPD) and lung cancer [11-13].

In this chapter, we critically reviewed the evidence available to date about the relationships between pesticide exposures, in both occupational and non-occupational settings, and respiratory health, including lung function, respiratory symptoms and diseases.

To review the literature, we searched English-language studies, reports and abstracts between 1990 and September 2013 in MEDLINE using key words (including synonyms and plural forms) and combinations of key words, including occupation, workplace, non-occupational, environmental, general population, pesticide, insecticide, herbicide, respiratory, pulmonary, airway, lung function, infection, asthma, bronchitis, chronic obstructive pulmonary disease (COPD), and lung cancer, Searching strategies also included cross-referencing of research and review papers.

2.2. Pesticides

2.2.1 Pesticide Classification

Pesticides are widely used in occupational settings and residential areas to prevent and control pests and pests-induced diseases [1]. Based on the target, pesticides are mainly grouped into herbicides, insecticides, fungicides, bactericides, and rodenticides (Table 2-1). Based on chemical properties, pesticides can also be categorized into organochlorines (OC), organophosphates (OP), carbamates, dithiocarbamates, pyrethroids, phenoxyl, triazine, amide, and coumadin compounds (Table 2-1). Other substances such as sulfur fumigants, arsenic compounds, urea derivatives and even botanic and biological products have also been used as

pesticides in human history. For example, the element sulfur was used as a fumigant by Chinese farmers around 1000 B.C. [14]. There are historical reports of the use of the seed of *strychnos nuxvomica* as a rodenticide, and the root of *Derris Eliptica* (a source of rotenone) as an insecticide [14]. In addition, a protein product expressed by microbe *Bacillus thuringiensis* has been used as an insecticide [15].

Pesticides can also be classified based on their mechanism of action. For example, OC, OP and pyrethroid insecticides are designed as neurotoxins. Phenoxyl herbicides are plant hormone analogues. Some pesticides are disruptors of normal metabolism and physiological processes, such as triazine and urea herbicides [16, 17]. The rodenticide coumadin and its derivatives have the potential to depress vitamin K synthesis and thus have anticoagulant property. Sodium fluoacetate, another rodenticide, is thought to interfere with the citric acid cycle [18]. There are also pesticides, such as dithiocarbamate fungicides and amide herbicides, which are disruptors of energy production and inducers of oxidative stress [19].

The acute toxidromes of pesticides in humans are mainly due to pesticide neurotoxicity, including interference with neural conduction by targeting voltage-gated ion channels or Na+/K+ ATPase, interference with neural transmission by inhibiting acetylcholine esterase, stimulating respiratory sensory neurons or initiating pro-inflammatory signals [14]. At high dose exposures, OC, OP and pyrethroids can affect both the central neural system (CNS) and peripheral neural system (PNS) in mammals [19]. As shown in Table 2-2, based on their toxic effects in mammals [14], pesticides can be classified into three groups.

2.2.2 Pesticide Exposures

2.2.2.1. Overview of Pesticides Exposures

Human exposure to pesticides can occur in either occupational or non-occupational settings. Occupational exposure to pesticides takes place during the production, transportation, preparation and application of pesticides in the workplace [1, 4]. Factors involved in occupational pesticide exposures usually include application intensity, frequency, duration and method, safety behaviors (e.g., use of personal protective equipment), as well as the physiochemical and toxicological profiles of the pesticides in use [20]. In occupational settings, persons working directly and frequently with pesticides are groups with the highest risk of exposure [4]. Additionally, family members of pesticide applicators can have substantial exposures to pesticides [21, 22]. In addition to the fact that occupational pesticide exposure is quite common among agricultural workers and their family members [5-7], accidental spills of pesticides, leakages, incorrect uses of equipment, and non-compliance with safety guidelines, are the leading causes of occupational pesticide exposures [4, 23]. Compared to environmental exposures where levels of exposure tend to be fairly low, occupational exposures to pesticides are often at relatively high doses, whether acute or chronic [1].

According to the US EPA Pesticide Program, about two billion pounds of pesticide are used in North America every year [2], but of this only approximately 1% will reach its target pests [24], suggesting the majority of the pesticides used goes into the environment. This would also suggests that people living close to the places where pesticides were constantly or intensively applied are at higher risk of being exposed. Run-off from farm land, e.g. OP pesticides, and movement into the water body can lead to the spread of OP exposures [1, 8]. Exposures to pesticides are also very common from the food supply. For example, it was also estimated that up to 50% of fruits, vegetables, and cereals grown in the EU contains pesticide residuals [25]. In addition, more than 50% of water streams in US contain five or more type of

pesticides [26]. Family members of pesticide applicators often also have substantial exposures to pesticides [21, 22]. Compared to occupational exposure, non-occupational or environmental exposure to pesticides is often chronic or sub-chronic and occurs at low doses [1].

2.2.2.2 Routes of Pesticide Exposures

Inhalation and dermal absorption are considered as the primary routes of exposures to pesticide in occupational settings [1, 27]. Respiratory exposures usually occur when applying highly volatile or aerosolized pesticide products, especially for those working with no respiratory protective equipment (e.g., mask with filter) or in a poorly ventilated working environment [28]. In agricultural occupations, typically about 10% of total pesticide exposure occurs via the respiratory route, with the rest through either dermal absorption or ingestion [28]. For non-volatile pesticides, inhalation also occurs when pesticides are sprayed as an inhalable form. Dermal absorption occurs through direct skin contact with pesticides or from clothing and tools that are contaminated with pesticide residues [11]. A study of Greek tobacco-growing farmers suggested that dermal exposure was the major route of exposure (58%) during occupational pesticide uses [29]. Dermal exposure and ingestion may also be relevant for systematic inflammation or sensitization after high level exposures to pesticide at the workplace [27].

While skin absorption and pulmonary inhalation appear the more important routes of exposures in occupational settings, oral ingestion is the major route of exposures to pesticides in non-occupational settings [1, 10]. For instance, pyrethroid exposures in the general population are mainly due to diet [30]. In Europe, one out of 20 food items is known to exceed the EU legal limit for pesticide residues [25]. In Canada, it is estimated that 68.5% of fruits and vegetables contained detectable levels of one or more pesticide residues [31]. The pesticide residue on food

items comes not only from agriculture but also from pesticides used during food storage and shipment [11].

The physiochemical properties of the particular pesticide, temperature, humidity, weather conditions, personal hygiene (e.g., hand washing), and use of personal protective equipment are all factors associated with pesticide exposures [32, 33]. For example, organophosphate and carbamate insecticides can be efficiently absorbed by the skin due to their high lipid solubility [34]. Certain organochlorine insecticides, such as DDT (dichlorodiphenyltrichloroethane), lindane, aldrin and chlordane, are more lipid soluble than others and thereby more efficiently absorbed by the skin [34]. In contrast, due to the low lipid solubility, pyrethroid insecticides are poorly absorbed though intact skin, but can be efficiently absorbed through inhalation and ingestion [34]. Chlorophenoxyl herbicides are often in a form of salts, which results in a low volatility and lipid solubility and makes phenoxyl compounds well absorbed by the gastrointestinal tract following ingestion, but less well absorbed by the lungs, and least well by the skin [34]. Methyl bromide, a halogenated fumigant, exists as a colorless and odorless volatile liquid and thus has poor olfactory warning properties, which increases the likelihood of exposure through inhalation [35]. Respiratory exposure also occurs through airway inhalation of pesticide-contaminated aerosols or particulate matters (PM) [36]. The hygroscopicity and mass-median aerodynamic diameter (MMAD) of pesticide-containing particles are important in determining their local deposition in airways, and hence potentially the site of toxicity [37].

2.2.2.3. Biomonitoring of Pesticide Levels

Due to variations in exposure magnitude and duration, routes of absorption (skin, respiratory tract, gastrointestinal tract), and physiological variability between exposed individuals, it is often difficult to quantitatively assess the effective dose of a pesticide an

individual has received either by measuring working hours or by monitoring the contamination level of the workplace. Biomonitoring of pesticide levels in biospecimen or using biomarkers has been considered as an alternative approach to assess pesticide exposures. It can provide an objective measure of the physiological burden of a pesticide on the human body. For example, using biomonitoring data, the US National Health And Nutrition Examination Survey (NHANES) has been able to identify that the majority of the US population had OP metabolites detectable in their urine samples [38]. It has also been shown that reduced levels of red blood cell (RBC) cholinesterase were significantly associated with the number of years of using OP pesticides after adjusting for age [39].

In the US Farm Family Exposure Study, researchers were able to assess pesticide exposure profiles by measuring urinary levels of the herbicides 2,4-D, atrazine, and TCP (3,5,6-trichloro-2-pyridinol), a metabolite of organophosphate insecticide chlorpyrifos [40-42]. As part of the Farm Family Exposure Study, Curwin *et al*. also found a significant correlation between urinary levels of the herbicide atrazine and the atrazine exposure levels measured by hand wipe samples [42]. In a study of pesticides with a relatively short half-life in the body, Perry *et al.* showed poor agreement between self-reported exposure and urinary measures of deethylatrazine (a major atrazine metabolite) level [43].

Although a biomonitoring approach has been accepted as a useful way to estimate exposures, a biomonitoring method often requires complex and costly sample collection, transportation, and analytical methods, sometime involving invasive procedures such as blood sample collections, which may make it less acceptable to participants [44-48].

2.2.3 Biotransformation of pesticides

2.2.3.1 Biotransformation of organophosphates

Many OPs are phosphorathionates, which can undergo oxidative desulfuration to generate an oxon, an intermediate compound of OP biotransformation (Figure 2-1), which is about 50-60 times more toxic than the parent OP [49, 50]. Oxidative desulfauration of OP pesticides is catalyzed by cytochrome P450 (CYP450) mono-oxygenase with NADPH or NADH as cofactors [49, 50]. The phosphotriester bond in oxon or in the parental OP compounds can be hydrolyzed by phosphotriesterase paraoxonase 1 (PON1), to form a dialkyl phosphate, such as DMP (dimethyl phosphate) or DEP (diethyl phosphate) (Figure 2-1) [51].

Other biotransformation pathways include dealkylation or dearylation of OP or oxons by CYP450 with NADPH/NADH as cofactors (Figure 2-2), and the hydrolysis of carboxylester bonds in OPs as well (Figure 2-3). Both parental OP and oxons can be dealkylated [e.g. the alkyl groups in malathion (R1 and R2 in Figure 2-2)] or dearylated [e.g. the aryl group in parathion (X in Figure 2-2)] by CYP450 with NADPH/NADH as cofactors. Dealkalytion or dearylation of OPs can also be coupled with hydrolysis to form dialkylphosphate or dialkylthiophosphate (Figure 2-2) [49, 50]. The carboxyl ester bonds in the alkyl chain in malathion (or malaoxon) can also be hydrolyzed by carboxyl esterase (Figure 2-3) [50]. CYP450 2B, 2C and 3A are predominant monooxygenase responsible for desulfuration and dealkylation, while CYP 2C9 and 2C19 are responsible for the dearylation [49].

2.2.3.2 Biotransformation of pyrethroids

In human, pyrethroid pesticides can be rapidly detoxified via ester hydrolysis in the blood and liver [52]. The major detoxification pathway of pyrethroid is the cleavage of ester bonds by carboxylesterase-mediated hydrolysis and CPY450-mediated oxidation (Figure 2-5) [53]. Two major types of metabolites are produced: i) carboxylic acids, including DCCA [3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid] and DBCA [3-(2,2-dibromovinyl)-

2,2-dimethylcyclopropane-1-carboxylic acid], ii) phenoxybenzoic acids (PBA), including 3-PBA and 4-F-3-PBA (Figure 2-5) [53].

Other minor pathways of biotransformation of pyrethroids include the CYP-mediated oxidation of methyl groups in pyrethroids and CYP-mediated hydroxylation, which are often followed by the hydrolysis of major carboxyl ester bonds in pyrethroids [54].

2.2.3.3 Biotransformation of organochlorines

In human, DDT can either be oxidized or reduced by CYP450 to form DDE (dichlorodiphenyldichloroethlene) or DDD (dichlorodiphenyldichloroethane), respectively [55]. DDE can be further epoxidated and undergo phase II conjugation with glutathione (GSH), or glucuronic acid, and DDD can be further oxidized to DDA (Figure 2-4) [55]. However, DDT, DDE and DDD are all highly lipophilic and therefore have high potential to bioaccumulate in human fat tissues. Consequently, the biotransformation rate of DDT and related compounds is low once they are accumulated in the human body [56].

Other organochlorine pesticides, such as lindane, aldrin and chlordane, can undergo dehydrogenation, dechlorination and hydroxylation by CYP450 and further phase II metabolism in the human body [57]. In addition, aldrin and chlordane, the chlorinated cyclodienes, can also undergo epoxidation to form epoxide dieldrin or expoxychlordene, a more toxic form of organochlorine insecticides [58]. These organochlorine pesticides are also highly resistant to metabolism in the human body [56].

2.2.3.4 Biotransformation of phenoxyl herbicides

Phenoxyl herbicides mainly include 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) and MCPA (2-methyl-4-chlorophenoxyacetic acid). These phenoxyl compounds are most often in the form of salts or acids, or esters, which can be easily

metabolized or excreted from the human body [59]. The chlorophenoxy compounds of esters or salts are usually dissociated or hydrolyzed by carboxyl esterase or many other hydrolases [59]. In addition, due to the structural similarity to acetic acid, phenoxy herbicides also have the potential to form analogues to acetyl-CoA, such as 2,4-D-CoA in the human body [59].

2.2.4 Pesticide toxicity

2.2.4.1 Neurotoxicity of pesticides

Most pesticides are capable of acting as neurotoxicants. For example, organochlorine (OC) pesticides can interfere with neural conduction by preventing the closure of voltage-gated sodium channels on neurons, which leads to an increased sodium influx and repeated firing in the peripheral neural system (PNS) [19]. Organochlorines lindane, endosulfan and chlordecone (kepone) can also decrease chloride efflux by inhibiting GABA-gated (GABA, an inhibiting neurotransmitter) chloride channels in the central neural system (CNS) [19]. The acute symptoms after high dose exposures to OC include headache, dizziness, nausea, incoordination, movement disorder, tremor, disorder of cognition, confusion [19].

Organophosphate and carbamate pesticides are anti-cholinerase insecticides inhibiting the activity of acetylcholine esterase (AchE) [19]. Exposures to OP will lead to the accumulation of neurotransmitter acetylcholine (Ach) and overstimulation of the postsynaptic cholinergic nerves by inhibiting the activity of acetylcholine esterase (AchE) [19, 60]. Acute symptoms after OP exposures include salivation, lacrimation, urination, diarrhea, gastrointestinal cramp and emesis (acronymed as SLUDGE) [19]. Toxidromes of carbamate are similar to OP but only last minutes or hours [19]. Respiratory paralysis and bradycardia are the major cause of OP related death [10]. The neurotoxic effects of OP can be potentially neutralized by administering atropine and pralidoxime [61]. The sub-chronic effect (2-5 weeks) of OP exposures is called OP-induced

delayed neuropathy (OPIDN), which is thought to be caused by the irreversible binding of OP to NTE (neuropathy target esterase) and subsequent neuron degeneration [19].

Pyrethroids are synthetic insecticides chemically similar to pyrethrin, a natural neurotoxicant to insects from chrysanthemum [34]. Pyrethroid insecticides can interfere with the voltage-gated sodium/chloride channel, which leads to a hyper-excitable state and prolonged depolarization of neurons [52]. Dermal paresthesia is the most common sign of pyrethroid poisoning [52]. At very high concentrations, pyrethroids can also target CNS GABA-gated chloride channels, which may result in pyrethroid-induced seizures [52]. Acute neurotoxic symptoms after high dose pyrethroid exposure include paresthesia (dermal route), gastrointestinal pain, upper respiratory tract irritation, dizziness, headache, movement coordinating dysfunction, and whole body tremor [19, 52].

2.2.4.2 Pesticide-induced oxidative stress and neurogenic inflammation

Almost all pesticides can cause local irritation of skin, eyes, respiratory and gastrointestinal linings [10, 34]. Pesticide-containing aerosols, gases or vapors also have the potential to irritate and even disrupt the airway epithelium due to their corrosive physicochemical properties [10, 59, 62, 63]. When exposed to high-dose, corrosive pesticides, oxidative stress occurs in the airways and produces reactive oxygen species (ROS) [64-66]. ROS, including superoxide anions (O2-) and hydroxyl radicals (OH-), will lead to lipid peroxidation of cellular membranes, the death of airway epithelial cells, and thus further oxidative stress [66, 67]. In a human study, levels of superoxide dismutase (SOD) and glutathione reductase (GR), the enzymes important for antioxidant defense, were lower among pesticide sprayers than normal controls [68].

In addition, pesticides and ROS produced from pesticide irritation will agonize transient receptor potential (TRP) ion channels expressed on C sensory fibers, respiratory epithelial cells and inflammatory cells [69-72], to induce neurogenic inflammation [71, 73]. Activated TRP ion channels will cause the production and release of inflammatory neuropeptides [74-76] and pro-inflammatory cytokines [71, 73] from the end of C fibers and inflammatory cells. Neurogenic inflammation and the subsequent release of pro-inflammatory neuropeptide and cytokines have been postulated as one of the potential mechanisms leading to the pesticide-induced asthma [77].

2.2.4.3. Immunotoxicity of pesticides

The immunotoxicity of pesticides can be either immunosuppressive or immunoenhansive, depending on chemical class, exposure dose, and target organ of the pesticides [78-80]. Table 2-3 summarizes the multiple immunological endpoints associated with pesticide exposures observed in human studies.

Studies have suggested that organochlorine pesticides have immunosuppressive effect [78, 79], which often leads to autoimmunity, recurrent infection of upper respiratory tract and cancer, such as Non-hodgkin's lymphoma (NHL) [81-86]. Both immunosuppressive and immunoenhansive effects have been suggested in studies of organophosphate and carbamate pesticides [78, 87]. Studies on people chronically exposed to chlorpyriphos (an organophosphate insecticide) showed that the exposed group had a higher frequency of atopy, antibiotic sensitivities and autoimmunity [88, 89].

In humans, acute exposure to phenoxyl herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (4-chloro-2-methylphenoxyacetic acid) can lead to immunosuppression, including inhibiting the innate cell-function and T-cell proliferation, reduction in CD4+ T helper cells, CD8+ Treg cells and nature killer cells [90]. In vitro study has shown that pyrethroids inhibited

production of IFN-alpha in monocytes, T-B- cell proliferation and antigen presenting cells (APC) [79]. However, little is known about the immunotoxicity of pyrethroid insecticides in human.

2.2.4.4. Endocrine-disrupting properties of pesticides

De Coster et al reviewed the endocrine-disrupting properties of pesticides [91]. Many pesticides, especially at low-dose, have endocrine-disrupting properties, i.e. alter hormone homeostasis (Table 2-4) [91]. For example, DDE, the main metabolite of the organochlorine pesticide DDT, binds to estrogen receptors, increasing glucose transport, glycolysis in mitochondria and fatty acid oxidation [92], which may lead to increased adipocytes and the development of obesity [93]. Organochlorine pesticides, such as pentachlorophenol (PCP) and lindane, can also function as an androgen antagonist and inhibit dehydrogen testosterone (DHT) from binding to androgen receptors (AR) [92]. In addition, hexachlorobenzene (HCB), as a corticoidsteroid hormone disruptor, interferes with the function of corticoids, which are critical for anti-inflammatory responses and Th1/2 balancing [94]. Dithiocarbamates can also interfere with hormone regulation and neuroendocrine homeostasis [92, 94]. The OP insecticide, chlorpyrifos, can inhibit thyroid hormone binding to its receptors and the synthesis of adrenal glucosteroid [92, 95]. Endocrine-disrupting properties of pesticides can potentially lead to the development of cancer, diabetes, obesity, metabolic diseases, fertility and diseases involved in the dysfunction of immune system, such as autoimmunity, allergy and asthma [91].

2.3 Occupational Pesticide Exposures and Respiratory Health

Due to their inherent biological reactivity, pesticides are potentially hazardous to human health. Globally, around 300,000 deaths per year are estimated to result from acute pesticide poisoning, with organophosphates, organochlorines and aluminium phosphide being reported most frequently as the cause [61]. According to the US Sentinel Event Notification System for

Occupational Risks (SENSOR)-pesticides surveillance program, the overall incidence rate of acute occupational pesticide-related illness was 1.17 per 100,000 full time equivalent workers (FTEs) and insecticides were responsible for 49% of all illnesses [9]. Moreover, the incidence rate among agricultural occupations, where pesticides are extensively and intensively used, was much higher (18.2/100,000 FTEs) compared to those employed in non-agricultural occupations (0.53/100,000 FTEs) [9]. A summary of the adverse health effects of environmental chemicals suggests that pesticide exposures may cause asthma (both new incidence and exacerbation of preexisting disease), chronic obstructive pulmonary disease and even lung cancer [11].

2.3.1 Respiratory Symptoms

Respiratory symptoms that have been reported in association with pesticide exposures include wheezing, airway irritation, dry/sore throat, cough, breathlessness and chest tightness. A cross-sectional study of workers in a bottling plant showed that in comparison with controls, pesticide processing workers had a significantly higher risk of developing respiratory symptoms, including chronic cough in females (OR = 1.29, 95% CI: 1.15–15.84), dyspnea grades 3 and 4 (OR = 1.11, 95% CI: 1.06–1.97 in females; OR = 2.35, 95% CI: 1.50–4.10 in males), throat irritation in males (OR = 1.36, 95% CI: 1.10–3.50), nasal catarrh (OR = 2.08, 95% CI: 1.12–3.40 in females; OR = 2.15, 95% CI: 1.15–4.10 in males), and nasal dryness (OR = 1.15, 95% CI: 1.05–2.91 in females; OR = 1.19, 95% CI: 1.10–3.15 in males) [96]. In addition, acute respiratory symptoms, such as cough, wheezing, chest tightness, dyspnea, throat irritation and dryness, nose secretion and dryness, were significantly increased across the work-shift among pesticide workers [96].

A number of other studies have also reported an excess of respiratory symptoms among farm workers exposed to pesticides. A study of livestock farm workers from Iowa reported that

after adjusting for age and smoking, respiratory symptoms including phlegm (OR = 1.91, 95% CI: 1.02–3.57), wheezing (OR = 3.92, 95% CI: 1.76–8.72) and flu-like symptoms (OR = 2.93, 95% CI: 1.69–5.12) were significantly associated with pesticide use, although the results may have been affected by concurrent exposures to other environmental agents such as ammonia and animal antigens [97]. In spite of non-significant results, Hashemi *et al*. in their study of work-related symptoms among Iranian farmers also reported that pesticide use was associated with an increased risk of wheezing and phlegm [98]. A study of the Farm Family Health and Hazard Surveillance Program (FFHHSP) among Ohio grain farmers in the US showed that personal involvement with pesticides was associated with a high prevalence of chronic cough [99].

In addition to these results suggesting the overall respiratory effect of unspecified pesticides, there have been some studies focusing on particular types of agent. For example, the neurological effects caused by cholinesterase inhibiting pesticides, such as OPs and carbamates, have been recognized to affect lungs and airways, leading to respiratory symptoms, impaired lung function and respiratory diseases. A matched case-control study of agricultural workers in Eastern India showed that compared to controls, agricultural workers who sprayed organophosphate and carbamate pesticides had significant depletion of red blood cell acetylcholinesterase (AchE), and the depletion of AchE was significantly associated with almost all respiratory symptoms, including runny or stuffy nose (OR = 2.85, 95% CI: 1.98–4.63), sore throat (OR = 1.76, 95% CI: 1.29–2.43), dry cough (OR = 2.83, 95% CI: 1.92–4.41), wheezing (OR = 1.78, 95% CI: 1.33–2.46), breathlessness (OR = 2.41, 95% CI: 2.06–3.82), chest tightness (OR = 3.26, 95% CI: 2.23–5.17) and dyspnea (OR = 2.63, 95% CI: 1.89–4.13), as well as chronic bronchitis (OR = 2.54, 95% CI: 1.48–3.74) and doctor diagnosed asthma (OR = 1.34, 95% CI: 1.09–1.79) [100]. Hoppin *et al.* reporting results from the Agricultural Health Study (AHS)

found that exposures to the OP pesticides dichlorvos and phorate in the past year were significantly associated with wheezing among commercial pesticide applicators (OR = 2.48, 95% CI: 1.08–5.66 and OR = 2.35, 95% CI: 1.36–4.06, respectively) after adjusting for age, BMI, smoking, asthma/atopy and previous use of pesticide [101]. Hoppin *et al*. also demonstrated a dose-response trend (p-value for trend < 0.01) for the association between organophosphate pesticide chlorpyrifos and wheezing [101]. In a study of Kenyan agricultural workers, Ohayo-Mitoko *et al*. reported that acetylcholinesterase inhibiting pesticides, including dimethoate, malathion, benomyi, mancozeb, methomyi, aldicarb, and propineb, were associated with a higher prevalence of respiratory symptoms, such as chest pain, cough, running nose, wheezing, difficulties in breathing, shortness of breath, and irritation of the throat [102]. Ciesielski *et al.* also found that pesticide exposure related cholinesterase inhibition was associated with chest pain and difficulty in breathing in a study of North Carolina migrant farmworkers [103]. However, neither study identified a significant dose-response effect of cholinesterase level on respiratory symptoms [102, 103].

Exposures to other types of pesticides, such as pyrethroid insecticides, certain herbicides and fumigants, can also lead to respiratory symptoms. Hoppin *et al*. in their study of farmers in the Agricultural Health Study (AHS) showed that herbicides alachlor (OR = 1.23, 95% CI: 1.06–1.41), atrazine (OR = 1.18, 95% CI: 1.05–1.32), S-ethyl-dipropylthiocarbamate (EPTC) (OR = 1.37, 95% CI: 1.08–1.73), petroleum oil (OR = 1.26, 95% CI: 1.09–1.47) and trifluralin (OR = 1.15, 95% CI: 1.02–1.30), and the insecticide permethrin (OR = 1.28, 95% CI: 1.06–1.55), were significantly associated with wheezing [101]. It has also been reported that office workers in California experienced shortness of breath and irritation of the respiratory tract after accidentally inhaling cypermethrin, a pyrethroid insecticide [104]. A case study in Tanzania

reported that inhabitants of houses sprayed with lambda-cyhalothrinnose, a pyrethroid insecticide, had nose or throat irritation accompanied by sneezing or coughing [105]. Although no dose-response relationship was found, in a study of Indonesian farmers, Kishi *et al*. showed that the respiratory symptoms, including dry throat, sore throat, difficulty in breathing and chest pain, were significantly associated with the pesticide spray season [106]. Moreover, a study of farmers in Nepal suggested that activities regarding to insecticide or fungicide uses, including the spraying duration and mixing pesticides were significant predictors of throat discomfort and respiratory depression [107].

Due to its neurotoxicity, occupational exposures (poisoning) to fumigant methyl bromide can also cause respiratory symptoms, including respiratory irritation, respiratory distress (shortness of breath), coughing and pulmonary injury (edema) [35, 108-110]. These respiratory symptoms often accompany other local or systematic symptoms including dizziness, vomiting, fatigue, headache, abdominal pain, tremor, seizures, ataxia paresthesia and dysfunction of other organs [109, 111, 112].

In summary, a large number of studies have identified associations between respiratory symptoms and pesticide exposure, but to date the findings have been relatively non-specific both in terms of the agents causing the risk and the symptoms caused, which makes interpretation of the data complex. Despite this, there does seem to be good evidence that at least some pesticides cause acute and chronic respiratory symptoms.

2.3.2 Lung Function

A number of papers have suggested that the use of pesticides in occupational settings is associated with impaired lung function. For example, in a patient reported as a case of occupational asthma related to chronic exposure to the fungicide captofol showed a substantial

and persistent decrease in forced expired volume in 1 s (FEV_1) [113]. A cross-sectional study among the pesticide-processing workers showed that there was significant reduction in forced vital capacity (FVC), FEV_1 and the forced expiratory flow between 25% and 75% of forced vital capacity ($FEF_{25\%–75\%}$) in comparison to controls [96]. Another cross-sectional study of 102 pesticide sprayers and 69 non-sprayers in state farms of Ethiopia showed that pesticide sprayers in the age group of 15–24 years had significantly reduced FEV_1 and FVC compared to controls [114]. A similar study conducted among agricultural pesticide sprayers in Spain suggested that short term exposure to pesticides was related to reduction in FEV_1, while long term pesticide exposure was associated with reduction in $FEF_{25\%–75\%}$ after adjusting for age, gender, smoking status, body mass index (BMI), height, alcohol consumption, paraoxonase 1 (PON1) polymorphism and cholinesterase levels [115]. Moreover, in a study of farm workers in Sri Lanka, Peiris-John *et al*. suggested that the OP insecticide related decrease in FEV_1 and FVC occurred among those in agricultural occupations [116]. When comparing the respiratory function of pesticide factory workers with controls in Lebanon, Salameh *et al*. also found a significant reduction in FEV_1, $FEF_{25\%-75\%}$, and FEV_1/FVC ratio [117]. In addition, in a study conducted among agricultural workers in Colorado and Nebraska, an interaction between pesticide and endotoxin was identified with those workers reporting both exposures having a significantly greater endotoxin-related reduction in FEV_1 [118].

Impaired lung function was also associated with organophosphate or carbamate insecticide induced cholinesterase inhibition. In a matched case-control study of agricultural workers in India, exposures to organophosphate and carbamate insecticides were significantly associated with reductions in FVC, FEV_1, FEV_1/FVC ratio, $FEF_{25\%-75\%}$ and peak expiratory flow rate (PEFR), which was also significantly correlated with cholinesterase inhibition [100]. In

addition, a cross-sectional study of pesticide sprayers in Indian mango orchards similarly showed a correlation between impaired lung function and reduction of acetylcholinesterase and butylcholinesterase activities [119].

Other than the adverse effect on dynamic lung volumes, occupational exposures to pesticides may also lead to impairment of gas exchange in the lung. Two studies among farmers in Costa Rican and Western Cape showed a relationship between long-term low level paraquat exposures and exercise-associated oxygen desaturation, suggesting paraquat may cause gas exchange abnormalities [120, 121].

Both obstructive and restrictive abnormalities have been reported in association with occupational pesticide exposures. In the Spanish study mentioned above, FEV_1/FVC ratio was decreased among farm pesticide sprayers (although the change was not significant) [115], suggesting an obstructive abnormality. In India, long-term exposure to cholinesterase-inhibiting insecticides among agricultural workers was also associated with a significant decrease in the FEV_1/FVC ratio [100]. In Sri Lanka, the acute seasonal low-level exposure to organophosphate pesticides among farmers was associated with a normal FEV_1/FVC ratio but a reduction of both FVC and FEV_1 [116], suggesting a restrictive abnormality. In a study of pesticide spraying workers in mango plantation in India, the author suggested that a restrictive type of impairment of lung function was related to the exposures to organophosphate and organochlorine insecticide [122]. Additionally, in a study of farm operator and their spouse in Colorado in US, pesticide poisoning was significantly associated with lower FVC and FEV_1 among current smokers, again suggesting a restrictive defect [123].

There were also studies reporting no clear association [124-126] between pesticide exposure and lung function. These results may be due to the uncontrolled social and environmental

factors [125, 126], a "healthy worker effect" [125], better awareness of pesticide associated hazards among some workers [124], or other inherent issues with study design [124-126].

2.3.3 Occupational Asthma

In the past decade, asthma has been recognized as the most commonly reported occupational lung diseases [127], although there are variations in the attributable fraction (defined as one minus the reciprocal of relative risk) for occupational asthma in different populations [128-131]. Occupational asthma can be associated with significant medical and socioeconomic consequences [132-134].

Pesticide exposures have been associated with asthma in a number of occupational settings [77, 135]. For example, in France, a case of persistent asthma was linked to acute inhalation of the organophosphate insecticide dichlorvos for 8 hours in a closed kitchen [136]. Two cases of occupational asthma were reported in UK following exposure to fungicides fluazinam and chlorothalonil [137]. In Belgium, a case of occupational asthma was linked to the chronic exposure to tetramethrin, a pyrethroid insecticide [138]. In addition, a case series of individuals with reactive airways dysfunction syndrome (RADS), a subtype of work-related irritant-induced asthma (IIA), were thought to be related to exposures to unspecified herbicide and insecticide diazinon [139].

In a population-based study of occupational risk factors for asthma and respiratory symptoms in Singapore, LeVan *et al*. found that vapor exposure from pesticides (unspecified) was associated with non-chronic cough or phlegm (OR = 1.14, 95% CI: 1.03–1.27), chronic dry cough (OR = 1.55, 95% CI: 1.19–2.01), and adult-onset asthma (OR = 1.34, 95% CI: 1.15–1.56)[140]. A cross-sectional study conducted among 1,379 Brazilian agricultural workers showed that pesticide (unspecified) exposure in agricultural occupations was associated with a

higher prevalence of adult-onset asthma (OR = 1.54, 95% CI: 1.04–2.58) [141]. In this study, the authors also suggested that the effect of pesticide exposures on asthma was stronger in women than in men [141]. In Canada, a cross-sectional study on male farmers in Saskatchewan suggested that self-reported asthma was associated with the use of carbamate insecticides (OR = 1.8, 95% CI: 1.1–3.1) [142]. This study was the first population-based study to report an association between asthma and use of carbamate insecticides. No significant association was found between asthma and use of organophosphate insecticides in this study [142].

The Agricultural Health Study (AHS) in the US reported that adult-onset asthma was associated with exposure to pesticides, including organophosphate insecticides, carbamate insecticides and herbicides alachlor, atrazine and paraquat [12, 101, 143, 144]. In these studies, dose-dependent relationships were also observed between asthma symptom of wheezing and application of organophosphate insecticides chlorpyrifos and parathion and herbicides atrazine and paraquat [101, 143]. Hoppin *et al*. showed highly significant associations between adult-onset atopic asthma among male farmers and agricultural uses of pesticides coumaphos (OR 2.34; 95% CI: 1.49–3.70), heptachlor (OR 2.01; 95% CI: 1.30–3.11), parathion (OR 2.05; 95% CI: 1.21–3.46), 80/20 mix (carbon tetrachloride/carbon disulfide) (OR 2.15; 95% CI: 1.23–3.76) and ethylene dibromide (OR 2.07; 95% CI: 1.02–4.20) [12]. For nonatopic asthma, Hoppin *et al*. showed that DDT had the strongest association (OR 1.41; 95% CI: 1.09–1.84) among male farmers [12]. Among female farmers in the AHS, agricultural pesticide exposures, including seven insecticides (carbaryl, coumaphos, DDT, malathion, parathion, permethrin, and phorate), two herbicides (2,4-D and glyphosate) and a fungicide (metalaxyl), were more associated with atopic asthma than non-atopic asthma [144]. Hoppin *et al*. also suggested that growing up on a farm might modify the association between pesticide use and atopic asthma [144]. Consistent with the AHS study, a

recent study of French farmers also suggested that pesticide exposures were more associated with allergic asthma (OR = 1.97; 95% CI: 1.43–2.73) than non-allergic asthma (OR = 1.24; 95% CI: 0.88–1.76) [145]. Given that there is little antigenicity of chemical pesticide, pesticide-induced or promoted allergic/atopic asthma may be due to the indirect effect of pesticides on the immune system, such as interfering with Th-1/Th-2 (T-helper) balance or pesticide-induced oxidative stress [27, 146].

Pesticide use has also been associated with asthma exacerbations and health outcomes of patients with occupational asthma. In a retrospective cohort study of outdoor pesticide applicators in Australia, the mortality rate from asthma was higher (SMR = 3.45; 95% CI: 1.39–7.10) in workers who were occupationally exposed to insecticides, including the organochlorine insecticide DDT and acetylcholine esterase inhibiting insecticides carbaryl and chlorpyrifos, compared to the general population [147].

Non-significant association [125, 148] and inverse-effect relationships [126, 149] between pesticide exposure and asthma have also been observed. For example, a cross-sectional study conducted among female indigenous plantation workers in Costa Rica found a protective relationship between organophosphate pesticide exposure (terbufos and chlorpyrifos) and asthma or lung function [126]. However, in these studies, often only a small number of subjects were investigated [125, 126, 149] or many important social and environmental risk factors of asthma, such as household income, educational levels and growing-up in the farming environment, were not considered [125, 126] when assessing the association between occupational pesticide exposures and asthma. Therefore, although weak and statistically non-significant relationships were reported [124, 125, 149], most epidemiological studies have suggested a significant association of occupational pesticide use with asthma [12, 101, 126, 136, 138, 141, 142, 144, 147].

2.3.4 Chronic Bronchitis and COPD

Many studies have suggested that exposure to pesticides, especially in occupational settings, is associated with chronic bronchitis and COPD. In a matched case-control study of agricultural workers in India, a higher prevalence of chronic bronchitis was associated with OP and carbamate pesticide exposures (OR = 2.54, 95% CI: 1.48–3.74) [100]. A study of pesticide producing workers in Poland showed that a higher prevalence of diagnosed COPD (19.3% *vs.* 3%; p = 0.002) was associated with pesticide exposures at work after adjusting for smoking status [150]. In addition, there was a negative correlation between FEV_1/FVC index and duration of pesticide exposures in this study [150]. A case-control study in Lebanon showed a similar positive relationship between pesticide exposure and chronic bronchitis [151]. In the American Agriculture Health Study (AHS), Hoppin *et al*. showed that 11 pesticides, including organochlorine pesticides (heptachlor chlordane, DDT, lindane and, toxaphene), organophosphate pesticides (coumaphos, diazinon, dichlorvos, malathion, parathion) carbamate pesticides (carbaryl and carbofuran), permethrin, chlorophenoxy herbicides (2,4,5-T and 2,4,5-TP) and two other herbicides (chlorimuron-ethyl and petroleum oil) were significantly associated with chronic bronchitis [13]. In addition, in the AHS study, farmers with a history of high exposures to pesticide had a higher prevalence of chronic bronchitis (OR = 1.85, 95% CI: 1.51–2.25) [13]. Another study using the same dataset found that the incidence of chronic bronchitis among female non-smoking farmers was significantly related to the application of five pesticides, including insecticides dichlorvos (OR = 1.63, 95% CI: 1.01–2.61) and DDT (OR = 1.67, 95% CI: 1.13–2.47), and herbicides cyanazine (OR = 1.88, 95% CI: 1.00–3.54), methyl bromide (OR = 1.82, 95% CI: 1.02–3.24) and paraquat (OR = 1.91, 95% CI: 1.02–3.55) [152]. A recent cross-sectional study of AGRIculture and CANcer (AGRICAN), a French agricultural cohort, showed

that risk of chronic bronchitis among farmers was significantly associated with pesticide poisoning (OR = 1.67, 95% CI: 1.08–2.58 for those without healthcare; OR = 1.64, 95% CI: 1.11–2.41 for those with healthcare), but not significantly with activities of using pesticides [153], suggesting that episodes of high-dose acute pesticide poisoning possibly contribute more to COPD than long term low level exposures.

2.3.5 Lung Cancer

Occupational pesticide exposure has been linked to lung cancer, especially in agricultural settings. For example, a study of pest control workers in Florida suggested that longer duration exposure to organophosphate and carbamate insecticides and phenyoxyacetic acid herbicides was associated with a higher mortality rate of lung cancer (OR = 1.4, 95% CI: 0.7–3.0 for subjects licensed 10–19 years; OR = 2.1, 95% CI: 0.8–5.5 for those licensed 20 years or more) [154]. In a nested case-control study arising from the Agriculture Health Study (AHS), after controlling for age and tobacco smoking, Alavanja *et al*. showed statistically significant dose-response relationships between pesticide exposures, including insecticides chlorpyrifos and diazinon and herbicides metolachlor and pendimethalin, and risk of lung cancer [155]. Similar results were replicated in later studies of the AHS cohort for chlorpyrifos [156], diazinon [157], metolachlor [158] and pendimethlin [159]. The organochlorine insecticide dieldrin and the carbamate insecticide carbofuran were also reported to be positively associated with the risk of lung cancer [160, 161]. Samanic *et al*. showed that the highest tertile of lifetime exposure to herbicide dicamba was significantly associated with an increased risk of lung cancer (RR = 2.16, *p*-value for trend = 0.02) [162]. Moreover, arsenical pesticides, which are not currently used, were linked to lung cancer [163-165]. However, there were also a number of studies showing non-significant

associations [166-174] or negative relationships [175-182] between occupational pesticide use and lung cancer.

Although a number of studies have controlled for age and smoking status when assessing the association between pesticide exposure and lung cancer, some important risk factors of lung cancer, such as indoor/outdoor air pollutants [183, 184], life styles and psychosocial factors[185] and genetic predisposition [186], have not been routinely taken into account. In addition, the impurities or promoting agents in pesticide formulae, such as dioxin and dioxin-like contaminants of phenoxy herbicides (2,4-D, 2,4,5-T and MCPA) [187, 188], might have contributed to the significant association found between some pesticide exposures and lung cancer [154, 187, 188]. Therefore, current studies did not provide conclusive evidence connecting occupational pesticide exposure with lung cancer.

2.3.6 Other Respiratory Diseases

In addition to asthma, COPD and lung cancer, other respiratory diseases have also been linked to occupational pesticide exposures. For example, in the analysis of A Case Control Etiologic Study of Sarcoidosis (ACCESS) data, Newman *et al*. found that occupational exposure to insecticides was associated with an increased risk of sarcoidosis [189]. Slager *et al*., using data from the Agricultural Health Study, found that herbicides 2,4-D, glyphosate and petroleum oil (an additive in the herbicide formula to increase the phytotoxocity), the insecticide diazinon and the fungicide benomyl were positively associated with current rhinitis [190, 191]. In a study of grape farmers in Greece, exposures to paraquat and other bipyridyl herbicides increased the risk of developing allergic rhinitis [192].

In a cross-sectional study of farm residents in northeastern Colorado, experience of pesticide poisoning was significantly associated with a number of respiratory problems including

cough, allergy, wheeze, and organic dust toxic syndrome (ODTS) among non-smokers [123]. Although no significant association was found between pesticide exposures and farmer's lung, Hoppin *et al.* suggested that pesticide exposures, especially to organochlorine and carbamate pesticides, along with the causative exposure to thermophilic fungi from farming activities, such as silage handling and animal exposures, may collectively contribute to the incidence of farmer's lung [193]. In addition, although respiratory infection has been linked to exposure to organochlorine pesticides in young children [194, 195], there is a lack of evidence clearly demonstrating such a link between pesticide exposures and respiratory tract infection in occupational settings.

2.4 Pesticide exposures and Respiratory Health in General Population

While associations between pesticide exposure in the workplace and respiratory health have been well documented, pesticide exposures arising from the environment or diet are also potentially linked to the incidence of respiratory diseases and symptoms among the general population, although this has not been extensively studied [11].

2.4.1 Respiratory symptoms

Respiratory symptoms associated with non-occupational or environmental pesticide exposures include wheezing, coughing, airway irritation, and airway infection in children and adults. A study in Tanzania, showed that the habitants in houses sprayed with lambda-cyhalothrinnose, a pyrethroid insecticide, had nose or throat irritation accompanied by sneezing or coughing [105]. A cross-sectional study in Lebanese public schools showed that residential and domestic exposure to pesticides, including organophosphates, pyrethroids, bipyridyl herbicides and fungicide, was significantly associated with chronic cough with phlegm (residential: OR=1.59, 95% CI: 1.03-2.45; domestic: OR=1.96, 95% CI: 1.32-2.92) and recurrent

wheezing (residential: OR=2.73, 95% CI: 1.85-4.05; domestic: OR=1.49, 95% CI: 1.03-2.16) in children aged 5-16 years [196].

Although there is no evidence that the majority of pesticide exposures are directly related to the development of respiratory infection, exposures to organochlorine pesticides have been linked to respiratory infection in young children. A population-based study among Canadian Inuit infants suggested that prenatal exposures to pesticides was associated with 1.56 times higher risk of developing upper respiratory tract infection, but this association was not observed for lower respiratory tract infection [194]. A cohort study in Spain showed that the recurrent lower respiratory tract infections in infants were associated with organochlorine exposure (RR=2.40, 95% CI: 1.19-4.83) [195]. The authors in this study suggested that the high prevalence of lower respiratory tract infection was due to the immunologic suppression effect of DDE [1,1-bis-(4-chlorophenyl)-2,2-dichloroethene] [195]. Long-term lower respiratory tract infection during childhood may be important in the development of chronic inflammatory diseases of airways, such as asthma, in the later life [197, 198].

2.4.2 Respiratory diseases

2.4.2.1 Asthma

Along with other social, environmental factors and changes in life styles, pesticide exposures have been linked to the increasing incidence of asthma in the past three decades [77, 135]. Although the majority of epidemiological studies suggest the association between pesticide uses and asthma in the occupational settings [12, 101, 126, 136, 138, 141-144, 147], non-occupational or environmental exposures to pesticides have also been reported to be associated with asthma among general populations.

In California, environmental spills of carbamate insecticides led to cases of irritant-induced asthma among community residents [199]. In Australia, asthmatic symptoms in subjects with asthma and/or a history of chest tightness were linked to acute exposure to household pyrethroid insecticides [200]. However, studies examining the health effect of the mosquito controlling program in New York City showed that spraying of malathion (an organophosphate insecticide) and resmethrin (a pyrethrin insecticide) did not increase the rate of asthma-related visits to the Emergency Department (ED) during the spraying season (rate ratio= 0.92, 95% CI: 0.80-1.07) indicating that low-dose seasonal exposure to environment pesticides has limited impact on asthma morbidity [201, 202].

The association of pesticide exposure with asthma morbidity in children has recently gained great attention. In the US, an 11-year-old girl diagnosed with asthma at the age of six years underwent a "respiratory arrest secondary to acute asthmatic attack" and died after giving a bath to her pet dog using an animal shampoo containing pyrethrin [203]. A cross-sectional study in Lebanon showed that both residential and domestic exposures to pesticides were significantly associated with asthma (residential: OR=2.10, 95% CI: 1.01-4.42; domestic: OR=1.99, 95% CI: 1.00-3.99) among children aged 5-16 years [196]. Birth cohort studies in Spain suggested that pre-natal and/or early life exposures to ogranochlorine insecticides, such as DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane], were positively associated with asthma prevalence (OR=2.63, 95% CI: 1.19-4.69) in children [204, 205]. A birth cohort study in the UK suggested that medium-to-high level of postnatal exposure to fungicides, including maternal occupational exposure, was associated with an increased risk of childhood asthma (OR=1.47, 95% CI: 1.14-1.88) and wheezing (OR=1.22, 95% CI: 1.02–2.05) [206]. A nested case-control study among participants of the Children's Health Study in Southern California reported that environmental

exposure to herbicide and insecticide in the first year of life was significantly associated with risk of developing childhood asthma (herbicides: OR=4.58, 95% CI: 1.36-15.43; pesticides: OR=2.39, 95% CI: 1.17-4.89) [207]. A case-control study of asthma patients and non-asthmatic controls in a hospital in Lebanon suggested that residential exposure to pesticides was significantly associated to asthma incidence (OR = 2.11, 95% CI: 2.11-5.85) [208].

These studies have provided evidence for an association of non-occupational or environmental exposure to pesticides with asthma in the general population, including children who are likely to be the most vulnerable groups of subjects. Nevertheless, some of the studies lacked the information on specific types of pesticide being exposed [196, 206-208] and only a few large population-based studies have been conducted [201, 202, 207].

2.4.2.2. Other respiratory diseases

In addition to asthma, COPD, sinusitis and bronchitis have also been linked to pesticide exposures among the general population. A case-control study from Lebanon showed a significant positive relationship between residential pesticide exposures and chronic bronchitis (OR=3.70, 95% CI: 2.05-6.70) [151]. A cross-sectional study suggested that the prevalence of sinusitis and bronchitis was higher among subjects exposed to chlordane termiticide in a residential area in the US [209].

2.4.3 Lung function

Although there is evidence suggesting an association of pesticide exposures with impaired lung function in occupational settings, especially in agricultural occupations [114-119], very few studies have investigated the effect of pesticides on lung function in non-occupational settings among general populations. In Australia, a reduction in lung function (FEV_1 and $FEF_{25\%-75\%}$) was observed among pre-existing asthmatic patients after acute exposure to household

pyrethroid insecticides [200]. In a study conducted among Hispanic children in grades 2-5 in New Jersey, self-reported use of pesticide at home was a predictor of having a peak expiratory flow rate (PEFR) less than 75% of the predicted values [210]. Although these studies suggest the effect of pesticide exposures on lung function, they were conducted either among a group of patients with pre-existing respiratory diseases [200], or in a specific age group [210].

In summary, the effect of pesticides on respiratory health among the general population has been understudied. Respiratory symptoms, including wheezing, coughing, airway irritation, and airway infection in children, have been associated with pesticide exposures [105, 194-198]. Although there is a lack of evidence for adult population, a few studies have demonstrated the association between pesticide exposures and respiratory diseases and symptoms in children [196, 203-208].

2.5 Potential Issues in Research

2.5.1 Pesticide Exposures and Doses

In epidemiological studies, accurate exposure assessment is crucial for identifying adverse health effects. In a review paper on pesticide exposures among farmworkers, Hoppin *et al*. suggested three approaches to measure pesticide exposures in current studies: (1) personal measurements; (2) scenario-based assessment; and (3) biomonitoring measurement [20].

Personal measurements, such as hand-wipe samples or samples from masks or respirators, measure pesticide concentration at the immediate contact interface between subjects and pesticides, but do not measure the actual pesticide doses into the human body [20]. In a scenario-based approach, pesticide exposures are often measured or modeled by questionnaire-based measurements or job titles, both liable to error and bias [211].

