

# **Persistent organic pollutants and type 2 diabetes mellitus**

**by**

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## Abstract

This study quantified the associations between plasma concentrations of persistent organic pollutants (POP) and type 2 diabetes (T2D) in cycle 1 (2007-2009) of the Canadian Health Measures Survey, a cross-sectional sample representative of 96% of Canadians. The sample included 1,612 participants aged 20 to 79. The analyses include nineteen POPs detectable in at least 60% of plasma samples. After adjusting for obesity, body mass index, daily leisure energy expenditure and age, a significant ( $p < 0.05$ ) association was observed with polychlorinated biphenyl (PCB) congeners 153, 170, 180, and with organochlorine pesticides including  $\beta$ -hexachlorocyclohexane, and hexachlorobenzene. The summed measures of non-dioxin-like PCBs, and PCB 1260 (Aroclor) were also significantly associated with T2D. The summed plasma levels did not interact with the association of obesity and T2D. Exposure to these POPs should be considered when assessing risk factors for and policies to reduce, T2D and potentially other chronic diseases.

**Keywords:** Canada; diabetes; environment and public health; environmental exposure; organochlorines; persistent organic pollutants

*In dedication to my parents, Gordon and Joyce  
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# Table of Contents

Approval .....	ii
Partial Copyright Licence .....	iii
Abstract .....	iv
Dedication .....	v
Acknowledgements .....	vi
Table of Contents .....	vii
List of Tables .....	x
List of Figures .....	xii
List of Symbols .....	xiii
List of Acronyms or Glossary .....	xiii
<b>1. Introduction .....</b>	<b>1</b>
1.1. Overview .....	1
1.2. The Epidemic of Type 2 Diabetes Mellitus .....	1
<b>2. The Association of POPs with IR and T2D.....</b>	<b>3</b>
2.1. What Are Persistent Organic Pollutants? .....	5
2.1.1. Organochlorine Pesticides .....	5
2.1.1.1. <i>p,p'</i> -Dichlorodiphenyldichloroethylene (CASRN 72-55-9).....	6
2.1.1.2. Chlordane (CASRN 57-74-9).....	9
2.1.1.3. Hexachlorobenzene (CASRN 118-74-1) .....	12
2.1.1.4. Hexachlorocyclohexane.....	14
2.1.2. Polychlorinated Biphenyls (PCBs).....	16
2.1.3. Polybrominated Diphenyl Ethers .....	19
2.2. Evidence of T2D Associated with Higher-Dose Exposures to POPs in Human Populations.....	22
2.2.1. Higher-Dose Exposures From Severe Occupational and Environmental Contamination .....	22
2.2.2. Consumer Product Higher-Dose Exposure to POPs .....	23
2.3. Evidence of T2D Associated with Lower-Dose Exposure to POPs.....	25
2.3.1. Evidence from the U.S. NHANES.....	25
2.3.2. Findings from CARDIA .....	27
2.3.3. Results from a cross-sectional population health survey in Belgium.....	28
2.3.4. Evidence from the Inuit population of Greenland .....	29
2.4. Biological and Genetic Evidence of the Relationship Between Exposure to POPs and Metabolic Outcomes .....	32
2.4.1. Suppression of Protein Kinase B Phosphorylation in Mouse Muscle Tissue.....	33
2.4.2. Increased intestinal fat absorption and obesity in mice .....	35
2.4.3. Obesity is associated with systemic inflammation .....	36
2.4.4. Human microarray studies.....	36