Using biomarkers or biomonitoring levels of pesticides or their metabolites is an objective measurement of pesticide exposures, and is also considered as an approach to measure doses or actual body burden arising from pesticide exposures [212]. However, the use of biomonitoring approach has limitations, especially when short time windows are required for accurately capturing the peak level of pesticide exposures. Bio-markers may be good for organochlorine (OC) pesticides, since they typically have a long half-life, and so serum levels can be used as a marker of past or cumulative OC exposure. However, for non-persistent pesticides, such as OPs, biomarkers have limitations if trying to estimate cumulative exposure and thus often studies of chronic exposure mainly rely on questionnaire-based approaches [213]. Moreover, in biological measurement of non-persistent pesticides, the sampling time frame and temporal variability are critical for the validity of data analyses and result interpretation [212]. For example, in measuring sub-chronicle OP exposures, the AChE level in blood or erythrocytes can be used as a biomarker, since this effect can last for 3–4 months [213], although it is considered relatively insensitive and prone to error. Nevertheless, it cannot be used to evaluate carbamate exposure, as AchE inhibition by carbamates lasts only a few minutes [213].

Biomonitoring measures have additional benefit of integrating pesticide exposures from all physiological pathways [212]. On occasions, multiple biomarkers can be used for assessing single exposures to pesticides. Urinary metabolites of pesticides such as dialkyl phosphates and TCP (3,5,6-trichloro-2-pyridinol, a metabolite of chlorpyrifos), and plasma butyl cholinesterase (BuChE) and erythrocyte acetyl cholinesterase (AChE) activities, can all be used as biomarkers for measuring OP exposures [214]. Studies of these three biomarkers have shown an inverse relationship between the acute concentration of urinary TCP and the sub-chronic activities of plasma BuChE and/or erythrocyte AChE when assessing chlorpyrifos exposures [215, 216].

Studies of pesticide exposures using personal measurements, such as hand-wipe samples or samples from the immediate contact interface, have suggested that pesticide exposures was higher among subjects at occupational risk than the general population [217, 218]. Moreover, the type of job being performed may also be associated with the biomonotoring levels of pesticide exposures for those working nominally in the same occupations. For example, a study in Egypt showed that among adolescent agricultural workers, those applying pesticide had a higher level of TCPy, a metabolite specific to the organophosphate insecticide chlorpyrifos, than individuals not performing that job [219]. However, a population-based study in the US using data from NHANES (1999–2002) showed no significant difference in the urinary concentrations of 3-PBA between subjects at higher occupational risk (farm and cleaning/building services) and those working at other occupations [220]. A study undertaken in France also suggested that there was no significant difference in biomonitoring concentrations of dialkyl phosphate metabolites of organophosphates between workers at higher occupational risk and the general population [221]. The inconsistent results between studies using a personal measurement approach [217, 218] and the biomonitoring approach [221] might be due to the limitations of the biomonitoring approach in estimating peak or cumulative exposures [212]. This issue is particularly a concern for the non-persistent chemicals when the relationship between external dose, i.e. personal measurement of pesticide exposures, and internal dose, i.e. measurement from the biomonitoring approach, are less clear [212].

Indirect exposures from “take-home” pesticides or overspray of residential areas as a consequence of occupational exposures may also have effect on human respiratory health [196, 206, 222]. For example, on the farm, pesticide applicators or sprayers often have substantial exposures to pesticides. However, farm workers who do not apply pesticide as part of their jobs

may still be exposed, and their family members might also be exposed from 'take-home' pesticides. People living close to farms are also likely at a high risk of being exposed. Therefore, some epidemiological studies may have underestimated the effect of pesticide exposure when assuming all farmers or pesticide applicators are exposed while non-farmers are un-exposed (non-differential misclassification) [213]. In addition, peak or average exposure intensity may be more relevant than cumulative exposure, especially for characterizing the dose-response relationships [213]. Nevertheless, for asthma, little is known about whether peak exposure has greater relevance than cumulative exposure [27].

2.5.2 Other Issues in the Association Studies

In 1998 pesticide exposure was listed as one of the top respiratory health hazards in agricultural by the American Thoracic Society [223]. Nevertheless, Kirkhorn and Garry in their review of agricultural lung diseases suggested that, although there were notable exceptions, pesticides may not be either a single or direct cause of chronic pulmonary diseases, such as asthma [224]. Other than pesticide exposures, many factors such as variations in genetic makeup, physiological states, socioeconomic and psychosocial factors, and other environmental factors may also contribute to the development of respiratory symptoms and diseases. These factors must be considered along with pesticide exposures when evaluating the effects of pesticide on respiratory health.

In occupational settings, it is often difficult to identify causes of disease because responses may be delayed and so occur at home, or may even occur many years later for diseases with considerable latency [127]. It is also critical when performing a study to use appropriate 'normal controls' to ensure results are compared between comparable groups. In studies of occupational disease, a 'healthy worker effect' is often observed, *i.e.*, workers usually have lower

rate of disease than the general population [225]. This phenomenon is due to the exclusion of persons with disease from employment [225]. Therefore, the effect of pesticides on respiratory health in occupational settings may be underestimated when comparing with general populations. Le Moual *et al*. showed that a "healthy worker effect" can also occur after employment commences when sick workers leave their jobs perceived as "risky" and find new jobs with less exposures, or become more careful to avoid being exposed within the same job [226]. The author suggested that many cross-sectional studies of occupational asthma lacked sufficient information of health and job status both before and after employment [226]. Spurious results can be obtained without taking account of the 'healthy worker effect' into study design, data collection and statistical analyses.

McCauley et al suggested that valid diagnosis or confirmation of symptoms, diseases, or biological markers of a health effect are critical for effectively studying the association between pesticide exposures and health outcomes [227]. Data sources on health outcomes of occupational pesticide exposures include workers' compensation (WC) systems, hospital and occupational medicine specialist admission and discharge data, and health insurance data. However, workers' compensation (WC) systems are generally different from region to region, and health insurance information may be incomplete or inaccurate, especially for those part-time workers without health insurance [227-230]. In addition, longitudinal studies are preferable for characterizing long-term respiratory health effect. Although it is challenging to track cohorts with high mobility, such as migrant and seasonal farmworkers, longitudinal data are especially critical for characterizing the association and establishing a temporal relationship between occupational exposures and health outcome [227, 231].

In summary, epidemiological studies require careful measurement of both exposures and outcomes when assessing the relationships between occupational pesticide exposures and respiratory health. In addition, proper evaluation of important biases, confounders, and effect modifiers, such as genetic predisposition, social, and psychological factors, are important for avoiding spurious results in association studies. It is also important to use longitudinal approaches when there were temporal variations in pesticide exposures.

2.6 Conclusions

Although this review is not exhaustive in its scope or depth, studies reviewed in this paper have strongly suggested an adverse effect of pesticide exposures on human respiratory health in occupational settings. Respiratory symptoms, including wheezing, airway irritation, dry/sore throat, cough, breathlessness and chest tightness, and respiratory diseases such as asthma and COPD, were associated with occupational pesticide exposures. Impaired lung function was also often observed among people occupationally exposed to pesticides. There is little evidence suggesting that occupational pesticide exposure is associated with respiratory tract infection, although an association has been described for organochlorine insecticide exposures in young children [194, 195]. Inconclusive results have been reported from studies of the association between occupational pesticide exposures and lung cancer [155, 157, 161, 162, 166-169].

There are some limitations to the data. Although there were studies of populations from developing countries, such as India, Sri Lanka and Ethiopia [100, 114, 116], most studies have taken place in more developed parts of the world, and many large (and important) areas remain unstudied. In addition, in many studies pesticide exposures were measured by questionnaire-based approaches or something as simple as job title, which has the potential to introduce error.

An exception are the studies performed by Sunyer, Boers, Del Prado-Lu, Chakraborty and Karmaus, which used biological measures of pesticide exposure in urine or blood samples [39, 100, 149, 204, 232].

Table 2-1. Categories of commonly used pesticides

Type of pesticide	Chemicals
Herbicide	Chlorophenoxyl (2,4-D, 2,4,5-T and MCPA), urea derivatives, triazines (atrazine), amide (propanil), bipyridils (paraquat and diquat), glyphosate
Insecticide	Organochlorines [dichlorodiphenylethanes (DDT, DDD, dicofol), chlorinated cyclohexanes and benzenes (lindane, HCB), cyclodienes (aldrin, endosulfan, chlordane and toxaphene) and chlordecone (mirex), organophosphates (chlorpyrifos, diazinon, parathion, malathion), carbamates (aldicarb, aminocarb), pyrethroids (pyrethrins, permethrin, deltamethrin, cypermethrin), rotenone, *Bacillus thuringiensis* (protein product)
Fungicide	Dithiocarbamate, captan, captofol, pentachlorophenol, iprodione, sulphur
Bactericide	Triazine-S-triones, chlorine-releasing agents, chlorine, dichloronitrobenzene
Rodenticide	Coumadin and derivatives, anticoagulants, strychnine, sodium fluoroacetate
Fumigant	Methyl bromide, aluminum/zinc phosphide, sulfur

Table 2-2. Classification of pesticides based on toxicity in mammals

Neural conduction interferer	
Organochlorine	dichlorodiphenylethanes (DDT, DDE)
	cyclodienes (aldrin, α-chlordane, γ-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, toxaphene parlar 26, toxaphene parlar 50) hexachlorocyclohexanes (hexachlorobenzene, β-hexachloro-cyclohexane, γ-hexachlorocyclohexane) chlordecone (Mirex)
Pyrethroid	pyrethrin, tetramethrin
Acetylcholine esterase inhibitor	
Organophosphate	parathion, malathion, methyl parathion, chlorpyrifos, diazinon,
Carbamate	aldicarb, carbofuran, carbaryl, ethienocarb, fenobucarb
Pro-inflammatory stimulator	
Chlorophenoxy herbicides	2,4-D (2,4-dichlorophenoxyacetic Acid)

Table 2-3. Immunological endpoints associated with pesticide exposure

Exposure	*Type of exposure*	*Effect*	*Reference*
DDT	Occupational	Increase in IL-4	[233]
DDT	Non-occupational	Increased in total lymphocytes and immunoglobulin A levels; decrease in CD16 cells, CD4/CD8 ratio, IgM and proliferative response to mitogen	[234, 235]
DDT	Occupational	Decrease in IgG	[236]
DDT and gamma-HCH	Occupational	Impaired neutrophil function, infectious diseases	[237]
HCB	Occupational	Impaired neutrophil function	[238, 239]
HCB	Occupational	Increase in IgM and IgG	[239]
HCB	Occupational	Decrease in IFN-gamma	[240]
HCH	Occupational	Increase in IgM	[241]
Lindane	Poisoning cases	Increase in IL-2,IL-4, TNF-alpha;decrease in IFN-gamma	[242]
Chlordane	Residential and occupational	Increase in IL-2, IL-3, IL-7, IL-12, IL-15, IFN-gamma, CD1 lymphocyte and auto antibody; decrease in CD45RA/T4 cells and proliferative response to mitogen	[243]
Chlordane and/or heptachlor	Non-occupational	Increase in IL-1, IL-6, IL-8, RANTES, TNF-alpha, increase in Th2 humoral immunity; the decrease in Th1-cytokines IL-2, IL-12, IL-15 and IFN-gamma and cellular immunity, allergic rhinitis, infection of upper airways.	[244]
PCP (pentachlorophenol)	Occupational	Decrease in proliferative response to mitogen	[245]
PCP	Non-occupational	Decrease in proliferative response to mitogen, MLR and IgG	[82]
PCP	Occupational	immature leucocytes and basophils	[81]

Table 2-3. Immunological endpoints associated with pesticide exposure (cont.)

Exposure	*Type of exposure*	*Effect*	*Reference*
Organochlorine compounds (chlorinated benzenes, organochlorine insecticides and PCB congeners)	Non-occupational	Increase in cord serum IgE, eczema	[246]
DDE and HCB	Non-occupational	Increase in serum IgE, infection and atopic diseases	[232]
Chlorpyrifos	Occupational	Increase in CD26 cells and autoantibodies; Decrease in CD5 cells and proliferative response to mitogen; atopic diseases	[88, 89]
Sarin (OP)	Poisoning cases	Decrease in NK and CTL cells	[247]
Aldicarb	Non-occupational	Increase in CD8 cells and percentage of CD8 cells; Decrease in CD4/CD8 ratio.	[248]
Aldicarb	Non-occupational	Increase in CD2 and CD8 cells	[249]
Mancozeb	Occupational	Increase in IL-2 and proliferative response to mitogen	[250]
Mancozeb	Occupational	Increase in CD19 B cells and proliferative response to mitogen; Decrease in TNF-alpha and CD25 cells	[251]
2,4-D and MCPA	Occupational	Decrease in T-cell proliferation, CD4+ T helper cells, CD8+ Treg cells, nature killer cells and CTL	[90]

Table 2-4. EDC properties of pesticides and their metabolites

Anti-estrogen
DCCA and 3-PBA (pyrethroid metabolites)
Xeno-estrogen
OC (DDT, chlordecone,taxophene), OPs (chlorpyrifos), pyrethroids, herbicide atrazine
Anti-androgen:
PCP, DDE, lindane
Xeno-androgen:
2,4,5-T
Thyroid hormone disruptors
Chlorpyrifos, PCB
Corticoid function disruptors
HCB

Figure 2-1. Phase I biotransformation of organophosphates

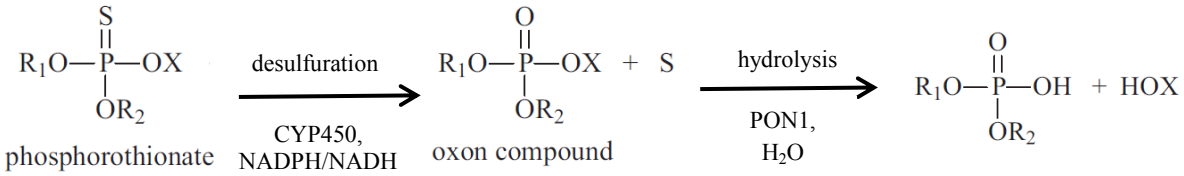


Figure 2-2. CYP-mediated O-dearylation of organophosphates

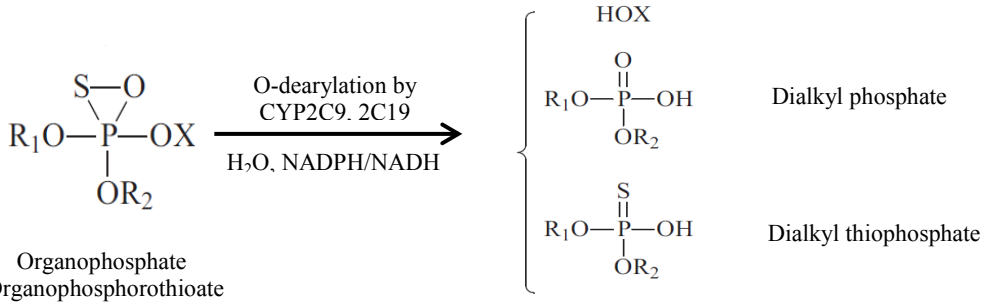
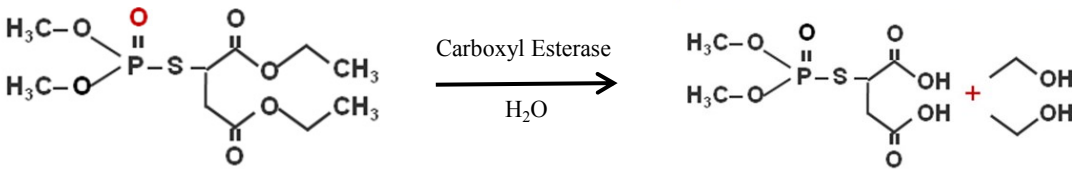
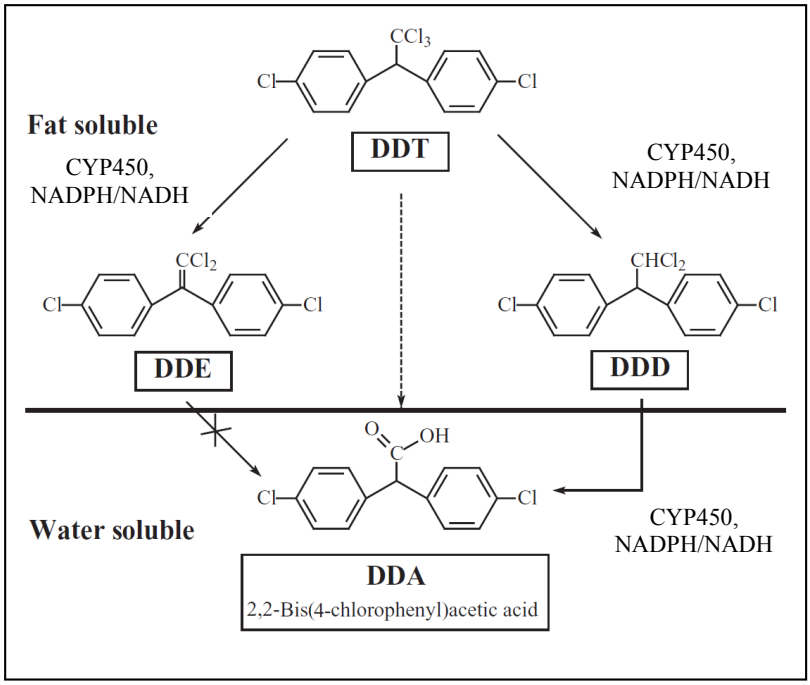


Figure 2-3. The hydrolysis pathway of organophosphate (malathion)



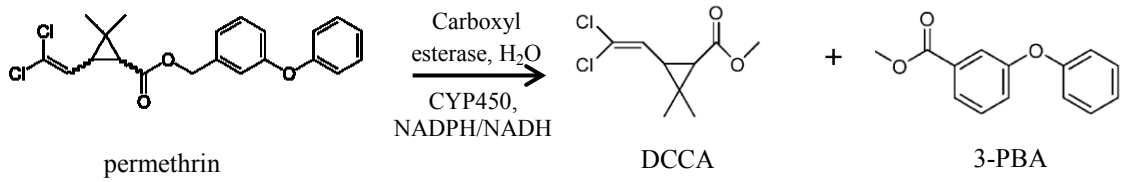
malathion

Figure 2-4. Biotransformation of DDT*



* Adapted from Chen Z et al *Int J Toxicol*. 2009 [55]

Figure 2-5. Oxidation and cleavage of ester bond in pyrethroid



permethrin DCCA 3-PBA

DCCA: 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid

3-PBA: 3-phenoxybenzoic acids

CHAPTER 3

ASSOCIATION BETWEEN DIETARY FACTORS AND URINARY CONCENTRATIONS OF ORGANOPHOSPHATE AND PYRETHROID METABOLITES IN A CANADIAN GENERAL POPULATION

3.1 Introduction

Organophosphate (OP) and pyrethroid (PYR) pesticides are currently widely used in large quantities in agriculture and residential areas to control insect pests [1]. According to the Environmental Protection Agency in the US, only 1% of the five billion pounds of pesticides applied annually actually reaches its target [2], suggesting the possibility for humans to be exposed to OP and pyrethroid pesticides through the environment.

Pesticide residues in food products are believed as a major source of pesticide exposure to humans. It has been estimated that approximately 33% of table-ready food items had detectable levels of one or more types of pesticides [3]. A study in Denmark showed that pesticide residues were detected in 60% of fruits and 18% of vegetables on the market [4]. Studies from the USA found that the OP pesticide, chlorpyrifos and its degradation products, were detected in up to 97% of solid food samples [5, 6]. Results from the US Children's Pesticide Exposure Study (CPES) showed that OP and pyrethroid insecticides were detected in 14% and 5% of children's food samples, respectively [7]. In addition, cypermethrin, a synthetic pyrethroid pesticide, was detected in 30% of the composite diet samples collected from an adult population in the USA [8].

Association studies have suggested relationships between dietary food intake and urinary concentrations of OP and PYR insecticides [9-14]. In an adult population in Israel, fruit consumption was significantly associated with urinary concentrations of OP metabolites [9].

Results from the New York City Health and Nutrition Examination Survey (NYC HANES) showed that urinary OP concentrations were positively associated with increasing frequency of fruit consumption [10]. Another study in Chile showed that residual levels of OP pesticides on fruits were significantly associated with higher urinary concentrations of OP metabolites among school-aged children [11]. A recent study of an adult population in Italy showed that consuming cruciferous and leafy vegetables were strongly associated with urinary levels of pyrethroid metabolite 3-phenoxybenzoic acid (3-PBA) [12]. In the US National Health and Nutrition Examination Survey (NHANES, 1999-2002), dietary factors were significantly associated with urinary concentrations of 3-PBA for children, adolescents and adults [13]. Moreover, results from the US CPES suggested that dietary food intake was one of the important predictors of urinary 3-PBA levels among children [14]. However, these epidemiological studies were typically conducted in specific age groups [9, 11, 14], and often in a small convenient sample [12] or specific region [10]. Only few large general population-based studies [10, 13], and none in Canada, have investigated the relationship between dietary factors and urinary concentrations of OP and PYR metabolites.

In the current study, associations between dietary factors and urinary concentrations of organophosphate and pyrethroid pesticide metabolites were examined in a Canadian general population using data from the Canadian Health Measures Survey (CHMS).

3.2 Methods

In 2007-2009, Statistics Canada conducted the Canadian Health Measures Survey (CHMS-Cycle 1), a population-based cross-sectional survey, to collect baseline health information of Canadians [15]. In the current study, we used data on 5, 604 participants aged 6-79 years in the CHMS-Cycle 1.

A multi-stage sampling strategy was used by Statistics Canada to select CHMS-Cycle1 participants: collection sites were selected after being stratified by geographic region and CMA (Census Metropolitan Area); dwellings within each collection site were sampled and then stratified based on inhabitants' ages; in each age stratum, participants were then sampled from the selected dwellings [15]. People living on reserves and Aboriginal settlements, residents of institutions, members of the Canadian Forces and those living in remote areas with population density less than 400 people per square kilometer, were not included [15]. Detailed information on the CHMS-Cycle 1 can be obtained from the online publication of Statistics Canada [15].

In the current study, urinary concentrations of OP and pyrethroid pesticide metabolites, and information on demographic, anthropometric, socioeconomic, household pesticide use and dietary food consumption of 5, 604 subjects were obtained from the CHMS-Cycle 1.

3.2.1 Urinary concentrations of OP and pyrethroid pesticide metabolites

Approximately 60mL of mid-stream urine was collected for each CHMS-Cycle 1 participant aged 6-79 years [15]. After collection, urine samples were refrigerated immediately and transported to an analytical laboratory at the National Public Health Institute of Quebec (INSPQ). Urine samples were analyzed for dialkyl phosphate (DAP) metabolites, including dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP), and for metabolites of pyrethroid pesticides [16], including *cis*-DCCA [*cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid], *trans*-DCCA, *cis*-DBCA [*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid], 3-PBA (3-phenoxybenzoic acid) and 4-fluoro-3-PBA [15]. Concentrations of DAPs and PYR metabolites were determined by gas chromatography–mass spectrometry (GC-MS) [15, 17]. Limits of detection (LOD) for

measuring DMP, DMTP, DMDTP, DEP, DETP, DEDTP, 3-PBA, 4-fluoro-3-PBA, *cis*-DCCA, *trans*-DCCA and *cis*-DBCA were 1.0 μg/L, 0.6 μg/L, 0.3 μg/L, 1.0 μg/L, 0.6 μg/L, 0.3 μg/L, 0.01 μg/L, 0.008 μg/L, 0.007 μg/L, 0.01 μg/L and 0.006 μg/L, respectively [17]. Urinary creatinine concentration (g/L) was measured using the colorimetric Jaffe method and was used to normalize pesticide metabolite concentrations for urine dilution [18].

3.2.2 Demographic, anthropometric and socioeconomic factors

Information on age, sex, ethnicity, immigration status and province of residence were collected by the CHMS-Cycle 1 using questionnaire [19]. Standing height and weight were measured using standard procedure of the Canadian Physical Activity, Fitness and Lifestyle Approach (CPAFLA) [20]. Body mass index (BMI) was calculated using the formula weight (kg) / [height (m)]^2 [20]. Subjects were classified, according to the US Center for Disease Control and Prevention (CDC) BMI-for-age growth charts for children [21] and the WHO International Classification of BMI for adults [22], into three BMI groups: underweight or normal [BMI < 85th percentile for children and <25 kg/m^2 for adults], overweight [BMI 85th to < 95th percentile for children and 25 to <30 kg/m^2 for adults] and obese [BMI >= 95th percentile for children and >=30 kg/m^2 for adults].

Information on socioeconomic factors included the highest education level with four categories (less than secondary, secondary, some post-secondary and completion of post-secondary education) and total household income with four categories (less than $30k, $30k-$50k, $50k-$80k, $80k and above) [19]. A dichotomous variable indicating whether or not a participant had ever smoked (Yes/No) was defined for subjects aged 12-79 years based on information collected on cigarette smoking [19].

3.2.3 Household pesticide use and dietary factors

Information on household pesticide use was collected for all 5,604 participants by the CHMS-Cycle 1 using questionnaire, including home/yard pesticide use (Yes/No) and the use of chemicals to control head lice or pet fleas (Yes/No) during the past month [19].

Information on food intake of 35 Canadian common dietary food items was collected in the CHMS-Cycle 1. Yearly frequency of consumption of each food item was calculated by Statistics Canada based on responses to the questions in the CHMS-Cycle 1 questionnaire: "How often [number of times] do you usually eat [food item]?" and "Select reporting period [per day/week/month/year]" [15].

Food items measured in the CHMS-Cycle 1 were classified into ten groups according to the food classification system (Foodex 2.0) of the European Food Safety Authority (EFSA) [23]: egg and egg products, meat and meat products (including red meat, organ meat, hot dog and sausage), fish and sea food (including salt water fish, fresh water fish and shell fish), pulses and nuts (including dried beans and nuts), milk and dairy products (including milks, cheese, yogurt and ice-cream), grains and grain-based products (including cereal, bread, pasta and rice), fruits and fruit juice, vegetable and vegetable products (including tomato, green leafy vegetables, spinach, other vegetables and vegetable juice), starchy roots (including potato and sweet potato), water and water based beverage. The weekly frequency of food consumption was calculated by adding the yearly frequency of food items within each of the ten groups and then dividing by the average number of weeks (52.2) in a year. In addition, a categorical variable based on the tertiles of the weekly frequency of food consumption was defined to indicate low, medium and high weekly frequency of food consumption for each of the ten groups.

3.2.4 Statistical analyses

We used the total concentration of six DAPs (ΣDAP, sum of DMP, DMTP, DMDTP, DEP, DETP and DEDTP) and the total concentration of five PYR metabolites (ΣPYR, sum of 3-PBA, 4-fluoro-3-PBA, *cis*-DCCA, *trans*-DCCA and *cis*-DBCA) to estimate the overall exposures to OP and pyrethroid pesticides, respectively. To calculate ΣDAP and ΣPYR, mass concentrations of metabolites in urine (μg/g creatinine or μg/L) were converted to molar concentrations (nmol/g creatinine or nmol/L) using respective molecular weights of DAPs and PYRs. Samples with pesticide metabolite concentrations less than LOD were assigned as 0.5*LOD [24]. DAP and PYR metabolite concentrations were dichotomized into detectable, or not detectable, according to their respective LODs. Natural log transformed ΣDAP, ΣPYR and urinary creatinine concentrations were also calculated to reduce the skewness of the distribution.

In the descriptive analyses, means, medians and inter quartile ranges (IQRs) of urinary concentrations (nmol/g creatinine) and proportions of subjects with detectable pesticide metabolites in urine were calculated for each DAP and PYR metabolite individually, as well as for ΣDAP and ΣPYR. Descriptive statistics were not calculated for a metabolite with more than 40% of samples having metabolite concentration less than the LOD [17].

Predictors of creatinine-adjusted urinary concentrations (nmol/g creatinine) of OP and pyrethroid pesticide metabolites were determined by both bivariate analyses and independent multiple linear regression analyses with natural log transformed concentrations of ΣDAPs and ΣPYRs as dependent variables and categorical variables indicating food consumption frequencies as independent variables, demographic and anthropometric characteristics, socioeconomic factors, household pesticide use as potential confounders. Sensitivity analyses were also performed using molar concentrations (nmol/L) of ΣDAPs and ΣPYRs as dependent variables and urinary creatinine concentrations (g/L) as an independent covariate to adjust for the

effect of the potential diet-related variation in urinary creatinine concentrations [18]. Clinically and biologically plausible interactions between predictors were examined. A significance level of ≤0.05 was used in the multiple regression analyses. The adjusted means of ΣDAP and ΣPYR concentrations were estimated using the fitted multiple linear regression models with significant covariates taking mean or reference values.

In all statistical analyses, sampling design weights provided by Statistics Canada were used to adjust for post-stratification in the multistage sampling, units with no responses and units with out of scope responses [15]. In addition, 500 bootstrap weights provided by Statistics Canada were applied in variance estimation in all the statistical analyses [15]. Statistical analyses were performed using complex survey procedures in the statistical software, STATA (StataCorp LP. 2007, Release 12) and SAS-callable SUDAAN (RTI International 2014, SUDAAN® 11.0 SAS Institute Inc. 2011, SAS® 9.3).

3.3 Results

3.3.1 Characteristics of the study participants

The demographic, anthropometric and socioeconomic characteristics, as well as the household pesticide use, of 5,604 participants are shown in Table 3-1. In addition, the distributions of weekly frequency of food consumption and tertiles of food consumption frequency (low, medium and high) for each of the ten food groups are listed in Table 3-2. Other than water and water based beverage, grain and grain-based products, milk, fruits and vegetables were the most frequently consumed food items in Canada (Table 3-2).

3.3.2 Urinary concentrations of DAPs and PYRs by demographic, socioeconomic factors and pesticide uses

Among the participants, 91.4% had at least one of the six dialkyl phosphate metabolites and almost all subjects (99.8%) had at least one of the PYR metabolites detectable in their urine samples (Table 3-3). The mean (geometric mean) concentrations of total DAPs (ΣDAP) and total pyrethroid metabolites (ΣPYR) in urine were 93.19 nmol/g creatinine (95% CI: 84.26-103.06 nmol/g) and 3.43 nmol/g creatinine (95% CI: 2.87-4.09 nmol/g), respectively (Table 3-3).

Children aged 6-11 years had higher mean urinary concentrations of ΣDAP and ΣPYR than adolescents and adults (Table 3-4). Female subjects had significantly higher mean ΣDAP and ΣPYR concentrations than males ($p<0.0001$). After adjusting for age, immigrants had significantly higher mean concentrations of both ΣDAP ($p<0.01$) and ΣPYR ($p<0.01$) than non-immigrants. There was no significant difference in ΣDAP and ΣPYR concentrations across household income levels. As shown in Table 3-4, mean concentration of ΣPYR was significantly higher among subjects who used chemicals to treat lice or fleas at home, or subjects who used pesticide at home or in the yard in the past month than those who did not ($p<0.05$). No significant association was observed between smoking status and ΣDAP or ΣPYR concentrations in a subgroup of CHMS participants aged 12 years or above (data not shown).

3.3.3 Frequency of food consumption and urinary ΣDAPs concentrations

In the bivariate analyses, urinary concentrations of ΣDAP were significantly higher among subjects who consumed fish/sea food, pulses/nuts, milk & dairy products, fruits or vegetables more frequently than those who consumed these foods less frequently ($p<0.05$, Table 3-5). In addition, results from bivariate analyses also showed that subjects who consumed egg products, meat or starchy roots more frequently had significantly lower urinary concentrations of ΣDAP than those who consumed these foods less frequently ($p<0.05$, Table 3-5).

In the multiple linear regression analysis, there was a dose-response relationship between weekly consumption of fruits and vegetables and ΣDAP concentrations after adjusting for age, sex, ethnicity, immigration status and BMI (Table 3-6). The adjusted means of urinary ΣDAP concentration for subjects with low, medium and high weekly frequency of fruit consumption were 79.88 nmol/g creatinine (95% CI: 69.38-91.96 nmol/g creatinine), 96.43 nmol/g (95% CI: 80.01-116.22 nmol/g creatinine) and 113.93 nmol/g (95% CI: 98.36-131.96 nmol/g creatinine), respectively. The adjusted means of urinary ΣDAP concentration for subjects with low, medium and high weekly frequency of vegetable consumption were 79.88 nmol/g (95% CI: 69.38-91.96 nmol/g creatinine), 91.34 nmol/g creatinine (95% CI: 77.13-108.17 nmol/g creatinine) and 106.23 nmol/g creatinine (95% CI: 91.91-122.79 nmol/g creatinine), respectively. No significant interactions were observed between age groups, sex, ethnicity and immigration status and weekly frequency of fruit and vegetable consumption in predicting urinary concentrations of ΣDAP.

In the sensitivity analysis (Table 3-7), when the urinary creatinine concentration was adjusted in the regression analysis as an independent variable, results regarding the associations between weekly frequency of fruit and vegetable consumption and urinary molar concentrations of ΣDAP were consistent with those reported in Table 3-6.

3.3.4 Frequency of food consumption and urinary ΣPYRs concentrations

In the bivariate analyses, as shown in Table 3-5, mean urinary concentrations of ΣPYR were significantly greater among subjects who consumed fish/sea food, pulses/nuts or vegetables more frequently in a week than those who consumed these foods less frequently per week ($p<0.05$). In addition, in the bivariate analyses, subjects who consumed meat or starchy roots more frequently in a week had significantly lower urinary concentrations of ΣPYR than those who consumed these foods less frequently per week ($p<0.05$).

In the multiple linear regression analyses, there was a suggestion of a dose-response relationship between weekly frequency consumption of pulses and nuts or vegetables and urinary concentrations of ΣPYR after adjusting for age, sex, ethnicity, immigration status, BMI and use of chemicals for lice/flea treatment (Table 3-6). The adjusted mean urinary ΣPYR concentrations for subjects with low, medium and high weekly frequency of pulses/nut consumption were 2.59 nmol/g creatinine (95% CI: 2.15-3.12 nmol/g creatinine), 2.74 nmol/g creatinine (95% CI: 2.24-3.36 nmol/g creatinine) and 3.21 nmol/g creatinine (95% CI: 2.51-4.09 nmol/g creatinine), respectively. The adjusted mean urinary ΣPYR concentrations for subjects with low, medium and high frequency vegetable consumption were 2.59 nmol/g creatinine (95% CI: 2.15-3.12 nmol/g creatinine), 3.33 nmol/g creatinine (95% CI: 2.84-3.90 nmol/g creatinine) and 3.68 nmol/g creatinine (95% CI: 3.07-4.43 nmol/g creatinine), respectively. No significant interactions were observed in the association of dietary factors with urinary concentrations of ΣPYR and age groups, sex, ethnicity and immigration status.

In the sensitivity analyses (Table 3-7), when the urinary creatinine concentration was adjusted in the regression analysis as an independent variable, results regarding the association between weekly frequencies of pulses/nuts or vegetable consumption and urinary molar concentrations of ΣPYR were consistent with those reported in Table 3-6.

3.4 Discussion

In the current study, we examined the relationship between weekly consumption of different types of foods and urinary concentrations of organophosphates and pyrethroid metabolites in a representative sample of the Canadian general population. Our results showed that urinary concentrations of ΣDAP were significantly associated with higher weekly frequency of fruit and vegetable consumption, which is consistent with other population-based studies in

the US and Israel [9, 10]. Pesticide residue on food items in Canada is likely the source of these pesticide metabolites detectable in urine. A study of food products in Ontario, Canada, showed that 68.5% of locally produced fruits and vegetables contained one or more pesticide residues [25]. This data, including our results, suggest that there is an increased likelihood of getting exposed to OP pesticides through fruit and vegetable consumption.

In addition, our results showed that weekly frequency of vegetable consumption, but not weekly frequency of fruit consumption, was significantly associated with urinary ΣPYR concentrations, which is consistent with a recent study in Italy that showed mainly an association of vegetable intake with urinary concentrations of 3-PBA [12]. In the German Environmental Survey on Children, urinary 3-PBA was found to be associated with the reported intake of boiled vegetables [26]. These data suggest that pesticide-sprayed or treated vegetables are significant sources of exposures to pyrethroid pesticides.

Our results also showed an association between the frequency of pulses/nut consumption and urinary ΣPYR concentration. This is plausible because of the use of pyrethroid insecticides in preserving and storage of pulses and nuts [27, 28]. To the best of our knowledge, this is the first report indicating the association between intake of nuts and urinary ΣPYR concentration in a population-based study.

Several food surveys have suggested there are lower pesticide levels on food items produced using organic farming [29, 30]. It has been found that children who consumed conventional diets had a median total dimethyl phosphate metabolite concentration six times higher than those consuming organic diets [31]. In addition, intervention studies in young children from the USA have shown that the urinary concentrations of OP metabolites were significantly reduced after substituting conventional food with organic food for 5 days [32, 33].

These results suggest that consumption of food products from organic farming could reduce exposures to OP and pyrethroid insecticides.

Several studies among children have suggested that household use of pyrethroid insecticides is more closely associated with the urinary concentrations of pesticide metabolites than dietary factors [14, 34]. In the bivariate analyses in our study, participants who used pesticide at home or in their yard during the previous month had a significant greater urinary concentration of ΣPYR than those who did not (p=0.044). A few pyrethroid insecticides, including pyrethrin, permethrin and phenothrin [35], are used as active ingredients in shampoo products and in prescribed medicine to treat head lice and pet fleas. In our study, we found a significantly greater urinary ΣPYR concentration among subjects using insecticidal products to treat lice and fleas at home in the past month, raising the possibility of another source of pyrethroid exposure in addition to dietary intake.

In the current study, we found female subjects had significantly higher urinary concentrations of both ΣDAP and ΣPYR than males, after adjusting for potential sex-related variation in creatinine. This result is consistent with previous studies of other populations for urinary concentrations of DAP [9] and pyrethroid metabolites [10, 12]. This gender-specific difference may be due to the relatively larger amount of fruits and vegetables consumed per diet by females than males [36]. Moreover, as many organophosphate and pyrethroid insecticides and their metabolites are estrogenic [37], health effects may differ between females and males. In addition, our analyses showed that immigrants had significantly greater concentrations of both ΣDAP and ΣPYR. Studies of Canadian immigrants have suggested that linguistic barriers, lack of information on specific food (ethnic, organic), and limited information on resources of

nutritional and organic food may lead to higher levels of OP and PYR metabolites among immigrants [38, 39].

There are several limitations to our study. First, while the urinary levels of DAP metabolites can be considered as an objective measure of actual body burden arising from OP pesticide exposures [40], they lack specificity in identifying corresponding pesticides, and therefore the current study was not able to provide information on specific OP pesticides that the participants were exposed to. Secondly, as suggested by several studies, the generation of DAPs and pyrethroid metabolites can occur naturally in the environment and exposures to the environmentally preformed metabolites can also lead to the detection of pesticide metabolites in urine samples [41, 42]. Therefore, in addition to exposures to the parental pesticides, urinary detection of pesticide metabolites in the current study could also be resulted from direct exposures to environmentally preformed pesticide metabolites [42]. Lastly, due to the cross-sectional nature of the CHMS, our data provide only a snapshot of urinary pesticide concentrations, which may not be directly related to peak or cumulative pesticide exposures [40].

3.5 Conclusions

In summary, our data showed significant associations between dietary factors and urinary concentrations of organophosphate and pyrethroid insecticide metabolites. To the best of our knowledge, the current study is the first nation-wide population-based investigation on predictors of organophosphate and pyrethroid insecticide exposures in the Canadian general population.

Although now banned for residential use, many OP pesticides, such as parathion, chlorpyrifos and azinphos-methyl, are still allowed for agricultural use in North America [43]. In addition, there has been increasing pyrethroid use in Canada during the last decade [44]. Results from the current study suggested common and ongoing exposures to these pesticides in the

Canadian population, and dietary intake as one of the major sources of exposures. While it is still unclear whether these exposures are important in terms of health, concerns remain about the chronic effects of low-level pesticide exposures [45], and the positive associations reported in the current study suggests a need for greater regulation of pesticide residue levels on food product to reduce human pesticide exposure and potential health risk.

Table 3-1. Characteristics of the study population

Characteristics (N=5,604)		**%, mean**(SE)	**95% CI** *
Age (%, years)			
	6-11	7.5	7.5-7.5
	12-19	11.5	11.5-11.6
	20-79	81.0	81.0-81.0
Sex (%)			
	Female	50.2	50.1-50.3
	Male	49.8	49.7-49.9
Height			
	cm	165.89 (0.24)	165.35-166.42
Weight			
	kg	72.67 (0.69)	71.14-74.19
BMI			
	kg/m^2	26.0 (0.21)	25.5-26.5
Ethnicity (%)			
	Caucasian	69.8	61.6-78.0
	Other	30.2	22.0-38.4
Immigrant (%)			
	No	79.1	70.6-87.5
	Yes	20.9	12.5-29.4
Province of residence (%)			
	New Brunswick	7.1	0-21.6
	Quebec	23.4	9.0-37.9
	Ontario	39.2	39.2-39.2
	Alberta	16.9	16.9-16.9
	British Columbia	13.4	13.4-13.4
Highest education (%)			
	Less than secondary	25.6	23.5-27.8
	Secondary school	15.9	12.1-19.7
	Some post-secondary	9.0	7.0-11.0
	Post-secondary	49.5	42.6-56.3
Household income (%)			
	<$30K	14.6	11.2-18.0
	$30K-$50K	17.8	16.0-19.6
	$50K-$80K	26.1	23.0-29.2
	>$80K	41.5	36.5-46.5
Lice/flea treatment (%)			
	No	96.5	94.9-98.2
	Yes	3.5	1.8-5.1
Home/yard pesticide use (%)			
	No	88.6	83.6-93.7
	Yes	11.4	6.3-16.4

* Survey design weights and 500 bootstrap weights were used in calculating percentages, mean values and standard errors (SE).

Table 3-2. Distribution of weekly frequency of food consumption among the study population

Food Categories	# times per week mean (SE)*	Low frequency mean (SE)*	Medium frequency mean (SE)*	High frequency mean (SE)*
Egg & egg products	1.92 (0.06)	0.66 (0.01)	1.96 (0.003)	4.27 (0.10)
Meat & meat products	4.56 (0.16)	1.67 (0.04)	4.03 (0.03)	8.16 (0.16)
Fish & sea food	1.65 (0.08)	0.28 (0.01)	1.19 (0.01)	3.38 (0.09)
Pulses & nuts	2.73 (0.12)	0.39 (0.01)	1.82 (0.03)	6.41 (0.13)
Milk & dairy products	12.15 (0.35)	4.24 (0.08)	11.89 (0.08)	24.40 (0.38)
Grain & grain-based products	15.33 (0.24)	9.43 (0.10)	14.91 (0.08)	22.98 (0.36)
Fruits	13.77 (0.31)	5.52 (0.10)	12.94 (0.08)	26.33 (0.33)
Vegetables	14.49 (0.36)	6.63 (0.10)	13.14 (0.08)	23.68 (0.30)
Starchy roots	2.91 (0.14)	1.15 (0.04)	2.83 (0.02)	5.60 (0.14)
Water & water based beverage	32.86 (0.54)	13.59 (0.20)	28.07 (0.13)	52.15 (0.61)

* Survey design weights and 500 bootstrap weights were used in calculating percentages, mean values and standard errors (SE).

Table 3-3. Distribution of urine concentrations of organophosphate and pyrethroid pesticide metabolites among the study population

(N=5,604)	**Detection limit** (nmol/L urine)	**Percentage >=LOD**[†] (%, 95% CI[*])	**Arithmetic mean**[‡] (nmol/g creatinine, SE[*])	**Geometric mean**[‡] (nmol/g creatinine, SE[*])	**Median** (nmol/g creatinine, SE[*])	**IQR** (nmol/g creatinine)
OP metabolites						
DMP	7.93	77.4 (71.6-83.1)	56.00 (3.12)	28.55 (1.90)	28.98 (1.96)	13.41-60.75
DMTP	4.22	67.0 (60.6-73.4)	71.15 (4.04)	17.35 (1.24)	14.87 (1.49)	4.88-56.57
DMDT	1.90	36.6 (29.5-43.7)	–	–	<LOD	<LOD-5.52
DEP	6.49	78.6 (70.4-86.7)	29.46 (0.89)	18.08 (0.90)	18.55 (0.96)	10.02-34.29
DETP	3.53	37.4 (30.1-44.7)	–	–	<LOD	<LOD-7.58
DEDT	1.61	2.6 (1.9-3.2)	–	–	<LOD	<LOD-<LOD
ΣDAP	n/a	91.4 (87.8-94.9)	177.19 (7.77)	93.19 (4.26)	86.79 (5.27)	44.16-181.98
PYR metabolites						
3-PBA	0.05	99.4 (99.1-99.7)	4.25 (0.55)	1.44 (0.13)	1.23 (0.11)	0.64-2.72
4-F-3-PBA	0.03	42.4 (36.4-48.3)	–	–	<LOD	<LOD-0.08
cis-DCCA	0.03	98.5 (97.5-99.5)	1.57 (0.19)	0.49 (0.04)	0.41 (0.03)	0.22-0.87
trans-DCCA	0.05	99.6 (99.2-99.9)	4.45 (0.58)	1.17 (0.08)	0.93 (0.06)	0.50-2.20
cis-DBCA	0.02	47.5 (39.7-55.2)	–	–	<LOD	<LOD-0.06
ΣPYR	n/a	99.8 (99.8-100.0)	10.48 (1.31)	3.43 (0.27)	2.82 (0.21)	1.53-6.09

[*] Survey design weights and 500 bootstrap weights were used in calculating percentages, mean values, standard errors (SE) and 95% confidence intervals.
[†] Percentage of participants with at least one of pesticide metabolites in the sum >= LOD
[‡] If <60% of samples had detectable organophosphate metabolites, means were not calculated

Table 3-4. Distribution of urine concentrations of organophosphate and pyrethroid metabolites by demographic factors, socioeconomic factors and pesticide use

Characteristics (N=5,604)	ΣDAP G.Mean (nmol/g creatinine, SE)*	ΣDAP p-value	ΣPYR G.Mean (nmol/g creatinine, SE)*	ΣPYR p-value
Age (years)				
6-11	152.03 (7.88)	-	3.56 (0.28)	-
12-19	83.81 (5.51)	<0.0001	2.80 (0.32)	0.014
20-79	90.49 (4.50)	<0.0001	3.51 (0.29)	0.82
Sex				
Female	112.86 (6.07)	-	3.93 (0.32)	-
Male	76.86 (3.54)	<0.0001	2.98 (0.25)	<0.0001
Ethnicity				
Caucasian	93.13 (4.18)	-	3.14 (0.25)	-
Other	94.13 (5.99)	0.84	4.23 (0.34)	0.002
Immigrant				
No	89.59 (3.72)	-	3.11 (0.22)	-
Yes	107.94 (7.08)	0.003	4.94 (0.58)	0.002
BMI categories				
Underweight or normal	106.87 (6.90)	-	3.80 (0.34)	-
Overweight	87.46 (4.89)	0.01	3.15 (0.19)	0.007
Obesity	73.40 (2.21)	<0.0001	3.03 (0.29)	0.003
Highest education				
Less than secondary	101.40 (4.80)	-	3.11 (0.28)	-
Secondary school	75.86 (3.19)	<0.0001	3.20 (0.35)	0.73
Some post-secondary	88.06 (8.96)	0.14	2.99 (0.26)	0.75
Post-secondary	95.38 (4.25)	0.048	3.75 (0.33)	0.028
Household income				
<$30K	99.13 (8.84)	-	3.92 (0.48)	-
$30K-$50K	94.43 (4.45)	0.61	3.28 (0.26)	0.035
$50K-$80K	95.85 (5.28)	0.75	3.49 (0.34)	0.20
>$80K	91.82 (6.40)	0.44	3.39 (0.28)	0.13
Lice/flea treatment				
No	93.52 (4.55)	-	3.40 (0.28)	-
Yes	87.45 (10.04)	0.64	4.70 (0.59)	0.013
Home/yard pesticide uses				
No	93.87 (4.56)	-	3.36 (0.27)	-
Yes	92.16 (8.86)	0.86	3.94 (0.36)	0.044

* Survey design weights and 500 bootstrap weights were used in calculating geometric mean values and standard errors (SE)

Table 3-5. Distribution of urine concentrations of organophosphate and pyrethroid metabolites by frequency of food consumption

	ΣDAP		ΣPYR	
Frequency (# times per week)	**G.Mean** (nmol/g creatinine, SE)*	**p-value**	**G.Means** (nmol/g creatinine, SE)*	**p-value**
Egg & egg products				
Low	97.76 (4.50)	-	3.39 (0.30)	-
Medium	93.61 (6.15)	0.47	3.41 (0.27)	0.93
High	84.71 (5.41)	0.028	3.51 (0.35)	0.63
Meat & meat products				
Low	108.52 (6.29)	-	4.06 (0.38)	-
Medium	91.13 (3.72)	0.002	3.34 (0.28)	0.011
High	80.70 (4.34)	<0.0001	2.92 (0.25)	0.005
Fish & sea food				
Low	85.88 (3.82)	-	2.97 (0.24)	-
Medium	93.37 (5.69)	0.18	3.68 (0.32)	0.004
High	100.47 (5.87)	0.032	3.68 (0.34)	0.016
Pulses & nuts				
Low	82.67 (4.46)	-	2.92 (0.21)	-
Medium	87.93 (4.75)	0.37	3.32 (0.27)	0.059
High	113.48 (8.12)	0.002	4.24 (0.47)	0.001
Milk & dairy products				
Low	82.78 (6.28)	-	3.37 (0.30)	-
Medium	100.17 (3.62)	0.011	3.53 (0.19)	0.31
High	100.50 (6.09)	0.013	3.37 (0.40)	0.99
Grain & grain-based products				
Low	93.57 (4.11)	-	3.45 (0.23)	-
Medium	93.70 (5.61)	0.98	3.29 (0.27)	0.44
High	94.82 (7.14)	0.84	3.71 (0.43)	0.31
Fruits				
Low	73.44 (3.74)	-	3.23 (0.30)	-
Medium	98.74 (6.18)	<0.0001	3.47 (0.29)	0.16
High	123.20 (6.78)	<0.0001	3.68 (0.31)	0.084
Vegetables				
Low	73.64 (3.53)	-	2.64 (0.24)	-
Medium	91.80 (5.42)	0.008	3.60 (0.31)	<0.0001
High	119.17 (7.07)	<0.0001	4.23 (0.33)	<0.0001
Starchy roots				
Low	104.83 (5.56)	-	4.03 (0.28)	-
Medium	89.30 (4.54)	0.021	3.14 (0.33)	0.002
High	80.44 (3.65)	<0.0001	2.86 (0.21)	<0.0001
Water & water based beverage				
Low	92.74 (4.03)	-	3.13 (0.24)	-
Medium	95.93 (5.00)	0.41	3.58 (0.31)	0.002
High	91.25 (5.40)	0.73	3.54 (0.33)	0.044

* Survey design weights and 500 bootstrap weights were used in calculating geometric mean values and standard errors (SE)

Table 3-6. Predictors of urinary concentrations (creatinine-adjusted) of ΣDAP and ΣPYR: results from the multiple linear regression*

Characteristics		ΣDAP (log-transformed)			ΣPYR (log-transformed)		
(N=5,604)		Beta	95% CI †	p-value	Beta	95% CI †	p-value
Constant		5.26	5.02, 5.49	<0.0001	1.29	1.05, 1.53	<0.0001
Age							
	6-11	0			0		
	12-19	-0.45	-0.58, -0.32	<0.0001	-0.18	-0.34, -0.03	0.024
	20-79	-0.34	-0.49, -0.20	<0.0001	0.01	-0.16, 0.18	0.88
Sex							
	Female	0			0		
	Male	-0.32	-0.42, -0.21	<0.0001	-0.22	-0.31, -0.12	<0.0001
Ethnicity							
	Caucasian	0			0		
	Other	0.12	0.01, 0.24	0.032	0.16	-0.04, 0.36	0.098
Immigrant							
	No	0			0		
	Yes	0.27	0.17, 0.37	0.001	0.37	0.08, 0.67	0.018
BMI							
	per kg/m^2	-0.02	-0.03, -0.01	0.001	-0.01	-0.02, -0.01	0.005
Pulses & nuts							
	Low	-	-	-	0		
	Medium	-	-	-	0.06	-0.07, 0.19	0.35
	High	-	-	-	0.21	0.07, 0.37	0.01
Fruits							
	Low	0			-	-	-
	Medium	0.19	0.06, 0.31	0.007	-	-	-
	High	0.36	0.23, 0.48	<0.0001	-	-	-
Vegetables							
	Low	0			0		
	Medium	0.13	-0.01, 0.27	0.058	0.25	0.12, 0.39	0.002
	High	0.29	0.15, 0.42	0.001	0.35	0.20, 0.50	<0.0001
Lice/flea treatment							
	No	-	-	-	0		
	Yes	-	-	-	0.43	0.20, 0.66	0.002

* Beta coefficients indicate the average differences [in ln(nmol/g cr)] from reference category. Constant term indicate the average insecticide concentration in log-scale when the all the variables take the value zero (reference category)

† Survey design weights and 500 bootstrap weights were included in calculating β coefficients and standard errors (SE).

Table 3-7 Predictors of urinary concentrations (volume-based) of ΣDAP and ΣPYR: results from the multiple linear regression*

Characteristics (N=5,604)		ΣDAP (log-transformed)			ΣPYR (log-transformed)		
		Beta	95% CI †	p-value	Beta	95% CI †	p-value
Constant		5.09	4.84, 5.35	<0.0001	1.20	0.96, 1.45	<0.0001
Age							
	6-11	0			0		
	12-19	-0.32	-0.48, -0.17	0.001	-0.11	-0.27, 0.06	0.171
	20-79	-0.30	-0.45, -0.15	0.001	0.04	-0.13, 0.21	0.626
Sex							
	Female	0			0		
	Male	-0.23	-0.34, -0.11	0.001	-0.17	-0.27, -0.06	0.006
Ethnicity							
	Caucasian	0			0		
	Other	0.13	0.02, 0.24	0.029	0.16	-0.03, 0.35	0.093
Immigrant							
	No	0			0		
	Yes	0.26	0.15, 0.36	<0.0001	0.37	0.06, 0.67	0.022
BMI							
	per kg/m^2	-0.02	-0.03, -0.01	<0.0001	-0.01	-0.02, -0.01	0.011
Pulses & nuts							
	Low	-	-	-	0		
	Medium	-	-	-	0.05	-0.07, 0.18	0.37
	High	-	-	-	0.21	0.05, 0.36	0.012
Fruits							
	Low	0			-	-	-
	Medium	0.20	0.07, 0.32	0.005	-	-	-
	High	0.37	0.24, 0.49	<0.0001	-	-	-
Vegetables							
	Low	0			0		
	Medium	0.11	-0.01, 0.24	0.075	0.24	0.10, 0.38	0.003
	High	0.23	0.10, 0.37	0.003	0.33	0.17, 0.49	0.001
Lice/flea treatment							
	No	-	-	-	0		
	Yes	-	-	-	0.45	0.21, 0.68	0.002
Urinary creatinine							
	per ln (g/L)	0.76	0.71, 0.81	<0.0001	0.86	0.77, 0.96	<0.0001

* Beta coefficients indicate the average differences [in ln(nmol/L)] from reference category. Constant term indicate the average insecticide concentration in log-scale when the all the variables take the value zero (reference category)

† Survey design weights and 500 bootstrap weights were included in calculating β coefficients and standard errors (SE).

CHAPTER 4

RELATIONSHIP BETWEEN URINARY DIALKYL PHOSPHATE CONCENTRATION AND LUNG FUNCTION IN ADOLESCENTS AND ADULTS: RESULTS FROM THE CANADIAN HEALTH MEASURES SURVEY

4.1 Introduction

Organophosphate (OP) pesticides are a group of structurally related neurotoxicants, which have been extensively used in agricultural and residential applications. Humans may be exposed in a number of ways, including occupational exposures, such as agricultural occupations, and environmental exposures, such as land run-off from the OP-treated areas [1].

OPs can be efficiently absorbed by the skin due to their high lipophilicity [2]. Ingestion of food and water contaminated with OPs is also a significant route of exposure for general populations [2]. After entering the body, OP and/or its activated desulfurated 'oxon' form [3], are rapidly hydrolyzed by phosphotriesterase paraoxonase 1 (PON1) to form dialkyl phosphate (DAP) metabolites that are subsequently excreted in the urine [4]. In the environment, generation of DAPs also occurs naturally when OP pesticides are degraded in soil, sediment and surface water [5].

There are six dialkyl phosphate metabolites: dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP). These dialkyl phosphates are common metabolites of OPs, but are not pesticide specific [6]. Blood or urinary levels of DAPs are often considered as biomarkers of exposure to parental OP pesticides or their metabolites in the environment. The detection of DAPs in urine samples is generally believed to reflect recent exposures to OPs over the past few days [6].

OP pesticides function as cholinesterase-inhibitors and thus interfere with neural transmission in the nervous system [7]. In humans, health concerns related to OP exposures mainly focus on their high acute neurotoxicity [8]. Exposures to high doses of OP can cause death due to OP-induced respiratory paralysis and bradycardia [8]. Other adverse health effects associated with OP exposures include chronic neurological effects [9], neurodevelopmental effects [10], immunological effects [11], endocrine disruptive effects [12] and respiratory effects [13]. Because of these health concerns, OP pesticides have been largely restricted to use in agricultural applications in many countries. For example, chlorpyrifos, has been banned for residential use in the US since 2001 [14].

Several studies on OP exposures in agriculture have examined the effect of OP pesticides on lung function. Peiris-John *et al*. showed that reductions in forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were associated with OP insecticide exposures among farm workers in Sri Lanka [15]. In a study from India, exposures to OP insecticides were significantly associated with lower FVC, FEV_1, FEV_1/FVC ratio, forced expiratory flow between 25% and 75% of FVC ($FEF_{25\%-75\%}$) and peak expiratory flow rate (PEFR) among agricultural workers [16]. In addition, impaired lung function has been found to be associated with OP-induced cholinesterase inhibition. A cross-sectional study of pesticide sprayers in India showed that impaired lung function was correlated with lower activities of acetylcholinesterase and butylcholinesterase, suggesting an adverse effect of OPs on lung function [17].

While there have been several studies on the association between OP exposures and lung function in agricultural occupations, few have reported its impact on lung function in the general population. In the current study, urinary concentrations of dialkyl phosphate metabolites and

their association with lung function were characterized among a Canadian general population using data from the Canadian Health Measures Survey (CHMS).

4.2 Methods

In this study, we used data from the first Canadian Health Measures Survey (CHMS-Cycle 1), a nation-wide cross-sectional survey conducted by the Statistics Canada in 2007-2009 [18]. The study participants were 4,446 CHMS participants, including 980 adolescents aged 12 to 19 years and 3,466 adults aged 20 to 79 years, who had data available on urinary concentrations of DAPs, spirometric measurements of lung function, smoking status and related predictors for lung function. These subjects were a representative sample of Canadian adolescents and adults [18].

The sampling method of the CHMS-Cycle 1 has been described in Chapter 3. The detailed information of the CHMS-Cycle 1 can also be obtained from Statistics Canada [18]. Participation in the CHMS was voluntary and all 4,446 subjects provided informed consent to storage and use of their urine samples [18]. This study was approved by the Health Research Ethics Board of the University of Alberta.

4.2.1 Urinary concentrations of dialkyl phosphates

The method for measuring urinary concentrations of DAP metabolites was same as those used in Chapter 3. In addition to calculating the total concentration of the six DAPs (ΣDAP) as in Chapter 3, total concentration of three dimethyl alkylphosphates (ΣDMAP) and total concentration of three diethyl alkylphosphates (ΣDEAP) were also calculated using the same method as calculating ΣDAPs to estimate the overall exposures to OPs with DMAP metabolites

and OPs with DEAP metabolites, respectively [19]. Samples with DAP concentrations less than LOD were assigned as 0.5*LOD [20].

4.2.2 Lung function measurements

Lung function parameters FVC, FEV_1, FEV_1/FVC ratio and $FEF_{25\%-75\%}$ were considered in the current study. For each participant, lung function tests were conducted at the same time as the urine sample was collected at the Mobile Examination Center (MEC).

Trained technologists measured the lung function of participants using a portable flow-based spirometer (Koko®, PDS Instrumentation Inc., Louisville, Colorado US). American Thoracic Society (ATS) recommendations for performance of spirometry were followed, including calibrating spirometers with a 3 liter syringe and obtaining a minimum of 3 acceptable trials from a maximum of 8 maneuvers based on the ATS definition of within- and between-manoeuvre criteria for usable and acceptable trials [18, 21]. Lung function measures were standardized to body temperature, barometric pressure and water saturation (BTPS) [18].

For FVC and FEV_1 measurements, the largest value of acceptable trials was used, and for $FEF_{25\%-75\%}$ measurements, the mean flow rate (L/s) of the acceptable trial with the largest sum of FVC and FEV_1 was used [18]. Subjects with difficulty in breathing at rest, acute (e.g., cold, bronchitis and flu) or chronic respiratory condition (e.g. persistent cough), taking medication for tuberculosis, recent surgery on eye (within 6 weeks), chest or abdomen (within 3 months), pregnancy (> 27 weeks), or with an important language barrier, were excluded from lung function testing [18].

4.2.3 Factors related to lung function

Information on demographic factors and socioeconomic status were also obstained using the same methods as in Chapter 3 [18].

Information on tobacco smoking was obtained for participants aged 12 years and above using the CHMS-Cycle 1 household questionnaire regarding the frequency and duration of cigarette smoking [18]. Based on the responses to the questionnaire, a variable with three categories was defined to indicate never, former smoker and current smoker, respectively.

Other lung function related factors, including environmental tobacco exposure (exposed to second-hand smoke inside their home, in their private vehicle, in public places, such as bars, restaurants, shopping malls, or at their place of work), types of heating source used at home (gas furnace/fireplace, oil furnace, electric heat, or wood burning fireplace/stove), and air quality at the time of the spirometry tests (ambient concentrations of $PM_{2.5}$, NO_2 and O_3), were also measured as part of CHMS-Cycle 1 [18].

4.2.4 Statistical analyses

Lung function parameters FVC, FEV_1, FEV_1/FVC and $FEF_{25\%-75\%}$ were considered as continuous outcome variables. Log transformed total concentrations of dialkly phosphates (ΣDAPs, ΣDMAPs, and ΣDEAPs) were also considered as continuous exposure variables in the analyses.

In the descriptive analyses, geometric means, medians, inter quarter ranges (IQRs) of urinary concentrations (nmol/g creatinine), and proportions of subjects with detectable urinary concentrations (≥LOD) of DAPs, were calculated for each DAP metabolite, as well as for ΣDAP, ΣDMAP, and ΣDEAP. Descriptive statistics were not calculated for any metabolite with > 40% of samples had concentrations < LOD [22]. Demographic and anthropomorphic characteristics and smoking status of participants were described by means with standard errors (SE) or proportions.

We incorporated sampling design weights provided by Statistics Canada in our statistical analyses to adjust for post-stratification in the multistage sampling, units with no responses and out of scope responses [18]. In order to allow for the complex sampling design, 500 bootstrap weights, provided by Statistics Canada, were applied in variance estimation for descriptive statistics, regression coefficients and 95% confidence intervals [18].

Relationships between urinary concentrations of DAPs (ΣDAP, ΣDMAP or ΣDEAP) and lung function were determined by linear regression analyses with lung function parameters as dependent variables and log (natural) transformed DAP concentrations as independent variables.

Univariate analyses were initially conducted to examine the relationship between risk factors, including urinary concentrations of DAPs, and lung function. In multiple regression models, a purposeful selection method was used in determine the final models. The variables that were previously established as risk factors of lung function, including age, sex, ethnicity, height and smoking, were forced into the final models. The variables that were non-significant at p=0.05 were excluded from the models. Interactions between urinary DAP concentrations and age, sex, ethnicity, smoking status on the association with lung function outcomes were also examined. Separate regression models were used to examine the association among adolescents (12-19 years) and adults (20-79 years) participants, respectively.

Sensitivity analyses were performed using mass volume concentrations (nmol/L) of ΣDAP as an exposure variable and urinary creatinine concentration (g/L) as a separate independent covariate to examine the effect of the potential covariates-related (such as age, sex, ethnicity and BMI) variation in urinary creatinine concentrations [23].

Statistical analyses were performed using STATA (StataCorp LP. 2007, Release 12) and SAS (SAS Institute Inc. 2011, SAS® 9.3) software with procedures for the complex survey data analysis.

4.3 Results

4.3.1 Characteristics of the study participants

Demographic and anthropometric characteristics and smoking status are summarized in Table 4-1. Among 4,446 participants, 980 (22.0%) were adolescents aged 12-19 years and 3,466 (78.0%) were adults aged 20-79 years. Overall, males and females were almost equally represented (Table 4-1). Approximately 62.7% of the participants aged 12-19 years and 71.7% of the participants aged 20-79 years self-identified as of Caucasian ethnicity. While the majority of adolescent participants (85.6%) had never smoked, approximately 50.0% of the adult participants ever smoked at some time during their lifetime (Table 4-1).

4.3.2 Lung function among the study participants

Lung function parameters across demographic groups and smoking status are summarized in Table 4-2. On average, lung function was greatest among young adults (age 20-29 years) and declined thereafter (Table 4-2). Among both adolescent and adult participants, former and current smokers had statistically significant lower mean values in FEV_1, FEV_1/FVC ratio and $FEF_{25\%-75\%}$ compared to non-smokers ($p<0.01$) after adjusting for age, sex and ethnicity. However, no significant associations were found between lung function parameters and other risk factors, including environmental tobacco smoke, types of heating source used at home, and ambient concentration of $PM_{2.5}$, NO_2, and O_3, among either adolescent or adult participants (data not shown).

4.3.3 Urinary concentrations of dialkyl phosphates in the study participants

Among the total study participants, 91.3% had at least one of the six dialkyl phosphate metabolites detectable in their urine samples. A greater percentage of adolescent participants (93.8%) with detectable DAP metabolites than adult participants (91.0%). The mean (both arithmetic and geometric means) concentrations (creatinine-adjusted) of DAP metabolites were higher among adults than adolescents (Table 4-3).

DMP and DEP were the most prevalent DAP metabolites (detected in approximately 80.0% of both adolescent and adult participants) while DEDTP was the least prevalent metabolite (detected in less than 5.0% of adolescent and adult participants) (Table 4-3). In addition, the mean concentrations (creatinine-adjusted) of total methyl DAPs (ΣDMAP) was significantly higher than the total ethyl DAPs (ΣDEAP) in both adolescent and adult participants (Table 4-3).

The mean (geometric) concentrations of total DAP (ΣDAP) were 83.8 (nmol/g creatinine) and 90.5 (nmol/g creatinine) for adolescent participants and adult participants, respectively. In both adolescent and adult participants, females had significantly higher mean concentrations of DAP metabolites, including ΣDAP, ΣDMAP and ΣDEAP, than male participants (p<0.05). In addition, among the adult participants, current smokers had statistically significant lower mean concentrations of DAP metabolites (ΣDAP, ΣDMAP and ΣDEAP) than former smokers and participants who never smoked (p<0.01, Table 4-4). In both adolescents and adults, no significant difference in the mean concentrations of DAP metabolites was observed across ethnic groups (Table 4-4).

4.3.4 Relationships between DAP concentrations and lung function

In the multiple regression analyses of adult participants aged 20-79 years, a one unit increase in log transformed concentration (nmol/g creatinine) of ΣDAP was associated with a 32.6 (95% CI: -57.2, -8.1) mL reduction in FVC, a 32.6 (95% CI: -59.0, -6.3) mL reduction in

FEV_1, 0.18% (95% CI: -0.61%, 0.24%) reduction in FEV_1/FVC ratio and 53.1 (95% CI: -113.9, 7.7) mL/s reduction in $FEF_{25\%-75\%}$ (Table 4-5). In addition, among the adult participants, urinary concentration of ΣDMAP was also negatively associated with lung function FVC and FEV_1 but not with ΣDEAP (Table 4-5). No interactions between DAP concentrations and age, sex, ethnicity, smoking status were significant at p=0.05 (data not shown). The linear relationship based on the multiple regression analyses between lung function parameters and log transformed concentration of ΣDAP is further illustrated in Figure 4-1 to Figure 4-8 by sex and by smoking categories (Never smoked, Former smoker and Current smoker).

In the multiple regression analyses of adolescent participants aged 12-19 years, none of the lung function parameters was significantly associated with urinary concentration of DAPs (Table 4-5).

In the sensitivity analyses, adjusting for the potential covariate-related variation in urinary creatinine concentrations, results regarding to the associations between DAP concentrations and lung function were consistent with the main models using the creatinine-adjusted concentrations for adolescent and adult participants (data not shown).

4.4 Discussion

In the current study, we examined the relationship between urinary concentration of DAPs and lung function among the Canadian Health Measures Survey-Cycle 1 participants aged 12-79 years, a representative sample of the Canadian adolescents and adults [18]. To the best of our knowledge, the current study is the first nation-wide population-based investigation on the relationships between DAP metabolites and lung function among the Canadian general population.

Results from the multiple regression analyses showed significant association between DAP concentrations and lung function in adult participants (20-79 years) but not in adolescent participants (12-19 years). The reason for this difference is unclear but may be due to the rapid growth of the lungs during the growth spurt in adolescents increasing variance in lung function parameters [24].

Among the adult participants aged 20-79 years, urinary concentrations of total DAPs (ΣDAP) were significantly associated with the reduction in FVC and FEV_1. With a one unit increase in the log transformed urinary concentrations of ΣDAP, i.e. with every 2.72 fold increase in the concentration of ΣDAP, FVC and FEV_1 was decreased by approximately 32.6 mL and 32.6 mL, respectively among adults. The magnitude of the DAP concentration associated lung function reduction in adult participants was similar to the natural age-related decline of lung function for healthy non-smoking adults (30mL/year in FVC and 20-30 mL/year in FEV_1) [25, 26]. Based on the multiple linear regression model, differences in FVC and FEV_1 between adult participants with 25^{th} (43.2 nmol/g creatinine) and 75^{th} (175.1 nmol/g creatinine) percentiles of urinary concentrations of total DAP would be 45.6 mL and 45.6 mL, respectively.

Although both methyl DAPs and ethyl DAPs were negatively associated with lung function among adults, they were only statistically significant for ΣDMAP. This result, in addition to the fact that the mean concentrations of ΣDMAP was higher than ΣDEAP, might suggest that the potential OP related lung function changes identified could be mainly from the OP insecticides with methyl DAP metabolites, although future work is required to confirm this.

Several studies in literature have suggested that exposures to OP pesticides in agricultural occupations were associated with the reduction in lung function parameters, including FEV_1 [15-17], FVC [15, 16], FEV_1/FVC ratio [16, 17], $FEF_{25\%-75\%}$ [16] and peak expiratory flow rate [16,

17]. In this study, the urinary ΣDAP level was associated with reductions in both FVC and FEV_1 among the adult general population, which is consistent with the findings in agricultural occupations [15-17].

OP pesticides are neurotoxicants that bind to the serine residue in acetylcholine esterase (AchE), resulting in an accumulation of acetylcholine (Ach) and overstimulation of postsynaptic cholinergic nerves [7]. This suggests a couple of possible mechanisms by which OP could affect lung function. Muscarinic 3 (M3) receptors, a stimulatory type of muscarinic Ach receptors, are expressed on both pulmonary nerves and smooth muscles [27]. Stimulation of M3 receptors by Ach would potentially lead to the contraction of airway smooth muscles [28]. Another muscarinic receptor, the M2 receptor located on the pulmonary prejunctional nerves and smooth muscles can inhibit further release of Ach from prejunctional nerve ends [29]. At a low-dose level, which may be particularly relevant among general populations, OP pesticides do not seem to inhibit AchE but have the potential to disrupt the auto-inhibitory function of pulmonary prejunctional M2 receptors [30, 31]. So leading to an unopposed release of ACh from prejunctional parasympathetic nerves again causes excessive bronchoconstriction [32].

Inhalation of OP-containing gases, vapors or aerosols into airways can lead to production of reactive oxygen species (ROS) and subsequent activation of ERK-MAPK, JNK and NFkB signalling pathways [33], which may in turn cause contraction of airway smooth muscles and airway narrowing [34, 35].

Despite our understanding of these actions of OPs, the biological mechanisms underlying the OP-related reduction of lung function are still unclear. The proposed biological mechanisms are not exclusive for a specific type of pulmonary diseases. Further study to characterize the biological plausibility of the OP-associated type of lung function impairment is necessary.

Our results also showed that over 90% of the CHMS Cycle 1 participants aged 12-79 years had at least one species of DAP metabolite detectable in their urine. This result is consistent with data from the US NHANES III [36]. Given the short half-life of OP pesticides in the environment [37] and *in vivo* [6], the current detection of DAP metabolites among the majority of the participants suggests that exposures to OP pesticides are common and ongoing in the Canadian general population.

For the general population, dietary intake of trace amounts of OPs from pesticide-sprayed or treated fruits and vegetables is a major source of OP exposures [2]. However, as suggested by Zhang et al, ingestion of environmentally preformed DAPs can also lead to the detection of DAP metabolites in urine samples [38]. Therefore, in addition to OP pesticides, DAP metabolites detected in urine samples in the current study may have resulted from the direct exposure to environmental DAPs.

There are several limitations in our data. Firstly, data from the CHMS were not representative of the entire Canadian population. Aboriginal people living on reserves and Aboriginal settlements, people living in remote areas, institutional residents and full-time members of the Canadian Force were excluded from the CHMS-Cycle 1 [18]. However, it is unlikely that the exclusion of these groups would change the relationships reported in this study, since the excluded populations in the CHMS-Cycle 1 represent less than 4% of the total Canadian population [18]. Secondly, participants with chronic or acute respiratory conditions and taking medication for tuberculosis [18], were excluded from the regression analyses, which might have led to a biased selection of a relatively healthier population in the study, which would have resulted in an underestimate of the true effect if any. Thirdly, while urinary levels of DAP metabolites can be considered as an objective measure of actual body burden arising from OP

pesticide exposures [6], they lack specificity in identifying corresponding pesticides, and therefore the current study was not able to provide information on specific OP pesticides that the participants were exposed to. In addition to DAP metabolites, serum BuChE (butyrylcholinesterase) and erythrocyte AChE (acetylcholinesterase) activities can also be used as biomarkers for OP exposures, especially for sub-chronicle (3-4 months) exposures [39]. Unfortunately, these were not measured among participants in the CHMS-Cycle1 [18]. Lastly, due to the cross-sectional nature of the CHMS, our data provide only a snapshot of urinary DAP concentrations, which may not be directly related to peak or cumulative OP exposures [6]. Moreover, the temporal sequence between changes in lung function and exposures to OPs cannot be determined in the current study, which limits the ability to determine causation from the findings.

4.5 Conclusions

Although many organophosphate (OP) pesticides, such as parathion and chlorpyrifos, have been restricted for agricultural uses only, exposure remains common and may still pose risks to public health [14]. In addition to neutrotoxicity of OPs [8], there are studies of their association with lung function in agricultural occupations. This study is the first population-based study investigating the association between urinary concentrations of DAP metabolites and lung function among the Canadian general population. Our results showed that urinary concentrations of total DAPs were significantly associated with reductions in FVC and FEV_1 among the adult participants aged 20-79 years.

Findings of the current study will raise awareness of the potentially effects of OP pesticides on lung function among the Canadian general population. Further research using prospective designs is warranted to confirm the associations reported in this study.

Table 4-1. Characteristics of the study population by age group[*]

Characteristics	Age groups (N=4,446)	
	12-19 years[†]	**20-79 years[†]**
Sex (%)		
Female	48.8	50.5
Male	51.2	49.5
Height (cm)		
	166.70 (0.24)	168.58 (0.28)
Weight (kg)		
	63.16 (0.98)	77.65 (0.76)
Ethnicity (%)		
Caucasian	62.7	71.7
Other	37.3	28.3
Province of residence (%)		
New Brunswick	7.1	7.2
Quebec	23.3	23.7
Ontario	39.2	38.7
Alberta	16.9	16.8
British Columbia	13.5	13.6
Smoking status (%)		
Never	85.6	47.9
Former smoker	2.4	30.5
Current smoker	11.9	21.5

[*] Survey design weights and 500 bootstrap weights were used in calculating percentages, mean values and standard errors (S.E.)
[†] Among 4,446 participants, 22.0% were adolescents aged 12-19 years, and 78.0% were adults aged 20-79 years.

Table 4-2. Distribution of lung function parameters by demographic factors and smoking status by age group

	FVC (L) Mean (SE) *	FEV_1 (L) Mean (SE) *	FEV_1/FVC (%) Mean (SE) *	$FEF_{25\%-75\%}$ (L/s) Mean (SE) *
		12-19 years (N=980)		
Total sample	4.14 (0.04)	3.46 (0.03)	84.0 (0.3)	3.55 (0.05)
Sex†				
Female	3.71 (0.03)	3.16 (0.03)	85.5 (0..3)	3.39 (0.06)
Male	4.54 (0.07)	3.74 (0.05)	82.6 (0.5)	3.69 (0.07)
Ethnicity†				
Caucasian	4.28 (0.03)	3.55 (0.02)	83.4 (0.3)	3.60 (0.06)
Others	3.88 (0.07)	3.29 (0.05)	85.2 (0.5)	3.45 (0.06)
Smoking status†				
Never	4.06 (0.03)	3.41 (0.03)	84.3 (0.3)	3.52 (0.05)
Former smoker	4.36 (0.13)	3.58 (0.07)	82.5 (2.0)	3.65 (0.17)
Current smoker	4.61 (0.10)	3.77 (0.09)	82.3 (1.0)	3.71 (0.20)
		20-79 years (N=3,466)		
Total sample	4.13 (0.04)	3.19 (0.03)	77.1 (0.3)	2.88 (0.04)
Age group †				
20-29 years	4.71 (0.10)	3.81 (0.08)	81.4 (0.5)	3.69 (0.12)
30-79 years	4.00 (0.03)	3.05 (0.02)	76.2 (0.3)	2.70 (0.03)
Sex †				
Female	3.45 (0.02)	2.68 (0.02)	77.6 (0.3)	2.48 (0.04)
Male	4.81 (0.05)	3.69 (0.04)	76.6 (0.4)	3.28 (0.06)
Ethnicity†				
Caucasian	4.19 (0.03)	3.20 (0.03)	76.3 (0.3)	2.84 (0.04)
Others	3.98 (0.06)	3.14 (0.04)	79.1 (0.5)	2.97 (0.04)
Smoking status†				
Never	4.12 (0.07)	3.26 (0.05)	79.2 (0.3)	3.09 (0.06)
Former smoker	4.02 (0.05)	3.06 (0.04)	75.9 (0.3)	2.67 (0.06)
Current smoker	4.30 (0.05)	3.21 (0.04)	74.4 (0.4)	2.72 (0.05)

* Survey design weights and 500 bootstrap weights were used in calculating arithmetic mean values and standard errors (S.E.).

† Statistically significant differences in lung function with $p<0.01$.

Table 4-3. Distribution of urinary concentrations of organophosphate metabolites in the study population by age group

Organophosphate pesticide metabolites	Detection limit (nmol/L)	Percentage >=LOD[†] (%, 95% CI[*])	A.mean[‡] (nmol/g creatinine, S.E.[*])	G.mean[‡] (nmol/g creatinine, S.E.[*])	Median (nmol/g creatinine, S.E.[*])	IQR (nmol/g creatinine)
12-19 years (N=980)						
DMP	7.9	82.3 (75.4-89.2)	51.5 (3.0)	27.1 (2.1)	29.0 (2.7)	13.2-63.0
DMTP	4.2	68.6 (60.7-76.5)	55.0 (6.3)	14.0 (1.3)	14.1 (1.5)	<LOD-42.9
DMDT	1.9	35.4 (27.6-43.1)	–	–	<LOD	<LOD-3.6
DEP	6.5	82.0 (73.5-90.5)	28.1 (2.0)	16.8 (1.3)	16.9 (1.3)	9.0-33.7
DETP	3.5	44.6 (33.5-55.7)	–	–	<LOD	<LOD-<LOD
DEDT	1.6	4.4 (2.6-6.2)	–	–	<LOD	<LOD-<LOD
ΣDAP	n/a	93.8 (89.6-98.0)	151.8 (12.0)	83.8 (5.5)	78.2 (7.8)	39.9-167.5
ΣDMAP	n/a	88.4 (82.4-94.4)	116.0 (9.9)	51.6 (3.6)	48.4 (4.6)	22.3-119.5
ΣDEAP	n/a	83.1 (74.9-91.3)	35.7 (2.5)	22.6 (1.5)	21.8 (1.8)	12.4-41.9
20-79 years (N=3,466)						
DMP	7.9	76.4 (70.5-82.2)	53.3 (3.5)	27.5 (2.0)	27.5 (2.0)	13.1-56.9
DMTP	4.2	66.7 (60.5-73.0)	68.8 (5.0)	17.2 (1.3)	14.5 (1.6)	<LOD-55.4
DMDT	1.9	36.4 (29.2-43.6)	–	–	<LOD	<LOD-5.5
DEP	6.5	77.9 (69.8-85.9)	27.6 (1.0)	17.5 (0.8)	17.9 (1.0)	9.8-33.4
DETP	3.5	36.1 (29.2-43.0)	–	–	<LOD	<LOD-<LOD
DEDT	1.6	2.3 (1.6-2.9)	–	–	<LOD	<LOD-<LOD
ΣDAP	n/a	91.0 (87.5-94.5)	169.7 (9.5)	90.5 (4.5)	84.8 (5.1)	43.2-175.1
ΣDMAP	n/a	82.4 (78.2-86.6)	132.7 (8.9)	55.5 (3.7)	50.7 (3.7)	23.2-125.0
ΣDEAP	n/a	78.6 (70.7-86.4)	36.9 (1.1)	24.3 (0.9)	24.3 (1.0)	13.6-43.4

[*] Survey design weights and 500 bootstrap weights were used in calculating percentages, mean values, standard errors (S.E.) and 95% confidence intervals.

[†] Percentage of participants with at least one of the organophosphate metabolites in the sum >= LOD

[‡] If <60% of samples had detectable organophosphate metabolites, means were not calculated. A.mean: Arithmetic means. G.mean: Geometric means

Table 4-4. Distribution of urinary concentrations of organophosphate metabolites by demographic factors and smoking status by age group

Characteristics	**Geometric means** (nmol/g creatinine, S.E.) [*†]					
	12-19 years (N=980)			**20-79 years** (N=3,466)		
	ΣDAP	**ΣDMAP**	**ΣDEAP**	**ΣDAP**	**ΣDMAP**	**ΣDEAP**
Average	83.8 (5.5)	51.6 (3.6)	22.6 (1.5)	90.5 (4.5)	55.5 (3.7)	24.3 (0.9)
Sex[‡]						
Female	95.3 (7.4)	59.9 (5.0)	24.3 (2.1)	112.2 (6.6)	68.5 (5.8)	29.8 (1.3)
Male	74.4 (5.3)	44.9 (3.7)	21.2 (1.2)	72.6 (3.7)	44.7 (2.9)	19.7 (0.7)
Ethnicity						
Caucasian	84.5 (7.3)	51.2 (4.7)	23.5 (2.0)	90.4 (4.4)	55.5 (3.6)	24.7 (1.0)
Others	83.6 (5.9)	53.2 (4.1)	21.2 (1.4)	91.4 (6.8)	56.1 (5.4)	23.4 (1.5)
Smoking status						
Never	87.8 (6.3)	54.3 (4.2)	23.4 (1.7)	96.7 (6.1)	60.2 (4.7)	25.3 (1.3)
Former smoker	70.8 (33.7)	46.7 (27.6)	16.7 (3.3)	104.0 (7.5)	64.5 (5.5)	26.0 (1.5)
Current smoker	61.8 (9.5)	36.3 (9.3)	18.7 (2.4)	63.8 (4.8)	37.3 (3.4)	20.2 (1.1)

[*] Survey design weights and 500 bootstrap weights were used in calculating geometric mean values and standard errors (S.E.)
[†] If <60% of samples had detectable organophosphate metabolites, means were not calculated
[‡] Statistically significant differences in DAP concentrations with $p<0.05$.

Table 4-5. Association between log transformed urinary concentrations of ΣDAP, ΣDMAP and ΣDEAP and lung function parameters by age group[†]

per nmol /g creatinine[*]	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
	Beta (S.E.)[‡]	*p*-value	Beta (S.E.)[‡]	*p*-value	Beta (S.E.)[‡]	*p*-value	Beta (S.E.)[‡]	*p*-value
				12-19 years (N=980)				
ΣDAP	13.9 (17.4)	0.44	-2.4 (15.2)	0.88	-0.3 (0.2)	0.20	-27.1 (39.9)	0.51
ΣDMAP	12.3 (13.9)	0.39	1.1 (11.0)	0.92	-0.2 (0.2)	0.31	-17.9 (31.7)	0.58
ΣDEAP	21.6 (30.7)	0.50	2.8 (23.7)	0.91	-0.3 (0.2)	0.18	-6.3 (39.7)	0.88
				20-79 years (N=3,466)				
ΣDAP	-32.6 (11.2)	0.014	-32.6 (12.0)	0.02	-0.2 (0.2)	0.36	-53.1 (27.6)	0.081
ΣDMAP	-24.3 (9.6)	0.028	-24.2 (9.7)	0.03	-0.1 (0.2)	0.39	-38.7 (23.4)	0.13
ΣDEAP	-20.4 (16.7)	0.25	-26.3 (15.8)	0.12	-0.2 (0.2)	0.22	-62.2 (23.4)	0.022

[*] Log (natural) transformed concentration adjusted for urine creatinine

[†] Associations were characterized by the multiple linear regression analyses with controlling for age groups, sex, ethnicity, height, weight and smoking status.

[‡] Survey design weights and 500 bootstrap weights were included in calculating β coefficients and standard errors (S.E.). Beta=β coefficients

Table 4-6. Results from the multiple linear regression of lung function parameters and ΣDAP concentration in urine among participants aged 20-79 years [*]

	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value
ΣDAP								
nmol /g creatinine[‡]	-32.6 (-57.2, -8.1)	0.014	-32.6 (-59.0, -6.3)	0.02	-0.2 (-0.6, 0.2)	0.36	-53.1 (-113.9, 7.7)	0.081
Age (years)								
20-29	0		0		0		0	
30-39	-134.1 (-241.3, -26.9)	0.019	-218.5 (-317.7, -119.3)	0.001	-2.6 (-3.7, -1.4)	0.001	-341.7 (-585.0, -98.3)	0.01
40-79	-614.6 (-716.4, -512.8)	<0.0001	-739.8 (-836.3, -643.2)	<0.0001	-6.6 (-7.4, -5.8)	<0.0001	-1099.2 (-1290.3, -908.0)	<0.0001
Sex								
Female	0		0		0		0	
Male	478.4 (389.4, 567.3)	<0.0001	389.5 (330.1, 448.9)	<0.0001	0.05 (-0.9, 1.0)	0.91	390.4 (214.9, 565.9)	<0.0001
Ethnicity								
Caucasian	0		0		0		0	
Others	-180.6 (-279.3, -81.9)	0.002	-76.2 (-140.8, -11.5)	0.025	1.8 (0.8, 2.7)	0.002	45.7 (-36.1, 127.5)	0.24
Height (cm)	71.6 (68.3, 75.0)	<0.0001	48.0 (44.6, 51.5)	<0.0001	-0.1 (-0.2, -0.07)	0.001	25.6 (16.2, 35.0)	<0.0001
Weight (kg)	-4.6 (-7.3, -1.8)	0.004	-1.6 (-3.6, 0.4)	0.10	0.05 (0.03, 0.07)	<0.0001	3.7 (1.1, 6.4)	0.01
Smoking status								
Never	0		0		0		0	
Former smoker	-37.5 (-119.8, 44.7)	0.34	-98.9 (-177.8, -20.0)	0.019	-1.9 (-2.8, -1.1)	<0.0001	-247.2 (-388.8, -105.5)	<0.0001
Current smoker	-3.7 (-92.2, 84.9)	0.93	-173.3 (-257.8, -88.8)	0.001	-4.7 (-5.4, -4.0)	<0.0001	-461.0 (-574.6, -347.4)	0.01
Cons.	-7226.5 (-7741.1, -6711.9)	<0.0001	-4253.6 (-4905.4, -3601.8)	<0.0001	102.3 (91.3, 113.2)	<0.0001	-775.3 (-2479.5, 928.8)	0.34

[*] Associations were characterized by the multiple linear regression analyses with controlling for age groups, sex, ethnicity, height, weight and smoking status.

[†] Survey design weights and 500 bootstrap weights were included in calculating β coefficients and standard errors (S.E.). Beta=β coefficients

[‡] Log (natural) transformed concentration adjusted for urine creatinine

Table 4-7. Results from the multiple linear regression of lung function parameters and ΣDMAP concentration in urine among participants aged 20-79 years [*]

	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value
ΣDMAP								
nmol /g creatinine[‡]	-24.3 (-45.4, -3.2)	0.028	-24.2 (-45.5, -2.8)	0.03	-0.1 (-0.5, 0.2)	0.39	-38.7 (-90.3, 12.8)	0.13
Age (years)								
20-29	0		0		0		0	
30-39	-135.1 (-242.6, -27.7)	<0.0001	-219.5 (-319.4, -119.5)	0.001	-2.6 (-3.7, -1.4)	0.001	-343.3 (-587.7, -98.8)	0.01
40-79	-616.9 (-718.3, -515.6)	<0.0001	-742.1 (-838.9, -645.4)	<0.0001	-6.6 (-7.4, -5.8)	<0.0001	-1103.2 (-1293.8, -912.6)	<0.0001
Sex								
Female	0		0		0		0	
Male	481.5 (390.4, 572.7)	<0.0001	392.8 (331.2, 454.3)	<0.0001	0.06 (-0.8, 1.0)	0.88	395.9 (221.7, 570.0)	<0.0001
Ethnicity								
Caucasian	0		0		0		0	
Others	-180.6 (-279.4, -81.7)	0.002	-76.1 (-141.1, -11.2)	0.026	1.8 (0.8, 2.7)	0.002	45.8 (-35.1, 126.7)	0.24
Height (cm)	71.6 (68.2, 75.1)	<0.0001	48.0 (44.6, 51.5)	<0.0001	-0.1 (-0.2, -0.07)	0.001	25.6 (16.2, 35.0)	<0.0001
Weight (kg)	-4.5 (-7.2, -1.8)	0.004	-1.6 (-3.5, 0.4)	0.11	0.04 (0.03, 0.07)	<0.0001	3.8 (1.1, 6.5)	0.01
Smoking status								
Never	0		0		0		0	
Former smoker	-38.3 (-121.2, 44.5)	0.33	-99.7 (-179.1, -20.2)	0.019	-2.0 (-2.8, -1.1)	<0.0001	-248.5 (-390.5, -106.5)	<0.0001
Current smoker	-5.3 (-93.4, 82.8)	0.90	-171.6 (-254.9, -88.3)	0.001	-4.7 (-5.4, -4.0)	<0.0001	-458.0 (-570.7, -345.2)	<0.0001
Cons.	-7280.2 (-7777.1, -6783.3)	<0.0001	-4307.7 (-4951.6, -3663.8)	<0.0001	102.0 (91.2, 112.8)	<0.0001	-864.9 (-2565.0, 835.3)	0.29

[*] Associations were characterized by the multiple linear regression analyses with controlling for age groups, sex, ethnicity, height, weight and smoking status.

[†] Survey design weights and 500 bootstrap weights were included in calculating β coefficients and standard errors (S.E.). Beta=β coefficients

[‡] Log (natural) transformed concentration adjusted for urine creatinine

Table 4-8. Results from the multiple linear regression of lung function parameters and ΣDEAP concentration in urine among participants aged 20-79 years [*]

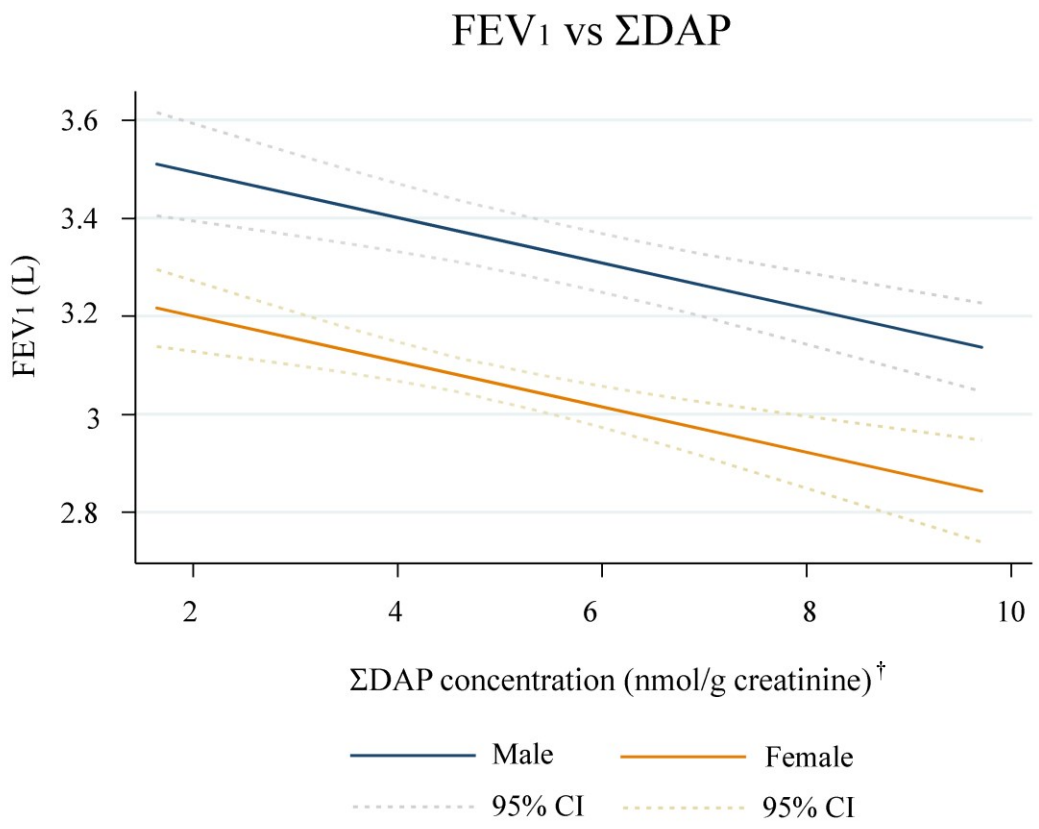
	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value
ΣDEAP								
nmol /g creatinine[‡]	-20.4 (-57.2, 16.4)	0.25	-26.3 (-61.1, 8.4)	0.12	-0.2 (-0.5, 1.4)	0.22	-62.2 (-113.6, -10.8)	0.022
Age (years)								
20-29	0		0		0		0	
30-39	-133.9 (-241.4, -26.4)	<0.0001	-218.2 (-317.2, -119.2)	<0.0001	-2.6 (-3.8, -1.4)	0.001	-340.0 (-584.4, -95.7)	0.011
40-79	-619.7 (-726.1, -513.3)	<0.0001	-743.4 (-840.4, -646.3)	<0.0001	-6.6 (-7.4, -5.7)	<0.0001	-1100.2 (-1296.5, -903.9)	<0.0001
Sex								
Female	0		0		0		0	
Male	485.5 (397.8, 573.3)	<0.0001	394.1 (336.0, 452.3)	<0.0001	0.05 (-0.9, 1.0)	0.91	389.9 (219.5, 560.2)	<0.0001
Ethnicity								
Caucasian	0		0		0		0	
Others	-182.1 (-282.5, -81.7)	0.002	-78.0 (-145.0, -11.0)	0.026	1.8 (0.8, 2.7)	0.002	41.6 (-40.1, 123.4)	0.29
Height (cm)	71.3 (67.9, 74.7)	<0.0001	47.7 (44.3, 51.2)	<0.0001	-0.1 (-0.2, -0.07)	0.001	25.2 (15.9, 34.5)	<0.0001
Weight (kg)	-4.3 (-5.7, -1.5)	0.006	-1.4 (-3.3, 0.5)	0.12	0.04 (0.03, 0.07)	<0.0001	3.8 (1.4, 6.3)	0.005
Smoking status								
Never	0		0		0		0	
Former smoker	-38.5 (-123.3, 46.3)	0.34	-99.4 (-180.7, -18.1)	0.021	-1.9 (-2.8, -1.1)	<0.0001	-247.1 (-389.7, -104.4)	0.003
Current smoker	-13.3 (-105.1, 78.5)	0.76	-164.7 (-251.6, -77.8)	0.002	-4.6 (-5.3, -3.9)	<0.0001	-450.6 (-563.6, -337.6)	<0.0001
Cons.	-7270.7 (-7791.7, -6749.7)	<0.0001	-4278.8 (-4918.6, -3638.9)	<0.0001	102.3 (91.6, 113.1)	<0.0001	-759.8 (-2431.7, 912.0)	0.34

[*] Associations were characterized by the multiple linear regression analyses with controlling for age groups, sex, ethnicity, height, weight and smoking status.