<b>3.</b>	<b>Hypothesis and Research Methods.....</b>	<b>38</b>
3.1.	Hypotheses and Research Question .....	38
3.2.	Limitation on Hypothesis Testing and Analysis.....	41
3.3.	Data Source and Sample Selection .....	43
3.4.	Ethics Review and Authorization to Access Confidential Microdata .....	45
3.5.	Identifying Respondents with T2D .....	46
3.5.1.	Self-Reported Diagnosis of Diabetes by a Health Professional .....	47
3.5.2.	Glycosylated Haemoglobin (HbA1c).....	48
3.5.3.	Diabetic Medication Use.....	49
3.6.	The Measures of POP Plasma Concentrations .....	49
3.6.1.	Imputation of values below the limit of detection .....	53
3.6.2.	Groupings of POP compounds and group summed measurement.....	56
3.7.	Parameter Selection for the T2D Model Without Adjustment for POP Plasma Concentration.....	57
3.7.1.	Measures of obesity .....	57
3.7.2.	Measures of physical activity, sedentariness and fitness .....	58
3.7.3.	Measure of age.....	60
3.8.	Model Specification .....	60
3.8.1.	Models Without a Pollutant Parameter .....	61
3.8.2.	Models Including a Pollutant Parameter.....	62
3.9.	Other Potential Confounders.....	64
3.10.	Design-based analysis .....	65
<b>4.</b>	<b>Findings from the analyses.....</b>	<b>67</b>
4.1.	Population Characteristics .....	67
4.2.	Plasma Concentrations of POPs among Canadian Adults .....	71
4.3.	Models for T2D without Adjustment for POP Plasma Concentration .....	75
4.4.	Models for T2D Including POP Compound Plasma Concentrations.....	78
4.5.	Models for T2D Including Plasma Concentrations of Molar Weight Sums of POP Groupings.....	83
4.5.1.	Multiple POP Groupings in the Models for T2D.....	86
4.6.	Do POP Plasma Concentrations Influence the Association of Obesity or Age with T2D?.....	87
4.7.	Model diagnostics .....	89
4.7.1.	Goodness of fit .....	89
4.7.2.	Likelihood ratio tests for nested models .....	90
4.7.3.	Linearity.....	90
4.7.4.	Influential observations.....	91
4.7.5.	Collinearity.....	91
<b>5.</b>	<b>Public Policy concerning POPs and Public Health.....</b>	<b>93</b>
5.1.	Public Policy for the 'Virtual Elimination' of POPs.....	93
5.2.	Disposal of POPs and Environmental Contamination.....	98
5.2.1.	Landfill Disposal of POP Contaminated Waste .....	99
5.2.2.	Discharge of POPs into Aquatic Systems .....	100
5.2.3.	Discharge of POPs through Thermal Processes.....	101
5.2.4.	Contamination from Recycling of Electronic and Metal Waste.....	102
5.2.5.	Future Challenges from Sites Contaminated with POPs.....	102

5.3. Assessing Risk Associated with Exposure to POPs .....	107
5.3.1. Lower-Dose Effects .....	107
5.3.2. TEQs and Risk Assessment.....	110
5.4. Reducing Human Exposure to POPs and Competing Risks.....	114
5.4.1. Fish consumption and T2D in Human Populations .....	114
5.4.2. Aboriginal Community Food Systems and POPs .....	116
5.5. Lessons Learned and New Chemical Products .....	120
<b>6. Summary and Limitations .....</b>	<b>122</b>
6.1. Limitation.....	122
6.2. Summary.....	125
<b>References.....</b>	<b>131</b>
<b>Appendices.....</b>	<b>162</b>
Appendix A. Supplementary Models for T2D Including POP Compound Plasma Concentrations without Interquartile Scaling .....	163
Appendix B. Supplementary Models for T2D Including POP Compound Plasma Concentrations without Lipid Adjustment .....	166
Appendix C. Environment Canada’s Toxic Substances Management Policy: Track 1: Virtual Elimination from the Environment .....	169

## List of Tables

Table 1.	POPs included in the analysis showing percentage of the sample below the limit of detection (<LOD).....	52
Table 2.	Groupings of Persistent Organic Pollutants Included in the Analysis.....	57
Table 3.	Criteria Used for Identifying Type 2 Diabetics .....	68
Table 4.	Population estimates of obesity, daily energy expenditure and age by diabetic status, 20-79 year-olds, Canada.....	70
Table 5.	Number and Percentage of Males and Females by T2D .....	71
Table 6.	Arithmetic and Geometric Means and Standard Deviations of Individual POP Compounds* .....	72
Table 7a.	Pearson Coefficients and (p-values) of Organochlorine pesticides.....	73
Table 7b.	Pearson Coefficients and (p-values) of Non Dioxin-Like PCBs.....	74
Table 7c.	Pearson Coefficients and (p-values) of Dioxin-Like PCBs .....	75
Table 7d.	Pearson Coefficients and (p-values) of POP Groupings <sup>a</sup> .....	75
Table 8a.	Model 1 OR of T2D with Selected Risk Factors .....	76
Table 8b.	Model 2 OR of T2D with Selected Risk Factors .....	76
Table 9a.	Model 1 OR of T2D with Selected Risk Factors including Sex.....	77
Table 9b.	Model 2 OR of T2D with Selected Risk Factors including Sex.....	78
Table 10a.	Model 3 OR of T2D by inter-quartile plasma POP concentrations in individual models.....	80
Table 10b.	Model 4 OR of T2D by inter-quartile plasma POP concentrations in individual models.....	81
Table 11a.	Model 5 OR of T2D by inter-quartile summed molar weights of POP grouping in individual models.....	85
Table 11b.	Model 6 OR of T2D by inter-quartile summed molar weights of POP grouping in individual models.....	86
Table 12.	OR of T2D Stratified by Age-Adjusted Summed OCs and PCBs Plasma Concentration .....	89