[†] Survey design weights and 500 bootstrap weights were included in calculating β coefficients and standard errors (S.E.). Beta=β coefficients

[‡] Log (natural) transformed concentration adjusted for urine creatinine

Figure 4-1. Relationship between urinary concentrations of total DAPs and FEV_1 for adult participants by sex[*‡]

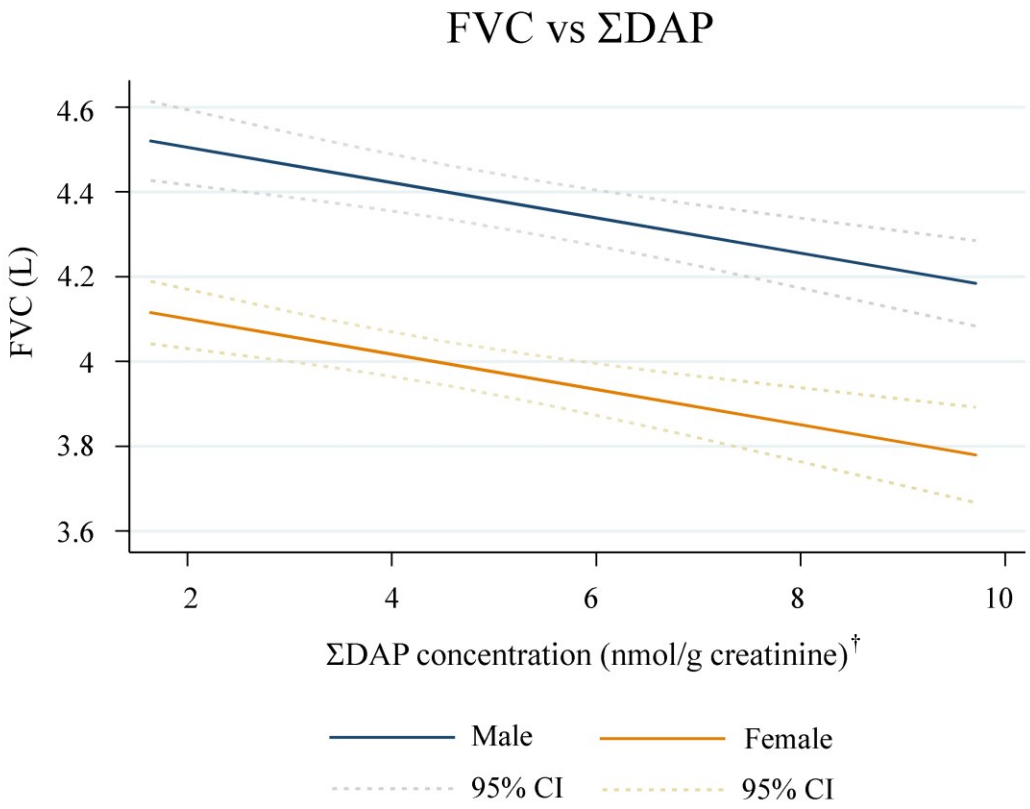


[*] Predicted lines were obtained based on the multiple linear regression models

[†] Concentration of ΣDAP was log transformed

[‡] Since there was no siginficant interaction between ΣDAP and sex, the predicted lines were parallel for males and females

Figure 4-2. Relationship between urinary concentrations of total DAPs and FVC for adult participants by sex[*‡]

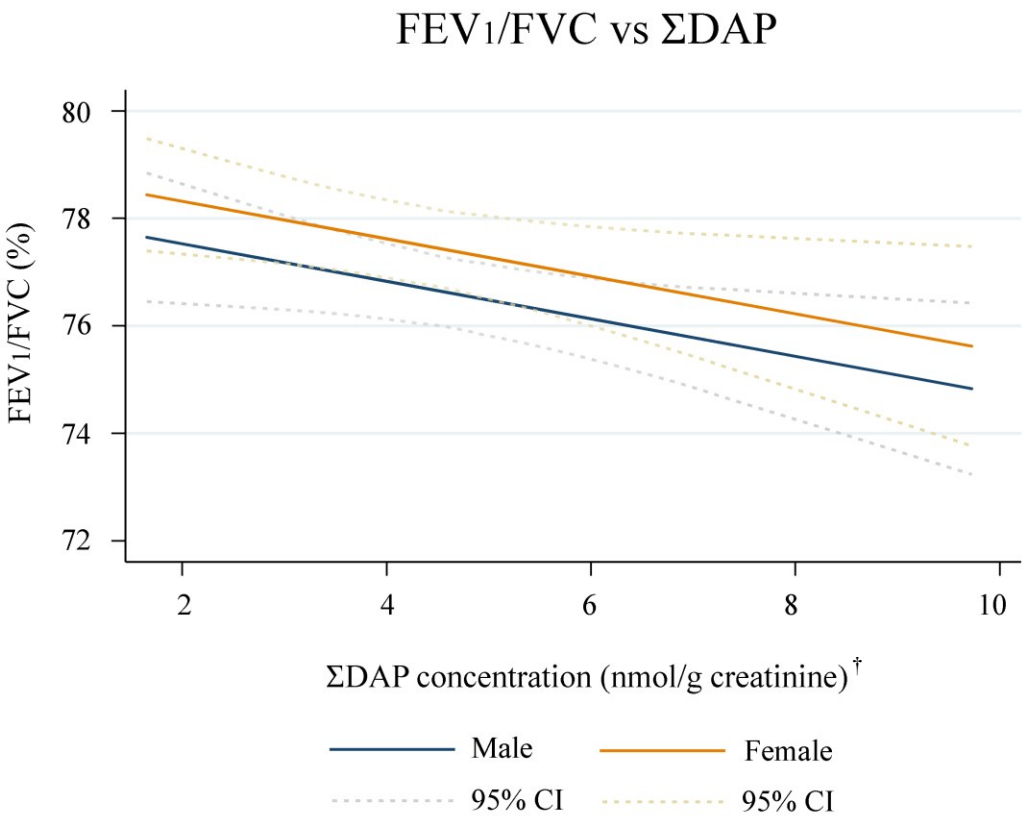


[*] Predicted lines were obtained based on the multiple linear regression models

[†] Concentration of ΣDAP was log transformed

[‡] Since there was no siginficant interaction between ΣDAP and sex, the predicted lines were parallel for males and females

Figure 4-3. Relationship between urinary concentrations of total DAPs and FEV_1/FVC ratio for adult participants by sex[*‡]

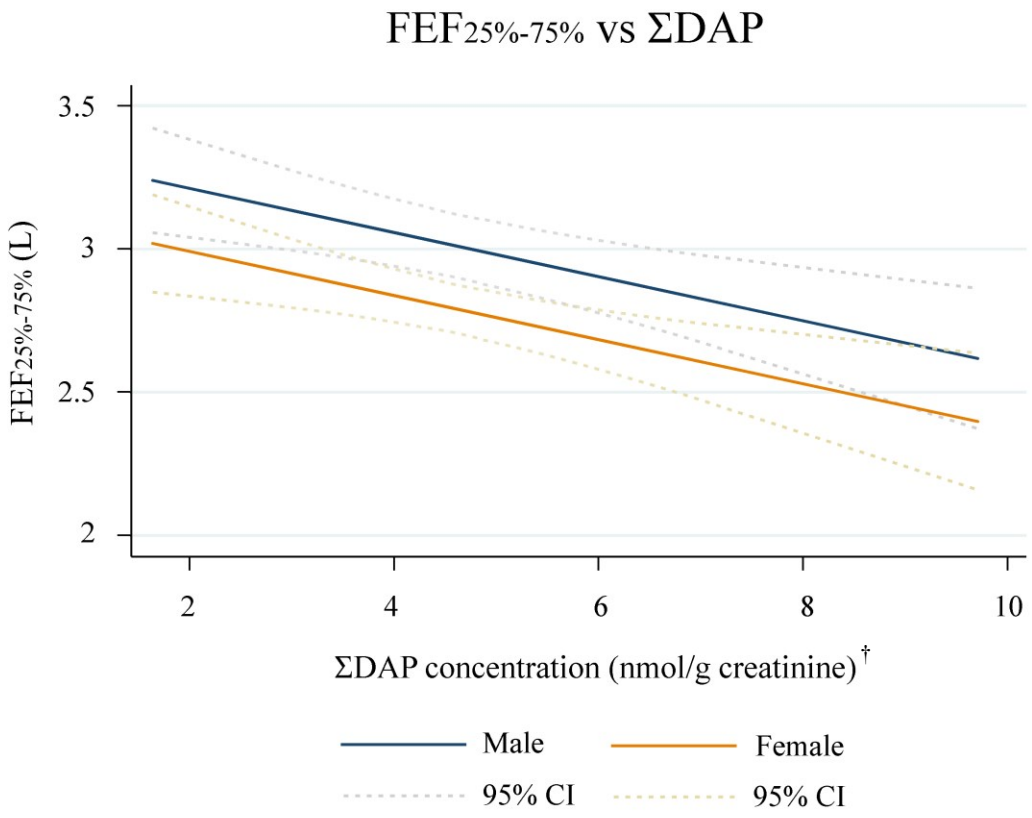


[*] Predicted lines were obtained based on the multiple linear regression models

[†] Concentration of ΣDAP was log transformed

[‡] Since there was no siginficant interaction between ΣDAP and sex, the predicted lines were parallel for males and females

Figure 4-4. Relationship between urinary concentrations of total DAPs and $FEF_{25\%-75\%}$ for adult participants by sex[*‡]

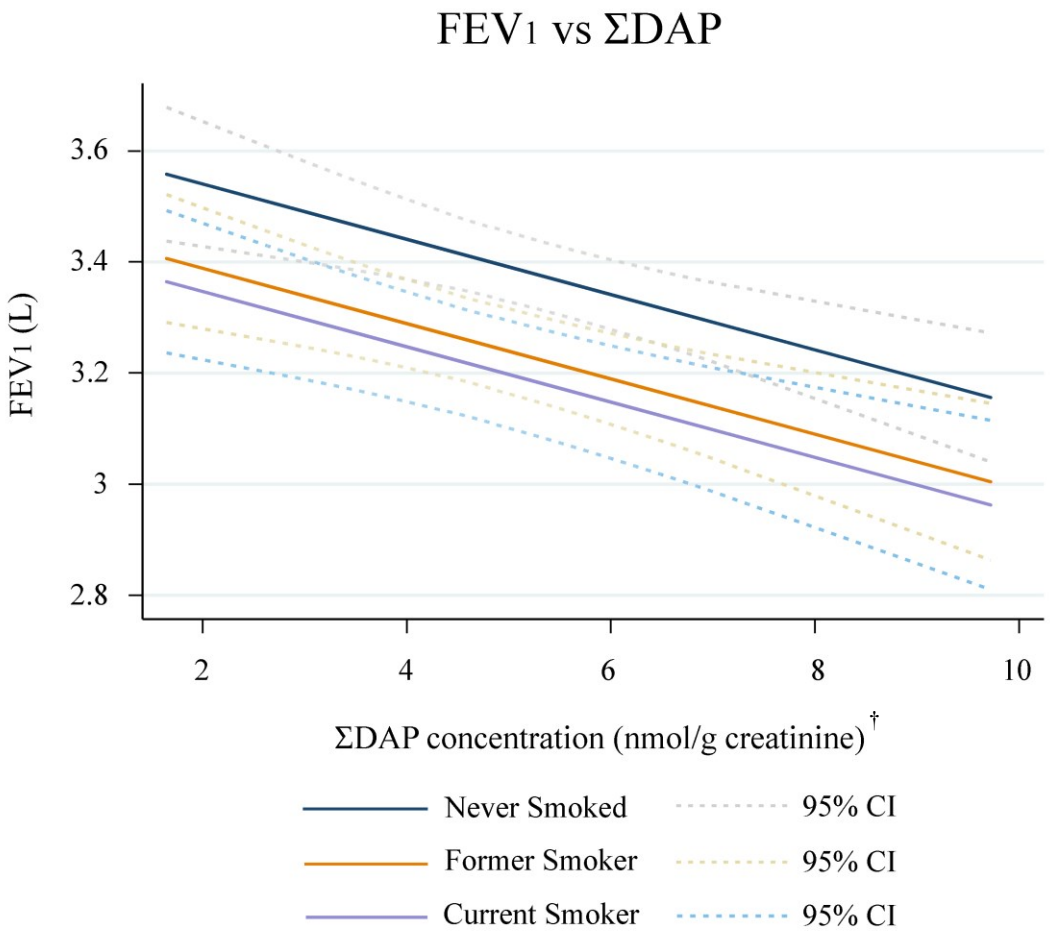


[*] Predicted lines were obtained based on the multiple linear regression models

[†] Concentration of ΣDAP was log transformed

[‡] Since there was no siginficant interaction between ΣDAP and sex, the predicted lines were parallel for males and females

Figure 4-5. Relationship between urinary concentrations of total DAPs and FEV_1 for adult participants by smoking status[*‡]

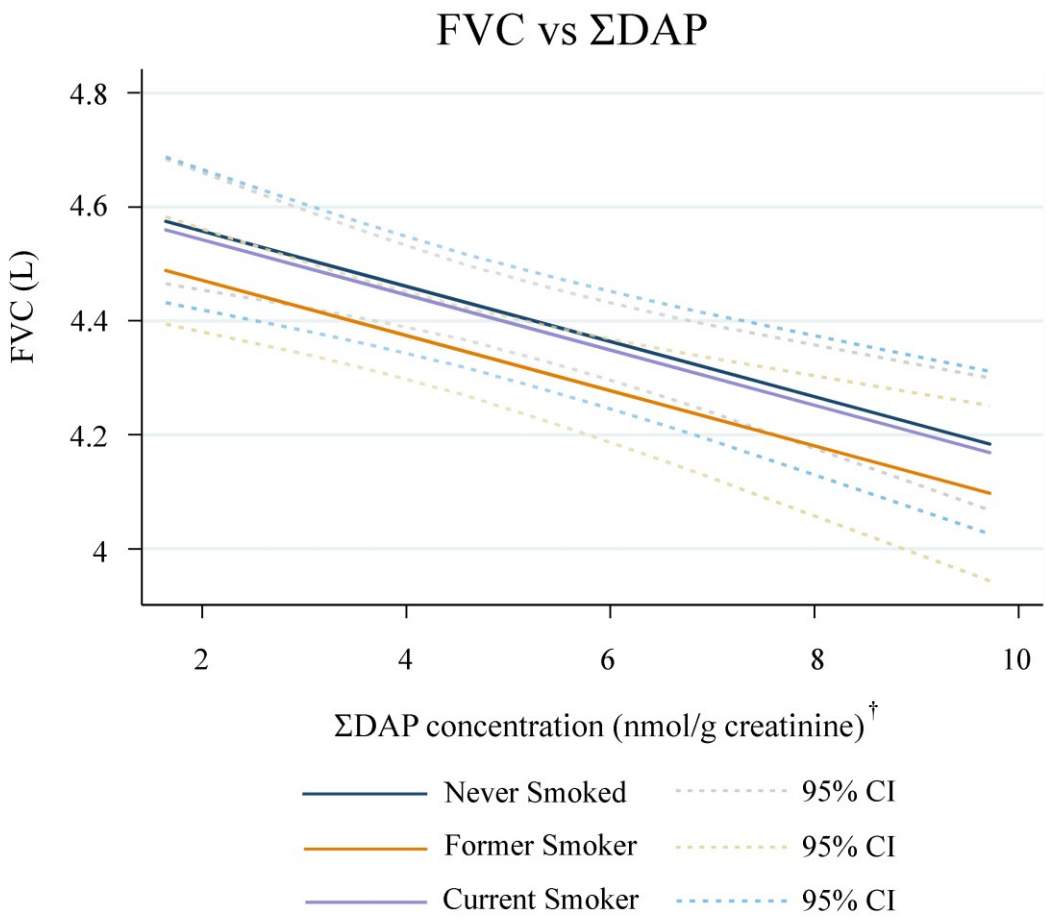


[*] Predicted lines were obtained based on the multiple linear regression models

[†] Concentration of ΣDAP was log transformed

[‡] Since there was no siginficant interaction between ΣDAP and smoking status, the predicted lines were parallel for the smoking categories

Figure 4-6. Relationship between urinary concentrations of total DAPs and FVC for adult participants by smoking status[*‡]

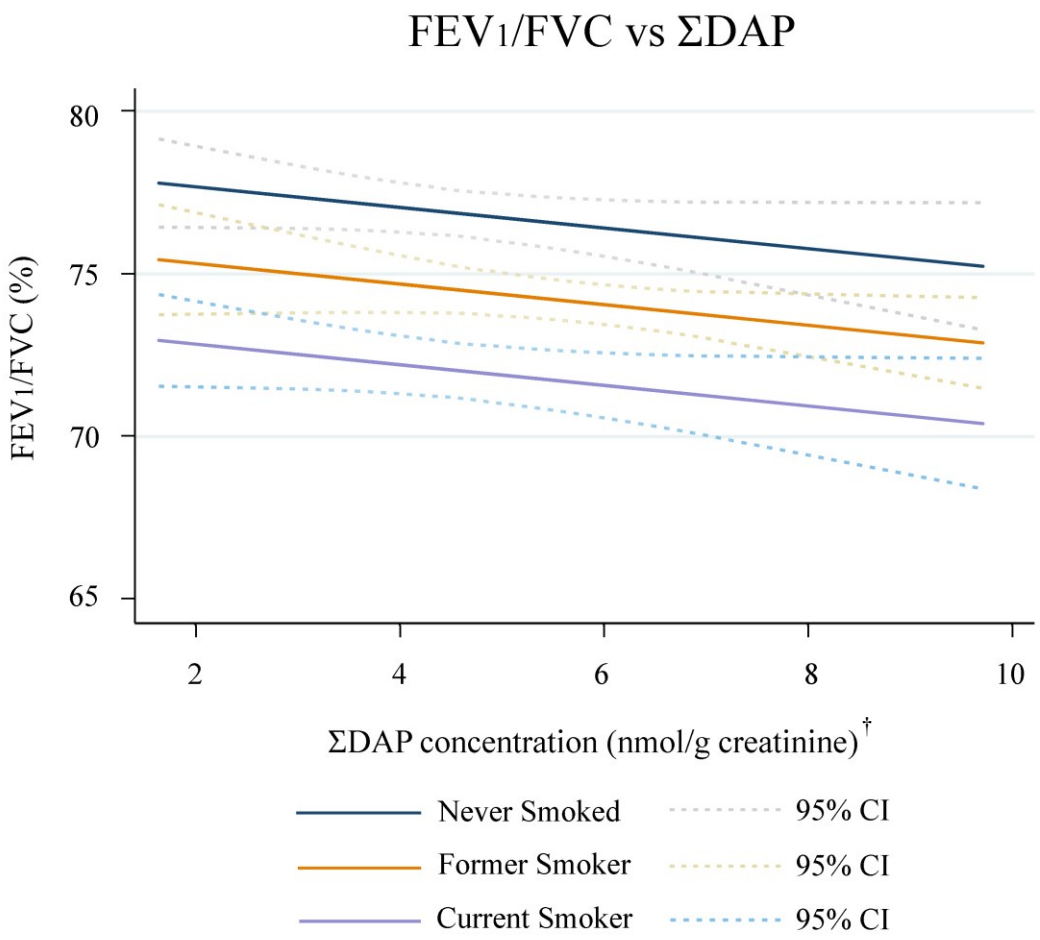


[*] Predicted lines were obtained based on the multiple linear regression models

[†] Concentration of ΣDAP was log transformed

[‡] Since there was no siginficant interaction between ΣDAP and smoking status, the predicted lines were parallel for the smoking categories

Figure 4-7. Relationship between urinary concentrations of total DAPs and FEV_1/FVC ratio for adult participants by smoking status[*‡]

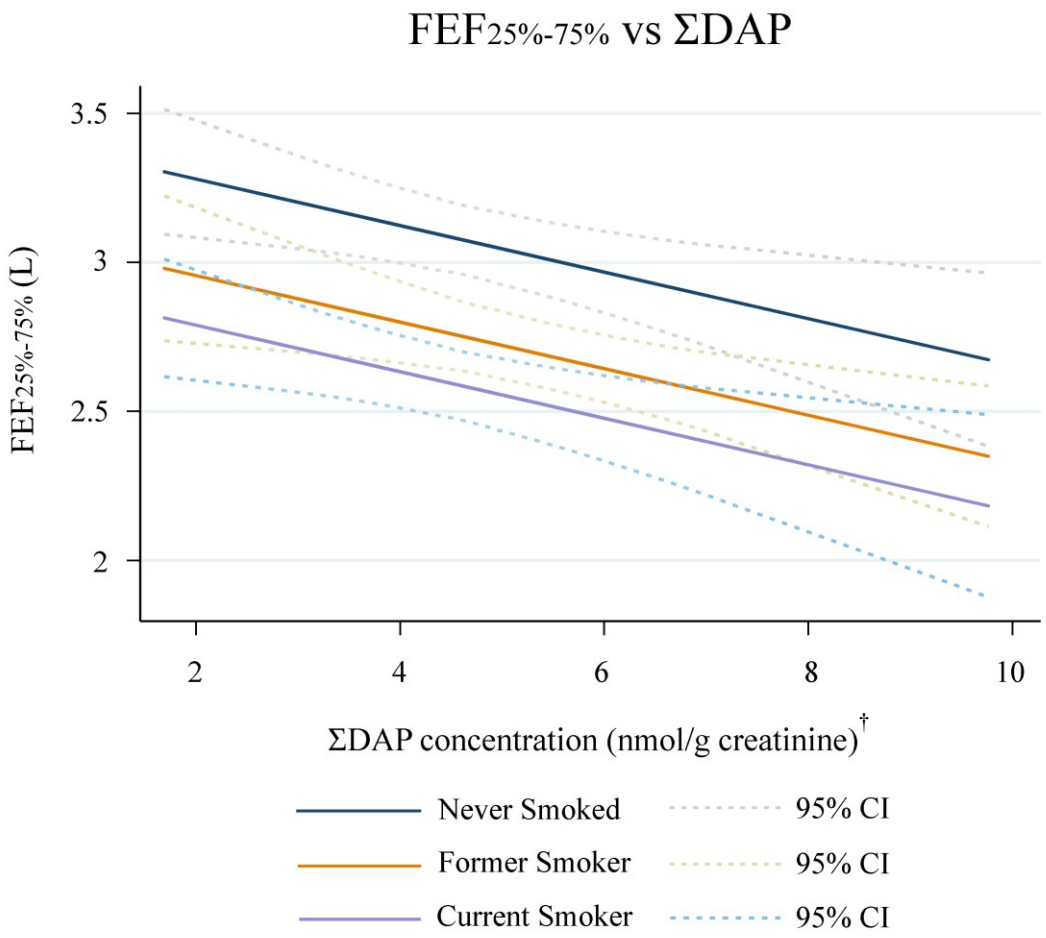


[*] Predicted lines were obtained based on the multiple linear regression models

[†] Concentration of ΣDAP was log transformed

[‡] Since there was no siginficant interaction between ΣDAP and smoking status, the predicted lines were parallel for the smoking categories

Figure 4-8. Relationship between urinary concentrations of total DAPs and $FEF_{25\%-75\%}$ for adult participants by smoking status[*‡]



[*] Predicted lines were obtained based on the multiple linear regression models

[†] Concentration of ΣDAP was log transformed

[‡] Since there was no siginficant interaction between ΣDAP and smoking status, the predicted lines were parallel for the smoking categories

CHAPTER 5

URINARY CONCENTRATIONS OF PYRETHROID METABOLITES AND ITS ASSOCIATION WITH LUNG FUNCTION IN A CANADIAN GENERAL POPULATION

5.1 Introduction

Pyrethroids are synthetic insecticides chemically similar to pyrethrin, a natural insecticide generated by chrysanthemum [1]. Synthetic pyrethroids are currently replacing organophosphates and carbamates as insecticides used in gardens, residential areas and agricultural applications [2]. Pyrethroids can be efficiently absorbed through airway inhalation, but only slightly through the skin due to their low lipid solubility [1, 3, 4]. Ingestion of food and water contaminated with pyrethroid insecticides is another route of pyrethroid exposure [3-5], especially for the general population [6].

In the blood and liver, pyrethroids can be rapidly detoxified by carboxylesterase mediated hydrolysis to produce metabolites DCCA [3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid], DBCA [3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid], 3-PBA and 4-fluoro-3-PBA [7-9]. These metabolites are not pesticide-specific [8, 9] and are rapidly eliminated in the urine after exposure [10]. A human biomonitoring approach, measuring pesticide metabolites in urine or blood samples, has been widely applied to estimate exposure to the parent pesticides [11-16]. Urinary detection of a measurable amount of pyrethroid metabolites indicates recent exposure to pyrethroid insecticides [9].

Pyrethroids insecticides are neurotoxicants, targeting the voltage-gated sodium/chloride channels and leading to a prolonged depolarization of neurons [7]. Acute neurotoxic symptoms after high dose pyrethroid exposures include dermal paresthesia, gastrointestinal pain, upper respiratory tract irritation, dizziness, headache, movement coordination dysfunction, tremor and

seizures [7, 17]. Other adverse health effects have also been linked to pyrethroid exposures, including the possible effects on neurodevelopment [18-20], reproductive function [18, 21], the immune system [22-27], the endocrine system [28, 29], and cancer [30, 31].

In the Agricultural Health Study (AHS), Hoppin *et al*. showed that exposures to the insecticide permethrin were significantly associated with wheezing (OR = 1.28, 95% CI: 1.06–1.55) among farmers in the US [32]. In addition, a cross-sectional study in Lebanon showed that there was a significant positive relationship between residential exposures to pesticides, including pyrethroid pesticides, and respiratory symptoms, such as chronic cough and recurrent wheezing, among school-aged children [33].

While a few of studies have suggested that there may be a relationship between pyrethroid exposures and respiratory symptoms [32, 33], little is known about the effect of pyrethroid pesticide on lung function [34]. The current study aims to address this question by characterizing the association between pyrethroid metabolite concentrations in urine and lung function among the Canadian general population using data from the Canadian Health Measures Survey (CHMS).

5.2 Methods

In the current study, we used data of 5,436 CHMS-Cycle 1 participants aged 6-79 years, including children (6-12 years), adolescents (12-19 years) and adults (20-79 years), to characterize the association between urinary concentrations of pyrethroid metabolites and lung function. The sampling method of the CHMS-Cycle 1 has been described in Chapter 3. The detailed information of the CHMS-Cycle 1 can also be obtained from Statistics Canada [35]. This study was approved by the Health Research Ethics Board of the University of Alberta.

5.2.1 Lung function measurements

This study used the same the method for measuring lung function as in Chapter 4.

5.2.2 Factors related to lung function

Information on lung function related factors, including demographic factors, socioeconomic status, tobacco smoking status and other environmental factors, was obtained using the same methods as in Chapter 3 [35]. Daily Energy Expenditure (DEE) value for all activities in a day was obtained using the formula below:

DEE (kcal/kg/day) = Sum of [(N_i *D_i * MET_i value) / 90],

where 'N_i' is the number of times a respondent engaged in an activity 'i' in the past 3 month, 'D_i' is the average duration in hours of the activity$_i$, and MET_i is the energy cost of the activity 'i' expressed as kilocalories expended per kilogram of body weight per hour of activity (kcal/kg per hour). The product of these terms were summed over each activity over 3 months (90 days) and then divided by 90 to convert to a mean daily value. Information on types of physical activities (MET_i), the number of times an individual engaged in an activity (N_i) and the average duration of the activity (D_i) were obtained using CHMS household questionnaire [36].

5.2.3 Urinary concentrations of pyrethroid metabolites

The method for measuring urinary concentrations of PYR metabolites was same as those used in Chapter 3.

5.2.4 Statistical analyses

Lung function parameters FVC, FEV_1, FEV_1/FVC and $FEF_{25\%-75\%}$ were analyzed as continuous outcome variables, and log-transformed total concentration of pyrethroid metabolites (ΣPYR) was used as exposure variable of interest.

In the descriptive analyses, means, medians and inter quarter ranges (IQRs) of urinary concentrations (nmol/g creatinine) and proportions of subjects with detectable pesticide

metabolites in urine were calculated for each PYR metabolite individually, as well as for ΣPYR. Same as in Chapter 3 and 4, summary statistics were not calculated when more than 40% of samples had concentrations for that metabolite less than the LOD [15].

Relationships between lung function parameters and urinary ΣPYR concentrations were examined using separate multiple linear regression analyses for children (6-11 years), adolescents (12-19 years) and adults (20-79 years). In the multiple linear regression analyses, lung function measures were dependent variables, log (natural) transformed creatinine-adjusted urinary concentration (nmol/g creatinine) of ΣPYR was the independent variable of interest, and age, sex, ethnicity, height, weight and smoking status were adjusted as potential confounders. Clinically and biologically plausible interactions, including the effect of age, sex, ethnicity, and smoking status on the associations, were examined. Covariates, including the interaction terms, that were non-significant at p=0.05, were removed from the regression models.

Sensitivity analyses were also performed using the mass volume concentrations (nmol/L) of ΣPYR as the independent variable with urinary creatinine concentrations (g/L) as a separate independent covariate to examine the effect of the potential creatinine-related variation on lung function [37].

Same as in Chapter 4, sampling design weights and 500 bootstrap weights were applied in variance estimation for descriptive statistics, regression coefficients and 95% confidence intervals [35]. Statistical analyses were performed using the same software as in Chapter 3 and 4.

5.3 Results

5.3.1 Characteristics of the study participants

Demographic characteristics, dwelling type, smoking status and daily energy expenditure of the study population are summarized in Table 5-1. Among the 5,604 CHMS participants, 7.5% were children, 11.5% were adolescents and 81.0% were adults. Overall, males and females were almost equally represented and 69.8% of the study subjects self-identified as of Caucasian ethnicity (Table 5-1). The majority of study subjects (73.7%-84.4% across different age groups) lived in houses. A higher proportion of adult subjects reported living in an apartment or mobile home than children and adolescents (Table 5-1). While most of the adolescent subjects (85.6%) had never smoked, approximately 50% of the adult subjects had smoked at some time during their lifetime (Table 5-1).

5.3.2 Lung function among the study participants

Lung function measures across demographic groups and smoking status are summarized in Table 5-2. In both adolescents and adults, smokers (former and current smokers) had significantly lower mean values in FEV_1, FEV_1/FVC ratio and $FEF_{25\%-75\%}$ compared with non-smokers (p<0.005) after adjusting for age, sex, ethnicity, weight and height. In addition, among adult subjects, one unit (kcal/kg/day) increase in daily energy expenditure was significantly associated with 37.6 (95% CI: 12.1-63.1) mL increase in FVC (p=0.008) and 29.0 (95% CI: 14.0-44.1) mL increase in FEV1 (p=0.001), after adjusting for age, sex, ethnicity, weight, height and smoking status.

No significant differences in lung function measures were observed between subjects with different socioeconomic status (household income and education levels) or other environmental factors, such as environmental tobacco smoke exposure (second-hand smoke) status, or types of heating source used at home. No significant association was observed between lung function measures and ambient concentrations of PM2.5, O_3, and NO_2 (data not shown).

5.3.3 Urinary concentrations of pyrethroid metabolites

The percentage of subjects with detectable concentrations of pyrethorid metabolites among 5,604 CHMS participants has been described in Chapter 3 (Table 3-3). Means (both arithmetic and geometric mean), medians and interquartile range (IQR) of each pyrethroid metabolite and total metabolites (ΣPYR) for three age groups are summarized in Table 5-3.

In Table 5-4, the geometric mean concentrations of ΣPYR are summarized by demographic factors, dwelling types and smoking status for each of the three age groups. Among the study subjects, adolescents aged 12-19 years had significantly lower mean (geometric) concentration of ΣPYR in urine than children and adults (p-value<0.01, Table 5-4). After adjusting for age, for both adolescents and adults, females had significantly greater mean concentrations of ΣPYR than male subjects (p-values<0.05). In children and adults, subjects who self-identified as non-Caucasian had significantly greater mean concentrations of ΣPYR than Caucasians after controlling for age and sex (p-values<0.02). In addition, after adjusting for age and sex, subjects living in a house had lower mean urinary concentrations of ΣPYR than those living in apartments or mobile homes, and this was significant among children and adult subjects (p-values<0.01). After adjusting for age and sex, no significant differences in the ΣPYR concentration was observed between subjects with different smoking history for both adolescents and adults (data not shown).

5.3.4 Relationships between ΣPYR concentrations and lung function

Among the child participants, after adjusting for age, sex, ethnicity, height and weight, one unit increase in log transformed urinary concentration (nmol/g creatinine) of ΣPYR was associated with a 17.4 mL reduction in FEV_1 (p=0.045) (Table 5-5). Among the adolescents aged

12-19 years, after adjusting for age, sex, ethnicity, height, weight and smoking status, a one unit increase in log transformed urinary concentration (nmol/g creatinine) of ΣPYR was associated with a 37.1 mL reduction in FVC (p=0.05). No significant association was observed between lung function measures and urinary concentration of ΣPYR among adult subjects (Table 5-5). In addition, there were no significant interactions between age, sex, ethnicity, and smoking status and ΣPYR concentrations in the multiple regression analysis of FEV_1 and FVC. Detailed results from the multiple linear regression analyses of lung function parameters and urinary ΣPYR concentrations in three age groups are shown in Table 5-6, 5-7 and 5-8.

In the sensitivity analyses, after adjusting for the potential covariate-related variation in urinary creatinine concentrations, results regarding associations between ΣPYR and lung function parameters FVC, FEV_1, FEV_1/FVC ratio and $FEF_{25\%-75\%}$ were consistent with the results reported in Table 5-5 (data not shown).

5.4 Discussion

In this study, we examined urinary concentrations of pyrethroid metabolites and their associations with lung function in a representative sample of the Canadian general population. Almost all (99.8%) CHMS-Cycle 1 participants had at least one type of pyrethroid metabolite detectable in their urine samples, which is consistent with previously published data [15, 16]. These analyses also showed that despite a similar mean urinary concentrations [14, 38], a higher percentage of the Canadian general population had detectable urinary pyrethroid metabolites (99.8%) than a US (75.4%, NHANES 1999-2002) or French (80.0%, ENNS 2006-2007) population [12, 14], which might result from increasing use of pyrethroid pesticides during the last decade in Canada [39]. Given the short half-life of pyrethroid pesticides *in vivo* [9, 10, 40,

41], current detection of pyrethroid metabolites at a high prevalence among the subjects suggests that pyrethroid exposures are common and ongoing among the Canadian general population.

There have been few studies in the literature showing the effect of pyrethroid insecticides on lung function. A case study in Australia did report that a patient with pre-existing asthma had a significant reduction in lung function FEV_1 and $FEF_{25\%-75\%}$ after an acute exposure to pyrethroid insecticides at home [42], but it was not clear that the pyrethroid itself was the cause.

In the current study, we found that the urinary concentrations of pyrethroid metabolites were significantly associated with a lower FEV_1 in children, and a lower FVC in adolescents, but not in adults. Based on the multiple linear regression models, difference in FEV_1 between children with lower and upper quartiles of urinary pyrethroid concentrations was 21.5 mL, and difference in FVC between adolescents with lower and upper quartiles of urinary pyrethroid concentrations was 52.5 mL. Although not statistically significant for each age group, the mean FVC and FEV_1 were consistently lower for subjects with greater pyrethroid exposures. The current study provides the first evidence suggesting the potential adverse effect of pyrethroid insecticides on lung function.

Child and adolescent lungs are not fully developed and the developing lungs are highly susceptible to environmental toxicants [43]. For example, previous studies have suggested that children and adolescents are more vulnerable than adults to the outdoor air pollutants [44-46]. In this study, the pyrethroid associated differences in lung function, particularly FEV_1, were larger in children and adolescents than adults. This result suggests a relatively higher risk of being affected by pyrethroid insecticides at younger ages, especially given that the mean (arithmetic) concentration of ΣPYR was lower in children and adolescents than adult subjects.

Nevertheless, the fact that only one of the four lung function variables, either FVC or FEV_1, was significantly associated with pyrethroid exposures in children and adolescent and it was different across the age groups, does raise the possibility that the associations reported in this study were in some way spurious. Uncontrolled confounding factors, including genetic predisposition and those environmental factors not measured in the CHMS-Cycle 1, such as indoor air pollutants [35], may lead to the inconsistent associations between age groups.

Several previous studies have suggested an effect of pyrethroid insecticides on respiratory symptoms and disease. Hoppin *et al*. showed that occupational exposures to permethrin, a pyrethroid insecticide, were significantly associated with wheezing (OR = 1.28, 95% CI: 1.06–1.55) among farmers in the US [32]. In Belgium, a case of occupational asthma was linked to the chronic exposure to tetramethrin, a pyrethroid insecticide [47]. In addition, an 11-year-old girl in the US with pre-existing asthma since the age of six years suffered a "respiratory arrest secondary to acute asthmatic attack" and died after acute exposures to an animal shampoo containing pyrethrin [48]. A case study in Tanzania showed that residential exposures to lambda-cyhalothrin, a pyrethroid insecticide, led to sneezing, coughing, and nose or throat irritation among residents [49]. It has also been reported that office workers in California experienced shortness of breath and irritation of the respiratory tract after accidentally inhaling cypermethrin, a pyrethroid insecticide [50]. Nevertheless, although not shown in the Results, we did not find any significant association between pyrethroid metabolite concentrations and a number of respiratory symptoms and diseases, including cough, night cough, dry cough, cough with phlegm, asthma and chronic bronchitis in our study populations, which suggests that the effect of pyrethroid on lung function may not be significant enough for individuals to develop overt symptoms or diseases.

There are several limitations to this study. Firstly, the study subjects from the CHMS Cycle 1 did not include Aboriginal people living on reserves and Aboriginal settlements, people living in remote areas, institutional residents and full-time members of the Canadian Forces [35]. Although the excluded population only represented about 4% of the total Canadians, it may limit the generalizability of our results to the whole Canadian population. Secondly, since parent pyrethroid insecticides can be naturally degraded into metabolites in the environment [51, 52], urinary detection of pyrethroid metabolites may be resulted from exposures to both parental insecticides and environmentally preformed pyrethroid metabolites. Our results may overestimate the exposure level to parent pyrethroid insecticides. Lastly, due to the cross-sectional nature of the CHMS Cycle 1, the current study is unable to determine whether the association identified between lung function changes and pyrethroid metabolite concentrations is causal. A different study design would be necessary to clarify this.

5.5 Conclusions

In summary, our data showed that urinary detection of pyrethroid metabolites was very common among the Canadian general population, suggesting a current widespread exposure to pyrethroid insecticides in Canada. While not completely consistent across age groups, our data suggested an adverse effect of pyrethroid insecticides on FVC and FEV_1 in children and adolescents. Further investigation using a different study design is necessary to confirm the possible adverse effect of pyrethroid insecticide on lung function reported in this study.

Table 5-1. Characteristics of the study population across three age groups[*]

Characteristics	Age groups (N=5,436)		
	6-11 years[†]	**12-19 years**[†]	**20-79 years**[†]
Sex (%)			
Female	48.7 (48.6, 48.9)	48.8 (48.8, 48.8)	50.5 (50.5, 50.6)
Male	51.3 (51.1, 51.4)	51.2 (51.2, 51.2)	49.5 (49.4, 49.5)
Height (cm)	135.47 (0.59)	166.70 (0.24)	168.58 (0.28)
Weight (kg)	33.43 (0.40)	63.16 (0.98)	77.65 (0.76)
Ethnicity (%)			
Caucasian	60.7 (48.4, 73.0)	62.7 (53.4, 72.0)	71.7 (63.8, 79.6)
Other	39.3 (27.0, 51.6)	37.3 (28.0, 46.6)	28.3 (20.4, 36.2)
Dwelling type (%)			
House	84.4 (74.5, 94.2)	85.1 (76.6, 93.5)	73.7 (61.3, 86.1)
Apartment or mobile home	15.6 (5.8, 25.5)	14.9 (6.5, 23.4)	26.3 (13.9, 38.7)
Daily energy expenditure (kcal/kg/day) [‡]	–	3.66 (0.15)	1.76 (0.08)
Smoking status (%) [‡]			
Never	–	85.6 (78.6, 92.6)	47.9 (44.7, 51.1)
Former smoker	–	2.4 (1.4, 3.4)	30.5 (27.3, 33.8)
Current smoker	–	11.9 (5.0, 18.9)	21.5 (19.3, 23.8)

[*] Descriptive statistics are presented as mean (S.E.) for continuous variables and percentage (95% CI) for categorical variables, where survey design weights and 500 bootstrap weights were applied in calculation.

[†] Among 5,604 subjects, 7.5% were children aged 6-11 years, 11.5% were adolescents aged 12-19 years, and 81.0% were adults aged 20-79 years.

[‡] Only measured for subjects aged 12-79 years

Table 5-2. Distribution of lung function parameters by age groups, demographic factors and smoking status[*]

	FVC (L) Mean (SE)	FEV_1 (L) Mean (SE)	FEV_1/FVC (%) Mean (SE)	$FEF_{25\%-75\%}$ (L/s) Mean (SE)
6-11 years				
Total sample	2.21 (0.03)	1.86 (0.02)	84.6 (0.3)	2.01 (0.03)
Sex[†]				
Female	2.15 (0.03)	1.84 (0.03)	85.6 (0.3)	2.04 (0.04)
Male	2.26 (0.04)	1.88 (0.03)	83.5 (0.5)	1.98 (0.05)
Ethnicity [†]				
Caucasian	2.25 (0.03)	1.89 (0.02)	84.4 (0.3)	2.03 (0.03)
Others	2.14 (0.03)	1.80 (0.03)	84.9 (0.7)	1.98 (0.05)
12-19 years				
Total sample	4.14 (0.04)	3.46 (0.03)	84.0 (0.3)	3.55 (0.05)
Sex [†]				
Female	3.71 (0.03)	3.16 (0.03)	85.5 (0..3)	3.39 (0.06)
Male	4.54 (0.07)	3.74 (0.05)	82.6 (0.5)	3.69 (0.07)
Ethnicity [†]				
Caucasian	4.28 (0.03)	3.55 (0.02)	83.4 (0.3)	3.60 (0.06)
Others	3.88 (0.07)	3.29 (0.05)	85.2 (0.5)	3.45 (0.06)
Smoking status[‡]				
Never	4.06 (0.03)	3.41 (0.03)	84.3 (0.3)	3.52 (0.05)
Former smoker	4.36 (0.13)	3.58 (0.07)	82.5 (2.0)	3.65 (0.17)
Current smoker	4.61 (0.10)	3.77 (0.09)	82.3 (1.0)	3.71 (0.20)
20-79 years				
Total sample	4.13 (0.04)	3.19 (0.03)	77.1 (0.3)	2.88 (0.04)
Age group [†]				
20-29 years	4.71 (0.10)	3.81 (0.08)	81.4 (0.5)	3.69 (0.12)
30-79 years	4.00 (0.03)	3.05 (0.02)	76.2 (0.3)	2.70 (0.03)
Sex [†]				
Female	3.45 (0.02)	2.68 (0.02)	77.6 (0.3)	2.48 (0.04)
Male	4.81 (0.05)	3.69 (0.04)	76.6 (0.4)	3.28 (0.06)
Ethnicity [†]				
Caucasian	4.19 (0.03)	3.20 (0.03)	76.3 (0.3)	2.84 (0.04)
Others	3.98 (0.06)	3.14 (0.04)	79.1 (0.5)	2.97 (0.04)
Smoking status[‡]				
Never	4.12 (0.07)	3.26 (0.05)	79.2 (0.3)	3.09 (0.06)
Former smoker	4.02 (0.05)	3.06 (0.04)	75.9 (0.3)	2.67 (0.06)
Current smoker	4.30 (0.05)	3.21 (0.04)	74.4 (0.4)	2.72 (0.05)

[*] Survey design weights and 500 bootstrap weights were used in calculating arithmetic mean values and standard errors (S.E.) of lung function parameters.
[†] Statistically significant differences in lung function with $p<0.0001$.
[‡] Only measured for subjects aged 12-79 years

Table 5-3. Urinary concentrations of pyrethroid pesticide metabolites among the study population

PYR metabolites	A.mean[†] (nmol/g, S.E.[*])	G.mean[†] (nmol/g, S.E.[*])	Median (nmol/g, S.E.[*])	IQR (nmol/g)
6-11 years				
3-PBA	3.55 (0.48)	1.51 (0.14)	1.28 (0.12)	0.74-2.62
4-F-3-PBA	–	–	<LOD	<LOD-0.08
cis-DCCA	0.93 (0.13)	0.40 (0.03)	0.34 (0.02)	0.20-0.64
trans-DCCA	3.36 (0.42)	1.28 (0.08)	1.04 (0.06)	0.61-2.20
cis-DBCA	–	–	<LOD	<LOD-0.08
ΣPYR	8.09 (1.02)	3.56 (0.28)	2.90 (0.20)	1.78-6.12
12-19 years				
3-PBA	3.02 (0.41)	1.16 (0.14)	0.93 (0.13)	0.50-2.11
4-F-3-PBA	–	–	<LOD	<LOD-0.06
cis-DCCA	1.07 (0.13)	0.38 (0.04)	0.29 (0.04)	0.16-0.71
trans-DCCA	3.13 (0.53)	1.02 (0.11)	0.76 (0.10)	0.42-2.07
cis-DBCA	–	–	<LOD	<LOD-0.05
ΣPYR	7.36 (1.07)	2.80 (0.32)	2.17 (0.31)	1.24-5.10
20-79 years				
3-PBA	4.48 (0.64)	1.48 (0.13)	1.27 (0.12)	0.66-2.79
4-F-3-PBA	–	–	<LOD	<LOD-0.08
cis-DCCA	1.69 (0.22)	0.52 (0.04)	0.43 (0.03)	0.23-0.91
trans-DCCA	4.73 (0.69)	1.19 (0.09)	0.94 (0.06)	0.50-2.20
cis-DBCA	–	–	<LOD	<LOD-0.06
ΣPYR	11.12 (1.55)	3.51 (0.29)	2.90 (0.23)	1.57-6.23

[*] Survey design weights and 500 bootstrap weights were used in calculating percentages, mean values, standard errors (S.E.) and 95% confidence intervals.

[†] If <60% of samples had detectable pyrethroid metabolites, means were not calculated

Table 5-4. Urinary concentrations of total pyrethroid metabolites by demographic factors, dwelling types and smoking status

Characteristics	ΣPYR (nmol/g creatinine) 6-11 years		12-19 years		20-79 years	
	G.Mean*†	p-value	G.Mean*†	p-value	G.Mean*†	p-value
Average	3.56 (0.28)	-	2.80 (0.32)	-	3.51 (0.29)	-
Sex						
Female	3.79 (0.37)	ref	3.15 (0.40)	ref	4.06 (0.35)	ref
Male	3.36 (0.31)	0.29	2.51 (0.30)	0.039	3.03 (0.27)	<0.0001
Ethnicity						
Caucasian	3.21 (0.23)	ref	2.62 (0.33)	ref	3.20 (0.27)	ref
Other	4.15 (0.37)	0.005	3.13 (0.41)	0.13	4.48 (0.36)	0.002
Smoking status‡						
Never		–	2.81 (0.32)	ref	3.60 (0.33)	ref
Former smoker		–	3.67 (1.55)	0.56	3.75 (0.39)	0.62
Current smoker		–	2.53 (0.45)	0.48	3.05 (0.24)	0.033
Dwelling type						
House	3.36 (0.26)	ref	2.71 (0.32)	ref	3.32 (0.23)	ref
Apartment or mobile home	4.81 (0.40)	0.002	3.36 (0.51)	0.13	4.11 (0.44)	0.012

* Survey design weights and 500 bootstrap weights were used in calculating geometric mean values of ΣPYR concentrations (nmol/g creatinine) and standard errors (S.E.)

† If <60% of samples had detectable pyrethroid metabolites, means were not calculated

‡ Only measured for subjects aged 12-79 years

Table 5-5. Association between log transformed urinary concentrations of ΣPYR and lung function parameters [*]

Unit increase in ΣPYR[‡]	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
	Beta (S.E.)[†]	*p*-value	Beta (S.E.)[†]	*p*-value	Beta (S.E.)[†]	*p*-value	Beta (S.E.)[†]	*p*-value
6-11 years	-14.7 (9.3)	0.14	-17.4 (7.7)	0.045	-0.2 (0.2)	0.34	-29.2 (20.2)	0.18
12-19 years	-37.1 (17.2)	0.05	-36.8 (18.2)	0.068	-0.05 (0.2)	0.83	-29.4 (33.0)	0.39
20-79 years	-20.1 (15.6)	0.23	-2.0 (12.4)	0.87	0.3 (0.1)	0.01	19.7 (19.3)	0.33

[*] Associations were characterized by the multiple linear regression analyses with controlling for age groups, sex, ethnicity, height, weight and smoking status.
[†] Survey design weights and 500 bootstrap weights were included in calculating β coefficients and standard errors (S.E.). Beta=β coefficients
[‡] Log (natural) transformed concentration adjusted for urine creatinine

Table 5-6. Results from the multiple linear regression of lung function parameters and urinary ΣPYR concentrations in children aged 6-11 years *

	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
	Beta (95% CI) †	*p*-value	Beta (95% CI) †	*p*-value	Beta (95% CI) †	*p*-value	Beta (95% CI) †	*p*-value
ΣPYR								
nmol /g creatinine‡	-14.7 (-35.2, 5.7)	0.14	-17.4 (-34.2, -0.5)	0.045	-0.2 (-0.6, 0.2)	0.34	-29.2 (-73.7, 15.4)	0.18
Age (years)								
	36.6 (-7.5, 80.8)	0.095	29.4 (2.3, 56.6)	0.036	-0.04 (-0.8, -0.7)	0.90	40.2 (6.6, 73.8)	0.023
Sex								
Female	0		0		0		0	
Male	89.1 (60.0, 118.3)	<0.0001	25.2 (-10.2, 60.6)	0.15	-2.1 (-3.3, -0.8)	0.004	-85.8 (-199.6, 28.0)	0.13
Ethnicity								
Caucasian	0		0		0		0	
Others	-86.4 (-159.1, -13.7)	0.007	-63.3 (-140.5, 13.9)	0.10	0.5 (-1.2, 2.2)	0.52	-27.5 (-174.4, 119.5)	0.69
Height (cm)	-36.1 (-79.4, 7.2)	0.094	-24.8 (-64.9, 15.4)	0.20	-0.2 (-1.4, 1.1)	0.75	-5.9 (-78.7, 66.8)	0.86
Height^2 (cm)	0.2 (0.08, 0.4)	0.007	0.2 (0.03, 0.3)	0.023	0.0008 (-0.003, 0.005)	0.69	0.1 (-0.2, 0.4)	0.39
Weight (kg)	10.3 (6.3, 14.3)	<0.0001	5.5 (-0.4, 11.4)	0.066	-0.1 (-0.3, 0.04)	0.13	3.4 (-73.7, 15.4)	0.18
Cons.	1968.3 (-946.5, 4882.2)	0.17	1349.7 (-1319.6, 4019.0)	0.289	100.8 (13.9, 187.6)	0.027	409.3 (-4478.8, 5297.4)	0.86

* Associations were characterized by the multiple linear regression analyses with controlling for age groups, sex, ethnicity, height, weight and smoking status.

† Survey design weights and 500 bootstrap weights were included in calculating β coefficients and standard errors (S.E.). Beta=β coefficients

‡ Log (natural) transformed concentration adjusted for urine creatinine

Table 5-7. Results from the multiple linear regression of lung function parameters and urinary ΣPYR concentrations in adolescents aged 11-19 years [*]

	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value
ΣPYR								
nmol /g creatinine [‡]	-37.1 (-75.1, 0.0008)	0.05	-36.8 (-76.7, 3.2)	0.068	-0.05 (-0.5, 0.4)	0.83	-29.4 (-102.1, 43.2)	0.39
Age (years)								
	77.2 (49.6, 104.7)	<0.0001	77.3 (55.4, 99.1)	<0.0001	0.3 (-0.04, 0.7)	0.08	88.8 (28.7, 148.9)	0.08
Sex								
Female	0		0		0		0	
Male	420.6 (307.0, 534.2)	<0.0001	260.5 (188.6, 332.3)	<0.0001	-2.6 (-4.2, -0.9)	0.006	27.2 (-185.2, 239.6)	0.78
Ethnicity								
Caucasian	0		0		0		0	
Others	-211.4 (-291.7, -131.2)	<0.0001	-96.5 (-173.4, -19.6)	0.019	2.0 (0.6, 3.4)	0.008	21.9 (-190.8, 234.6)	0.83
Height (cm)	51.4 (44.4, 58.4)	<0.0001	45.3 (39.9, 50.7)	<0.0001	0.07 (-0.03, 0.2)	0.15	43.7 (27.6, 59.9)	<0.0001
Weight (kg)	10.2 (6.2, 14.1)	<0.0001	4.9 (1.7, 8.2)	0.006	-0.09 (-0.1, -0.04)	0.002	3.6 (-5.2, 12.3)	0.39
Smoking status								
Never	0		0		0		0	
Former smoker	112.5 (-141.9, 366.8)	0.35	-11.2 (-276.0, 253.7)	0.93	-2.8 (-7.1, 1.5)	0.18	-142.4 (-766.5, 481.6)	0.63
Current smoker	213.3 (15.9, 410.7)	0.037	71.1 (-48.1, 190.3)	0.22	-2.3 (-4.5, -0.2)	0.032	-103.8 (-480.9, 273.3)	0.56
Cons.	-6412.0 (-7299.5, -5524.4)	<0.0001	-5684.1 (-6368.0, -5000.2)	<0.0001	74.2 (62.6, 85.8)	<0.0001	-5333.4 (-7313.7, -3353.1)	<0.0001

[*] Associations were characterized by the multiple linear regression analyses with controlling for age groups, sex, ethnicity, height, weight and smoking status.

[†] Survey design weights and 500 bootstrap weights were included in calculating β coefficients and standard errors (S.E.). Beta=β coefficients

[‡] Log (natural) transformed concentration adjusted for urine creatinine

Table 5-8. Results from the multiple linear regression of lung function parameters and urinary ΣPYR concentrations in adults aged 20-79 years [*]

	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value	Beta (95% CI) [†]	*p*-value
ΣPYR								
nmol /g creatinine [‡]	-20.1 (-54.4, 14.3)	0.23	-2.0 (-29.3, 25.2)	0.87	0.3 (0.09, 0.6)	0.011	19.7 (-22.7, 62.2)	0.33
Age (years)								
19-29	0		0		0		0	
30-39	-133.9 (-242.2, -25.6)	0.02	-220.2 (-321.8, -118.6)	0.001	-2.6 (-3.8, -1.4)	0.001	342.1 (-587.0, -97.1)	0.011
40-79	-632.0 (-739.4, -524.5)	<0.0001	-759.9 (-864.0, -655.8)	<0.0001	-6.7 (-7.6, -5.9)	<0.0001	-1132.1 (-1328.2, -935.9)	<0.0001
Sex								
Female	0		0		0		0	
Male	495.8 (421.9, 569.7)	<0.0001	410.1 (354.2, 466.0)	<0.0001	0.2 (-0.7, 1.2)	0.59	422.2 (249.1, 595.3)	<0.0001
Ethnicity								
Caucasian	0		0		0		0	
Others	-151.6 (-238.2, -64.9)	0.003	-59.8 (-118.5, -1.2)	0.046	1.6 (0.7, 2.6)	0.003	47.9 (-34.5, 130.3)	0.23
Height (cm)	69.7 (65.9, 73.4)	<0.0001	46.4 (42.3, 50.5)	<0.0001	-0.1 (-0.2, -0.08)	<0.0001	23.6 (14.8, 32.5)	<0.0001
Weight (kg)	-4.1 (-6.7, -1.5)	0.005	-1.0 (-2.7, 0.6)	0.19	0.05 (0.03, 0.07)	<0.0001	4.7 (1.9, 7.4)	0.003
Smoking status								
Never	0		0		0		0	
Former smoker	-32.8 (-123.3, 57.7)	0.44	-96.4 (-181.4, -11.3)	0.03	-2.0 (-2.8, -1.1)	<0.0001	-247.9 (-396.5, -99.3)	0.004
Current smoker	51.4 (-53.2, 156.0)	0.30	-134.2 (-223.6, -44.8)	0.007	-4.6 (-5.3, -3.8)	<0.0001	-414.4 (-531.1, -297.7)	<0.0001
Dwelling type								
House	0		0		0		0	
Apartment	-134.7 (-246.9, -22.5)	0.023	-94.2 (-183.5, -4.9)	0.04	0.2 (-0.7, 1.1)	0.63	-56.3 (-159.3, 46.7)	0.25
Daily energy expenditure kcal/kg/day	40.1 (19.5, 60.7)	0.001	29.3 (14.4, 44.3)	0.001	0.02 (-0.3, 0.3)	0.88	30.8 (-10.2, 71.8)	0.13
Cons.	-7107.8 (-7684.1, -6531.6)	<0.0001	-4202.4 (-4937.9, -3466.8)	<0.0001	101.5 (91.6, 111.4)	<0.0001	-8303.0 (-2448.6, 788.0)	0.28

[*] Associations were characterized by the multiple linear regression analyses with controlling for age groups, sex, ethnicity, height, weight and smoking status.

[†] Survey design weights and 500 bootstrap weights were included in calculating β coefficients and standard errors (S.E.). Beta=β coefficients

[‡] Log (natural) transformed concentration adjusted for urine creatinine

CHAPTER 6

ASSOCIATION BETWEEN LUNG FUNCTION IN ADULTS AND PLASMA DDT AND DDE LEVELS: RESULTS FROM THE CANADIAN HEALTH MEASURES SURVEY

[This chapter is a version of the paper, M. Ye, J. Beach, J.W. Martin and A. Senthilselvan, *Association between Lung Function in Adults and Plasma DDT and DDE Levels: Results from the Canadian Health Measures Survey,* that has been published in *Environmental Health Perspective* 2014 Dec 23; DOI:10.1289.]

6.1 Introduction

DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane], an organochlorine insecticide, was once widely used to control insects in agriculture [1] as well as insect-transmitted diseases, such as malaria and typhus [2]. DDT can naturally break down into DDE [1,1-bis-(4-chlorophenyl)-2,2-dichloroethene] and DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)ethane] through photolysis and microbial biodegradation [3]. In humans, DDT can be either oxidized or reduced by cytochrome P450 enzymes (CYP450) to form DDE or DDD [4]. DDE can further undergo epoxidation and phase II metabolism and DDD can be further oxidized to DDA [2,2-bis(4-chlorophenyl)acetic acid] [4].

Both DDT and its breakdown products DDE and DDD are highly persistent in the environment. In the soil, DDT, DDE, and DDD can persist as long as 40 years or more [3]. In addition, DDT and its breakdown products are highly lipophilic and have the potential to bioaccumulate in fat tissue of exposed animals [5]. DDT and its breakdown compounds can enter the human body through contaminated water, soil and food [6, 7]. In humans, DDT and DDE have half-lives of 6 years, and up to 10 years, respectively [8, 9]. Previous studies have shown

that DDT and/or DDE were detectable in almost all human blood and breast milk samples, which were collected mainly in the 1990s and 2010s from a number of global regions [6, 10, 11].

As a result of such environmental concerns, the use of DDT was greatly restricted or banned in most developed countries, including the US, Canada, and many European countries, in the 1970s. A worldwide ban of DDT for agricultural use began in 2004 after the Stockholm Convention classified DDT as a persistent organic pollutant (POP) [12]. Nevertheless, due to its ongoing use for disease vector control in some countries, high environmental persistence, and bioaccumulative properties, DDT and its breakdown compounds still pose potential risks to human health. Many adverse effects on human health DDT exposures have been associated with a variety of outcomes, including neurological [13], immunological [14], reproductive[15], and respiratory outcomes [16], and some cancers [15]. In addition, there is experimental evidence that DDT has endocrine disrupting effects [17].

A number of associations between respiratory health outcomes and DDT have been reported among agricultural pesticide applicators. For example, results from the US Agricultural Health Study demonstrated that adult-onset asthma was associated with exposures to DDT among farmers [18, 19]. The authors further suggested associations appeared to be more specific for atopic asthma among women [18]. Another report based on the Agricultural Health Study suggested that duration of DDT exposure was significantly associated with chronic bronchitis [20]. A retrospective cohort study of outdoor pesticide applicators in Australia also reported that asthma mortality was higher among workers who were occupationally exposed to insecticides, including DDT [21].

While there have been some studies of the effects of DDT exposure on respiratory diseases, few have focussed on its impact on lung function. In the current study, association of

DDT and its metabolite DDE with lung function was estimated using data from the Canadian Health Measures Survey (CHMS).

6.2 Methods

6.2.1 Study population

In the current study, we used data of 1,696 participants aged 20-79 years, which is a subgroup from the CHMS-Cycle 1 participants who provided fasting blood samples for DDT and DDE measurement. Participation in the CHMS-Cycle 1 was voluntary and all 1,696 subjects have provided informed consent to storage of their blood samples for future studies [22].

The sampling method of the CHMS-Cycle 1 has been described in Chapter 3. The detailed information of the CHMS-Cycle 1 can also be obtained from Statistics Canada [22]. This study was approved by the Health Research Ethics Board of the University of Alberta.

6.2.2 Lung function measures

This study used the same the method for measuring lung function as in Chapter 4.

6.2.3 *p,p'*-DDT and *p,p'*-DDE concentrations in plasma

In the current study, concentrations of *p,p'*-DDT and its major metabolite *p,p'*-DDE were measured in blood plasma. All blood samples were centrifuged within two hours and aliquotted within four hours after the blood was drawn [22]. Blood samples were then stored frozen at -20°C until concentrations of *p,p'*-DDT and *p,p'*-DDE were measured. Concentrations of *p,p'*-DDT and *p,p'*-DDE in blood plasma (μg/L) were measured using gas chromatography–mass spectrometry (GC-MS) [22, 23]. Detailed laboratory standard operating procedures (SOP) are described at the INSPQ website [24]. Limits of detection (LOD) for *p,p'*-DDT and *p,p'*-DDE were 0.05 μg/L plasma and 0.09 μg/L plasma, respectively [23]. Concentrations of *p,p'*-DDT or

p,p'-DDE (μg/L plasma) were normalized to total blood lipids and converted to ng/g lipid [23, 25], with total blood lipids calculated as: total lipids (g/L) = 2.27 x 386.65 x cholesterol (mol/L) + 885.45 x triglycerides (mol/L) + 0.623, where 386.65 and 855.45 are the average molecular weights (g/mol) of cholesterol and triglycerides, respectively [23].

6.2.4 Factors related to lung function

A number of potential confounders affecting lung function were considered in this study, including demographic factors (age, sex, ethnicity and immigration status), anthropomorphic data (standing height, weight), physical activity (daily energy expenditure) and tobacco smoking status. Detailed methods for obtaining information on these confounding factors have been described in Chapter 3 and 5.

For smoking status, in addition to a categorical variable defining the types of smokers, which is the same variable used in Chapter 4 and 5, another variable 'pack-years' was also considered in this study. Pack-years, defined as number of packs of cigarettes smoked per day multiplied by number of years of smoking, were also calculated using detailed information collected on smoking in the CHMS-Cycle 1 [26]. In the pack-years calculation, never smokers and former occasional smokers (< 1 cigarettes smoked / day in the past) were assigned a value of 0 pack-years.

6.2.5 Statistical analyses

Lung function measures FVC, FEV_1, FEV_1/FVC and $FEF_{25\%-75\%}$ were modeled as continuous health outcome variables in the analyses. In regression analyses, plasma *p,p'*-DDT was dichotomized as detectable (>LOD) or not detectable (≤ LOD = 0.05 μg/L plasma) as samples for 90% of participants were ≤ LOD. Plasma *p,p'*-DDE was modeled as a continuous variable because only a small proportion (0.7%) had a concentration ≤ LOD. For participants

with *p,p'*-DDE concentrations ≤ LOD, a substitution of 0.5*LOD was used [27]. Chi-square test and student-t test were used to examine the difference in the proportion of detecting *p,p'*-DDT in blood and the mean concentrations of *p,p'*-DDE across demographic factors, respectively.

Univariate analyses were initially conducted to examine the relationship between risk factors and lung function. Factors that were significantly at p=0.1 were considered in the multiple regression models. In multiple regression models, a purposeful selection method was used in determine the final models, i.e. the known risk factors of lung function, including age, sex, ethnicity, height and smoking, were forced into the final models, and other variables that were non-significant at p=0.05, were excluded from the models.

Associations between lung function parameters and dichotomous *p,p'*-DDT or lipid-normalized *p,p'*-DDE concentrations were estimated by the final multiple linear regression analyses, with lung function as the dependent variable, adjusting for age (continuous), sex, ethnicity (Caucasian or other), height (continuous), smoking status (never, former, current) and daily energy expenditure (continuous). In addition, interactions were not included in final models because none of the interactions between exposures and other covariates on association with lung function outcomes were significant at p=0.05.

Same as in Chapter 4 and 5, sampling design weights and 500 bootstrap weights were applied in variance estimation for descriptive statistics, regression coefficients and 95% confidence intervals [22]. Statistical analyses were performed using the same software as in Chapter 3, 4 and 5.

6.3 Results

6.3.1 Characteristics of the study population

Fasting blood samples for *p,p'*-DDT and *p,p'*-DDE analysis were collected from 1,696 participants aged 20-79 years from five Canadian provinces (Table 6-1). Among these participants, males and females were almost equally distributed, 22.9% were immigrants, and more than two thirds had Caucasian ethnicity. The study population had an average height of 169.0 cm and average weight of 77.4 kg. In addition, among this study population, 45.8% never smoked, 31.3% were former smokers and 22.9% were current smokers.

6.3.2 *p,p'*-DDT and *p,p'*-DDE concentrations in the study population

Of 1,696 participants, 10.0% (95% CI: 4.6, 15.4%) had detectable plasma *p,p'*-DDT (Table 6-2). A significantly higher proportion of non-Caucasians had detectable *p,p'*-DDT compared with Caucasians (25.6% vs. 3.8%), and immigrants were significantly more likely to have detectable *p,p'*-DDT than non-immigrants (34.1% vs. 2.9%).

In this study, more than 99.0% of participants (95% CI: 99.2, 100) had detectable plasma *p,p'*-DDE (Table 6-2). The average concentration of *p,p'*-DDE was 326.9 ng/g lipid with a median value of 151.9 ng/g lipid (95% CI: 126.9, 191.8) and an interquartile range of (71.5-284.6) ng/g lipid. On average, females had higher plasma *p,p'*-DDE than males (Table 6-2). Participants aged 60 years and above had a mean concentration of *p,p'*-DDE three times of that for participants aged 20-39 years.

The proportion of participants with detectable *p,p'*-DDT was greater in never smokers than in former and current smokers, and the mean concentration of *p,p'*-DDE was greater in non-smokers than in current and former smokers (Table 6-2). In addition, participants with detectable *p,p'*-DDT had a significantly greater mean *p,p'*-DDE concentration compared to those with no detectable *p,p'*-DDT (1493.3 ng/g lipid; 95% CI: 540.4, 2446.1 vs. 196.1 ng/g lipid; 95% CI: 171.6, 220.6; p =0.012).

6.3.3 Relationship between lung function and *p,p'*-DDT concentration

After adjusting for age, sex, ethnicity, height, smoking status and daily energy expenditure, participants with detectable *p,p'*-DDT had a significantly lower mean FVC (diff=311 mL; 95% CI: –492, –130; p=0.003) and FEV_1 (diff=232 mL; 95% CI: –408, –55; p=0.015) than those with non-detectable *p,p'*-DDT (Table 6-3). No significant differences were observed in FEV_1/FVC ratio and $FEF_{25\%-75\%}$ between subjects with different concentrations of *p,p'*-DDT in the multiple linear regression analysis (Table 6-3). Model estimates were similar when adjusted for pack-years instead of smoking status.

6.3.4 Relationship between lung function *p,p'*-DDE concentration

In a multiple linear regression analysis, after adjusting for age, sex, ethnicity, height, smoking status and daily energy expenditure, each 100 ng/g increase in plasma concentration of *p,p'*-DDE was associated with a 18.8 mL reduction in mean FVC (p=0.002) and an 11.8 mL reduction in mean FEV_1 (p=0.013) (Table 6-4). No significant associations were observed between FEV_1/FVC ratio or $FEF_{25\%-75\%}$ and plasma concentration of *p,p'*-DDE (Table 6-4). The linear relationship based on multiple regression models between *p,p'*-DDE concentrations and lung function parameters was further illustrated in Figure 4-1 to Figure 4-8. Model estimates were similar when adjusting for pack-years instead of categorical smoking status.

6.4 Discussion

DDT was widely used in agriculture and in the control of malaria and typhus before its use was restricted in the 1970s. Although it has been out of use now for many years, the current results from the CHMS-Cycle 1 (2007-2009) show that almost all Canadian adults aged 20-79 years still had *p,p'*-DDT and/or *p,p'*-DDE detectable in their blood plasma, which is consistent with the data reported by Health Canada using the same survey data (99.6% and 9.3% had

detectable plasma *p,p'*-DDE and *p,p'*-DDT, respectively) [23]. In addition, for participants who had plasma *p,p'*-DDE concentrations less than the LOD also had *p,p'*-DDT non-detectable. Ongoing exposure may arise due to the high persistence of DDT and DDE in the environment [3]. DDT and its metabolites are also highly persistent in the human body, and so our results could also be partially, or wholly, a consequence of exposures some time ago [8, 9]. The mean plasma concentration of *p,p'*-DDE reported in this study (152 ng/g lipid adjusted) was lower than that reported from the US National Health And Nutrition Examination Survey (NHANES III, 1999-2004, 238-260 ng/g lipid adjusted) [28, 29], indicating a lower exposure to DDT and its related compounds in Canada than in the US.

Although there have been a number of studies suggesting an adverse effect of pesticides on pulmonary function [30-38], most have lacked information on the specific types of pesticides used [30-32, 38], while others focused on pesticides other than DDT, such as organophosphate or carbamate insecticides [34-36].

We estimated that among a representative sample of Canadian adults aged 20-79 years, participants with detectable plasma *p,p'*-DDT had significantly lower mean FVC and FEV_1 than those with plasma *p,p'*-DDT $\leq$ LOD. The estimated magnitude of FVC and FEV_1 reduction associated with DDT exposure reported in this study (310.7 mL and 231.8 mL, respectively) is similar to the natural decline of lung function (30mL/year in FVC and 20-30 mL/year in FEV_1) for a healthy non-smoker adults over a 10 years period [39, 40]. In addition, lipid normalized plasma *p,p'*-DDE concentrations were negatively associated with FVC and FEV_1 when modeled as a continuous variable. Based on the multiple linear regression models, differences in FVC and FEV_1 between subjects with lower and upper quartiles of *p,p'*-DDE concentrations were 40.1 mL and 25.1 mL, respectively. To the best of our knowledge, this study is the first population-based

investigation of the association of DDT and its metabolite DDE with lung function among Canadian adults.

Several studies in the literature have also reported that exposures to other organochlorine pesticides are associated with reductions in lung function. For example, a study among agricultural pesticide sprayers in Spain reported that exposures to endosulfan were negatively associated with FEV_1 and $FEF_{25\%-75\%}$ [33]. Another study among pesticide spraying workers in India reported that a restrictive type of impairment of lung function was associated with exposures to unspecified organochlorine insecticides [37], which is consistent with the negative association between DDT/DDE and lung function estimated in the present study.

Exposure to DDT has also been associated with the prevalence of respiratory diseases. Hoppin et al in the Agricultural Health Study reported that DDT exposures were associated with nonatopic asthma among male farmers [19] and atopic asthma among female farmers [18]. In addition, Hoppin et al. reported that the lifetime number of days of occupational application of DDT in agriculture was significantly associated with higher prevalence of chronic bronchitis [20]. Another study using the same dataset found that the prevalence of chronic bronchitis among female non-smoking farmers was significantly associated with the use of DDT [41].

DDT and related compounds are neurotoxicants that bind to voltage-gated sodium channels to prevent their closure, which leads to increased sodium influx and repeated firing of neurons [13]. In addition, physiological studies of animal models have shown that sodium influx and subsequent depolarization of neurons in general can cause contractile responses of airway smooth muscles [42, 43]. Moreover, DDT and its metabolite DDE have been shown to be able to activate stress-response signalling *in vitro*, including ERK-MAPK, JNK and NFkB signalling pathways, which result in an intracellular release of calcium [44, 45]. Calcium release into

cytoplasm has also been shown to lead to contraction of airway smooth muscles in studies of airway smooth muscle cells and animal model rat [46-49]. Airway narrowing in response to DDT/DDE exposures would be consistent with the negative associations between exposures and lung function in our study population.

Changes in immune system parameters and markers of immune function have been associated with DDT/DDE exposures in observational studies [50-52], which suggest that DDT/DDE exposures might contribute to impaired lung function by increasing airway sensitization or inflammation.

Previous studies of pesticides and respiratory outcomes often used questionnaire-based approaches or job titles to classify pesticide exposures [30-33, 53], both of which are liable to errors and bias. In this study, we used a biomonitoring approach to measure DDT/DDE exposures, i.e. objectively testing the concentration of *p,p'*-DDT/DDE in blood plasma [54]. The concentration of pesticide measured by biomonitoring method is likely a good estimate of actual body burden arising from exposures to bioaccumulative chemicals, and hence is a good alternative for measuring cumulative exposures. For DDT and DDE, this is particularly so because of their long half-life in the human body, which makes them a good marker of past or cumulative exposure in research and environmental surveillance projects [55, 56].

There are several limitations in this study. Firstly, the CHMS survey was not fully representative of the Canadian population. Aboriginal people living on reserves and Aboriginal settlements, people living in remote areas, residents of institutions and full-time members of the Canadian Forces were not included in the CHMS [22]. However, the excluded populations in the CHMS represent less than 4% of the total Canadian populations [22]. Secondly, in the current study, only one of the 13 isomers of the insecticide DDT [57], *p,p'*-DDT and its metabolite *p,p'*-

DDE, were measured. The rest of the 12 isomers might have been present in blood samples and were not monitored [22]. Thirdly, in the current study, associations between DDT/DDE and lung function parameters were characterized among participants aged 20-79 years. Potential effects of DDT and DDE on respiratory health may also be critical for subjects with younger ages. For example, birth cohort studies in Spain suggested that perinatal exposure to DDT was positively associated with asthma prevalence and persistent wheezing in children [58, 59]. In addition, associations between respiratory tract infection and DDT/DDE exposures have also been reported among young children [60, 61]. A future study of the effect of DDT/DDE on lung function among children and youth is necessary. Lastly, due to the cross-sectional nature of the CHMS, the temporal sequence between changes in lung function and DDT exposures is not clear. In addition, analyses of DDT using a dichotomous exposure, due to the large proportion of participants having no detectable level of DDT, may lead to a potential bias due to uncontrolled confounding or misclassification.

6.5 Conclusions

Although a worldwide ban of DDT for agricultural use has been in place since 2004 when the Stockholm Convention classified DDT as persistent organic pollutant (POP), DDT is still currently produced and used in many countries, including China, India, South Africa, Ethiopia and North Korea [12]. Our results show that *p,p'*-DDE, the metabolite of insecticide DDT was detectable in almost all blood samples of Canadian adults aged 20-79 years, indicating that exposure to DDT is still a health concern, despite a ban in Canada many decades ago. Issues related to the health impact of DDT have been raised since Rachel Carson's well known book 'Silent Spring' was published in the early 1960s [62]. However, there is still limited evidence for an effect of DDT on respiratory health. Our study is the first population-based study of Canadian

adults demonstrating that plasma DDT, and its metabolite DDE, were negatively associated with two measures of lung function, specifically FVC and FEV_1.

Table 6-1. Characteristics of the study population

Characteristics	% or mean* (95% CI or ± S.E.†)
Total sample (N=1,696)	
Age (%, years)	
20-39	37.9 (37.9, 37.9)
40-59	41.3 (41.3, 41.3)
60-79	20.7 (20.7, 20.7)
Sex (%)	
Female	50.6 (50.4, 50.9)
Male	49.4 (49.1, 49.6)
Height (mean, cm)	169.0 ± 0.4
Weight (mean, kg)	77.4 ± 0.9
Ethnicity (%)	
Caucasian	71.4 (62.7, 80.1)
Others	28.6 (19.9, 37.3)
Immigrant (%)	
No	77.1 (66.6, 87.6)
Yes	22.9 (12.4, 33.4)
Province of residence (%)	
New Brunswick	7.2 (0, 22.1)
Quebec	23.8 (8.9, 38.6)
Ontario	38.9 (38.9, 38.9)
Alberta	16.6 (16.6, 16.6)
British Columbia	13.6 (13.6, 13.6)
Smoking status (%)	
Never	45.8 (42.0, 49.6)
Former smoker	31.3 (28.0, 34.6)
Current smoker	22.9 (20.4, 25.4)

* Survey design weights were used in calculating percentages and mean values of the study population, a representative sample of the Canadian adults.

† Survey design weights and 500 bootstrap weights were included in calculating the standard errors (S.E.) and 95% CI.

Table 6-2. Plasma *p,p'*-DDT and *p,p'*-DDE among the study population by demographic factors and smoking status[*†]

Characteristics	*p,p'*-DDT		*p,p'*-DDE	
	% ≥LOD[*] (95% CI[†])	*p*-value	Mean[*] (ng/g lipid, 95% CI[†])	*p*-value
Total sample	10.0 (4.6, 15.4)		326.9 (210.7, 443.0)	
Age (years)				
20-39	9.1 (3.9, 14.2)		198.6 (115.1, 282.1)	
40-59	9.5 (2.6, 16.4)	0.53	281.9 (188.8, 374.9)	0.023
60-79	12.8 (7.4, 18.2)	0.10	648.0 (280.4, 1015.6)	0.014
Sex				
Female	11.3 (5.8, 16.8)		418.7 (235.0, 602.5)	
Male	8.7 (2.7, 14.8)	0.23	235.4 (169.0, 301.8)	0.021
Ethnicity				
Caucasian	3.8 (2.5, 5.1)		197.6 (171.2, 224.1)	
Others	25.6 (13.9, 37.3)	<0.0001	648.4 (305.4, 991.5)	0.015
Immigrant				
No	2.9 (1.4, 4.4)		173.9 (153.9, 193.8)	
Yes	34.1 (19.5, 48.7)	<0.0001	650.1 (452.6, 847.7)	<0.0001
Smoking status				
Never	15.3 (6.6, 24.1)		432.4 (217.7, 647.0)	
Former smoker	7.1 (2.6, 11.6)	0.056	273.8 (221.6, 326.0)	0.060
Current smoker	3.1 (0.5, 5.7)	0.003	183.0 (141.8, 224.2)	0.033

[*] Mean concentrations of *p,p'*-DDT were lower than LOD as a higher proportion of participants had no *p,p'*-DDT detectable in plasma. Mean concentrations of *p,p'*-DDE were calculated among all participants and for participants with concentrations less than LOD (less than 1.0%), 0.5*LOD was used.

[†] Survey design weights and 500 bootstrap weights were used in calculating percentages, mean values and the 95% confidence intervals.

Table 6-3. Results from the multiple linear regression of lung function parameters and concentrations of *p,p'*-DDT in blood[†]

	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value
***p,p'*-DDT**								
<LOD	0		0		0		0	
≥LOD	-310.7 (-491.8, -129.6)	0.003	-231.8 (-408.3, -55.3)	0.015	0.08 (-1.71, 1.87)	0.925	-98.6 (-435.7, 238.5)	0.533
Age (years)	-25.3 (-29.4, -21.1)	<0.0001	-27.4 (-30.2, -24.6)	<0.0001	-0.21 (-0.24, -0.19)	<0.0001	-39.0 (-43.3, -34.7)	<0.0001
Sex								
Female	0		0		0		0	
Male	537.4 (440.8, 634.2)	<0.0001	463.0 (379.6, 546.4)	<0.0001	0.93 (-0.50, 2.37)	0.181	512.4 (281.7, 743.0)	<0.0001
Ethnicity								
Caucasian	0		0		0		0	
Others	-159.3 (-269.9, -48.8)	0.009	-85.5 (-158.4, -12.7)	0.025	1.21 (0.19, 2.22)	0.024	-40.1 (-198.4, 118.2)	0.589
Height (cm)	60.8 (55.4, 66.2)	<0.0001	39.1 (34.9, 43.4)	<0.0001	-0.17 (-0.24, -0.10)	<0.0001	15.7 (6.1, 25.3)	0.004
Smoking status								
Never	0		0		0		0	
Former smoker	8.1 (-75.0, 91.2)	0.835	-49.1 (-101.5, 3.3)	0.064	-1.57 (-2.70, -0.43)	0.011	-127.3 (-285.6, 31.0)	0.104
Current smoker	-19.4 (-145.2, 106.5)	0.741	-187.4 (-308.0, -66.9)	0.006	-4.85 (-6.33, -3.37)	<0.0001	-448.7 (-692.9, -204.4)	0.002
Daily energy expenditure	34.7 (10.7, 58.8)	0.009	34.9 (14.1, 55.7)	0.004	0.20 (-0.17, 0.58)	0.254	57.7 (8.8, 106.6)	0.025
Cons.	-5279.3	<0.0001	-2383.5	<0.0001	115.6	<0.0001	1779.3	0.038

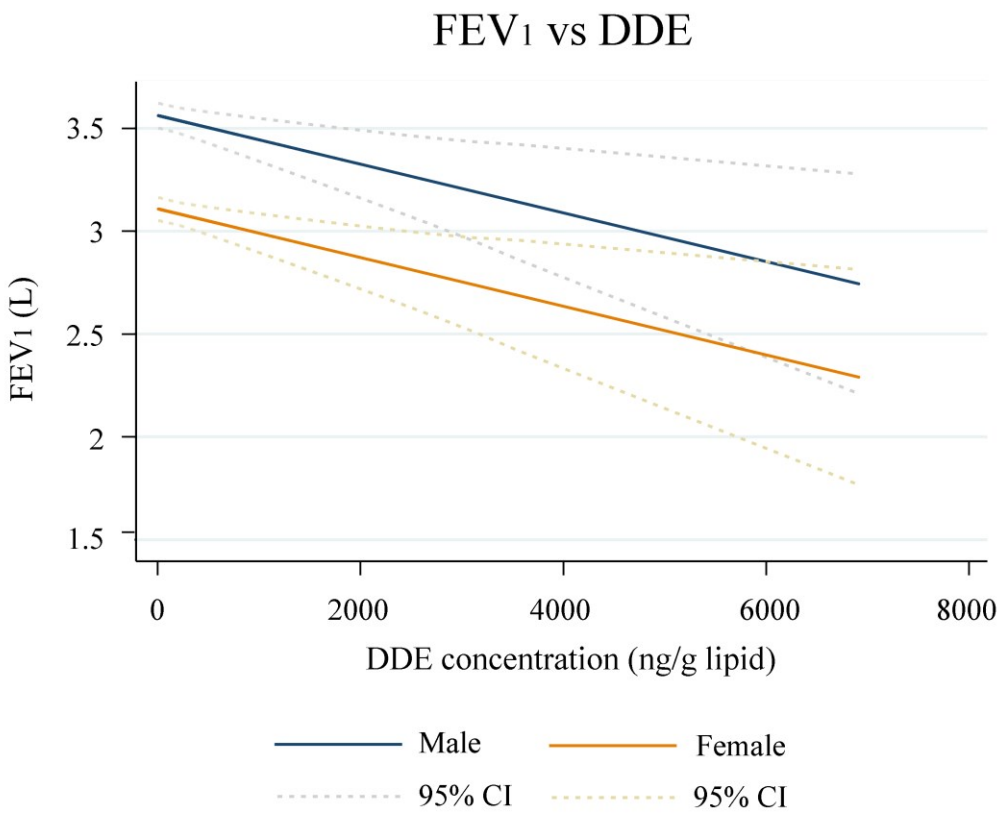
[†]Survey design weights and 500 bootstrap weights were included in calculating beta coefficients (β), 95% confidence intervals and variance estimation.

Table 6-4 Results from the multiple linear regression of lung function parameters and *p,p'*-DDE concentration in blood[†]

	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value
***p,p'*-DDE**								
per 100 ng/g lipid	-18.8 (-28.7, -8.9)	0.002	-11.8 (-20.6, -3.1)	0.013	0.09 (-0.11, 0.28)	0.363	-2.2 (-27.3, 22.9)	0.850
Age (years)	-24.4 (-28.4, -20.4)	<0.0001	-27.0 (-30.0, -24.0)	<0.0001	-0.22 (-0.25, -0.19)	<0.0001	-39.2 (-44.1, -34.4)	<0.0001
Sex								
Female	0		0		0		0	
Male	523.1 (415.4, 630.8)	<0.0001	453.9 (359.0, 548.7)	<0.0001	1.02 (-0.39, 2.42)	0.139	513.6 (285.2, 741.9)	<0.0001
Ethnicity								
Caucasian	0		0		0		0	
Others	-157.8 (-299.7, -15.9)	0.032	-92.7 (-194.7, 9.2)	0.07	0.85 (-0.39, 2.10)	0.160	-59.7 (-236.6, 117.2)	0.473
Height (cm)	60.9 (55.7, 66.2)	<0.0001	39.2 (35.2, 43.3)	<0.0001	-0.17 (-0.24, -0.10)	<0.0001	15.7 (6.2, 25.2)	0.004
Smoking status								
Never	0		0		0		0	
Former smoker	10.1 (-74.7, 95.0)	0.797	-44.4 (-101.9, 13.0)	0.117	-1.43 (-2.51, -0.34)	0.015	-117.3 (-274.5, 39.8)	0.129
Current smoker	-15.7 (-144.2, 112.7)	0.792	-180.7 (-305.8, -55.5)	0.009	-4.69 (-6.12, -3.26)	<0.0001	-437.4 (-675.6, -199.3)	0.002
Daily energy expenditure	33.2 (8.6, 57.8)	0.013	34.2 (12.2, 56.3)	0.006	0.23 (-0.14, 0.59)	0.203	58.7 (9.6, 107.8)	0.023
Cons.	-5300.6	<0.0001	-2405.3	<0.0001	115.5	<0.0001	1776.3	0.036

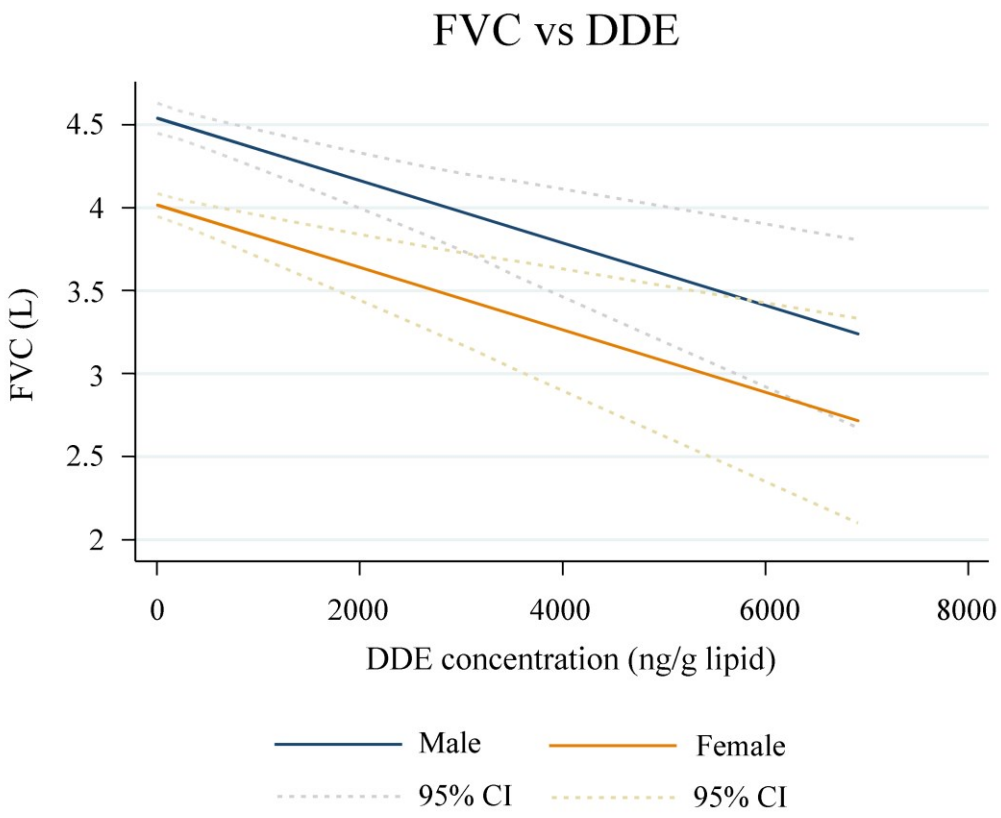
[†]Survey design weights and 500 bootstrap weights were included in calculating beta coefficients (β), 95% confidence intervals and variance estimation.

Figure 6-1. Relationship between urinary concentrations of *p,p'*-DDE (ng/g lipid) and FEV_1 by sex[*†]



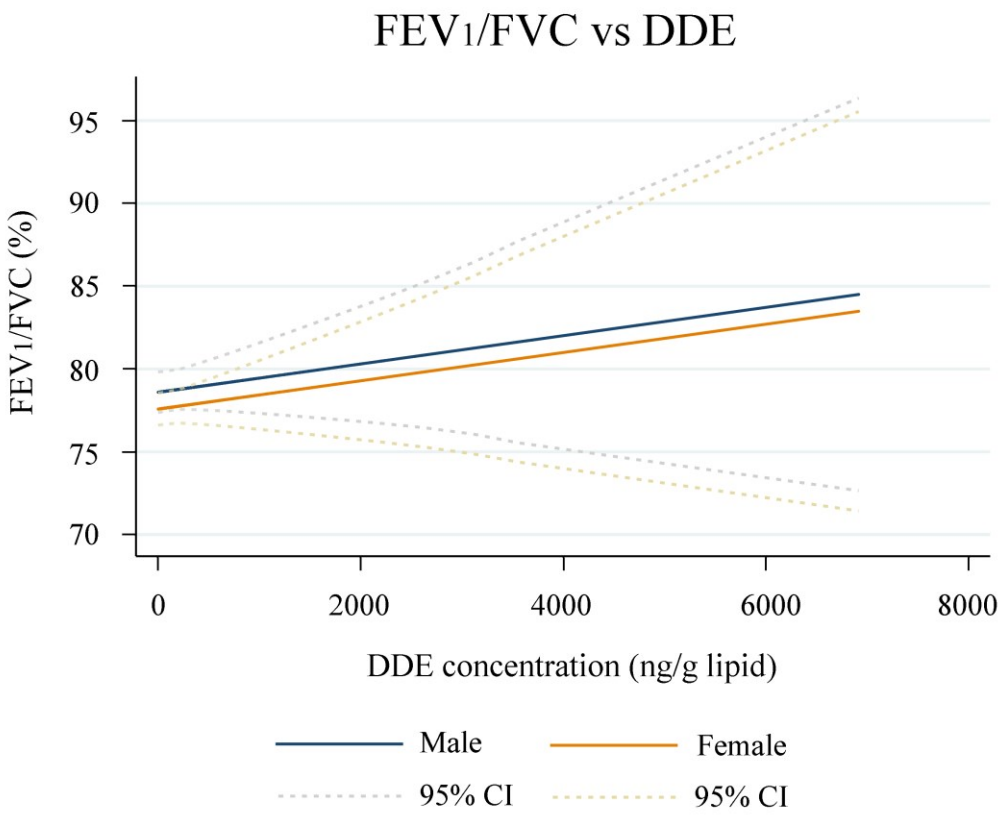
[*] Predicted lines were obtained based on the multiple linear regression models
[†] Since there was no siginficant interaction between DDE and sex**,** the predicted lines were parallel for males and females

Figure 6-2. Relationship between urinary concentrations of *p,p'*-DDE (ng/g lipid) and FVC by sex[*†]



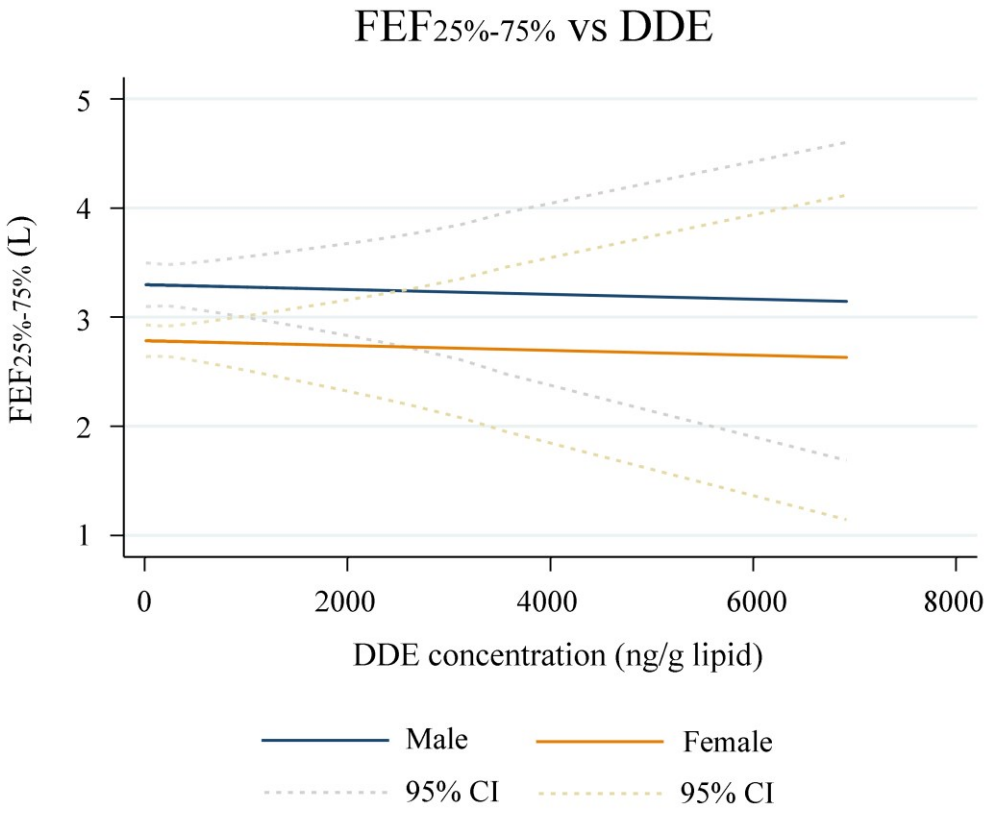
[*] Predicted lines were obtained based on the multiple linear regression models
[†] Since there was no siginficant interaction between DDE and sex**,** the predicted lines were parallel for males and females

Figure 6-3. Relationship between urinary concentrations of *p,p'*-DDE (ng/g lipid) and FEV_1/FVC ratio by sex[*†]



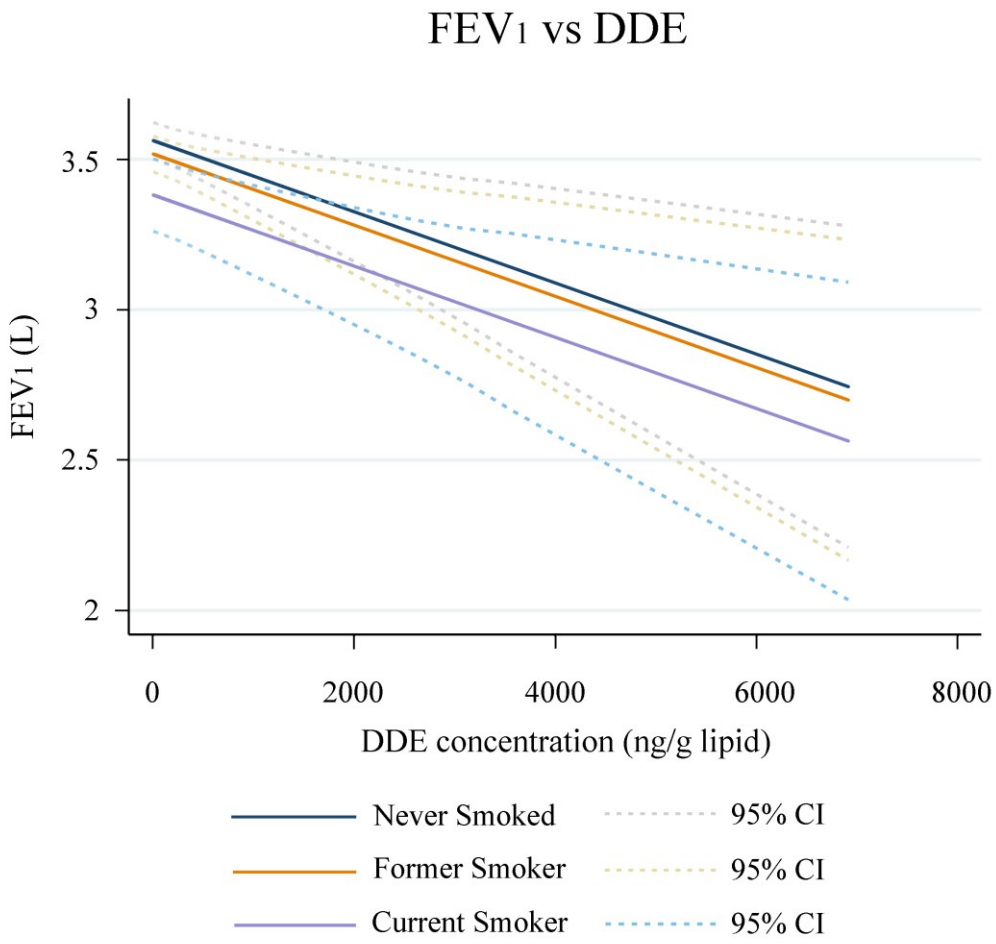
[*] Predicted lines were obtained based on the multiple linear regression models
[†] Since there was no siginficant interaction between DDE and sex**,** the predicted lines were parallel for males and females

Figure 6-4. Relationship between urinary concentrations of *p,p'*-DDE (ng/g lipid) and $FEF_{25\%-75\%}$ by sex[*†]



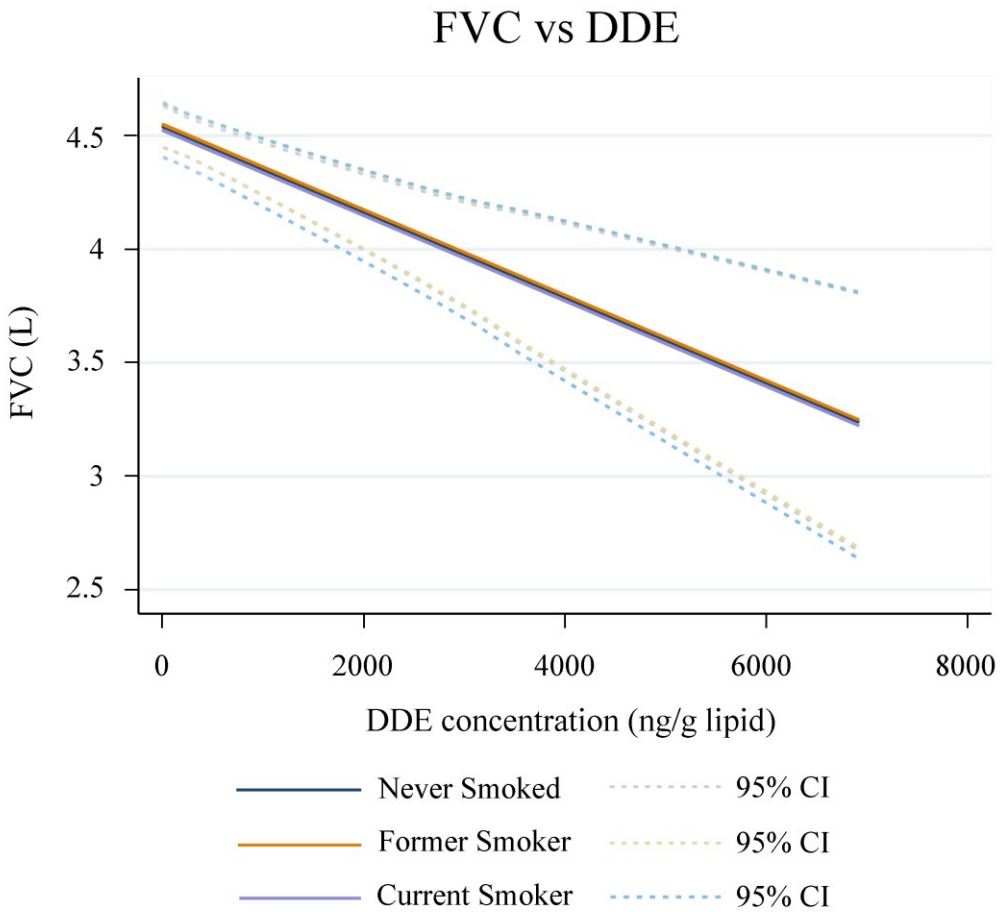
[*] Predicted lines were obtained based on the multiple linear regression models
[†] Since there was no siginficant interaction between DDE and sex**,** the predicted lines were parallel for males and females

Figure 6-5. Relationship between urinary concentrations of *p,p'*-DDE (ng/g lipid) and FEV_1 by smoking status[*†]



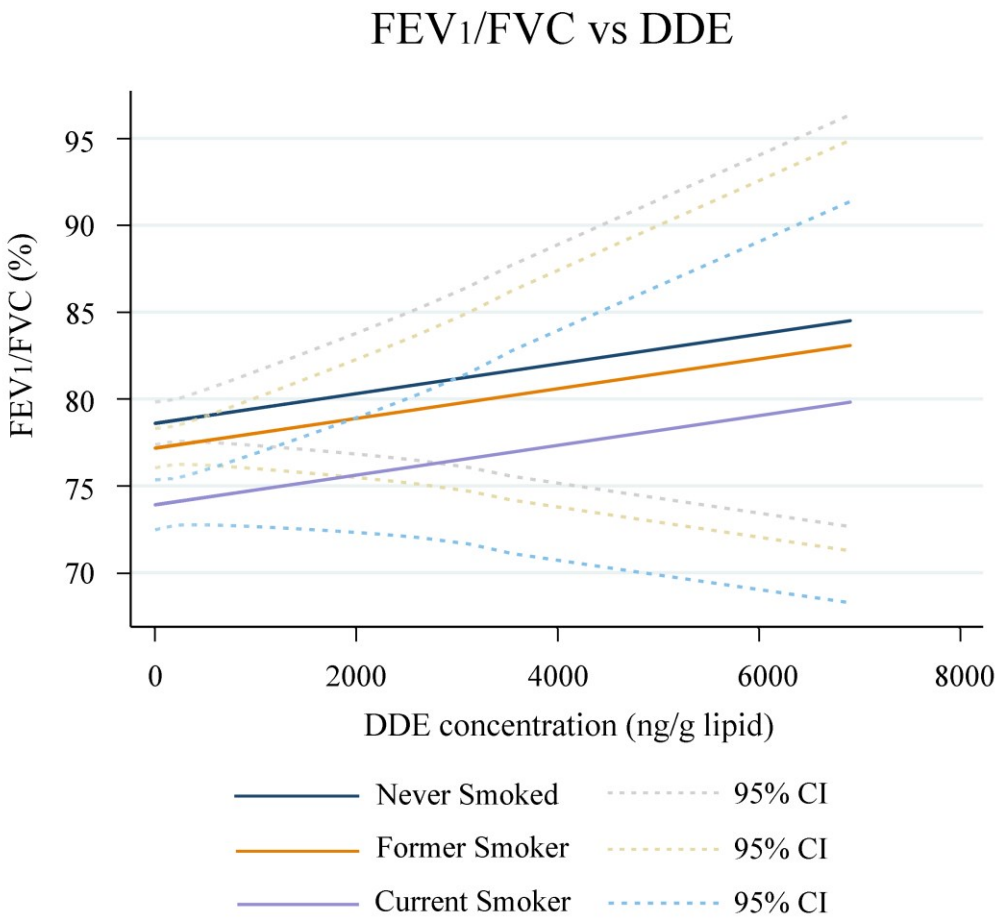
[*] Predicted lines were obtained based on the multiple linear regression models
[†] Since there was no siginficant interaction between DDE and smoking status, the predicted lines were parallel for the smoking categories

Figure 6-6. Relationship between urinary concentrations of *p,p'*-DDE (ng/g lipid) and FVC by smoking status[*†]



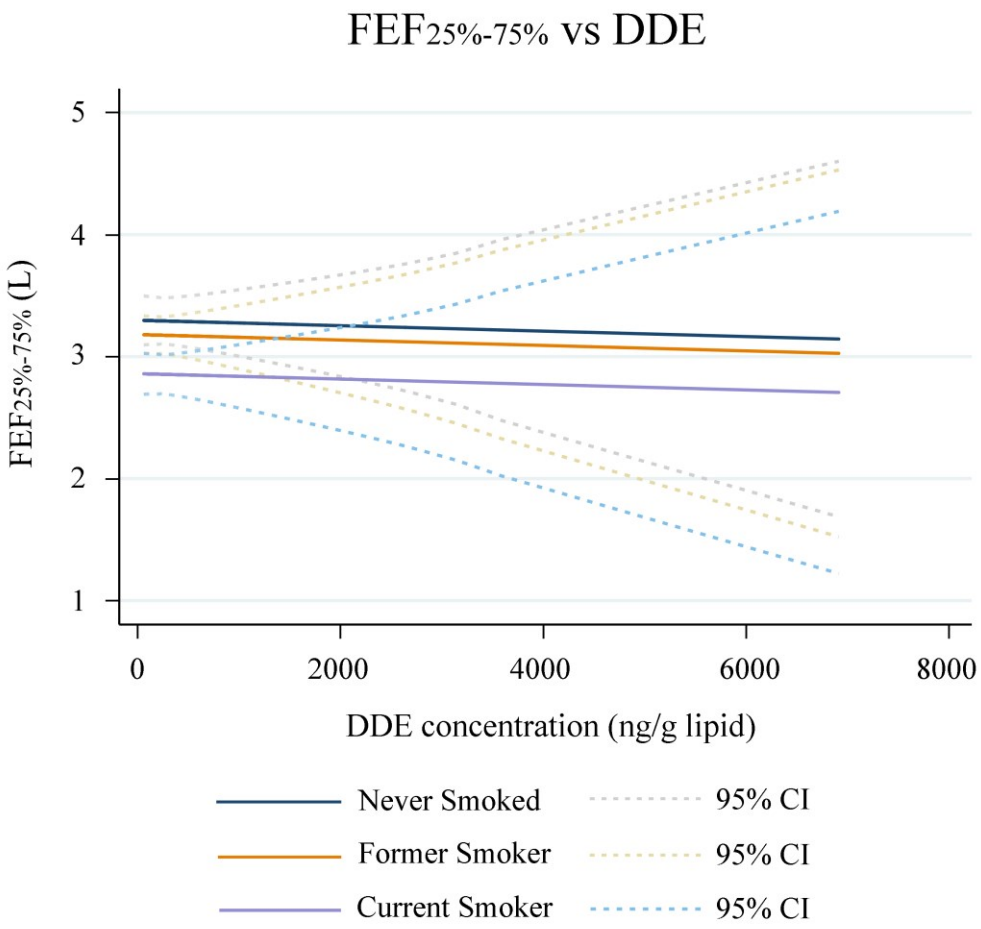
[*] Predicted lines were obtained based on the multiple linear regression models
[†] Since there was no siginficant interaction between DDE and smoking status, the predicted lines were parallel for the smoking categories

Figure 6-7. Relationship between urinary concentrations of *p,p'*-DDE (ng/g lipid) and FEV_1/FVC ratio by smoking status[*†]



[*] Predicted lines were obtained based on the multiple linear regression models
[†] Since there was no siginficant interaction between DDE and smoking status, the predicted lines were parallel for the smoking categories

Figure 6-8. Relationship between urinary concentrations of *p,p'*-DDE (ng/g lipid) and $FEF_{25\%-75\%}$ by smoking status[*†]



[*] Predicted lines were obtained based on the multiple linear regression models
[†] Since there was no siginficant interaction between DDE and smoking status, the predicted lines were parallel for the smoking categories

CHAPTER 7

REPORT ON URINARY CONCENTRATIONS OF 2,4-D AND ITS ASSOCIATION WITH LUNG FUNCTION IN THE CANADIAN GENERAL POPULATIONS

7.1 Introduction

2,4-D (2,4-dichlorophenoxyacetic acid) is a widely used herbicide to control broadleaf weeds in many settings, including in agriculture, for commercial and residential lawns, as well as highway and railroad rights-of-way. The herbicide 2,4-D is often formulated in the form of salts or acids [1], which can be easily absorbed by the gastrointestinal tract after ingestion, but less absorbed by the lungs and least by the skin [2]. Active ingredients in sunscreen products are able to increase dermal absorption of 2,4-D [3, 4]. Dermal contact of contaminated dust [5] and surfaces [6], as well as dietary intake of food items contaminated with herbicides [7], have been suggested as the main routes of exposures to 2,4-D in the general population.