Table 13a. Model 3 OR of T2D by plasma POP concentrations in individual models.....	163
Table 13b. Model 4 OR of T2D by plasma POP concentrations in individual models.....	164
Table 14a. Model 5 OR of T2D by summed molar weights of POP grouping in individual models.....	165
Table 14b. Model 6 OR of T2D by summed molar weights of POP grouping in individual models.....	165
Table 15a. Model 3 OR of T2D by inter-quartile plasma POP concentrations without lipid adjustment in individual models .....	166
Table 15b. Model 4 OR of T2D by inter-quartile plasma POP concentrations without lipid adjustment in individual models .....	167
Table 16a. Model 5 OR of T2D by inter-quartile summed molar weights of POP groupings without lipid adjustment in individual models.....	168
Table 16b. Model 6 OR of T2D by inter-quartile summed molar weights of POP groupings without lipid adjustment in individual models.....	168
Table 17. Criteria for the Selection of Substances for Track 1 .....	169

## List of Figures

Figure 1. Population Percentiles of Lipid-Adjusted Plasma Concentration of PCB 153.....	4
Figure 2. Directed Acyclic Graph of Type 2 Diabetes Mellitus and Risk Factors.....	39
Figure 3. Sample size of the CHMS and the analysis .....	45

## List of Symbols

$\alpha$	alpha
$\beta$	beta
$\gamma$	gamma
‡	$p \leq 0.001$
†	$p \leq 0.01$
*	$p \leq 0.05$

## List of Acronyms or Glossary

ARET	Accelerated Reduction/Elimination of Toxics Program
ATC	anatomical therapeutic chemical classification
$\beta$ HCH	$\beta$ -hexachlorocyclohexane
BMI	body mass index
CASRN	Chemical Abstract Service Registry Number of the American Chemical Society
CEPA	Canadian Environmental Protection Act
CHMS	Canadian Health Measures Survey
CL	95% confidence limits
CVD	cardio-vascular disease
DAG	directed acyclic graph
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DL-PCB	dioxin-like polychlorinated biphenyl
EDC	endocrine disrupting chemical
GLBTS	Great Lakes Binational Toxics Strategy
HbA1c	glycosylated haemoglobin
HCB	hexachlorobenzene
HOMA	homeostasis model adjustment

HxCDD	1,2,3,4,7,8-hexachlorodibenzo- <i>p</i> -dioxin
IARC	International Agency for Research on Cancer
IGT	impaired glucose tolerance
IGTT	impaired glucose tolerance test
IJC	International Joint Commission
IR	insulin resistance
IUPAC	International Union of Pure and Applied Chemistry
LOAEL	lowest observed adverse effect level
MRL	Maximum Residue Limits in foods as established by Health Canada's Food and Drug Regulations
NARAP	North American Regional Action Plans
NDL-PCB	non-dioxin-like polychlorinated biphenyl
NHANES	National Health, Nutrition and Examination Survey
NOAEL	no observed adverse effect level
OCP	organochlorine pesticide
OCHL	oxychlorane
OR	odds ratio
PBB	polybrominated biphenyls
PBDE	polybrominated diphenyl ethers
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo- <i>p</i> -dioxin
PCDF	polychlorinated dibenzofuran
PFC	perfluorinated compounds
PKB	protein kinase B
POP	persistent organic pollutant
PPT	parts per trillion
PUFA	<i>n</i> -3 polyunsaturated fatty acids
SMCI	Sound Management of Chemicals Initiative
T2D	type 2 diabetes mellitus
TCDD	tetrachlorodibenzo- <i>p</i> -dioxin
TDI	total daily intake
TDS	Canadian Total Diet Study
TEF	toxic equivalent factor
TEQ	toxic equivalence quotient

TNAC	trans-nonachlor
TSMP	Toxic Substances Management Policy
UNEP	United Nations Environment Program
WC	waist circumference
WHR	waist to hip ratio

# **1. Introduction**

## **1.1. Overview**

The introductory chapter describes the current epidemic of type 2 diabetes mellitus (T2D) and the etiologic factors underlying this epidemic. This chapter concludes with the hypothesis that environmental pollutants may be a factor in this epidemic. Previously published evidence on the possible relationship between levels of persistent organic pollutants (POP) and impaired glucose tolerance (IGT, also known as pre-diabetes) or T2D is presented in chapter 2. This review discusses the epidemiological and experimental studies of this relationship. A possible biological pathway from exposure to POPs to impairment of glucose homeostasis is considered here.

Chapter 3 presents a hypothesis of how the bioaccumulation of POPs in human is associated with the odds ratio (OR) of having T2D in the population. The research design, methods and data used to address the proposed research questions are also presented in chapter 3. The findings from the analyses are provided in chapter 4. Finally, a discussion of public health implications and policy considerations arising from the hypothesis and findings concludes this study in chapter 5.