In the human body, 2,4-D undergoes little metabolism and can be eliminated as the parent compound in urine with an average half-life of 17.7 hours [8]. High-dose exposures to 2,4-D potentially induce oxidative stress responses, including glutathione (GSH) depletion, lipid peroxidation and cell necrosis [1, 9]. Moreover, due to the presence of its ethanoic acid group, (i.e. similarity to acetic acid), 2,4-D has the potential to disrupt cellular processes involving acetyl groups. For example, 2,4-D can interfere with energy metabolism by forming analogues to acetyl-CoA, such as 2,4-D-CoA, which will disrupt the Krebs cycle and ATP synthesis [1]. It has also been found that 2,4-D can interfere with neurotransmitter acetylcholine (Ach) synthesis and produces a false competitor 2,4-D-Ach, which can cause neural poisoning in the peripheral neural system [1, 10]. In addition, acute exposure to 2,4-D can lead to the reduction in CD4+ T helper cells, CD8+ Treg cells and nature killer cells [11]

Poisonings by 2,4-D are uncommon in humans [9]. However, ingestion of large amounts of 2,4-D can cause symptoms like vomiting, abdomen pain, diarrhea, coma, hypertonia, ataxia, and miosis [1]. In addition, inhalational exposures to 2,4-D can cause airway irritation, peripheral neuromuscular symptoms, such as hypertonia and respiratory failure [1]. Chronic exposure to 2,4-D has also been associated with higher risk of developing non-Hodgkin's lymphoma (NHL) [12].

A few studies suggest the possible adverse effect of 2,4-D on respiratory health. In a study of female farmers in the Agricultural Health Study (AHS), exposures to 2,4-D were significantly associated with atopic asthma [13]. Slager *et al*., using the same data from the AHS, found that exposures to 2,4-D were positively associated with current rhinitis [14, 15]. Nevertheless, little is known about the effect of 2,4-D on lung function [16].

In the current study, urinary concentrations of 2,4-D and their associations with lung function were examined among the Canadian general population using data from the Canadian Health Measures Survey (CHMS).

7.2 Methods

To characterize the effect of 2,4-D on lung function, the same methods as in Chapter 5 were used, including the study design, selection of the study population, spirometric tests, biomonitoring measurement of urinary concentrations of chemicals, and the statistical analyses. Detailed information on these methods was described in Chapter 4 and 5. Measurement of urinary concentrations of 2,4-D and related analyses is described in the following paragraphs.

7.2.1 Urinary concentrations of 2,4-D and related analyses

Approximately 60 mL of mid-stream spot urine sample was collected for each CHMS-Cycle 1 participant aged 6-79 years. Concentrations of 2,4-D in the urine were measured using

gas chromatography–mass spectrometry (GC-MS) at the National Public Health Institute of Quebec (INSPQ) [17, 18]. Limit of detection (LOD) for measuring 2,4-D was 0.2 μg/L [19]. Urinary creatinine concentration (g/L) was also measured and used to adjust for urine dilution when calculating urinary concentrations of 2,4-D [18, 20].

In the multiple linear regression analyses, lung function parameters FVC, FEV_1, FEV_1/FVC and $FEF_{25\%-75\%}$ were considered as continuous outcome variables. The proportions of subjects having detectable 2,4-D concentrations in urine were estimated. Since more than 50% of participants had urinary concentrations 2,4-D less than LOD [18], concentrations of 2,4-D were dichotomized (detectable or not) according to its LOD and used as a categorical exposure variable in the analyses. In addition, a subgroup analysis among those subjects who had detectable concentrations of 2,4-D was conducted with urinary concentrations of 2,4-D as continuous exposure variable in the multiple regression analysis.

7.3 Results

7.3.1 Characteristics and lung function of the study participants

Subjects in this study were the same as the study population considered in Chapter 5. The distribution of the demographic characteristics and lung function are seen in Table 5-1 and Table 5-2 in Chapter 5.

7.3.2 Urinary levels of 2,4-D in the study population

Among the study subjects, 41.3% had detectable levels of 2,4-D in their urine. Although adolescents (12-19 years) had the highest percentage (44.1%) compared to children and adults, there was no significant difference in the proportion of detectable 2,4-D in urine samples across age groups (Table 7-1). In addition, although there were variations, the percentages of detectable

levels of 2,4-D in urine samples were not significantly different across sex and ethnicity groups and smoking status (Table 7-1). Although not statistically significant, on average, the prevalence of having detectable 2,4-D in urine was consistently higher among subjects living in houses than those who lived in apartments or mobile dwellings across three age groups (Table 7-1). Since less than 50% of subjects had detectable 2,4-D in urine, urinary concentrations of 2,4-D were only considered on a continuous scale in the subgroup analysis among participants with detectable levels of 2,4-D.

7.3.3 Relationships between urinary levels of 2,4-D and lung function

In the independent multiple linear regression analyses conducted for children, adolescents and adults, after adjusting for age, sex, ethnicity, height, weight, smoking status (adolescents and adults), daily energy expenditure (adults) and dwelling type (adults), there was no significant differences observed in lung function FVC, FEV_1, FEV_1/FVC ratio and $FEF_{25\%-75\%}$ between subjects with and without detectable levels of 2,4-D in all three age groups (Table 7-2).

In the subgroup analysis among those subjects who had detectable 2,4-D concentrations, no significant association was observed between 2,4-D concentrations and any of the lung function paramerters FVC, FEV_1, FEV_1/FVC ratio and $FEF_{25\%-75\%}$ after adjusting for age, sex, ethnicity, height, weight, smoking status (adolescents and adults), daily energy expenditure (adults) and dwelling type (adults).

Moreover, among the study participants, urinary levels of 2,4-D were not associated with respiratory symptoms and diseases as well, including cough regularly, night cough, dry cough, cough with phlegm, asthma and chronic bronchitis (data not shown).

7.4 Discussion

In the current study, we examined urinary levels of the herbicide 2,4-D and its associations with lung function in a representative sample of the Canadian general population. Approximately 40% of the CHMS-Cycle 1 participants (6-79 years) had 2,4-D detectable in their urine samples, which is consistent with previously published results using the same survey data [19].

Although two studies have shown an association between herbicide exposures and asthma and rhinitis in agricultural occupations [13, 15], no study has been identifiable in the literature specifically investigating the possible effect of 2,4-D on lung function. In the current study, no significant association was observed between urinary concentrations of 2,4-D and any of the lung function parameters FVC, FEV_1, FEV_1/FVC ratio and $FEF_{25\%-75\%}$ and respiratory symptoms and diseases, such as asthma and COPD, which suggests that the effect of 2,4-D on human respiratory health, if any, is minimal among the Canadian general populations.

Measuring urinary concentrations of 2,4-D has been shown as a valid biomonitoring approach to estimate the relative levels of 2,4-D exposures in agriculture and farms. For example, in the US Farm Family Exposure Study, researchers were able to assess exposure profiles of 2,4-D by measuring its urinary levels [21]. Nevertheless, in the current study, the majority of study subjects (58.7%) had a urinary concentration of 2,4-D lower than the limit of detection (LOD). For those subjects, other than a sign designated as “less than LOD”, no actual numeric concentrations of 2,4-D were reported in CHMS [17]. In addition to the analysis using a dichotomized 2,4-D level (detectable or not), a subgroup analysis using 2,4-D concentrations as a continuous variable was also conducted among subjects with detectable levels of 2,4-D. However, neither of these two approaches showed significant associations between 2,4-D and lung function in the present study. While statistical imputatation technicques can be applied to

convert partially complete data into a complete data, imputing 2,4-D data for approximately 60% of study participants in this study might compromise the validity of the results [22], and imputing was not carried out in the present study.

Except for the respiratory health, other adverse health effects of 2,4-D have also been suggested, including cancer, neurologic disease, reproductive disease, and diseases in immune system [23]. Nevertheless, in a review of epidemiological studies on the association between 2,4-D and chronic diseases, Burn et al suggested that current studies using the biomonitoring data of 2,4-D did not provide "convincing or consistent evidence for any chronic adverse effect of 2,4-D in humans" [24]. Additionally, dioxin and dioxin-like impurities in herbicide 2,4-D [25] might also be a factor confounding the associations found in literature between 2,4-D exposures and diseases, especially for cancer [26-28]. Therefore, it is challenging for epidemiologists to accurately characterize the health effect of the herbicide 2,4-D mixed with other compounds.

There are also other limitations in this study, including the exclusion of Aboriginal people living on reserves and Aboriginal settlements, people living in remote areas, institutional residents and full-time members of the Canadian Force from the CHMS Cycle 1 and the cross-sectional nature of the study [17]. Nevertheless, the excluded populations only represented approximately 4% of the total Canadian population [17], which is unlikely to have significant impact on results reported in this study. In addition, the use of 2,4-D as a weed-and-feed products has been banned recently in several provinces in Canada, including Alberta, New Brunswick, Ontario, Prince Edward Island and Quebec [29-31], which may mean that the biomonitoring level of 2,4-D reported in this study using data from the CHMS-Cycle 1 (2007-2009) may not reflect the current exposures to 2,4-D in the Canadian general population.

7.5 Conclusions

In summary, our data showed that approximately 40% of the Canadian general population had herbicide 2,4-D detectable in urine. Results from the current study did not show significant association between urinary concentrations of 2,4-D and lung function. Future studies on the potential effect of 2,4-D on lung function is warranted.

Table 7-1. Urine levels of 2,4-D in the study population by demographic factors and smoking status [*]

Characteristics	2,4-D levels (%>=LOD)					
	6-11 years		12-19 years		20-79 years	
	%	95% CI[†]	%	95% CI[†]	%	95% CI[†]
Average	41.3	29.5-53.1	44.1	30.6-57.7	40.9	31.5-50.3
Sex						
Female	40.0	28.0-52.0	45.4	28.3-62.5	35.9	27.1-44.7
Male	42.5	29.0-55.9	42.9	29.9-56.0	46.0	35.5-56.6
Ethnicity						
Caucasian	39.6	24.3-54.8	46.2	30.7-61.6	41.3	30.8-51.7
Other	43.9	33.0-54.9	40.0	27.6-52.4	40.2	31.4-48.9
Smoking status[‡]						
Never	-	-	44.9	30.6-59.3	39.5	30.0-49.0
Former smoker	-	-	40.9	3.1-78.8	45.5	35.0-56.0
Current smoker	-	-	39.4	23.9-54.8	37.6	27.5-47.5
Dwelling type						
House	42.5	30.0-54.9	46.3	30.8-61.9	41.9	31.6-52.2
Apartment	32.8	18.4-47.3	30.9	15.5-46.4	38.2	26.1-50.3

[*] If <60% of samples had detectable 2,4-D, mean and medians were not calculated

[†] Survey design weights and 500 bootstrap weights were used in calculating the percentage of having detectable 2,4-D and 95% confidence intervals (CI)

[‡] Only measured for subjects aged 12-79 years

Table 7-2. Association between log transformed urinary levels of 2,4-D and lung function parameters [*]

w/ detectable 2,4-D[†]	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
	Beta (S.E.) [‡]	*p*-value	Beta (S.E.) [‡]	*p*-value	Beta (S.E.) [‡]	*p*-value	Beta (S.E.) [‡]	*p*-value
6-11 years	25.6	0.27	26.4	0.24	0.3	0.43	48.1	0.38
12-19 years	71.4	0.20	39.5	0.19	-0.4	0.52	-6.0	0.94
20-79 years	-19.3	0.65	-26.3	0.39	-0.3	0.35	-27.7	0.53

[*] Associations were characterized by the multiple linear regression analyses with controlling for age groups, sex, ethnicity, height, weight and smoking status.

[†] Reference groups were subjects with 2,4-D concentrations less than level of detection (LOD).

[‡] Survey design weights and 500 bootstrap weights were included in calculating β coefficients and standard errors (S.E.). Beta=β coefficients

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

8.1 Summary of the Results

In this thesis, using data from the CHMS-Cycle 1, urinary (or plasma) concentrations of pesticides or pesticides metabolites were examined, dietary predictors of pesticide exposure were investigated, and the relationships between the biomonitoring level of pesticides and lung function were characterized in the Canadian general population.

The results in Chapter 3-7 showed that over 90% of CHMS-Cycle 1 participants aged 6-79 years had OP metabolites, almost all CHMS participants (99.8%) in the same age group had pyrethroid metabolites, and approximately 40% of the same subjects had herbicide 2,4-D, detectable in their urine samples. In addition, almost all CHMS participants aged 20-79 years (over 99.0%) had DDT-related compounds in their blood samples.

In Chapter 3, fruit and vegetable consumption was significantly associated with the urinary concentrations of total dialkyl phosphate (DAP) metabolites (p-values<0.001), while nuts/pulse and vegetable consumptions and household use of chemicals to control head lice or pet flea infestations were significantly associated with urinary concentrations of total pyrethroid metabolites (p-values<0.01).

In Chapter 4, among the CHMS-Cycle 1 participants aged 20-79 years, a one unit increase in log transformed urinary concentration (nmol/g creatinine) of total DAP (ΣDAP) was associated with a 32.6 mL reduction in FVC (p=0.014) and a 32.6 mL reduction in FEV_1 (p=0.02). There was no statistically significant association between urinary concentrations of DAP and lung function among children aged 6-11 years and adolescent aged 12-19 years.

In Chapter 5, multiple linear regression analyses showed that a one unit increase in log transformed urinary concentration (nmol/g creatinine) of total pyrethorid metabolites (ΣPYR) were associated with a 17.4 mL reduction in FEV_1 (p=0.045) in children (6-11 years) and a 37.1 mL reduction in FVC (p=0.05) in adolescents (12-19 years). No significant association was observed between concentrations of ΣPYR and lung function among adult subjects.

In Chapter 6, among the CHMS adult participants (20-79 years), subjects with detectable *p,p'*-DDT in the blood plasma had a 310 mL lower mean FVC (p=0.003) and a 231 mL lower mean FEV_1 (p=0.015) than those without. Moreover, among the group of subjects, every 100 units (μg/kg lipid) increase in the *p,p'*-DDE concentration in plasma was associated with an 18.8mL decrease in FVC (p-value=0.002) and an 11.8mL decrease in FEV_1 (p-value=0.013). No significant association was observed between *p,p'*-DDT and *p,p'*-DDE concentrations and FEV_1/FVC ratio or $FEF_{25\%-75\%}$.

In Chapter 7, no significant differences in lung function FVC, FEV_1, FEV_1/FVC ratio and $FEF_{25\%-75\%}$ were observed between subjects with and without detectable concentrations of 2,4-D.

In summary, exposure to pesticides, especially to organophosphate insecticides and pyrethroid insecticides, was prevalent among the Canadian general population. Fruit and vegetable consumptions were significantly associated with organophospate and pyrethroid insecticide exposures among the Canadian general population. Urinary concentrations of OP metabolites and pyrethroid metabolites were associated with lower lung function. In addition, plasma levels of DDT and DDE were significantly associated with low lung function in the Canadian adult population.

8.2 Importance of the Study

Due to the toxic nature of pesticides, many adverse health effects of pesticides have been characterized, including acute neurotoxic effects [1-3], chronic neurological effects [4], neurodevelopmental effects [5-9], immunologic effects [10-15], endocrine disruptive effects [16, 17], effects on reproductive health [7, 18], respiratory effects [19] and carcinogenic effects [20-22]. However, the relationship between pesticides exposures and respiratory health has been mainly characterized in occupational settings [19] and there was little evidence showing the effect of pesticide on lung function in the general populations.

Significant associations between organophosphate insecticide exposures and lung function among the Canadian general population reported in this thesis provide new evidence of the adverse effects of OP insecticides on respiratory health. The magnitude of lung function reduction associated with a one unit increase in log transformed urinary DAP concentration among adult participants was close to the annual age-related decline of lung function [23, 24], which suggests a significant extra loss of lung function for those exposed to OP pesticides. This is the first nation-wide population-based investigation on the effect of OP pesticides on lung function in Canada.

The relationship between pyrethroid metabolites and lung function reported in this thesis provides the first population-based evidence showing the potential adverse effect of pyrethroid insecticide exposures on lung function in the Canadian general population. While no significant association was observed among adult subjects, urinary concentration of pyrethroid metabolites was significantly associated with reduction in FEV_1 among children and reduction in FVC among adolescents, which suggests a relatively higher risk of being affected by pyrethroid insecticides at younger ages.

In this thesis, a significant reduction in lung function was associated with pesticide DDT, suggesting a persistent adverse health impact of DDT even after it was banned a long time ago. The relationship between DDT/DDE and lung function reported in this thesis, is the first study showing plasma concentrations of the *p,p'*-DDT and *p,p'*-DDE were associated with a reduction in FVC and FEV_1 in the general population. The magnitude of lung function decrement associated with DDT exposures is similar to a 10-year natural decline of lung function for normal adults [23, 24], suggesting a clinically relevant effect. In addition, there was a potential dose-response relationship between *p,p'*-DDE concentration and lung function. The novel findings in this study will add one more piece of evidence to the long list of adverse effects of DDT on human health.

This thesis also showed that exposure to pesticides was prevalent among the Canadian general population by detecting pesticide metabolites at a high frequency in urine and blood samples, especially for organophosphate insecticides and pyrethroid insecticides. The association between dietary factors and pesticide exposures observed in the thesis is one of the several [25, 26], but the first population-based study in Canada, showing the positive relationship between dietary consumptions of fruits, vegetables and/or nuts and exposure to OP and pyrethroid insecticides. In addition, results of this study based on the analysis of a nation-wide health survey, the CHMS-Cycle 1, will give the best evidence for future population-based interventions aiming to reduce pesticide exposures.

8.3 Strength of the Study

The main goal of research studies reported in this thesis was to characterize the effect of pesticides, including organophosphate insecticides, pyrethroid insecticides, the organochlorine pesticide DDT, and the phenoxyl herbicide 2.4-D, on lung function in the Canadian general

population. To examine the association between pesticide exposures and lung function, lung function parameters (the health outcome of the study), and urinary concentrations of pesticide or pesticide metabolites (the exposure estimate of the study), were objectively measured, which gives less measurement error and better validity of the study.

Lung function parameters were objectively measured using flow-sensing spirometers as part of the CHMS-Cycle 1. The whole procedure of measuring lung function followed American Thoracic Society (ATS) standards [27, 28], with a volume accuracy of ± 3% or ± 50 mL (whichever was greater), and a volume resolution of no more than 30 mL, and an error of timing accuracy of less than ± 1% of each reading [27].

In some epidemiological studies, pesticide exposures are measured by approaches such as questionnaire-based measures, job titles, or the relative distance to chemical sources [29]. Biomarkers or biomonitoring measurements, which measure pesticide metabolite concentrations in biospecimen, provide an objective approach to estimate the actual level of pesticide exposures from all potential routes [30]. In this study, concentrations of pesticides or their metabolites in urine or blood samples were objectively measured using gas chromatography-mass spectrometry (GC-MS), which gives the best possible estimate of actual physiological burden of pesticides exposures from all sources on the human body [31].

For each CHMS-Cycle 1 participant, spirometry tests and urine/blood sample collection were conducted at the same time when the subjects visited the Mobile Examination Center (MEC) [32]. This would typically be considered a cross-sectional study design which in principle cannot give information about the sequence of exposure and outcome. However, it may be possible to speculate to an extent on the temporal sequence of exposure and outcome in this instance. The biomonitoring measurement of pesticide metabolites estimated the level of pesticide exposures

that had occurred in the past, which for some pesticides may have been a considerable time ago. For example, plasma detection of DDT and its metabolite DDE were used as estimates of previous exposure to DDT or DDE. As the use of DDT has been banned for over 30 years in Canada [33], it is unlikely that the DDT-associated changes in lung function reported in this thesis occurred prior to DDT exposures. In addition, due to its bioaccumulative property [34, 35], the biomonitoring level of DDT/DDE estimated in this thesis also reflects the cumulative level of DDT exposures for each CHMS participant. For pesticides with a shorter half-life, such as OPs and pyrethroids, it is more difficult to characterize the temoral relationship between exposure and outcome using cross-sectional data, although urinary detection of dialkly phosphate (DAP) metabolites or pyrethroid metabolites reflects recent exposures to OPs or pyrethorid insecticides over the past 3-5 days [36, 37].

According to Statistics Canada, subjects in the CHMS-Cycle 1 with acute and/or chronic respiratory conditions, persistent cough, taking medication for tuberculosis, and recent surgery on the chest or abdomen, were excluded from spirometry testing [32], which minimizes the potential confounding effect of respiratory medications on the associations reported in the thesis, although it may also have led to an underestimate of the true effect for some outcomes.

Findings of research studies in this thesis resulted from the analysis of 5,604 CHMS-Cycle 1 participants provided adequate statistical power to examine the association between pesticide concentrations and lung function [38, 39]. In addition, the CHMS-Cycle 1 participants were selected from five provinces across Canada and considered to be representative of 96.3% of the Canadian general population [32], which makes these research findings the best evidence at the population-based level for the Canadian general populations.

8.4 Limitations

There were some limitations in the studies reported in this thesis. Firstly, although the CHMS-Cycle 1 provided information on the biomonitoring level of pesticides and objective measures of lung function, it was not specifically designed for the purpose of the studies reported in the thesis. Data used for this thesis were secondary data from the CHMS-Cycle 1. This limits our ability to use objective measures for some important confounders of interest. For example, in the CHMS-Cycle 1, environmental tobacco smoke, an important risk factor for lung function, was simply measured by self-reporting or parental reporting the presence (or absence) of smokers inside home, at work place or in private vehicle. Secondly, although the CHMS-Cycle 1 participants were intented to be representative of the majority of the Canadian general population [32], they were not representative of the entire Canadian population. Aboriginal people living on reserves and aboriginal settlements, people living in remote areas, institutional residents and full-time members of the Canadian Force were excluded from the CHMS-Cycle 1 [32]. However, it is unlikely that the exclusion of these groups would greatly change the results reported in this study, since the excluded populations in the CHMS-Cycle 1 represented less than 4% of the total Canadian population [32]. In addition, in the studies of organophosphate insecticides, pyrethroid insecticides, the organochlorine pesticide DDT/DDE, and the phenoxyl herbicide 2,4-D, there were approximately 6.3%, 7.0%, 5.3% and 5.2% of CHMS-Cycle 1 participants excluded respectively from the analyses due to the missing values in spirometry tests and/or the biomonitoring measures of pesticides. There were slightly more female than male subjects with missing values and no significant difference in other demongraphic factors and socioeconomic status between subjects excluded from and subjects in the analyses. More importantly, as we have discussed before, excluding participants with acute and/or chronic respiratory conditions, who therefore had missing values in lung function measures [32], may underestimate the true

effect of pesticides on lung function. Thirdly, this study used pesticide metabolite concentrations to estimate the exposure level of OP and pyrethroid pesticides, which makes this study unable to identify the specific pesticide(s) that subjects were actually exposed to. Lastly, the cross-sectional nature of CHMS does not allow us to identify the temoral sequences between pesticide exposures and decrease in lung function.

8.5 Implications for Future Research

Studies reported in this thesis showed the association between DAP concentrations and lung function were significant among adults, but not in children and adolescents even though children (6-11 years) had the highest mean concentration of total DAPs among three age groups (Chapter 3). In addition, this study also showed a reduction in lung function was associated with pyrethroid insecticides among children and adolescents, but not in adult participants. These observations suggest there is an age-specific effect of pesticides on lung function and this age-specific effect also depends on the type of insecticide, i.e. whether organophosphate or pyrethroid.

An age-specific effect of pyrethroid insecticides on lung function might result from differences attributable to age-related stages of lung development. Children and adolescents lungs are not fully developed and may be more susceptible to environmental toxicants than adults [40]. There are data suggesting children and youth are more susceptible than adults to the insult of some outdoor air pollutants [41-43]. In addition, except for dietary ingestion [44, 45], airway inhalation is another major route of exposures to pyrethroid insecticides [2, 44, 46], which can lead to a direct local damage on the respiratory system. Consequently, impaired lung function reported in this study appeared to be one of the main adverse health effects of pyrethroid insecticides and this effect was predominantly seen among children and adolescents.

However, the effect of organophosphate insecticides on lung function, which was more evident among adults than children and adolescents, had a different age-related pattern compared to pyrethroid insecticides. As both organophosphate and pyrethroid insecticides can be efficiently absorbed by ingestion and inhalation [44, 46], a non-neurotoxic mechanism may lead to the different pattern of the age-related effect of OP insecticides observed in this study. For example, some non-cholinergic mechanisms of OP insecticides, including endocrine disruptive effects [16] and the effect on lymphocytic cholinergic system, a cholinergic-receptor mediated modulation on immune function [47, 48], have been proposed as potential alternative mechanisms for the repeated low-dose exposures to organophosphates [49-51]. Moreover, the toxicodynamics of organophosphate metabolites, such as dialkyl phosphates (DAP), which may be different from their parent insecticides, are still unclear [52]. To better understand the age-specific effect of organophosphate insecticides, further studies on the non-cholinergic effects of OP insecticides, especially with the repeated low-dose exposures, and studies aiming for separating the effect of parent OP insecticdes from their metabolites, are necessary.

While the studies reported in this thesis have demonstrated the adverse effect of pesticides, including organophosphate insecticides, pyrethroid insecticides and organochlorine pesticide (DDT) on lung function among the Canadian general populations, no significant associations were observed between pesticide concentrations and respiratory symptoms and diseases in these studies. One possible reason is that the pesticide-associated loss of lung function reported in this thesis was not adequate to develop overt respiratory symptoms or diseases, especially when pesticide exposures are often at the low-dose in the general population [46].

However, a few studies have suggested a link between pesticide exposures and respiratory symptoms [53, 54] and diseases [53, 55-62], especially for childhood asthma [53, 57-60], in

general populations. Given the limitation of this thesis using cross-sectional data of the CHMS-Cycle 1, future prospective studies using the biomarkers of pesticides measured over time will be able to better characterize the chronic effect of pesticides on incidence of respiratory symptoms and diseases since both the time-related variations in biomonitoring levels of pesticide exposures and incidence rate of health outcomes will be captured. In addition, causal associations will also be better characterized in future investigations using a longitudinal study design.

Currently, approximately 7,600 pesticide products and 400 active ingredients in the products are registered for use in Canada [63]. Before any pesticide is registered for use in Canada, Health Canada reviews the toxicological profile of the pesticide and assesses the related health and environmental impacts [64]. The review process will be conducted every 15 years to make sure that evaluations meet Canada's current health and environmental standards [64].

The CHMS is a population-based national survey collecting baseline environmental chemical exposures and related health outcomes in every two years [65, 66]. In addition to the current studies in this thesis using data from the CHMS-Cycle 1 (2007-2009), the multi-phasic design of the CHMS will also allow future studies on the effect of pesticides on respiratory health using data of the following cycles, including the CHMS-Cycle 2 (2009-2011) and CHMS-Cycle 3 (2011-2013). Results from future studies will not only be useful to confirm the current findings regarding the adverse effect of pesticides on lung function, but also generate new knowledge regarding the health impact of pesticides, which might be particularly important for re-evaluating pesticides that have been registered for some time, such as the OP insecticides marathion and diazinon [67, 68].

The biomonitoring approach has been widely used in population-based surveillance programs monitoring environmental chemicals due to its accuracy and cost-effectiveness [69, 70].

Other than the US National Health and Nutritional Examination Survey (NHANES III) [71-73] and the French National Survey on Nutrition and Health (ENNS-Étude nationale nutrition santé) [74], the Canadian Health Measures Survey [65, 66] is currently one of the three national population-based surveys using the human biomonitoring approach to estimate pesticide exposures. With increasing concerns about the chronic health impact of pesticides [18, 20, 75], including the adverse effects on lung function reported in this thesis, it is necessary to conduct longitudinal surveillance programs monitoring pesticide exposure levels and the related health outcomes over time in the Canadian general population.

Findings in this thesis have suggested ongoing and prevalent exposure to pesticides among the Canadian general population. Nevertheless, pesticide exposure levels will change over time. For example, the use of pyrethroid insecticides in Canada has been increasing over the last decade [76]. In addition, there were also regional variations in pesticide exposure levels among the Canadian general population possibly in part due to the differential regulations across provinces in Canada on pesticide use and sale. For example, since 2008, three provinces in Canada including Quebec, Ontario and New Brunswick have banned the cosmetic use of synthetic lawn pesticide "as a result of health and environmental concerns" [77]. Moreover, the herbicide 2,4-D has been banned as weed-and-feed lawn products for sale and use in Alberta since January 1, 2010 [78, 79]. Meanwhile, there is little regulation in place on the use of lawn pesticides in the rest of the Canadian provinces, such as Saskatchenwan and Manitoba [77]. Provincial health surveys using a biomonitoring approach may be necessary to assess pesticide exposures and related health outcomes, complementing data from the CHMS.

It has been estimated that annual pesticide use in Canada is over 30,000 tons [80, 81] and only 1% of pesticides consumed reached target pests [82], which suggests considerable risk of

exposure to pesticides from the environment. Ingestion of trace amount of pesticide residues on food products is believed to be the major source of pesticide exposures in the general population [83, 84]. The results of this thesis raised the possiblity of being exposed to pesticides from consuming of fruits, vegetables, pulses and nuts. Future population-based interventions aiming to reduce pesticide exposures among the general population should focus on greater regulation of pesticides used on these food items. Promoting organic farming of fruits, vegetables, pulses and nuts may also help to reduce pesticide exposures among the Canadian general population.

Lastly, although health impacts of DDT have been recognized since Rachel Carson's book, 'Silent spring', was published half a century ago [85], our understanding of its adverse effect on human health is still far from thorough. Results reported in this thesis showed that blood concentrations of DDT and its metabolites were associated with reduced lung function, which provides the first population-based evidence showing the effect of DDT on lung function in the general population. Given the environmental persistent and bioaccumulative nature of DDT, future studies should focus on its long-term effect on human health, including respiratory health. In addition, although DDT has been banned in Canada since the 1970s and the worldwide ban of DDT has also been in place since 2004 [33], atmospheric transportation and distillation, especially those occurring at the Arctic region, still makes DDT related environmental and health issues relevant globally [33]. Worldwide cooperative actions are necessary to reduce DDT-related environmental and health problems.

8.6 Conclusions

In this thesis, the adverse health effects of OP insecticide, pyrethrod insecticide and the organochlorine pesticide DDT on lung function were reported. It was also suggested that exposures to pesticides were common, ongoing and widespread among the Canadian general

population, and dietary factors, including consuming fruits and vegetables, were significantly associated with pesticide exposures. Findings of this thesis will provide a population-based evidence of ongoing exposures to pesticides and their impact on human respiratory health in the Canadian general population.

The Canadian general public needs to be aware the potential adverse effect of pesticides on respiratory health, including the effect on lung function reported in this thesis. To reduce the health impact of pesticide exposures, efforts from government, public health agencies, non-governmental organizations, agriculture and industry, are required to control the source of pesticide exposures, such as the dietary sources, which is potentially the major route of pesticide exposures in the Canadian general populations. Incorporating scientific evidence from population-based epidemiological studies, including current studies in this thesis, into decision-making, will help to improve the current regulatory policies and laws on pesticide use and pest controls.

BIBLIOGRAPHY

References for Chapter 1

1. *Food and Agriculture Organization of the United Nations (FAO)-Database on pesticides consumption*. 2006 [cited 2013 July 8, 2013]; Available from: http://www.fao.org/waicent/FAOINFO/economic/pesticid.htm.
2. Damalas, C.A. and I.G. Eleftherohorinos, *Pesticide exposure, safety issues, and risk assessment indicators.* Int J Environ Res Public Health, 2011. **8**(5): p. 1402-19.
3. Davis, J.R., R.C. Brownson, and R. Garcia, *Family pesticide use in the home, garden, orchard, and yard.* Arch Environ Contam Toxicol, 1992. **22**(3): p. 260-6.
4. *Pesticide Market Estimates: 2006-2007.* . 2011 [cited 2012 Dec 14]; Available from: http://www.epa.gov/pesticides/pestsales/07pestsales/table_of_contents2007.htm.
5. O'Malley, M., *Clinical evaluation of pesticide exposure and poisonings.* Lancet, 1997. **349**(9059): p. 1161-6.
6. Sanborn, M.D., et al., *Identifying and managing adverse environmental health effects: 4. Pesticides.* CMAJ, 2002. **166**(11): p. 1431-6.
7. Deschamps, D., et al., *Persistent asthma after acute inhalation of organophosphate insecticide.* Lancet, 1994. **344**(8938): p. 1712.
8. Hoppin, J.A., et al., *Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study.* Am J Respir Crit Care Med, 2008. **177**(1): p. 11-8.
9. Hoppin, J.A., et al., *Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study.* Eur Respir J, 2009. **34**(6): p. 1296-303.
10. Hoppin, J.A., et al., *Pesticides and adult respiratory outcomes in the agricultural health study.* Ann N Y Acad Sci, 2006. **1076**: p. 343-54.

11. Senthilselvan, A., H.H. McDuffie, and J.A. Dosman, *Association of asthma with use of pesticides. Results of a cross-sectional survey of farmers.* Am Rev Respir Dis, 1992. **146**(4): p. 884-7.

12. Beard, J., et al., *Health impacts of pesticide exposure in a cohort of outdoor workers.* Environ Health Perspect, 2003. **111**(5): p. 724-30.

13. Salam, M.T., et al., *Early-life environmental risk factors for asthma: findings from the Children's Health Study.* Environ Health Perspect, 2004. **112**(6): p. 760-5.

14. Salameh, P., et al., *Respiratory diseases and pesticide exposure: a case-control study in Lebanon.* J Epidemiol Community Health, 2006. **60**(3): p. 256-61.

15. Chakraborty, S., et al., *Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India.* J Occup Health, 2009. **51**(6): p. 488-97.

16. Salameh, P.R., et al., *Chronic bronchitis and pesticide exposure: a case-control study in Lebanon.* Eur J Epidemiol, 2006. **21**(9): p. 681-8.

17. Hoppin, J.A., et al., *Pesticide use and chronic bronchitis among farmers in the Agricultural Health Study.* Am J Ind Med, 2007. **50**(12): p. 969-79.

18. Valcin, M., et al., *Chronic bronchitis among nonsmoking farm women in the agricultural health study.* J Occup Environ Med, 2007. **49**(5): p. 574-83.

19. Tual, S., et al., *Agricultural exposures and chronic bronchitis: findings from the AGRICAN (AGRIculture and CANcer) cohort.* Ann Epidemiol, 2013. **23**(9): p. 539-45.

20. Pesatori, A.C., et al., *Cohort mortality and nested case-control study of lung cancer among structural pest control workers in Florida (United States).* Cancer Causes Control, 1994. **5**(4): p. 310-8.

21. Alavanja, M.C., et al., *Pesticides and lung cancer risk in the agricultural health study cohort.* Am J Epidemiol, 2004. **160**(9): p. 876-85.

22. Lee, W.J., et al., *Cancer incidence among pesticide applicators exposed to chlorpyrifos in the Agricultural Health Study.* J Natl Cancer Inst, 2004. **96**(23): p. 1781-9.

23. Beane Freeman, L.E., et al., *Cancer incidence among male pesticide applicators in the Agricultural Health Study cohort exposed to diazinon.* Am J Epidemiol, 2005. **162**(11): p. 1070-9.

24. Rusiecki, J.A., et al., *Cancer incidence among pesticide applicators exposed to metolachlor in the Agricultural Health Study.* Int J Cancer, 2006. **118**(12): p. 3118-23.

25. Hou, L., et al., *Pendimethalin exposure and cancer incidence among pesticide applicators.* Epidemiology, 2006. **17**(3): p. 302-7.

26. Purdue, M.P., et al., *Occupational exposure to organochlorine insecticides and cancer incidence in the Agricultural Health Study.* Int J Cancer, 2007. **120**(3): p. 642-9.

27. Bonner, M.R., et al., *Occupational exposure to carbofuran and the incidence of cancer in the Agricultural Health Study.* Environ Health Perspect, 2005. **113**(3): p. 285-9.

28. Samanic, C., et al., *Cancer incidence among pesticide applicators exposed to dicamba in the agricultural health study.* Environ Health Perspect, 2006. **114**(10): p. 1521-6.

29. Ye, M., et al., *Occupational pesticide exposures and respiratory health.* Int J Environ Res Public Health, 2013. **10**(12): p. 6442-71.

30. Hutchinson, J., *On the capacity of the lungs, and on the respiratory functions, with a view of establishing a precise and easy method of detecting disease by the spirometer.* Med Chir Trans, 1846. **29**: p. 137-252.

31. Liang, B.M., D.C. Lam, and Y.L. Feng, *Clinical applications of lung function tests: a revisit.* Respirology, 2012. **17**(4): p. 611-9.

32. Hendrick, D., et al., *Occupational Disorders of The Lung: Recognition, Management, and Prevention*. 1st ed. 2002, London: WB Saunders.

33. Miller, M.R., et al., *Standardisation of spirometry.* Eur Respir J, 2005. **26**(2): p. 319-38.

34. Pellegrino, R., et al., *Interpretative strategies for lung function tests*. Eur Respir J, 2005. **26**(5): p. 948-68.

35. Statistics Canada, *Canadian Health Measures Survey: Cycle 1 Data Tables 2011* [cited 2012 Feb 14, 2012]; Available from: http://www5.statcan.gc.ca/olc-cel/olc.action?objId=82-623-X&objType=2&lang=en&limit=0.

36. Landau, L.I., *Tobacco smoke exposure and tracking of lung function into adult life*. Paediatr Respir Rev, 2008. **9**(1): p. 39-43; quiz 43-4.

37. Uysal, N. and R.M. Schapira, *Effects of ozone on lung function and lung diseases*. Curr Opin Pulm Med, 2003. **9**(2): p. 144-50.

38. Hegewald, M.J. and R.O. Crapo, *Socioeconomic status and lung function*. Chest, 2007. **132**(5): p. 1608-14.

39. Zuskin, E., et al., *Respiratory function in pesticide workers.* J Occup Environ Med, 2008. **50**(11): p. 1299-305.

40. Mekonnen, Y. and T. Agonafir, *Lung function and respiratory symptoms of pesticide sprayers in state farms of Ethiopia.* Ethiop Med J, 2004. **42**(4): p. 261-6.

41. Hernandez, A.F., et al., *Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects.* Inhal Toxicol, 2008. **20**(9): p. 839-49.

42. Peiris-John, R.J., et al., *Low-level exposure to organophosphate pesticides leads to restrictive lung dysfunction.* Respir Med, 2005. **99**(10): p. 1319-24.

43. Salameh, P., et al., *Spirometric changes following the use of pesticides.* East Mediterr Health J, 2005. **11**(1-2): p. 126-36.

44. Royce, S., et al., *Occupational asthma in a pesticides manufacturing worker.* Chest, 1993. **103**(1): p. 295-6.

45. Reynolds, S.J., et al., *Pulmonary Function Reductions Among Potentially Susceptible Subgroups of Agricultural Workers in Colorado and Nebraska.* J Occup Environ Med, 2012. **54**(5): p. 632-641.

46. Schneider, D., N.C. Freeman, and P. McGarvey, *Asthma and respiratory dysfunction among urban, primarily Hispanic school children.* Arch Environ Health, 2004. **59**(1): p. 4-13.

47. Statistics Canada. *Canadian Health Measures Survey (CHMS) Data User Guide: Cycle 1*. 2011 [cited 2012 Feb 14, 2012]; Available from: http://www23.statcan.gc.ca/imdb-bmdi/document/5071_D2_T1_V1-eng.htm.

48. Ye, M., et al., *Association between lung function in adults and plasma DDT and DDE levels: results from the Canadian Health Measures Survey*. Environ Health Perspect, 2014. DOI: 10.1289

References for Chapter 2

1. Damalas, C.A. and I.G. Eleftherohorinos, *Pesticide exposure, safety issues, and risk assessment indicators.* Int J Environ Res Public Health, 2011. **8**(5): p. 1402-19.

2. *Pesticide Market Estimates: 2006-2007*. . 2011 [cited 2012 Dec 14]; Available from: http://www.epa.gov/pesticides/pestsales/07pestsales/table_of_contents2007.htm.

3. *Food and Agriculture Organization of the United Nations (FAO)-Database on pesticides consumption*. 2006 [cited 2013 July 8, 2013]; Available from: http://www.fao.org/waicent/FAOINFO/economic/pesticid.htm.

4. Maroni, M., A.C. Fanetti, and F. Metruccio, *Risk assessment and management of occupational exposure to pesticides in agriculture.* Med Lav, 2006. **97**(2): p. 430-7.

5. Bradman, A., et al., *Community-based intervention to reduce pesticide exposure to farmworkers and potential take-home exposure to their families.* J Expo Sci Environ Epidemiol, 2009. **19**(1): p. 79-89.

6. Coronado, G.D., et al., *Agricultural task and exposure to organophosphate pesticides among farmworkers.* Environ Health Perspect, 2004. **112**(2): p. 142-7.

7. Quandt, S.A., et al., *Agricultural and residential pesticides in wipe samples from farmworker family residences in North Carolina and Virginia.* Environ Health Perspect, 2004. **112**(3): p. 382-7.

8. Davis, J.R., R.C. Brownson, and R. Garcia, *Family pesticide use in the home, garden, orchard, and yard.* Arch Environ Contam Toxicol, 1992. **22**(3): p. 260-6.

9. Calvert, G.M., et al., *Acute occupational pesticide-related illness in the US, 1998-1999: surveillance findings from the SENSOR-pesticides program.* Am J Ind Med, 2004. **45**(1): p. 14-23.

10. O'Malley, M., *Clinical evaluation of pesticide exposure and poisonings.* Lancet, 1997. **349**(9059): p. 1161-6.

11. Sanborn, M.D., et al., *Identifying and managing adverse environmental health effects: 4. Pesticides.* CMAJ, 2002. **166**(11): p. 1431-6.

12. Hoppin, J.A., et al., *Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study.* Eur Respir J, 2009. **34**(6): p. 1296-303.

13. Hoppin, J.A., et al., *Pesticide use and chronic bronchitis among farmers in the Agricultural Health Study.* Am J Ind Med, 2007. **50**(12): p. 969-79.

14. Louis J. Casarett, J.D., Curtis D. Klaassen, *Casarett and Doull's toxicology: The Basic Science of Poisons* 7th ed. Toxic Effects of Pesticides 2008: McGraw-Hill Professional.

15. Pearce, M., et al., *The effects of aerial spraying with Bacillus thuringiensis Kurstaki on children with asthma.* Can J Public Health, 2002. **93**(1): p. 21-5.

16. Oettmeier, W., *Herbicide resistance and supersensitivity in photosystem II.* Cell Mol Life Sci, 1999. **55**(10): p. 1255-77.

17. Sinning, I., *Herbicide binding in the bacterial photosynthetic reaction center.* Trends Biochem Sci, 1992. **17**(4): p. 150-4.

18. Proudfoot, A.T., S.M. Bradberry, and J.A. Vale, *Sodium fluoroacetate poisoning.* Toxicol Rev, 2006. **25**(4): p. 213-9.

19. Keifer, M.C. and J. Firestone, *Neurotoxicity of pesticides.* J Agromedicine, 2007. **12**(1): p. 17-25.

20. Hoppin, J.A., et al., *Environmental exposure assessment of pesticides in farmworker homes.* Environ Health Perspect, 2006. **114**(6): p. 929-35.

21. Fenske, R.A., *Pesticide exposure assessment of workers and their families.* Occup Med, 1997. **12**(2): p. 221-37.

22. Gladen, B.C., et al., *Exposure opportunities of families of farmer pesticide applicators.* Am J Ind Med, 1998. **34**(6): p. 581-7.

23. Jaga, K. and C. Dharmani, *Sources of exposure to and public health implications of organophosphate pesticides.* Rev Panam Salud Publica, 2003. **14**(3): p. 171-85.

24. Gavrilescu, M., *Fate of Pesticides in the Environment and its Bioremediation.* Engineering in Life Sciences, 2005. **5**(6): p. 497–526.

25. *Monitoring of Pesticide Residues in Products of Plant Origin in the European Union, Norway, Iceland and Liechtenstein, 2005. Commision staff working document*. 2005; Available from: http://ec.europa.eu/food/fvo/specialreports/pesticide_residues/report_2005_en.pdf.

26. Zeliger, H.I., *Human Toxicology of Chemical Mixtures: Toxic consequences Beyond the impact of One-Component Product and Environmental Exposures*. 2011, Oxford: William Andrew Inc.

27. Maestrelli, P., et al., *Mechanisms of occupational asthma.* J Allergy Clin Immunol, 2009. **123**(3): p. 531-42; quiz 543-4.

28. Dowling, K.C. and J.N. Seiber, *Importance of respiratory exposure to pesticides among agricultural populations.* Int J Toxicol, 2002. **21**(5): p. 371-81.

29. Damalas, C.A., E.B. Georgiou, and M.G. Theodorou, *Pesticide use and safety practices among Greek tobacco farmers: a survey.* Int J Environ Health Res, 2006. **16**(5): p. 339-48.

30. Schettgen, T., et al., *Pyrethroid exposure of the general population-is this due to diet.* Toxicol Lett, 2002. **134**(1-3): p. 141-5.

31. Ripley, B.D., et al., *Pesticide residues on fruits and vegetables from Ontario, Canada, 1991-1995.* J AOAC Int, 2000. **83**(1): p. 196-213.

32. Jindal, T., D.K. Singh, and H.C. Agarwal, *Effect of UV radiation and temperature on mineralization and volatilization of coumaphos in water.* J Environ Sci Health B, 2007. **42**(4): p. 367-72.

33. Gomes, J., O.L. Lloyd, and D.M. Revitt, *The influence of personal protection, environmental hygiene and exposure to pesticides on the health of immigrant farm workers in a desert country.* Int Arch Occup Environ Health, 1999. **72**(1): p. 40-5.

34. J. Routt Reigart, J.R.R. *Recognition and Management of Pesticide Poisonings*. 1999 [cited 2012 Feb 14, 2012]; Available from: http://npic.orst.edu/rmpp.htm.

35. Bulathsinghala, A. and I. Shaw, *The toxic chemistry of methyl bromide.* Hum Exp Toxicol, 2013.

36. Bemer, D., et al., *Pesticide aerosol characteristics in the vicinity of an agricultural vehicle cab during application.* J Occup Environ Hyg, 2007. **4**(7): p. 476-82.

37. Carvalho, T.C., J.I. Peters, and R.O. Williams, 3rd, *Influence of particle size on regional lung deposition--what evidence is there?* Int J Pharm, 2011. **406**(1-2): p. 1-10.

38. Terry, A.V., Jr., *Functional consequences of repeated organophosphate exposure: potential non-cholinergic mechanisms.* Pharmacol Ther, 2012. **134**(3): p. 355-65.

39. Del Prado-Lu, J.L., *Pesticide exposure, risk factors and health problems among cutflower farmers: a cross sectional study.* J Occup Med Toxicol, 2007. **2**: p. 9.

40. Alexander, B.H., et al., *Chlorpyrifos exposure in farm families: results from the farm family exposure study.* J Expo Sci Environ Epidemiol, 2006. **16**(5): p. 447-56.

41. Baker, B.A., et al., *Farm Family Exposure Study: methods and recruitment practices for a biomonitoring study of pesticide exposure.* J Expo Anal Environ Epidemiol, 2005. **15**(6): p. 491-9.

42. Curwin, B.D., et al., *Urinary and hand wipe pesticide levels among farmers and nonfarmers in Iowa.* J Expo Anal Environ Epidemiol, 2005. **15**(6): p. 500-8.

43. Perry, M.J., A. Marbella, and P.M. Layde, *Nonpersistent pesticide exposure self-report versus biomonitoring in farm pesticide applicators.* Ann Epidemiol, 2006. **16**(9): p. 701-7.

44. He, F., *Biological monitoring of exposure to pesticides: current issues.* Toxicol Lett, 1999. **108**(2-3): p. 277-83.

45. Curwin, B.D., et al., *Comparison of immunoassay and HPLC-MS/MS used to measure urinary metabolites of atrazine, metolachlor, and chlorpyrifos from farmers and non-farmers in Iowa.* J Expo Sci Environ Epidemiol, 2010. **20**(2): p. 205-12.

46. Lucas, A.D., et al., *Determination of atrazine metabolites in human urine: development of a biomarker of exposure.* Chem Res Toxicol, 1993. **6**(1): p. 107-16.

47. Perry, M.J., et al., *Urinalysis of atrazine exposure in farm pesticide applicators.* Toxicol Ind Health, 2000. **16**(7-8): p. 285-90.

48. Mage, D.T., R.H. Allen, and A. Kodali, *Creatinine corrections for estimating children's and adult's pesticide intake doses in equilibrium with urinary pesticide and creatinine concentrations.* J Expo Sci Environ Epidemiol, 2008. **18**(4): p. 360-8.

49. Flaskos, J., *The developmental neurotoxicity of organophosphorus insecticides: a direct role for the oxon metabolites.* Toxicol Lett, 2012. **209**(1): p. 86-93.

50. Hernandez, A.F., et al., *Toxic effects of pesticide mixtures at a molecular level: Their relevance to human health.* Toxicology, 2012.

51. Jansen, K.L., et al., *Paraoxonase 1 (PON1) modulates the toxicity of mixed organophosphorus compounds.* Toxicol Appl Pharmacol, 2009. **236**(2): p. 142-53.

52. Ray, D.E. and J.R. Fry, *A reassessment of the neurotoxicity of pyrethroid insecticides.* Pharmacol Ther, 2006. **111**(1): p. 174-93.

53. Mikata, K., N. Isobe, and H. Kaneko, *Biotransformation and enzymatic reactions of synthetic pyrethroids in mammals.* Top Curr Chem, 2012. **314**: p. 113-35.

54. Kaneko, H.M., J, *Handbook of Pesticide Toxicology: Pyrethroid chemistry and metabolism*. 2001, San Diego.

55. Chen, Z., et al., *2,2-bis(4-Chlorophenyl)acetic acid (DDA), a water-soluble urine biomarker of DDT metabolism in humans.* Int J Toxicol, 2009. **28**(6): p. 528-33.

56. Anderson, H.A., *Utilization of adipose tissue biopsy in characterizing human halogenated hydrocarbon exposure.* Environ Health Perspect, 1985. **60**: p. 127-31.

57. Fitzloff, J.F., J. Portig, and K. Stein, *Lindane metabolism by human and rat liver microsomes.* Xenobiotica, 1982. **12**(3): p. 197-202.

58. *Public Health Goal for Heptachlor and Heptachlor Epoxide In Drinking Water*. 1999 [cited 2012 Dec 19]; Available from: http://oehha.ca.gov/water/phg/pdf/hepandox.pdf.

59. Bradberry, S.M., et al., *Mechanisms of toxicity, clinical features, and management of acute chlorophenoxy herbicide poisoning: a review.* J Toxicol Clin Toxicol, 2000. **38**(2): p. 111-22.

60. Fukuto, T.R., *Mechanism of action of organophosphorus and carbamate insecticides.* Environ Health Perspect, 1990. **87**: p. 245-54.

61. Goel, A. and P. Aggarwal, *Pesticide poisoning.* Natl Med J India, 2007. **20**(4): p. 182-91.

62. Spencer, J. and M. O'Malley, *Pyrethroid illnesses in California, 1996-2002.* Rev Environ Contam Toxicol, 2006. **186**: p. 57-72.

63. Proudfoot, A.T., *Pentachlorophenol poisoning.* Toxicol Rev, 2003. **22**(1): p. 3-11.

64. Abdollahi, M., et al., *Pesticides and oxidative stress: a review.* Med Sci Monit, 2004. **10**(6): p. RA141-7.

65. Karami-Mohajeri, S. and M. Abdollahi, *Toxic influence of organophosphate, carbamate, and organochlorine pesticides on cellular metabolism of lipids, proteins, and carbohydrates: a systematic review.* Hum Exp Toxicol, 2011. **30**(9): p. 1119-40.

66. Kesavachandran, C., et al., *Possible mechanism of pesticide toxicity-related oxidative stress leading to airway narrowing.* Redox Rep, 2006. **11**(4): p. 159-62.

67. Chung, K.F. and J.A. Marwick, *Molecular mechanisms of oxidative stress in airways and lungs with reference to asthma and chronic obstructive pulmonary disease.* Ann N Y Acad Sci, 2010. **1203**: p. 85-91.

68. Lopez, O., et al., *Changes in antioxidant enzymes in humans with long-term exposure to pesticides.* Toxicol Lett, 2007. **171**(3): p. 146-53.

69. Montell, C., L. Birnbaumer, and V. Flockerzi, *The TRP channels, a remarkably functional family.* Cell, 2002. **108**(5): p. 595-8.

70. Caceres, A.I., et al., *A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma.* Proc Natl Acad Sci U S A, 2009. **106**(22): p. 9099-104.

71. Canning, B.J. and D. Spina, *Sensory nerves and airway irritability.* Handb Exp Pharmacol, 2009(194): p. 139-83.

72. Veronesi, B. and M. Oortgiesen, *The TRPV1 receptor: target of toxicants and therapeutics.* Toxicol Sci, 2006. **89**(1): p. 1-3.

73. Banner, K.H., F. Igney, and C. Poll, *TRP channels: emerging targets for respiratory disease.* Pharmacol Ther, 2011. **130**(3): p. 371-84.

74. Joachim, R.A., et al., *Stress induces substance P in vagal sensory neurons innervating the mouse airways.* Clin Exp Allergy, 2006. **36**(8): p. 1001-10.

75. Butler, C.A. and L.G. Heaney, *Neurogenic inflammation and asthma.* Inflamm Allergy Drug Targets, 2007. **6**(2): p. 127-32.

76. Barnes, P.J., *Neurogenic inflammation in the airways.* Respir Physiol, 2001. **125**(1-2): p. 145-54.

77. Hernandez, A.F., T. Parron, and R. Alarcon, *Pesticides and asthma.* Curr Opin Allergy Clin Immunol, 2011. **11**(2): p. 90-6.

78. Voccia, I., et al., *Immunotoxicity of pesticides: a review.* Toxicol Ind Health, 1999. **15**(1-2): p. 119-32.

79. Corsini, E., et al., *Effects of pesticide exposure on the human immune system.* Hum Exp Toxicol, 2008. **27**(9): p. 671-80.

80. Colosio, C., et al., *Low level exposure to chemicals and immune system.* Toxicol Appl Pharmacol, 2005. **207**(2 Suppl): p. 320-8.

81. Klemmer, H.W., et al., *Clinical findings in workers exposed to pentachlorophenol.* Arch Environ Contam Toxicol, 1980. **9**(6): p. 715-25.

82. McConnachie, P.R. and A.C. Zahalsky, *Immunological consequences of exposure to pentachlorophenol.* Arch Environ Health, 1991. **46**(4): p. 249-53.

83. Daniel, V., et al., *Association of elevated blood levels of pentachlorophenol (PCP) with cellular and humoral immunodeficiencies.* Arch Environ Health, 2001. **56**(1): p. 77-83.

84. Jaga, K. and D. Brosius, *Pesticide exposure: human cancers on the horizon.* Rev Environ Health, 1999. **14**(1): p. 39-50.

85. Eskenazi, B., et al., *The Pine River statement: human health consequences of DDT use.* Environ Health Perspect, 2009. **117**(9): p. 1359-67.

86. Grulich, A.E. and C.M. Vajdic, *The epidemiology of non-Hodgkin lymphoma.* Pathology, 2005. **37**(6): p. 409-19.

87. Vial, T., B. Nicolas, and J. Descotes, *Clinical immunotoxicity of pesticides.* J Toxicol Environ Health, 1996. **48**(3): p. 215-29.

88. Thrasher, J.D., G. Heuser, and A. Broughton, *Immunological abnormalities in humans chronically exposed to chlorpyrifos.* Arch Environ Health, 2002. **57**(3): p. 181-7.

89. Thrasher, J.D., R. Madison, and A. Broughton, *Immunologic abnormalities in humans exposed to chlorpyrifos: preliminary observations.* Arch Environ Health, 1993. **48**(2): p. 89-93.

90. Faustini, A., et al., *Immunological changes among farmers exposed to phenoxy herbicides: preliminary observations.* Occup Environ Med, 1996. **53**(9): p. 583-5.

91. De Coster, S. and N. van Larebeke, *Endocrine-disrupting chemicals: associated disorders and mechanisms of action.* J Environ Public Health, 2012. **2012**: p. 713696.

92. Androutsopoulos, V.P., et al., *A mechanistic overview of health associated effects of low levels of organochlorine and organophosphorous pesticides.* Toxicology, 2012.

93. Lee, D.H., et al., *Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes.* PLoS One, 2011. **6**(1): p. e15977.

94. Cutolo, M., et al., *The hypothalamic-pituitary-adrenal and gonadal axes in rheumatoid arthritis.* Ann N Y Acad Sci, 2000. **917**: p. 835-43.

95. Zaidi, S.S., et al., *Assessment of thyroid function in pesticide formulators.* Hum Exp Toxicol, 2000. **19**(9): p. 497-501.

96. Zuskin, E., et al., *Respiratory function in pesticide workers.* J Occup Environ Med, 2008. **50**(11): p. 1299-305.

97. Sprince, N.L., et al., *Respiratory symptoms: associations with pesticides, silos, and animal confinement in the Iowa Farm Family Health and Hazard Surveillance Project.* Am J Ind Med, 2000. **38**(4): p. 455-62.

98. Hashemi, N., et al., *Prevalence of work-related respiratory symptoms in Iranian farmers.* Can Respir J, 2006. **13**(4): p. 198-202.

99. Wilkins, J.R., 3rd, et al., *Prevalence of chronic respiratory symptoms among Ohio cash grain farmers.* Am J Ind Med, 1999. **35**(2): p. 150-63.

100. Chakraborty, S., et al., *Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India.* J Occup Health, 2009. **51**(6): p. 488-97.

101. Hoppin, J.A., et al., *Pesticides and adult respiratory outcomes in the agricultural health study.* Ann N Y Acad Sci, 2006. **1076**: p. 343-54.

102. Ohayo-Mitoko, G.J., et al., *Self reported symptoms and inhibition of acetylcholinesterase activity among Kenyan agricultural workers.* Occup Environ Med, 2000. **57**(3): p. 195-200.

103. Ciesielski, S., et al., *Pesticide exposures, cholinesterase depression, and symptoms among North Carolina migrant farmworkers.* Am J Public Health, 1994. **84**(3): p. 446-51.

104. Lessenger, J.E., *Five office workers inadvertently exposed to cypermethrin.* J Toxicol Environ Health, 1992. **35**(4): p. 261-7.

105. Moretto, A., *Indoor spraying with the pyrethroid insecticide lambda-cyhalothrin: effects on spraymen and inhabitants of sprayed houses.* Bull World Health Organ, 1991. **69**(5): p. 591-4.

106. Kishi, M., et al., *Relationship of pesticide spraying to signs and symptoms in Indonesian farmers.* Scand J Work Environ Health, 1995. **21**(2): p. 124-33.

107. Atreya, K., *Probabilistic assessment of acute health symptoms related to pesticide use under intensified Nepalese agriculture.* Int J Environ Health Res, 2008. **18**(3): p. 187-208.

108. Burgess, J.L., et al., *Fumigant-related illnesses: Washington State's five-year experience.* J Toxicol Clin Toxicol, 2000. **38**(1): p. 7-14.

109. Herzstein, J. and M.R. Cullen, *Methyl bromide intoxication in four field-workers during removal of soil fumigation sheets.* Am J Ind Med, 1990. **17**(3): p. 321-6.

110. Stromberg, P.E. and K.L. Cumpston, *Cases from NACCT acute and intensive care symposium: altered mental status, seizures, and rash in a fumigation company employee.* Clin Toxicol (Phila), 2013. **51**(3): p. 182-5.

111. Deschamps, F.J. and J.C. Turpin, *Methyl bromide intoxication during grain store fumigation.* Occup Med (Lond), 1996. **46**(1): p. 89-90.

112. Hustinx, W.N., et al., *Systemic effects of inhalational methyl bromide poisoning: a study of nine cases occupationally exposed due to inadvertent spread during fumigation.* Br J Ind Med, 1993. **50**(2): p. 155-9.

113. Royce, S., et al., *Occupational asthma in a pesticides manufacturing worker.* Chest, 1993. **103**(1): p. 295-6.

114. Mekonnen, Y. and T. Agonafir, *Lung function and respiratory symptoms of pesticide sprayers in state farms of Ethiopia.* Ethiop Med J, 2004. **42**(4): p. 261-6.

115. Hernandez, A.F., et al., *Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects.* Inhal Toxicol, 2008. **20**(9): p. 839-49.

116. Peiris-John, R.J., et al., *Low-level exposure to organophosphate pesticides leads to restrictive lung dysfunction.* Respir Med, 2005. **99**(10): p. 1319-24.

117. Salameh, P., et al., *Spirometric changes following the use of pesticides.* East Mediterr Health J, 2005. **11**(1-2): p. 126-36.

118. Reynolds, S.J., et al., *Pulmonary Function Reductions Among Potentially Susceptible Subgroups of Agricultural Workers in Colorado and Nebraska.* J Occup Environ Med, 2012. **54**(5): p. 632-641.

119. Fareed, M., et al., *Adverse respiratory health and hematological alterations among agricultural workers occupationally exposed to organophosphate pesticides: a cross-sectional study in North India.* PLoS One, 2013. **8**(7): p. e69755.

120. Schenker, M.B., et al., *Pulmonary function and exercise-associated changes with chronic low-level paraquat exposure.* Am J Respir Crit Care Med, 2004. **170**(7): p. 773-9.

121. Dalvie, M.A., et al., *Long-term respiratory health effects of the herbicide, paraquat, among workers in the Western Cape.* Occup Environ Med, 1999. **56**(6): p. 391-6.

122. Rastogi, S.K., et al., *Study of respiratory impairment among pesticide sprayers in Mango plantations.* Am J Ind Med, 1989. **16**(5): p. 529-38.

123. Beseler, C.L. and L. Stallones, *Pesticide poisoning and respiratory disorders in Colorado farm residents.* J Agric Saf Health, 2009. **15**(4): p. 327-34.

124. Abu Sham'a, F., et al., *Lung function and respiratory symptoms in male Palestinian farmers.* Arch Environ Occup Health, 2010. **65**(4): p. 191-200.

125. Jones, S.M., et al., *Occupational asthma symptoms and respiratory function among aerial pesticide applicators.* Am J Ind Med, 2003. **43**(4): p. 407-17.

126. Fieten, K.B., et al., *Pesticide exposure and respiratory health of indigenous women in Costa Rica.* Am J Epidemiol, 2009. **169**(12): p. 1500-6.

127. Beckett, W.S., *Occupational respiratory diseases.* N Engl J Med, 2000. **342**(6): p. 406-13.

128. Ezzati, M.L., Alan D; Rodgers, Anthony; Murray, Christopher J. L *Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors.* WHO Geneva, 2004.

129. Kogevinas, M., et al., *Exposure to substances in the workplace and new-onset asthma: an international prospective population-based study (ECRHS-II).* Lancet, 2007. **370**(9584): p. 336-41.

130. Toren, K. and P.D. Blanc, *Asthma caused by occupational exposures is common - a systematic analysis of estimates of the population-attributable fraction.* BMC Pulm Med, 2009. **9**: p. 7.

131. Kogevinas, M., et al., *Occupational asthma in Europe and other industrialised areas: a population-based study. European Community Respiratory Health Survey Study Group.* Lancet, 1999. **353**(9166): p. 1750-4.

132. Ayres, J.G., et al., *Costs of occupational asthma in the UK.* Thorax, 2011. **66**(2): p. 128-33.

133. Moscato, G. and C. Rampulla, *Costs of occupational asthma and of occupational chronic obstructive pulmonary disease.* Curr Opin Allergy Clin Immunol, 2003. **3**(2): p. 109-14.

134. Vandenplas, O., K. Toren, and P.D. Blanc, *Health and socioeconomic impact of work-related asthma.* Eur Respir J, 2003. **22**(4): p. 689-97.

135. Selgrade, M.K., et al., *Induction of asthma and the environment: what we know and need to know.* Environ Health Perspect, 2006. **114**(4): p. 615-9.

136. Deschamps, D., et al., *Persistent asthma after acute inhalation of organophosphate insecticide.* Lancet, 1994. **344**(8938): p. 1712.

137. Draper, A., et al., *Occupational asthma from fungicides fluazinam and chlorothalonil.* Occup Environ Med, 2003. **60**(1): p. 76-7.

138. Vandenplas, O., et al., *Asthma to tetramethrin.* Allergy, 2000. **55**(4): p. 417-8.

139. Henneberger, P.K., et al., *Work-related reactive airways dysfunction syndrome cases from surveillance in selected US states.* J Occup Environ Med, 2003. **45**(4): p. 360-8.

140. LeVan, T.D., et al., *Vapor, dust, and smoke exposure in relation to adult-onset asthma and chronic respiratory symptoms: the Singapore Chinese Health Study.* Am J Epidemiol, 2006. **163**(12): p. 1118-28.

141. Faria, N.M., et al., *Pesticides and respiratory symptoms among farmers.* Rev Saude Publica, 2005. **39**(6): p. 973-81.

142. Senthilselvan, A., H.H. McDuffie, and J.A. Dosman, *Association of asthma with use of pesticides. Results of a cross-sectional survey of farmers.* Am Rev Respir Dis, 1992. **146**(4): p. 884-7.

143. Hoppin, J.A., et al., *Chemical predictors of wheeze among farmer pesticide applicators in the Agricultural Health Study.* Am J Respir Crit Care Med, 2002. **165**(5): p. 683-9.

144. Hoppin, J.A., et al., *Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study.* Am J Respir Crit Care Med, 2008. **177**(1): p. 11-8.

145. Baldi, I., et al., *Agricultural exposure and asthma risk in the AGRICAN French cohort.* Int J Hyg Environ Health, 2013.

146. Crinnion, W.J., *Do environmental toxicants contribute to allergy and asthma?* Altern Med Rev, 2012. **17**(1): p. 6-18.

147. Beard, J., et al., *Health impacts of pesticide exposure in a cohort of outdoor workers.* Environ Health Perspect, 2003. **111**(5): p. 724-30.

148. *A Review of the Relationship between Pyrethrins, Pyrethroid Exposure and Asthma and Allergies*. 2009 January 13, 2012 [cited 2012 January 13, 2012]; Available from: http://nepis.epa.gov/EPA/html/DLwait.htm?url=/Adobe/PDF/P1006R0U.PDF.

149. Boers, D., et al., *Asthmatic symptoms after exposure to ethylenebisdithiocarbamates and other pesticides in the Europit field studies.* Hum Exp Toxicol, 2008. **27**(9): p. 721-7.

150. Barczyk, A., E. Sozanska, and W. Pierzchala, *[The influence of occupational exposure to pesticides on the frequency of chronic obstructive pulmonary diseases].* Wiad Lek, 2006. **59**(9-10): p. 596-600.

151. Salameh, P.R., et al., *Chronic bronchitis and pesticide exposure: a case-control study in Lebanon.* Eur J Epidemiol, 2006. **21**(9): p. 681-8.

152. Valcin, M., et al., *Chronic bronchitis among nonsmoking farm women in the agricultural health study.* J Occup Environ Med, 2007. **49**(5): p. 574-83.

153. Tual, S., et al., *Agricultural exposures and chronic bronchitis: findings from the AGRICAN (AGRIculture and CANcer) cohort.* Ann Epidemiol, 2013. **23**(9): p. 539-45.

154. Pesatori, A.C., et al., *Cohort mortality and nested case-control study of lung cancer among structural pest control workers in Florida (United States).* Cancer Causes Control, 1994. **5**(4): p. 310-8.

155. Alavanja, M.C., et al., *Pesticides and lung cancer risk in the agricultural health study cohort.* Am J Epidemiol, 2004. **160**(9): p. 876-85.

156. Lee, W.J., et al., *Cancer incidence among pesticide applicators exposed to chlorpyrifos in the Agricultural Health Study.* J Natl Cancer Inst, 2004. **96**(23): p. 1781-9.

157. Beane Freeman, L.E., et al., *Cancer incidence among male pesticide applicators in the Agricultural Health Study cohort exposed to diazinon.* Am J Epidemiol, 2005. **162**(11): p. 1070-9.

158. Rusiecki, J.A., et al., *Cancer incidence among pesticide applicators exposed to metolachlor in the Agricultural Health Study.* Int J Cancer, 2006. **118**(12): p. 3118-23.

159. Hou, L., et al., *Pendimethalin exposure and cancer incidence among pesticide applicators.* Epidemiology, 2006. **17**(3): p. 302-7.

160. Purdue, M.P., et al., *Occupational exposure to organochlorine insecticides and cancer incidence in the Agricultural Health Study.* Int J Cancer, 2007. **120**(3): p. 642-9.

161. Bonner, M.R., et al., *Occupational exposure to carbofuran and the incidence of cancer in the Agricultural Health Study.* Environ Health Perspect, 2005. **113**(3): p. 285-9.

162. Samanic, C., et al., *Cancer incidence among pesticide applicators exposed to dicamba in the agricultural health study.* Environ Health Perspect, 2006. **114**(10): p. 1521-6.

163. Davies, J.M., *Lung cancer among pesticide workers exposed to inorganic arsenicals.* Arch Environ Health, 1980. **35**(2): p. 123-4.

164. Luchtrath, H., *The consequences of chronic arsenic poisoning among Moselle wine growers. Pathoanatomical investigations of post-mortem examinations performed between 1960 and 1977.* J Cancer Res Clin Oncol, 1983. **105**(2): p. 173-82.

165. Mabuchi, K., A.M. Lilienfeld, and L.M. Snell, *Lung cancer among pesticide workers exposed to inorganic arsenicals.* Arch Environ Health, 1979. **34**(5): p. 312-20.

166. Bonner, M.R., et al., *Malathion exposure and the incidence of cancer in the agricultural health study.* Am J Epidemiol, 2007. **166**(9): p. 1023-34.

167. Rusiecki, J.A., et al., *Cancer incidence among pesticide applicators exposed to atrazine in the Agricultural Health Study.* J Natl Cancer Inst, 2004. **96**(18): p. 1375-82.

168. Freeman, L.E., et al., *Atrazine and cancer incidence among pesticide applicators in the agricultural health study (1994-2007).* Environ Health Perspect, 2011. **119**(9): p. 1253-9.

169. Settimi, L., et al., *Cancer risk among male farmers: a multi-site case-control study.* Int J Occup Med Environ Health, 2001. **14**(4): p. 339-47.

170. Christensen, C.H., et al., *Coumaphos exposure and incident cancer among male participants in the Agricultural Health Study (AHS).* Environ Health Perspect, 2010. **118**(1): p. 92-6.

171. Lee, W.J., et al., *Cancer incidence among pesticide applicators exposed to alachlor in the Agricultural Health Study.* Am J Epidemiol, 2004. **159**(4): p. 373-80.

172. Kang, D., et al., *Cancer incidence among pesticide applicators exposed to trifluralin in the Agricultural Health Study.* Environ Res, 2008. **107**(2): p. 271-6.

173. Mozzachio, A.M., et al., *Chlorothalonil exposure and cancer incidence among pesticide applicator participants in the agricultural health study.* Environ Res, 2008. **108**(3): p. 400-3.

174. van Bemmel, D.M., et al., *S-ethyl-N,N-dipropylthiocarbamate exposure and cancer incidence among male pesticide applicators in the agricultural health study: a prospective cohort.* Environ Health Perspect, 2008. **116**(11): p. 1541-6.

175. Lynch, S.M., et al., *Cancer incidence among pesticide applicators exposed to butylate in the Agricultural Health Study (AHS).* Environ Res, 2009. **109**(7): p. 860-8.

176. Lynch, S.M., et al., *Cancer incidence among pesticide applicators exposed to cyanazine in the agricultural health study.* Environ Health Perspect, 2006. **114**(8): p. 1248-52.

177. Mahajan, R., et al., *Fonofos exposure and cancer incidence in the agricultural health study.* Environ Health Perspect, 2006. **114**(12): p. 1838-42.

178. Mahajan, R., et al., *Phorate exposure and incidence of cancer in the agricultural health study.* Environ Health Perspect, 2006. **114**(8): p. 1205-9.

179. De Roos, A.J., et al., *Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study.* Environ Health Perspect, 2005. **113**(1): p. 49-54.

180. Koutros, S., et al., *Dichlorvos exposure and human cancer risk: results from the Agricultural Health Study.* Cancer Causes Control, 2008. **19**(1): p. 59-65.

181. Greenburg, D.L., et al., *Cancer incidence among pesticide applicators exposed to captan in the Agricultural Health Study.* Cancer Causes Control, 2008. **19**(10): p. 1401-7.

182. Rusiecki, J.A., et al., *Cancer incidence among pesticide applicators exposed to permethrin in the Agricultural Health Study.* Environ Health Perspect, 2009. **117**(4): p. 581-6.

183. Enomoto, M., W.J. Tierney, and K. Nozaki, *Risk of human health by particulate matter as a source of air pollution--comparison with tobacco smoking.* J Toxicol Sci, 2008. **33**(3): p. 251-67.

184. Samet, J.M. and G.R. Eradze, *Radon and lung cancer risk: taking stock at the millenium.* Environ Health Perspect, 2000. **108 Suppl 4**: p. 635-41.

185. Yang, P., *Lung cancer in never smokers.* Semin Respir Crit Care Med. **32**(1): p. 10-21.

186. Brennan, P., P. Hainaut, and P. Boffetta, *Genetics of lung-cancer susceptibility.* Lancet Oncol, 2011. **12**(4): p. 399-408.

187. Becher, H., et al., *Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins.* Cancer Causes Control, 1996. **7**(3): p. 312-21.

188. Kogevinas, M., et al., *Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study.* Am J Epidemiol, 1997. **145**(12): p. 1061-75.

189. Newman, L.S., et al., *A case control etiologic study of sarcoidosis: environmental and occupational risk factors.* Am J Respir Crit Care Med, 2004. **170**(12): p. 1324-30.

190. Slager, R.E., et al., *Rhinitis associated with pesticide exposure among commercial pesticide applicators in the Agricultural Health Study.* Occup Environ Med, 2009. **66**(11): p. 718-24.

191. Slager, R.E., et al., *Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study.* J Toxicol Environ Health A, 2010. **73**(20): p. 1382-93.

192. Chatzi, L., et al., *Association of allergic rhinitis with pesticide use among grape farmers in Crete, Greece.* Occup Environ Med, 2007. **64**(6): p. 417-21.

193. Hoppin, J.A., et al., *Pesticides and other agricultural factors associated with self-reported farmer's lung among farm residents in the Agricultural Health Study.* Occup Environ Med, 2007. **64**(5): p. 334-41.

194. Dallaire, F., et al., *Acute infections and environmental exposure to organochlorines in Inuit infants from Nunavik.* Environ Health Perspect, 2004. **112**(14): p. 1359-65.

195. Sunyer, J., et al., *DDE in mothers' blood during pregnancy and lower respiratory tract infections in their infants.* Epidemiology, 2010. **21**(5): p. 729-35.

196. Salameh, P.R., et al., *Respiratory symptoms in children and exposure to pesticides.* Eur Respir J, 2003. **22**(3): p. 507-12.

197. Sigurs, N., et al., *Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7.* Am J Respir Crit Care Med, 2000. **161**(5): p. 1501-7.

198. Sigurs, N., et al., *Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13.* Am J Respir Crit Care Med, 2005. **171**(2): p. 137-41.

199. Cone, J.E., et al., *Persistent respiratory health effects after a metam sodium pesticide spill.* Chest, 1994. **106**(2): p. 500-8.

200. Newton, J.G. and A.B. Breslin, *Asthmatic reactions to a commonly used aerosol insect killer.* Med J Aust, 1983. **1**(8): p. 378-80.

201. Karpati, A.M., et al., *Pesticide spraying for West Nile virus control and emergency department asthma visits in New York City, 2000.* Environ Health Perspect, 2004. **112**(11): p. 1183-7.

202. O'Sullivan, B.C., et al., *The effect of pesticide spraying on the rate and severity of ED asthma.* Am J Emerg Med, 2005. **23**(4): p. 463-7.

203. Wagner, S.L., *Fatal asthma in a child after use of an animal shampoo containing pyrethrin.* West J Med, 2000. **173**(2): p. 86-7.

204. Sunyer, J., et al., *Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six.* Clin Exp Allergy, 2006. **36**(10): p. 1236-41.

205. Sunyer, J., et al., *Prenatal dichlorodiphenyldichloroethylene (DDE) and asthma in children.* Environ Health Perspect, 2005. **113**(12): p. 1787-90.

206. Tagiyeva, N., et al., *Parental occupation is a risk factor for childhood wheeze and asthma.* Eur Respir J, 2010. **35**(5): p. 987-93.

207. Salam, M.T., et al., *Early-life environmental risk factors for asthma: findings from the Children's Health Study.* Environ Health Perspect, 2004. **112**(6): p. 760-5.

208. Salameh, P., et al., *Respiratory diseases and pesticide exposure: a case-control study in Lebanon.* J Epidemiol Community Health, 2006. **60**(3): p. 256-61.

209. Menconi, S., et al., *A preliminary study of potential human health effects in private residences following chlordane applications for termite control.* Arch Environ Health, 1988. **43**(5): p. 349-52.

210. Schneider, D., N.C. Freeman, and P. McGarvey, *Asthma and respiratory dysfunction among urban, primarily Hispanic school children.* Arch Environ Health, 2004. **59**(1): p. 4-13.

211. Ejigu, D. and Y. Mekonnen, *Pesticide use on agricultural fields and health problems in various activities.* East Afr Med J, 2005. **82**(8): p. 427-32.

212. Barr, D.B., et al., *Biomonitoring of exposure in farmworker studies.* Environ Health Perspect, 2006. **114**(6): p. 936-42.

213. Kamel, F. and J.A. Hoppin, *Association of pesticide exposure with neurologic dysfunction and disease.* Environ Health Perspect, 2004. **112**(9): p. 950-8.

214. Cocker, J., et al., *Biological monitoring of exposure to organophosphate pesticides.* Toxicol Lett, 2002. **134**(1-3): p. 97-103.

215. Farahat, F.M., et al., *Biomarkers of chlorpyrifos exposure and effect in Egyptian cotton field workers.* Environ Health Perspect, 2011. **119**(6): p. 801-6.

216. Garabrant, D.H., et al., *Cholinesterase inhibition in chlorpyrifos workers: Characterization of biomarkers of exposure and response in relation to urinary TCPy.* J Expo Sci Environ Epidemiol, 2009. **19**(7): p. 634-42.

217. Curwin, B.D., et al., *Pesticide contamination inside farm and nonfarm homes*. J Occup Environ Hyg, 2005. **2**(7): p. 357-67.

218. Bouvier, G., et al., *Pesticide exposure of non-occupationally exposed subjects compared to some occupational exposure: a French pilot study*. Sci Total Environ, 2006. **366**(1): p. 74-91.

219. Callahan, C.L., et al., *Chlorpyrifos exposure and respiratory health among adolescent agricultural workers*. Int J Environ Res Public Health, 2014. **11**(12): p. 13117-29.

220. Riederer, A.M., et al., *Diet and nondiet predictors of urinary 3-phenoxybenzoic acid in NHANES 1999-2002.* Environ Health Perspect, 2008. **116**(8): p. 1015-22.

221. Bouvier, G., et al., *Environmental and biological monitoring of exposure to organophosphorus pesticides: application to occupationally and non-occupationally exposed adult populations*. J Expo Sci Environ Epidemiol, 2006. **16**(5): p. 417-26.217.

222. Duramad, P., et al., *Early environmental exposures and intracellular Th1/Th2 cytokine profiles in 24-month-old children living in an agricultural area.* Environ Health Perspect, 2006. **114**(12): p. 1916-22.

223. *Respiratory health hazards in agriculture.* Am J Respir Crit Care Med, 1998. **158**(5 Pt 2): p. S1-S76.

224. Kirkhorn, S.R. and V.F. Garry, *Agricultural lung diseases.* Environ Health Perspect, 2000. **108 Suppl 4**: p. 705-12.

225. Last, J., *A Dictionary of Epidemiology*. 4th ed. 2001, Oxford, UK: Oxford University Press.

226. Le Moual, N., et al., *The healthy worker effect in asthma: work may cause asthma, but asthma may also influence work.* Am J Respir Crit Care Med, 2008. **177**(1): p. 4-10.

227. McCauley, L.A., et al., *Studying health outcomes in farmworker populations exposed to pesticides.* Environ Health Perspect, 2006. **114**(6): p. 953-60.

228. Beach, J., I. Burstyn, and N. Cherry, *Estimating the extent and distribution of new-onset adult asthma in British Columbia using frequentist and Bayesian approaches.* Ann Occup Hyg, 2012. **56**(6): p. 719-27.

229. Groenewold, M.R. and S.L. Baron, *The Proportion of Work-Related Emergency Department Visits Not Expected to Be Paid by Workers' Compensation: Implications for Occupational Health Surveillance, Research, Policy, and Health Equity.* Health Serv Res, 2013.

230. Cherry, N., et al., *Data linkage to estimate the extent and distribution of occupational disease: new onset adult asthma in Alberta, Canada.* Am J Ind Med, 2009. **52**(11): p. 831-40.

231. Quandt SA , P.J., Arcury TA *Mobility Patterns of Migrant Farmworkers in North Carolina: Implications for Occupational Health Research and Policy.* Human Organization, 2002. **61**(1): p. 21-29.

232. Karmaus, W., J. Kuehr, and H. Kruse, *Infections and atopic disorders in childhood and organochlorine exposure.* Arch Environ Health, 2001. **56**(6): p. 485-92.

233. Daniel, V., et al., *Associations of dichlorodiphenyltrichloroethane (DDT) 4.4 and dichlorodiphenyldichloroethylene (DDE) 4.4 blood levels with plasma IL-4.* Arch Environ Health, 2002. **57**(6): p. 541-7.

234. Vine, M.F., et al., *Plasma 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) levels and immune response.* Am J Epidemiol, 2001. **153**(1): p. 53-63.

235. Vine, M.F., et al., *Effects on the immune system associated with living near a pesticide dump site.* Environ Health Perspect, 2000. **108**(12): p. 1113-24.

236. Cooper, G.S., et al., *Associations between plasma DDE levels and immunologic measures in African-American farmers in North Carolina.* Environ Health Perspect, 2004. **112**(10): p. 1080-4.

237. Hermanowicz, A., et al., *The neutrophil function and infectious diseases in workers occupationally exposed to organochloride insecticides.* Int Arch Occup Environ Health, 1982. **50**(4): p. 329-40.

238. Queiroz, M.L., et al., *Defective neutrophil function in workers occupationally exposed to hexachlorobenzene.* Hum Exp Toxicol, 1997. **16**(6): p. 322-6.

239. Queiroz, M.L., et al., *Immunoglobulin levels in workers exposed to hexachlorobenzene.* Hum Exp Toxicol, 1998. **17**(3): p. 172-5.

240. Daniel, V., et al., *Associations of blood levels of PCB, HCHS, and HCB with numbers of lymphocyte subpopulations, in vitro lymphocyte response, plasma cytokine levels, and immunoglobulin autoantibodies.* Environ Health Perspect, 2001. **109**(2): p. 173-8.

241. Kashyap, S.K., *Health surveillance and biological monitoring of pesticide formulators in India.* Toxicol Lett, 1986. **33**(1-3): p. 107-14.

242. Seth, V., et al., *Lindane-induced immunological alterations in human poisoning cases.* Clin Biochem, 2005. **38**(7): p. 678-80.

243. McConnachie, P.R. and A.C. Zahalsky, *Immune alterations in humans exposed to the termiticide technical chlordane.* Arch Environ Health, 1992. **47**(4): p. 295-301.

244. Phillips, T.M., *Assessing environmental exposure in children: immunotoxicology screening.* J Expo Anal Environ Epidemiol, 2000. **10**(6 Pt 2): p. 769-75.

245. Colosio, C., et al., *Toxicological and immune findings in workers exposed to pentachlorophenol (PCP).* Arch Environ Health, 1993. **48**(2): p. 81-8.

246. Reichrtova, E., et al., *Cord serum immunoglobulin E related to the environmental contamination of human placentas with organochlorine compounds.* Environ Health Perspect, 1999. **107**(11): p. 895-9.

247. Li, Q., et al., *The by-products generated during sarin synthesis in the Tokyo sarin disaster induced inhibition of natural killer and cytotoxic T lymphocyte activity.* Toxicology, 2000. **146**(2-3): p. 209-20.

248. Fiore, M.C., et al., *Chronic exposure to aldicarb-contaminated groundwater and human immune function.* Environ Res, 1986. **41**(2): p. 633-45.

249. Mirkin, I.R., et al., *Changes in T-lymphocyte distribution associated with ingestion of aldicarb-contaminated drinking water: a follow-up study.* Environ Res, 1990. **51**(1): p. 35-50.

250. Colosio, C., et al., *Immunomodulatory effects of occupational exposure to mancozeb.* Arch Environ Health, 1996. **51**(6): p. 445-51.

251. Corsini, E., et al., *Immunomodulatory effects of the fungicide Mancozeb in agricultural workers.* Toxicol Appl Pharmacol, 2005. **208**(2): p. 178-85.

References for Chapter 3

1. *Food and Agriculture Organization of the United Nations (FAO)-Database on pesticides consumption*. 2006 [cited 2013 July 8, 2013]; Available from: http://www.fao.org/waicent/FAOINFO/economic/pesticid.htm.
2. *Pesticide Market Estimates: 2006-2007.* . 2011 [cited 2012 Dec 14]; Available from: http://www.epa.gov/pesticides/pestsales/07pestsales/table_of_contents2007.htm.
3. Nougadere, A., et al., *Total diet study on pesticide residues in France: levels in food as consumed and chronic dietary risk to consumers.* Environ Int, 2012. **45**: p. 135-50.
4. Poulsen, M.E. and J.H. Andersen, *Results from the monitoring of pesticide residues in fruit and vegetables on the Danish market, 2000-01.* Food Addit Contam, 2003. **20**(8): p. 742-57.
5. Morgan, M.K., et al., *Exposures of preschool children to chlorpyrifos and its degradation product 3,5,6-trichloro-2-pyridinol in their everyday environments.* J Expo Anal Environ Epidemiol, 2005. **15**(4): p. 297-309.
6. MacIntosh, D.L., C.W. Kabiru, and P.B. Ryan, *Longitudinal investigation of dietary exposure to selected pesticides.* Environ Health Perspect, 2001. **109**(2): p. 145-50.
7. Lu, C., et al., *Assessing children's dietary pesticide exposure: direct measurement of pesticide residues in 24-hr duplicate food samples.* Environ Health Perspect, 2010. **118**(11): p. 1625-30.
8. Riederer, A.M., et al., *Pyrethroid and organophosphorus pesticides in composite diet samples from Atlanta, USA adults.* Environ Sci Technol, 2010. **44**(1): p. 483-90.
9. Berman, T., et al., *Urinary concentrations of organophosphate pesticide metabolites in adults in Israel: demographic and dietary predictors.* Environ Int, 2013. **60**: p. 183-9.

10. McKelvey, W., et al., *Population-based biomonitoring of exposure to organophosphate and pyrethroid pesticides in New York City.* Environ Health Perspect, 2013. **121**(11-12): p. 1349-56.

11. Munoz-Quezada, M.T., et al., *Predictors of exposure to organophosphate pesticides in schoolchildren in the Province of Talca, Chile.* Environ Int, 2012. **47**: p. 28-36.

12. Fortes, C., et al., *The relation between dietary habits and urinary levels of 3-phenoxybenzoic acid, a pyrethroid metabolite.* Food Chem Toxicol, 2013. **52**: p. 91-6.

13. Riederer, A.M., et al., *Diet and nondiet predictors of urinary 3-phenoxybenzoic acid in NHANES 1999-2002.* Environ Health Perspect, 2008. **116**(8): p. 1015-22.

14. Lu, C., et al., *The attribution of urban and suburban children's exposure to synthetic pyrethroid insecticides: a longitudinal assessment.* J Expo Sci Environ Epidemiol, 2009. **19**(1): p. 69-78.

15. Statistics Canada. *Canadian Health Measures Survey (CHMS) Data User Guide: Cycle 1*. 2011 [cited 2012 Feb 14, 2012]; Available from: http://www23.statcan.gc.ca/imdb-bmdi/document/5071_D2_T1_V1-eng.htm.

16. Mikata, K., N. Isobe, and H. Kaneko, *Biotransformation and enzymatic reactions of synthetic pyrethroids in mammals.* Top Curr Chem, 2012. **314**: p. 113-35.

17. Health Canada. *Report on Human Biomonitoring of Environmental Chemicals in Canada-Results of the Canadian Health Measures Survey Cycle 1 (2007-2009)*. 2010 [cited 2012; Available from: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms/index-eng.php.

18. Barr, D.B., et al., *Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements.* Environ Health Perspect, 2005. **113**(2): p. 192-200.

19. Statistics Canada. *Canadian Health Measures Survey (Cycle 1) - Household Questionnaire* 2010 [cited 2012 Feb 14, 2012]; Available from: http://www23.statcan.gc.ca/imdb-bmdi/pub/instrument/5071_Q2_V1-eng.htm.

20. CSEP, *Canadian Physical Activity, Fitness and Lifestyle Approach. 3rd Ed. Canadian Society for Exercise Physiology.* 2003, Ottawa.

21. Kuczmarski, R.J., et al., *2000 CDC Growth Charts for the United States: methods and development.* Vital Health Stat 11, 2002(246): p. 1-190.

22. WHO. *The International Classification of adult underweight, overweight and obesity according to BMI* 2004 [cited 2014 April 11, 2014]; Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html.

23. EFSA. *Food classification*. 2014 [cited 2014 March 19, 2014]; Available from: http://www.efsa.europa.eu/en/datex/datexfoodclass.htm.

24. Hornung, R.W. and L.D. Reed, *Estimation of average concentration in the presence of nondetectable values.* Applied Occupational and Environmental Hygiene, 1990. **5**(1): p. 46-51.

25. Ripley, B.D., et al., *Pesticide residues on fruits and vegetables from Ontario, Canada, 1991-1995.* J AOAC Int, 2000. **83**(1): p. 196-213.

26. Becker, K., et al., *GerES IV pilot study: assessment of the exposure of German children to organophosphorus and pyrethroid pesticides.* Int J Hyg Environ Health, 2006. **209**(3): p. 221-33.

27. Arthur, F.H., *Efficacy of Cyfluthrin, Cyfluthrin + Piperonyl Butoxide, and Cyfluthrin + Piperonyl Butoxide + Chlorpyrifos-Methyl as Protectants of Stored Peanuts.* Peanut Science, 1994. **21**(1): p. 44-48.

28. Mukherjee, I., R. Singh, and J.N. Govil, *Risk assessment of a synthetic pyrethroid, bifenthrin on pulses.* Bull Environ Contam Toxicol, 2010. **84**(3): p. 294-300.

29. Lairon, D., *Nutritional quality and safety of organic food. A review. .* Agron. Sustain. Dev., 2010. **30**: p. 33-41.

30. Baker, B.P., et al., *Pesticide residues in conventional, integrated pest management (IPM)-grown and organic foods: insights from three US data sets.* Food Addit Contam, 2002. **19**(5): p. 427-46.

31. Curl, C.L., R.A. Fenske, and K. Elgethun, *Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets.* Environ Health Perspect, 2003. **111**(3): p. 377-82.

32. Lu, C., et al., *Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children.* Environ Health Perspect, 2008. **116**(4): p. 537-42.

33. Lu, C., et al., *Organic diets significantly lower children's dietary exposure to organophosphorus pesticides.* Environ Health Perspect, 2006. **114**(2): p. 260-3.

34. Morgan, M.K., et al., *An observational study of 127 preschool children at their homes and daycare centers in Ohio: environmental pathways to cis- and trans-permethrin exposure.* Environ Res, 2007. **104**(2): p. 266-74.

35. *Prevention and Treatment-Head Lice*. 2013 [cited 2014 May 06, 2014]; Available from: http://www.health.gov.sk.ca/head-lice.

36. Emanuel, A.S., et al., *Theory of Planned Behavior explains gender difference in fruit and vegetable consumption.* Appetite, 2012. **59**(3): p. 693-7.

37. De Coster, S. and N. van Larebeke, *Endocrine-disrupting chemicals: associated disorders and mechanisms of action.* J Environ Public Health, 2012. **2012**: p. 713696.

38. Vahabi, M. and C. Damba, *Perceived barriers in accessing food among recent Latin American immigrants in Toronto.* Int J Equity Health, 2013. **12**: p. 1.

39. Girard, A. and P. Sercia, *Immigration and food insecurity: social and nutritional issues for recent immigrants in Montreal, Canada.* International Journal of Migration, Health and Social Care, 2013. **9**(1): p. 32-45.

40. Barr, D.B., et al., *Biomonitoring of exposure in farmworker studies.* Environ Health Perspect, 2006. **114**(6): p. 936-42.

41. Starr, J., et al., *Pyrethroid pesticides and their metabolites in vacuum cleaner dust collected from homes and day-care centers.* Environ Res, 2008. **108**(3): p. 271-9.

42. Zhang, X., et al., *Dialkylphosphates (DAPs) in fruits and vegetables may confound biomonitoring in organophosphorus insecticide exposure and risk assessment.* J Agric Food Chem, 2008. **56**(22): p. 10638-45.

43. PMRA, *Re-evaluation note: Update on re-evaluation of azinphos-methyl.* 2007, Pest Management Regulatory Agency.

44. van Balen, E.C., M.J. Wolansky, and T. Kosatsky, *Increasing use of pyrethroids in Canadian households: should we be concerned?* Can J Public Health, 2012. **103**(6): p. e404-7.

45. Androutsopoulos, V.P., et al., *A mechanistic overview of health associated effects of low levels of organochlorine and organophosphorous pesticides.* Toxicology, 2012.

References for Chapter 4

1. Kolpin, D., J. Barbash, and R. Gilliom, *Occurrence of pesticides in shallow groundwater of the United States: initial results from the National Water-Quality Assessment program.* Environ Sci Technol, 1998. **32**(5): p. 558-566.
2. Reigart, J.R., *Recognition and Management of Pesticide Poisonings*. 1999 [cited 2012 Feb 14, 2012]; Available from: http://npic.orst.edu/rmpp.htm.
3. *Reregistration Eligibility Decision(RED) for Malathion*. 2006 [cited 2012 Dec 19]; Available from: http://www.epa.gov/oppsrrd1/REDs/malathion_red.pdf.
4. Jansen, K.L., et al., *Paraoxonase 1 (PON1) modulates the toxicity of mixed organophosphorus compounds.* Toxicol Appl Pharmacol, 2009. **236**(2): p. 142-53.
5. Walker, C., *Organic pollutants, an ecotoxicological perspectives.* 2001, London: Taylor and Francis.
6. Kapka-Skrzypczak, L., et al., *Biomonitoring and biomarkers of organophosphate pesticides exposure - state of the art.* Ann Agric Environ Med, 2011. **18**(2): p. 294-303.
7. Androutsopoulos, V.P., et al., *A mechanistic overview of health associated effects of low levels of organochlorine and organophosphorous pesticides.* Toxicology, 2012.
8. Keifer, M.C. and J. Firestone, *Neurotoxicity of pesticides.* J Agromedicine, 2007. **12**(1): p. 17-25.
9. Abou-Donia, M.B., *Organophosphorus ester-induced chronic neurotoxicity.* Arch Environ Health, 2003. **58**(8): p. 484-97.
10. Eskenazi, B., et al., *Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children.* Environ Health Perspect, 2007. **115**(5): p. 792-8.

11. Corsini, E., et al., *Effects of pesticide exposure on the human immune system.* Hum Exp Toxicol, 2008. **27**(9): p. 671-80.

12. De Coster, S. and N. van Larebeke, *Endocrine-disrupting chemicals: associated disorders and mechanisms of action.* J Environ Public Health, 2012. **2012**: p. 713696.

13. Ye, M., et al., *Occupational pesticide exposures and respiratory health.* Int J Environ Res Public Health, 2013. **10**(12): p. 6442-71.

14. *Reregistration Eligibility Decision for Chlorpyrifos*. 2006; Available from: http://www.epa.gov/oppsrrd1/REDs/chlorpyrifos_ired.pdf.

15. Peiris-John, R.J., et al., *Low-level exposure to organophosphate pesticides leads to restrictive lung dysfunction.* Respir Med, 2005. **99**(10): p. 1319-24.

16. Chakraborty, S., et al., *Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India.* J Occup Health, 2009. **51**(6): p. 488-97.

17. Fareed, M., et al., *Adverse respiratory health and hematological alterations among agricultural workers occupationally exposed to organophosphate pesticides: a cross-sectional study in North India.* PLoS One, 2013. **8**(7): p. e69755.

18. Statistics Canada. *Canadian Health Measures Survey (CHMS) Data User Guide: Cycle 1*. 2011 [cited 2012 Feb 14, 2012]; Available from: http://www23.statcan.gc.ca/imdb-bmdi/document/5071_D2_T1_V1-eng.htm.

19. Oulhote, Y. and M.F. Bouchard, *Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in canadian children.* Environ Health Perspect, 2013. **121**(11-12): p. 1378-84.

20. Hornung, R.W. and L.D. Reed, *Estimation of average concentration in the presence of nondetectable values.* Applied Occupational and Environmental Hygiene, 1990. **5**(1): p. 46-51.

21. David J. Hendrick, P.S.B., William S. Beckett, Andrew Churg, *Occupational Disorders of The Lung: Recognition, Management, and Prevention.* 2002.

22. Health Canada. *Report on Human Biomonitoring of Environmental Chemicals in Canada-Results of the Canadian Health Measures Survey Cycle 1 (2007-2009)*. 2010 [cited 2012; Available from: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms/index-eng.php.

23. Barr, D.B., et al., *Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements.* Environ Health Perspect, 2005. **113**(2): p. 192-200.

24. Pellegrino, R., et al., *Interpretative strategies for lung function tests.* Eur Respir J, 2005. **26**(5): p. 948-68.

25. Burrows, B., et al., *A descriptive analysis of the growth and decline of the FVC and FEV1.* Chest, 1983. **83**(5): p. 717-24.

26. Peat, J.K., A.J. Woolcock, and K. Cullen, *Decline of lung function and development of chronic airflow limitation: a longitudinal study of non-smokers and smokers in Busselton, Western Australia.* Thorax, 1990. **45**(1): p. 32-7.

27. Racke, K. and S. Matthiesen, *The airway cholinergic system: physiology and pharmacology.* Pulm Pharmacol Ther, 2004. **17**(4): p. 181-98.

28. Fryer, A.D. and D.B. Jacoby, *Muscarinic receptors and control of airway smooth muscle.* Am J Respir Crit Care Med, 1998. **158**(5 Pt 3): p. S154-60.

29. Costello, R.W., D.B. Jacoby, and A.D. Fryer, *Pulmonary neuronal M2 muscarinic receptor function in asthma and animal models of hyperreactivity.* Thorax, 1998. **53**(7): p. 613-6.

30. Lein, P.J. and A.D. Fryer, *Organophosphorus insecticides induce airway hyperreactivity by decreasing neuronal M2 muscarinic receptor function independent of acetylcholinesterase inhibition.* Toxicol Sci, 2005. **83**(1): p. 166-76.

31. Proskocil, B.J., et al., *Organophosphorus pesticides decrease M2 muscarinic receptor function in guinea pig airway nerves via indirect mechanisms.* PLoS One, 2010. **5**(5): p. e10562.

32. Minette, P.A. and P.J. Barnes, *Prejunctional inhibitory muscarinic receptors on cholinergic nerves in human and guinea pig airways.* J Appl Physiol, 1988. **64**(6): p. 2532-7.

33. Terry, A.V., Jr., *Functional consequences of repeated organophosphate exposure: potential non-cholinergic mechanisms.* Pharmacol Ther, 2012. **134**(3): p. 355-65.

34. Abdollahi, M., et al., *Pesticides and oxidative stress: a review.* Med Sci Monit, 2004. **10**(6): p. RA141-7.

35. Tomasic, M., et al., *Contractile agonists activate voltage-dependent calcium channels in airway smooth muscle cells.* Am J Physiol, 1992. **263**(1 Pt 1): p. C106-13.

36. Barr, D.B., et al., *Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the U.S. population.* Environ Health Perspect, 2004. **112**(2): p. 186-200.

37. Pehkonen S, Z.Q., *The Degradation of Organophosphorus Pesticides in Natural Waters: A Critical Review.* Critical Reviews in Environmental Science and Technology, 2002. **32**(1): p. 17-72.

38. Zhang, X., et al., *Dialkylphosphates (DAPs) in fruits and vegetables may confound biomonitoring in organophosphorus insecticide exposure and risk assessment.* J Agric Food Chem, 2008. **56**(22): p. 10638-45.

39. Cocker, J., et al., *Biological monitoring of exposure to organophosphate pesticides.* Toxicol Lett, 2002. **134**(1-3): p. 97-103.

References for Chapter 5

1. Reigart JR., *Recognition and Management of Pesticide Poisonings*. 1999 [cited 2012 Feb 14, 2012]; Available from: http://npic.orst.edu/rmpp.htm.

2. *Food and Agriculture Organization of the United Nations (FAO)-Database on pesticides consumption*. 2006 [cited 2013 July 8, 2013]; Available from: http://www.fao.org/waicent/FAOINFO/economic/pesticid.htm.

3. Damalas, C.A. and I.G. Eleftherohorinos, *Pesticide exposure, safety issues, and risk assessment indicators.* Int J Environ Res Public Health, 2011. **8**(5): p. 1402-19.

4. O'Malley, M., *Clinical evaluation of pesticide exposure and poisonings.* Lancet, 1997. **349**(9059): p. 1161-6.

5. ATSDR. *Toxicological Profile for Chlorpyrifos*. 1997 [cited 2014 Feb 21, 2014]; Available from: www.atsdr.cdc.gov/toxprofiles/tp84.html.

6. Schettgen, T., et al., *Pyrethroid exposure of the general population-is this due to diet.* Toxicol Lett, 2002. **134**(1-3): p. 141-5.

7. Ray, D.E. and J.R. Fry, *A reassessment of the neurotoxicity of pyrethroid insecticides.* Pharmacol Ther, 2006. **111**(1): p. 174-93.

8. Mikata, K., N. Isobe, and H. Kaneko, *Biotransformation and enzymatic reactions of synthetic pyrethroids in mammals.* Top Curr Chem, 2012. **314**: p. 113-35.

9. Sudakin, D.L., *Pyrethroid insecticides: advances and challenges in biomonitoring.* Clin Toxicol (Phila), 2006. **44**(1): p. 31-7.

10. Eadsforth, C.V. and M.K. Baldwin, *Human dose-excretion studies with the pyrethroid insecticide, cypermethrin.* Xenobiotica, 1983. **13**(2): p. 67-72.

11. Barr, D.B., et al., *Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the U.S. population.* Environ Health Perspect, 2004. **112**(2): p. 186-200.

12. Barr, D.B., et al., *Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999-2002.* Environ Health Perspect, 2010. **118**(6): p. 742-8.

13. Barr, D.B., et al., *Concentrations of selective metabolites of organophosphorus pesticides in the United States population.* Environ Res, 2005. **99**(3): p. 314-26.

14. Fréry N, S.A., Garnier R, Zeghnoun A, Falq G, Guldner L., *Exposure of the French population to environmental pollutants — environmental components of the French National Survey on Nutrition and Health — initial results. Saint-Maurice (Fra)*, in *French Institute for Public Health Surveillance*. 2010.

15. Health Canada. *Report on Human Biomonitoring of Environmental Chemicals in Canada-Results of the Canadian Health Measures Survey Cycle 1 (2007-2009)*. 2010 [cited 2012; Available from: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms/index-eng.php.

16. Haines, D.A. and J. Murray, *Human biomonitoring of environmental chemicals--early results of the 2007-2009 Canadian Health Measures Survey for males and females.* Int J Hyg Environ Health, 2012. **215**(2): p. 133-7.

17. Keifer, M.C. and J. Firestone, *Neurotoxicity of pesticides.* J Agromedicine, 2007. **12**(1): p. 17-25.

18. Hanke, W. and J. Jurewicz, *The risk of adverse reproductive and developmental disorders due to occupational pesticide exposure: an overview of current epidemiological evidence.* Int J Occup Med Environ Health, 2004. **17**(2): p. 223-43.

19. Shafer, T.J., D.A. Meyer, and K.M. Crofton, *Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs.* Environ Health Perspect, 2005. **113**(2): p. 123-36.

20. DeMicco, A., et al., *Developmental neurotoxicity of pyrethroid insecticides in zebrafish embryos.* Toxicol Sci, 2010. **113**(1): p. 177-86.

21. Koureas, M., et al., *Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes.* Toxicol Lett, 2012. **210**(2): p. 155-68.

22. Corsini, E., et al., *Effects of pesticide exposure on the human immune system.* Hum Exp Toxicol, 2008. **27**(9): p. 671-80.

23. Diel, F., et al., *Pyrethroids and piperonyl-butoxide affect human T-lymphocytes in vitro.* Toxicol Lett, 1999. **107**(1-3): p. 65-74.

24. Prater, M.R., et al., *Single-dose topical exposure to the pyrethroid insecticide, permethrin in C57BL/6N mice: effects on thymus and spleen.* Food Chem Toxicol, 2002. **40**(12): p. 1863-73.

25. Punareewattana, K., et al., *Topical permethrin exposure inhibits antibody production and macrophage function in C57Bl/6N mice.* Food Chem Toxicol, 2001. **39**(2): p. 133-9.

26. Emara, A.M. and E.I. Draz, *Immunotoxicological study of one of the most common over-the-counter pyrethroid insecticide products in Egypt.* Inhal Toxicol, 2007. **19**(12): p. 997-1009.

27. Fuortes, L., *Urticaria due to airborne permethrin exposure.* Vet Hum Toxicol, 1999. **41**(2): p. 92-3.

28. De Coster, S. and N. van Larebeke, *Endocrine-disrupting chemicals: associated disorders and mechanisms of action.* J Environ Public Health, 2012. **2012**: p. 713696.

29. McCarthy, A.R., et al., *Estrogenicity of pyrethroid insecticide metabolites.* J Environ Monit, 2006. **8**(1): p. 197-202.

30. Koutros, S., et al., *An update of cancer incidence in the Agricultural Health Study.* J Occup Environ Med, 2010. **52**(11): p. 1098-105.

31. Rusiecki, J.A., et al., *Cancer incidence among pesticide applicators exposed to permethrin in the Agricultural Health Study.* Environ Health Perspect, 2009. **117**(4): p. 581-6.

32. Hoppin, J.A., et al., *Pesticides and adult respiratory outcomes in the agricultural health study.* Ann N Y Acad Sci, 2006. **1076**: p. 343-54.

33. Salameh, P.R., et al., *Respiratory symptoms in children and exposure to pesticides.* Eur Respir J, 2003. **22**(3): p. 507-12.

34. Ye, M., et al., *Occupational pesticide exposures and respiratory health.* Int J Environ Res Public Health, 2013. **10**(12): p. 6442-71.

35. Statistics Canada. *Canadian Health Measures Survey (CHMS) Data User Guide: Cycle 1*. 2011 [cited 2012 Feb 14, 2012]; Available from: http://www23.statcan.gc.ca/imdb-bmdi/document/5071_D2_T1_V1-eng.htm.

36. Statistics Canada. *Canadian Health Measures Survey (Cycle 1) - Household Questionnaire* 2010 [cited 2012 Feb 14, 2012]; Available from: http://www23.statcan.gc.ca/imdb-bmdi/pub/instrument/5071_Q2_V1-eng.htm.

37. Barr, D.B., et al., *Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements.* Environ Health Perspect, 2005. **113**(2): p. 192-200.

38. CDC. *Fourth National Report on Human Exposure to Environmental Chemicals-Updated Tables. Centers for Disease Control and Prevention*. 2013 [cited 2013 August 1st]; Available from: http://www.cdc.gov/exposurereport/.

39. van Balen, E.C., M.J. Wolansky, and T. Kosatsky, *Increasing use of pyrethroids in Canadian households: should we be concerned?* Can J Public Health, 2012. **103**(6): p. e404-7.

40. Garfitt, S.J., et al., *Exposure to the organophosphate diazinon: data from a human volunteer study with oral and dermal doses.* Toxicol Lett, 2002. **134**(1-3): p. 105-13.

41. Kapka-Skrzypczak, L., et al., *Biomonitoring and biomarkers of organophosphate pesticides exposure - state of the art.* Ann Agric Environ Med, 2011. **18**(2): p. 294-303.

42. Newton, J.G. and A.B. Breslin, *Asthmatic reactions to a commonly used aerosol insect killer.* Med J Aust, 1983. **1**(8): p. 378-80.

43. Kajekar, R., *Environmental factors and developmental outcomes in the lung.* Pharmacol Ther, 2007. **114**(2): p. 129-45.

44. Roy, A., et al., *Ambient particulate matter and lung function growth in Chinese children.* Epidemiology, 2012. **23**(3): p. 464-72.

45. Forbes, L.J., et al., *Chronic exposure to outdoor air pollution and lung function in adults.* Thorax, 2009. **64**(8): p. 657-63.

46. Schwartz, J., *Lung function and chronic exposure to air pollution: a cross-sectional analysis of NHANES II.* Environ Res, 1989. **50**(2): p. 309-21.

47. Vandenplas, O., et al., *Asthma to tetramethrin.* Allergy, 2000. **55**(4): p. 417-8.

48. Wagner, S.L., *Fatal asthma in a child after use of an animal shampoo containing pyrethrin.* West J Med, 2000. **173**(2): p. 86-7.

49. Moretto, A., *Indoor spraying with the pyrethroid insecticide lambda-cyhalothrin: effects on spraymen and inhabitants of sprayed houses.* Bull World Health Organ, 1991. **69**(5): p. 591-4.

50. Lessenger, J.E., *Five office workers inadvertently exposed to cypermethrin.* J Toxicol Environ Health, 1992. **35**(4): p. 261-7.

51. Starr, J., et al., *Pyrethroid pesticides and their metabolites in vacuum cleaner dust collected from homes and day-care centers.* Environ Res, 2008. **108**(3): p. 271-9.

52. *Permethrin, Resmethrin, Sumithrin: Synthetic Pyrethroids For Mosquito Control*. 2002, United States-Environmental Protection Agency.

References for Chapter 6

1. WHO, *DDT and its derivatives. World Health Organization*. Vol. 9. 1979, Geneva.

2. Attaran, A. and R. Maharaj, *Ethical debate: doctoring malaria, badly: the global campaign to ban DDT.* BMJ, 2000. **321**(7273): p. 1403-5.

3. ATSDR. *Toxicological Profile for DDT, DDE, and DDD. Agency for Toxic Substances and Disease Registry*. 2002 [cited 2014 Dec 08]; Available: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=81&tid=20.

4. Chen, Z., et al., *2,2-bis(4-Chlorophenyl)acetic acid (DDA), a water-soluble urine biomarker of DDT metabolism in humans.* Int J Toxicol, 2009. **28**(6): p. 528-33.

5. Anderson, H.A., *Utilization of adipose tissue biopsy in characterizing human halogenated hydrocarbon exposure.* Environ Health Perspect, 1985. **60**: p. 127-31.

6. Perez-Maldonado, I.N., et al., *Assessment of DDT levels in selected environmental media and biological samples from Mexico and Central America.* Chemosphere, 2010. **78**(10): p. 1244-9.

7. Bayen, S., et al., *Bioaccumulation of DDT pesticide in cultured Asian seabass following dietary exposure.* J Toxicol Environ Health A, 2005. **68**(1): p. 51-65.

8. Longnecker, M.P., *Invited Commentary: Why DDT matters now.* Am J Epidemiol, 2005. **162**(8): p. 726-8.

9. Wolff, M.S., et al., *Risk of breast cancer and organochlorine exposure.* Cancer Epidemiol Biomarkers Prev, 2000. **9**(3): p. 271-7.

10. Smith, D., *Worldwide trends in DDT levels in human breast milk.* Int J Epidemiol, 1999. **28**(2): p. 179-88.

11. Eskenazi, B., et al., *The Pine River statement: human health consequences of DDT use.* Environ Health Perspect, 2009. **117**(9): p. 1359-67.

12. UNEP. *Report of the Third DDT Expert Group meeting-Stochholm Convention*. 2010 [cited 2014 Dec 08]; Available:

http://chm.pops.int/Implementation/DDT/Meetings/DDTEG32010/tabid/1108/mctl/ViewDetails/EventModID/1421/EventID/116/xmid/4037/Default.aspx.

13. Keifer, M.C. and J. Firestone, *Neurotoxicity of pesticides.* J Agromedicine, 2007. **12**(1): p. 17-25.

14. Corsini, E., et al., *Effects of pesticide exposure on the human immune system.* Hum Exp Toxicol, 2008. **27**(9): p. 671-80.

15. Beard, J., *DDT and human health.* Sci Total Environ, 2006. **355**(1-3): p. 78-89.

16. Ye, M., et al., *Occupational pesticide exposures and respiratory health.* Int J Environ Res Public Health, 2013. **10**(12): p. 6442-71.

17. De Coster, S. and N. van Larebeke, *Endocrine-disrupting chemicals: associated disorders and mechanisms of action.* J Environ Public Health, 2012. **2012**: p. 713696.

18. Hoppin, J.A., et al., *Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study.* Am J Respir Crit Care Med, 2008. **177**(1): p. 11-8.

19. Hoppin, J.A., et al., *Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study.* Eur Respir J, 2009. **34**(6): p. 1296-303.

20. Hoppin, J.A., et al., *Pesticide use and chronic bronchitis among farmers in the Agricultural Health Study.* Am J Ind Med, 2007. **50**(12): p. 969-79.

21. Beard, J., et al., *Health impacts of pesticide exposure in a cohort of outdoor workers.* Environ Health Perspect, 2003. **111**(5): p. 724-30.

22. Statistics Canada. *Canadian Health Measures Survey (CHMS) Data User Guide: Cycle 1.* 2011 [cited 2014 Dec 08]; Available: http://www23.statcan.gc.ca/imdb-bmdi/pub/document/5071_D2_T1_V1-eng.htm.

23. Health Canada. *Report on Human Biomonitoring of Environmental Chemicals in Canada-Results of the Canadian Health Measures Survey Cycle 1 (2007-2009)*. 2010 [cited 2014 Dec 08]; Available: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms/index-eng.php.

24. INSPQ, *Analytical method for the determination of polychlorinated biphenyl congeners, polybrominated congeners, toxaphene congeners, and organochlorinated pesticides in plasma by GC-MS (E-446), condensed version*. 2009, Laboratoire de Toxicologie, Institut National de Santé Publique du Québec.

25. Aylward, L.L., et al., *Biomonitoring equivalents for hexachlorobenzene.* Regul Toxicol Pharmacol, 2010. **58**(1): p. 25-32.

26. Statistics Canada. *Canadian Health Measures Survey (Cycle 1) - Household Questionnaire* 2010 [cited 2014 Dec 08]; Available: http://www23.statcan.gc.ca/imdb/pIX.pl?Function=showStaticArchiveHTML&a=1&fl=http://www23.statcan.gc.ca/imdb-bmdi/instrument/5071_Q2_V1-eng.htm&Item_Id=64294.

27. Rollin, H.B., et al., *Concentration of selected persistent organic pollutants in blood from delivering women in South Africa.* Sci Total Environ, 2009. **408**(1): p. 146-52.

28. CDC. *Fourth National Report on Human Exposure to Environmental Chemicals. Centers for Disease Control and Prevention*. 2009 [cited 2014 Dec 08]; Available from: http://www.cdc.gov/exposurereport/.

29. EPA. *U.S. EPA's 2008 Report on the Environment (Final Report). Environment Protection Agency*. 2008 [cited 2014 Dec 08]; Available from: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=190806.

30. Salameh, P., et al., *Spirometric changes following the use of pesticides.* East Mediterr Health J, 2005. **11**(1-2): p. 126-36.

31. Mekonnen, Y. and T. Agonafir, *Lung function and respiratory symptoms of pesticide sprayers in state farms of Ethiopia.* Ethiop Med J, 2004. **42**(4): p. 261-6.

32. Zuskin, E., et al., *Respiratory function in pesticide workers.* J Occup Environ Med, 2008. **50**(11): p. 1299-305.

33. Hernandez, A.F., et al., *Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects.* Inhal Toxicol, 2008. **20**(9): p. 839-49.

34. Peiris-John, R.J., et al., *Low-level exposure to organophosphate pesticides leads to restrictive lung dysfunction.* Respir Med, 2005. **99**(10): p. 1319-24.

35. Chakraborty, S., et al., *Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India.* J Occup Health, 2009. **51**(6): p. 488-97.

36. Fareed, M., et al., *Adverse respiratory health and hematological alterations among agricultural workers occupationally exposed to organophosphate pesticides: a cross-sectional study in North India.* PLoS One, 2013. **8**(7): p. e69755.

37. Rastogi, S.K., et al., *Study of respiratory impairment among pesticide sprayers in Mango plantations.* Am J Ind Med, 1989. **16**(5): p. 529-38.

38. Beseler, C.L. and L. Stallones, *Pesticide poisoning and respiratory disorders in Colorado farm residents.* J Agric Saf Health, 2009. **15**(4): p. 327-34.

39. Burrows, B., et al., *A descriptive analysis of the growth and decline of the FVC and FEV1.* Chest, 1983. **83**(5): p. 717-24.

40. Peat, J.K., A.J. Woolcock, and K. Cullen, *Decline of lung function and development of chronic airflow limitation: a longitudinal study of non-smokers and smokers in Busselton, Western Australia.* Thorax, 1990. **45**(1): p. 32-7.

41. Valcin, M., et al., *Chronic bronchitis among nonsmoking farm women in the agricultural health study.* J Occup Environ Med, 2007. **49**(5): p. 574-83.

42. Souhrada, M. and J.F. Souhrada, *Sodium and calcium influx induced by phorbol esters in airway smooth muscle cells.* Am Rev Respir Dis, 1989. **139**(4): p. 927-32.

43. Souhrada, M., M.H. Souhrada, and J.F. Souhrada, *The inhibition of sodium influx attenuates airway response to a specific antigen challenge.* Br J Pharmacol, 1988. **93**(4): p. 884-92.

44. Androutsopoulos, V.P., et al., *A mechanistic overview of health associated effects of low levels of organochlorine and organophosphorous pesticides.* Toxicology, 2012.

45. Abdollahi, M., et al., *Pesticides and oxidative stress: a review.* Med Sci Monit, 2004. **10**(6): p. RA141-7.

46. Berridge, M.J., *Smooth muscle cell calcium activation mechanisms.* J Physiol, 2008. **586**(Pt 21): p. 5047-61.

47. Sakai, H., et al., *Involvement of the Tyr kinase/JNK pathway in carbachol-induced bronchial smooth muscle contraction in the rat.* Anesthesiology, 2013. **118**(5): p. 1076-85.

48. Tomasic, M., et al., *Contractile agonists activate voltage-dependent calcium channels in airway smooth muscle cells.* Am J Physiol, 1992. **263**(1 Pt 1): p. C106-13.

49. Menshikova, E.V., et al., *Pulmonary microsomes contain a Ca(2+)-transport system sensitive to oxidative stress.* Biochim Biophys Acta, 1995. **1228**(2-3): p. 165-74.

50. Vine, M.F., et al., *Plasma 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) levels and immune response.* Am J Epidemiol, 2001. **153**(1): p. 53-63.

51. Daniel, V., et al., *Associations of blood levels of PCB, HCHS, and HCB with numbers of lymphocyte subpopulations, in vitro lymphocyte response, plasma cytokine levels, and immunoglobulin autoantibodies.* Environ Health Perspect, 2001. **109**(2): p. 173-8.

52. Daniel, V., et al., *Associations of dichlorodiphenyltrichloroethane (DDT) 4.4 and dichlorodiphenyldichloroethylene (DDE) 4.4 blood levels with plasma IL-4.* Arch Environ Health, 2002. **57**(6): p. 541-7.

53. Ejigu, D. and Y. Mekonnen, *Pesticide use on agricultural fields and health problems in various activities.* East Afr Med J, 2005. **82**(8): p. 427-32.

54. Kapka-Skrzypczak, L., et al., *Biomonitoring and biomarkers of organophosphate pesticides exposure - state of the art.* Ann Agric Environ Med, 2011. **18**(2): p. 294-303.

55. Reigart, J.R. and J.R. Roberts. *Recognition and Management of Pesticide Poisonings*. 1999 [cited 2012 Feb 14, 2012]; Available from: http://npic.orst.edu/rmpp.htm.

56. CDC, *Third National Report on Human Exposure to Environmental Chemicals. Centers for Disease Control and Prevention*, C.C.f.D.C.a. Prevention), Editor. 2005, CDC (Centers for Disease Control and Prevention): Atlanta, GA.

57. Kroschwitz, J., M. Howe-Grant, and R. Metcalf, *Kirk-Othmer encyclopedia of chemical technology*. 1995, New York, NY: John Wiley and Sons, Inc.

58. Sunyer, J., et al., *Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six.* Clin Exp Allergy, 2006. **36**(10): p. 1236-41.

59. Sunyer, J., et al., *Prenatal dichlorodiphenyldichloroethylene (DDE) and asthma in children.* Environ Health Perspect, 2005. **113**(12): p. 1787-90.

60. Dallaire, F., et al., *Acute infections and environmental exposure to organochlorines in Inuit infants from Nunavik.* Environ Health Perspect, 2004. **112**(14): p. 1359-65.

61. Sunyer, J., et al., *DDE in mothers' blood during pregnancy and lower respiratory tract infections in their infants.* Epidemiology, 2010. **21**(5): p. 729-35.

62. Carson, R., *Silent Spring*. 1st ed. 1962, Boston: Houghton Mifflin.

References for Chapter 7

1. Bradberry, S.M., et al., *Mechanisms of toxicity, clinical features, and management of acute chlorophenoxy herbicide poisoning: a review.* J Toxicol Clin Toxicol, 2000. **38**(2): p. 111-22.

2. Reigart JR., *Recognition and Management of Pesticide Poisonings*. 1999 [cited 2012 Feb 14, 2012]; Available from: http://npic.orst.edu/rmpp.htm.

3. Brand, R.M., M. Spalding, and C. Mueller, *Sunscreens can increase dermal penetration of 2,4-dichlorophenoxyacetic acid.* J Toxicol Clin Toxicol, 2002. **40**(7): p. 827-32.

4. Pont, A.R., A.R. Charron, and R.M. Brand, *Active ingredients in sunscreens act as topical penetration enhancers for the herbicide 2,4-dichlorophenoxyacetic acid.* Toxicol Appl Pharmacol, 2004. **195**(3): p. 348-54.

5. Curwin, B.D., et al., *Pesticide contamination inside farm and nonfarm homes.* J Occup Environ Hyg, 2005. **2**(7): p. 357-67.

6. Nishioka, M.G., et al., *Distribution of 2,4-D in air and on surfaces inside residences after lawn applications: comparing exposure estimates from various media for young children.* Environ Health Perspect, 2001. **109**(11): p. 1185-91.

7. Morgan, M.K., N.K. Wilson, and J.C. Chuang, *Exposures of 129 preschool children to organochlorines, organophosphates, pyrethroids, and acid herbicides at their homes and daycares in North Carolina.* Int J Environ Res Public Health, 2014. **11**(4): p. 3743-64.

8. Sauerhoff, M.W., et al., *The fate of 2,4-dichlorophenoxyacetic acid (2,4-D) following oral administration to man.* Toxicology, 1977. **8**(1): p. 3-11.

9. Bjorling-Poulsen, M., H.R. Andersen, and P. Grandjean, *Potential developmental neurotoxicity of pesticides used in Europe.* Environ Health, 2008. **7**: p. 50.

10. Keifer, M.C. and J. Firestone, *Neurotoxicity of pesticides.* J Agromedicine, 2007. **12**(1): p. 17-25.

11. Faustini, A., et al., *Immunological changes among farmers exposed to phenoxy herbicides: preliminary observations.* Occup Environ Med, 1996. **53**(9): p. 583-5.

12. Grulich, A.E. and C.M. Vajdic, *The epidemiology of non-Hodgkin lymphoma.* Pathology, 2005. **37**(6): p. 409-19.

13. Hoppin, J.A., et al., *Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study.* Am J Respir Crit Care Med, 2008. **177**(1): p. 11-8.

14. Slager, R.E., et al., *Rhinitis associated with pesticide exposure among commercial pesticide applicators in the Agricultural Health Study.* Occup Environ Med, 2009. **66**(11): p. 718-24.

15. Slager, R.E., et al., *Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study.* J Toxicol Environ Health A, 2010. **73**(20): p. 1382-93.

16. Ye, M., et al., *Occupational pesticide exposures and respiratory health.* Int J Environ Res Public Health, 2013. **10**(12): p. 6442-71.

17. Statistics Canada. *Canadian Health Measures Survey (CHMS) Data User Guide: Cycle 1*. 2011 [cited 2012 Feb 14, 2012]; Available from: http://www23.statcan.gc.ca/imdb-bmdi/document/5071_D2_T1_V1-eng.htm.

18. Health Canada. *Report on Human Biomonitoring of Environmental Chemicals in Canada-Results of the Canadian Health Measures Survey Cycle 1 (2007-2009)*. 2010 [cited 2012; Available from: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms/index-eng.php.

19. Health Canada. *Second Report on Human Biomonitoring of Environmental Chemicals in Canada-Results of the Canadian Health Measures Survey Cycle 2 (2009-2011)*. 2013 [cited 2013; Available from: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms-cycle2/index-eng.php.

20. Barr, D.B., et al., *Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements.* Environ Health Perspect, 2005. **113**(2): p. 192-200.

21. Curwin, B.D., et al., *Urinary and hand wipe pesticide levels among farmers and nonfarmers in Iowa.* J Expo Anal Environ Epidemiol, 2005. **15**(6): p. 500-8.

22. Succop, P.A., et al., *Imputation of data values that are less than a detection limit.* J Occup Environ Hyg, 2004. **1**(7): p. 436-41.

23. Garabrant, D.H. and M.A. Philbert, *Review of 2,4-dichlorophenoxyacetic acid (2,4-D) epidemiology and toxicology.* Crit Rev Toxicol, 2002. **32**(4): p. 233-57.

24. Burns, C.J. and G.M. Swaen, *Review of 2,4-dichlorophenoxyacetic acid (2,4-D) biomonitoring and epidemiology.* Crit Rev Toxicol, 2012. **42**(9): p. 768-86.

25. Holt, E., et al., *Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) impurities in pesticides: a neglected source of contemporary relevance.* Environ Sci Technol, 2010. **44**(14): p. 5409-15.

26. Pesatori, A.C., et al., *Cohort mortality and nested case-control study of lung cancer among structural pest control workers in Florida (United States).* Cancer Causes Control, 1994. **5**(4): p. 310-8.

27. Becher, H., et al., *Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins.* Cancer Causes Control, 1996. **7**(3): p. 312-21.

28. Kogevinas, M., et al., *Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study.* Am J Epidemiol, 1997. **145**(12): p. 1061-75.

29. *Private Property Pesticide By-laws In Canada* 2010 [cited 2012 March 30, 2012]; Available from: www.cbc.ca/marketplace/relatedlinks/pesticide_by_laws.pdf.

30. *Ban on the sale of weed and feed lawn products in the province, effective January 1, 2010*. 2010; Available from: http://esrd.alberta.ca/focus/state-of-the-environment/water/surface-water/condition-indicators/river-water-quality-indices/pesticide-index.aspx.

31. *Alberta bans weed and feed lawn products*. 2008; Available from: http://www.cbc.ca/news/canada/calgary/alberta-bans-weed-and-feed-lawn-products-1.757076.

References for Chapter 8

1. Keifer, M.C. and J. Firestone, *Neurotoxicity of pesticides.* J Agromedicine, 2007. **12**(1): p. 17-25.

2. O'Malley, M., *Clinical evaluation of pesticide exposure and poisonings.* Lancet, 1997. **349**(9059): p. 1161-6.

3. Ray, D.E. and J.R. Fry, *A reassessment of the neurotoxicity of pyrethroid insecticides.* Pharmacol Ther, 2006. **111**(1): p. 174-93.

4. Abou-Donia, M.B., *Organophosphorus ester-induced chronic neurotoxicity.* Arch Environ Health, 2003. **58**(8): p. 484-97.

5. Eskenazi, B., et al., *Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children.* Environ Health Perspect, 2007. **115**(5): p. 792-8.

6. Guodong, D., et al., *Organophosphate pesticide exposure and neurodevelopment in young Shanghai children.* Environ Sci Technol, 2012. **46**(5): p. 2911-7.

7. Hanke, W. and J. Jurewicz, *The risk of adverse reproductive and developmental disorders due to occupational pesticide exposure: an overview of current epidemiological evidence.* Int J Occup Med Environ Health, 2004. **17**(2): p. 223-43.

8. Shafer, T.J., D.A. Meyer, and K.M. Crofton, *Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs.* Environ Health Perspect, 2005. **113**(2): p. 123-36.

9. DeMicco, A., et al., *Developmental neurotoxicity of pyrethroid insecticides in zebrafish embryos.* Toxicol Sci, 2010. **113**(1): p. 177-86.

10. Corsini, E., et al., *Effects of pesticide exposure on the human immune system.* Hum Exp Toxicol, 2008. **27**(9): p. 671-80.

11. Diel, F., et al., *Pyrethroids and piperonyl-butoxide affect human T-lymphocytes in vitro.* Toxicol Lett, 1999. **107**(1-3): p. 65-74.

12. Prater, M.R., et al., *Single-dose topical exposure to the pyrethroid insecticide, permethrin in C57BL/6N mice: effects on thymus and spleen.* Food Chem Toxicol, 2002. **40**(12): p. 1863-73.

13. Punareewattana, K., et al., *Topical permethrin exposure inhibits antibody production and macrophage function in C57Bl/6N mice.* Food Chem Toxicol, 2001. **39**(2): p. 133-9.

14. Emara, A.M. and E.I. Draz, *Immunotoxicological study of one of the most common over-the-counter pyrethroid insecticide products in Egypt.* Inhal Toxicol, 2007. **19**(12): p. 997-1009.

15. Fuortes, L., *Urticaria due to airborne permethrin exposure.* Vet Hum Toxicol, 1999. **41**(2): p. 92-3.

16. De Coster, S. and N. van Larebeke, *Endocrine-disrupting chemicals: associated disorders and mechanisms of action.* J Environ Public Health, 2012. **2012**: p. 713696.

17. McCarthy, A.R., et al., *Estrogenicity of pyrethroid insecticide metabolites.* J Environ Monit, 2006. **8**(1): p. 197-202.

18. Koureas, M., et al., *Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes.* Toxicol Lett, 2012. **210**(2): p. 155-68.

19. Ye, M., et al., *Occupational pesticide exposures and respiratory health.* Int J Environ Res Public Health, 2013. **10**(12): p. 6442-71.

20. Alavanja, M.C. and M.R. Bonner, *Occupational pesticide exposures and cancer risk: a review.* J Toxicol Environ Health B Crit Rev, 2012. **15**(4): p. 238-63.

21. Koutros, S., et al., *An update of cancer incidence in the Agricultural Health Study.* J Occup Environ Med, 2010. **52**(11): p. 1098-105.

22. Rusiecki, J.A., et al., *Cancer incidence among pesticide applicators exposed to permethrin in the Agricultural Health Study.* Environ Health Perspect, 2009. **117**(4): p. 581-6.

23. Burrows, B., et al., *A descriptive analysis of the growth and decline of the FVC and FEV1.* Chest, 1983. **83**(5): p. 717-24.

24. Peat, J.K., A.J. Woolcock, and K. Cullen, *Decline of lung function and development of chronic airflow limitation: a longitudinal study of non-smokers and smokers in Busselton, Western Australia.* Thorax, 1990. **45**(1): p. 32-7.

25. McKelvey, W., et al., *Population-based biomonitoring of exposure to organophosphate and pyrethroid pesticides in New York City.* Environ Health Perspect, 2013. **121**(11-12): p. 1349-56.

26. Riederer, A.M., et al., *Diet and nondiet predictors of urinary 3-phenoxybenzoic acid in NHANES 1999-2002.* Environ Health Perspect, 2008. **116**(8): p. 1015-22.

27. Miller, M.R., et al., *Standardisation of spirometry.* Eur Respir J, 2005. **26**(2): p. 319-38.

28. Hendrick, D., et al., *Occupational Disorders of The Lung: Recognition, Management, and Prevention*. 1st ed. 2002, London: WB Saunders.

29. Ejigu, D. and Y. Mekonnen, *Pesticide use on agricultural fields and health problems in various activities.* East Afr Med J, 2005. **82**(8): p. 427-32.

30. Kapka-Skrzypczak, L., et al., *Biomonitoring and biomarkers of organophosphate pesticides exposure - state of the art.* Ann Agric Environ Med, 2011. **18**(2): p. 294-303.

31. Barr, D.B., et al., *Biomonitoring of exposure in farmworker studies.* Environ Health Perspect, 2006. **114**(6): p. 936-42.

32. Statistics Canada. *Canadian Health Measures Survey (CHMS) Data User Guide: Cycle 1*. 2011 [cited 2012 Feb 14, 2012]; Available from: http://www23.statcan.gc.ca/imdb-bmdi/document/5071_D2_T1_V1-eng.htm.

33. UNEP. *Report of the Third DDT Expert Group meeting-Stochholm Convention*. 2010 [cited 2012 Dec 19]; Available from: http://chm.pops.int/Implementation/DDT/Meetings/DDTEG32010/tabid/1108/mctl/ViewDetails/EventModID/1421/EventID/116/xmid/4037/Default.aspx.

34. *National Biomonitoring Program-DDT Factsheet 2013* [cited 2013 August 1st]; Available from: http://www.cdc.gov/biomonitoring/DDT_FactSheet.html.

35. Longnecker, M.P., *Invited Commentary: Why DDT matters now*. Am J Epidemiol, 2005. **162**(8): p. 726-8.

36. Cocker, J., et al., *Biological monitoring of exposure to organophosphate pesticides*. Toxicol Lett, 2002. **134**(1-3): p. 97-103.

37. Sudakin, D.L., *Pyrethroid insecticides: advances and challenges in biomonitoring*. Clin Toxicol (Phila), 2006. **44**(1): p. 31-7.

38. Hintz, J. *PASS 2008 NCSS, LLC*. 2008; Available from: www.ncss.com.

39. Cohen, J., *Statistical Power Analysis for the Behavioral Sciences*. 1988, Hillsdale, New Jersey: Lawrence Erlbaum Associates.

40. Kajekar, R., *Environmental factors and developmental outcomes in the lung.* Pharmacol Ther, 2007. **114**(2): p. 129-45.

41. Roy, A., et al., *Ambient particulate matter and lung function growth in Chinese children.* Epidemiology, 2012. **23**(3): p. 464-72.

42. Forbes, L.J., et al., *Chronic exposure to outdoor air pollution and lung function in adults.* Thorax, 2009. **64**(8): p. 657-63.

43. Schwartz, J., *Lung function and chronic exposure to air pollution: a cross-sectional analysis of NHANES II.* Environ Res, 1989. **50**(2): p. 309-21.

44. Reigart JR., *Recognition and Management of Pesticide Poisonings*. 1999 [cited 2012 Feb 14, 2012]; Available from: http://npic.orst.edu/rmpp.htm.

45. Schettgen, T., et al., *Pyrethroid exposure of the general population-is this due to diet.* Toxicol Lett, 2002. **134**(1-3): p. 141-5.

46. Damalas, C.A. and I.G. Eleftherohorinos, *Pesticide exposure, safety issues, and risk assessment indicators.* Int J Environ Res Public Health, 2011. **8**(5): p. 1402-19.

47. Kawashima, K. and T. Fujii, *The lymphocytic cholinergic system and its contribution to the regulation of immune activity.* Life Sci, 2003. **74**(6): p. 675-96.

48. Tarkowski, M., W. Lutz, and S. Birindelli, *The lymphocytic cholinergic system and its modulation by organophosphorus pesticides.* Int J Occup Med Environ Health, 2004. **17**(3): p. 325-37.

49. Terry, A.V., Jr., *Functional consequences of repeated organophosphate exposure: potential non-cholinergic mechanisms.* Pharmacol Ther, 2012. **134**(3): p. 355-65.

50. Ray, D.E. and P.G. Richards, *The potential for toxic effects of chronic, low-dose exposure to organophosphates.* Toxicol Lett, 2001. **120**(1-3): p. 343-51.

51. Costa, L.G., *Current issues in organophosphate toxicology.* Clin Chim Acta, 2006. **366**(1-2): p. 1-13.

52. Sudakin, D.L. and D.L. Stone, *Dialkyl phosphates as biomarkers of organophosphates: the current divide between epidemiology and clinical toxicology.* Clin Toxicol (Phila), 2011. **49**(9): p. 771-81.

53. Salameh, P.R., et al., *Respiratory symptoms in children and exposure to pesticides.* Eur Respir J, 2003. **22**(3): p. 507-12.

54. Sunyer, J., et al., *DDE in mothers' blood during pregnancy and lower respiratory tract infections in their infants.* Epidemiology, 2010. **21**(5): p. 729-35.

55. Karpati, A.M., et al., *Pesticide spraying for West Nile virus control and emergency department asthma visits in New York City, 2000.* Environ Health Perspect, 2004. **112**(11): p. 1183-7.

56. O'Sullivan, B.C., et al., *The effect of pesticide spraying on the rate and severity of ED asthma.* Am J Emerg Med, 2005. **23**(4): p. 463-7.

57. Sunyer, J., et al., *Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six.* Clin Exp Allergy, 2006. **36**(10): p. 1236-41.

58. Sunyer, J., et al., *Prenatal dichlorodiphenyldichloroethylene (DDE) and asthma in children.* Environ Health Perspect, 2005. **113**(12): p. 1787-90.

59. Tagiyeva, N., et al., *Parental occupation is a risk factor for childhood wheeze and asthma.* Eur Respir J, 2010. **35**(5): p. 987-93.

60. Salam, M.T., et al., *Early-life environmental risk factors for asthma: findings from the Children's Health Study.* Environ Health Perspect, 2004. **112**(6): p. 760-5.

61. Salameh, P., et al., *Respiratory diseases and pesticide exposure: a case-control study in Lebanon.* J Epidemiol Community Health, 2006. **60**(3): p. 256-61.

62. Salameh, P.R., et al., *Chronic bronchitis and pesticide exposure: a case-control study in Lebanon.* Eur J Epidemiol, 2006. **21**(9): p. 681-8.

63. *About Pesticides-Ministry of Agriculture, British Columbia, Canada*. [cited 2013 July 8, 2013]; Available from: http://www.agf.gov.bc.ca/pesticides/a_3.htm.

64. *Pest Management Regulatory Agency*. 2014 [cited 2014 July 24, 2014]; Available from: http://www.hc-sc.gc.ca/ahc-asc/branch-dirgen/pmra-arla/index-eng.php.

65. Health Canada. *Report on Human Biomonitoring of Environmental Chemicals in Canada-Results of the Canadian Health Measures Survey Cycle 1 (2007-2009)*. 2010 [cited 2012; Available from: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms/index-eng.php.

66. Haines, D.A. and J. Murray, *Human biomonitoring of environmental chemicals--early results of the 2007-2009 Canadian Health Measures Survey for males and females.* Int J Hyg Environ Health, 2012. **215**(2): p. 133-7.

67. *Reregistration Eligibility Decision for Chlorpyrifos*. 2006; Available from: http://www.epa.gov/oppsrrd1/REDs/chlorpyrifos_ired.pdf.

68. PMRA, *Re-evaluation note: Update on re-evaluation of azinphos-methyl.* 2007, Pest Management Regulatory Agency.

69. Manno, M., et al., *Biomonitoring for occupational health risk assessment (BOHRA).* Toxicol Lett, 2010. **192**(1): p. 3-16.

70. Angerer, J., U. Ewers, and M. Wilhelm, *Human biomonitoring: state of the art.* Int J Hyg Environ Health, 2007. **210**(3-4): p. 201-28.

71. Barr, D.B., et al., *Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the U.S. population.* Environ Health Perspect, 2004. **112**(2): p. 186-200.

72. Barr, D.B., et al., *Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999-2002.* Environ Health Perspect, 2010. **118**(6): p. 742-8.

73. Barr, D.B., et al., *Concentrations of selective metabolites of organophosphorus pesticides in the United States population.* Environ Res, 2005. **99**(3): p. 314-26.

74. Fréry N, S.A., Garnier R, Zeghnoun A, Falq G, Guldner L., *Exposure of the French population to environmental pollutants — environmental components of the French National Survey on Nutrition and Health — initial results. Saint-Maurice (Fra)*, in *French Institute for Public Health Surveillance*. 2010.

75. Hernandez, A.F., T. Parron, and R. Alarcon, *Pesticides and asthma.* Curr Opin Allergy Clin Immunol, 2011. **11**(2): p. 90-6.

76. van Balen, E.C., M.J. Wolansky, and T. Kosatsky, *Increasing use of pyrethroids in Canadian households: should we be concerned?* Can J Public Health, 2012. **103**(6): p. e404-7.

77. *Private Property Pesticide By-laws In Canada*. 2010 [cited 2014 September 16, 2014]; Available from: www.flora.org/healthyottawa/BylawList.pdf.

78. *Ban on the sale of weed and feed lawn products in the province, effective January 1, 2010*. 2010; Available from: http://esrd.alberta.ca/focus/state-of-the-environment/water/surface-water/condition-indicators/river-water-quality-indices/pesticide-index.aspx.

79. *Alberta bans weed and feed lawn products*. 2008; Available from: http://www.cbc.ca/news/canada/calgary/alberta-bans-weed-and-feed-lawn-products-1.757076.

80. *Food and Agriculture Organization of the United Nations (FAO)-Database on pesticides consumption*. 2006 [cited 2013 July 8, 2013]; Available from: http://www.fao.org/waicent/FAOINFO/economic/pesticid.htm.

81. *Food and Agriculture Organization of the United Nations (FAO)-FAOSTAT*. 2006 [cited 2013 July 8, 2013]; Available from: http://faostat.fao.org/site/424/default.aspx#ancor.

82. *Pesticide Market Estimates: 2006-2007*. . 2011 [cited 2012 Dec 14]; Available from: http://www.epa.gov/pesticides/pestsales/07pestsales/table_of_contents2007.htm.

83. Boobis, A.R., et al., *Cumulative risk assessment of pesticide residues in food.* Toxicol Lett, 2008. **180**(2): p. 137-50.

84. Neidert E, G., Havelock G, *Report on levels and incidences of pesticide residues in selected agricultural food commodities available in Canada during 1994–1998*. 1998, Canadian Food Inspection Agency: Ottawa.

85. Carson, R., *Silent Spring*. 1st ed. 1962, Boston: Houghton Mifflin.

APPENDIX

ETHICS APPROVAL FOR THE STUDY FROM THE UNIVERSITY OF ALBERTA

Notification of Approval

Date:	March 24, 2014
Study ID:	Pro00045536
Principal Investigator:	Ambikaipakan Senthilselvan
Study Title:	Effect of environmental chemicals on respiratory diseases and lung function in Canadian general populations: a cross-sectional study using data from CHMS Cycles 1 and 2
Approval Expiry Date:	March 23, 2015

Thank you for submitting the above study to the Health Research Ethics Board - Health Panel. Your application has been reviewed and approved on behalf of the committee.

- This study involves the secondary analysis of anonymous Canadian Measures Health Survey (CMHS) data obtained from StatsCan.

A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date, you will have to re-submit an ethics application.

Approval by the Health Research Ethics Board does not encompass authorization to access the patients, staff or resources of Alberta Health Services or other local health care institutions for the purposes of the research. Enquiries regarding Alberta Health Services approvals should be directed to (780) 407-6041. Enquiries regarding Covenant Health should be directed to (780) 735-2274.

Sincerely,

Anthony S. Joyce, Ph.D.
Chair, Health Research Ethics Board - Health Panel

Note: This correspondence includes an electronic signature (validation and approval via an online system).