## **1.2. The Epidemic of Type 2 Diabetes Mellitus**

Impairment of glucose homeostasis increases the risk for developing T2D, cardiovascular diseases, non-alcoholic fatty liver disease, polycystic ovarian disease, and certain types of cancer.<sup>1,2</sup> The inhibition of glucose and lipid metabolism is often referred to as insulin resistance (IR).<sup>3</sup> IR is commonly associated with other conditions including abdominal obesity, dyslipidemia, and hypertension. When these conditions are experienced together, they are collectively known as the metabolic syndrome.<sup>4</sup> The

metabolic syndrome is a common risk factor for the development of T2D and cardiovascular diseases such as atherosclerosis.<sup>4</sup>

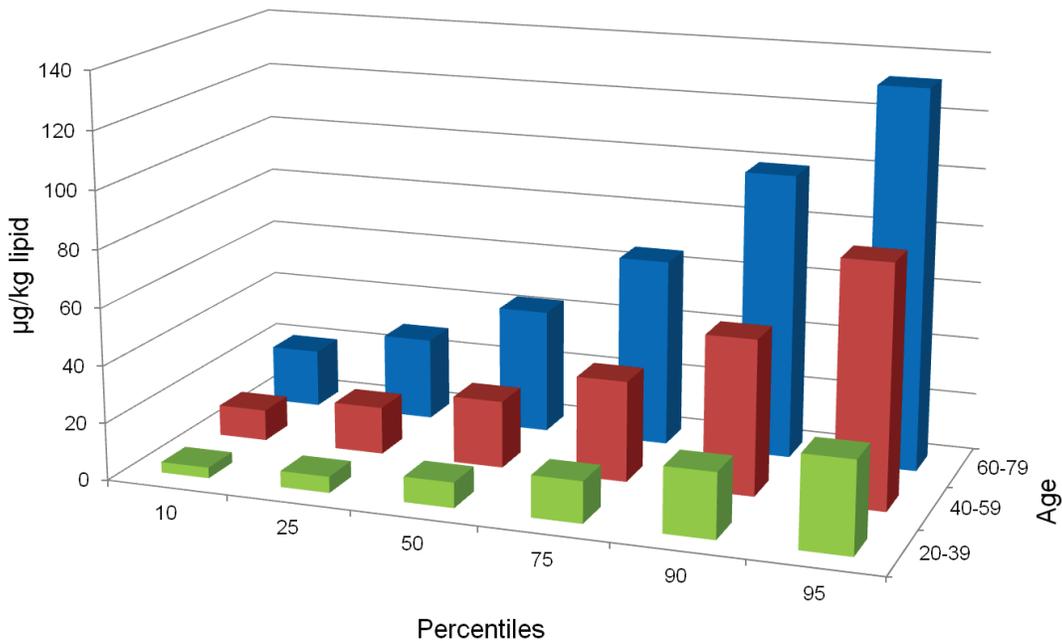
The incidence of IR, T2D, and the metabolic syndrome is increasing throughout the world and presents substantial public health challenges.<sup>3,5,6</sup> Globally, the prevalence of T2D among adults from 20 to 79 years-old was estimated to be 6.4% or 285 million people in 2010. This prevalence is expected to rise to 7.7% or 439 million adults by 2030. This increase is predicted to be higher in developing countries at 69% compared with a 20% rise in developed countries.<sup>7</sup> More than one-quarter of American adults have been estimated to be affected by metabolic abnormalities associated with IR.<sup>6</sup> Understanding the aetiology of T2D is important for identifying the possible factors driving the increasing prevalence of T2D.

Identifying the complex aetiology of T2D requires a comprehensive examination of genetic, physiological, nutritional, behavioural, and environment factors and their interactions. Evidence of the genetic, physiological and nutritional factors associated with T2D is well established. Factors such as a sedentary lifestyle and a high-fat diet are major contributors to both T2D and obesity.<sup>8,9</sup> The effects of environmental factors may further explain the rising incidence of T2D and related metabolic conditions.<sup>10-17</sup> There is a growing body of epidemiological and toxicological evidence to support the hypothesis that persistent organic pollutants (POPs) also contribute to increased diabetes risk. Positive associations between POPs and diabetes or insulin resistance have been identified in multiple epidemiological studies in diverse populations<sup>18</sup>. Recent experimental evidence suggests that environmentally relevant concentrations of POPs may antagonize insulin signalling through the important Akt/PI3 pathway<sup>10,11</sup>. The aim of this analysis was to quantify associations between plasma concentrations of POPs and T2D in the Canadian adult population (20-79 years-old) using data from Cycle 1 (2007-2009) of the Canadian Health Measures Survey (CHMS). These data were well-suited to test the hypothesis that some halogenated organic substances (especially organochlorines) would be positively associated with the OR of T2D in Canadians after controlling for important potentially confounding variables. The following chapter discusses the empirical evidence concerning the association of POPs with the metabolic conditions of IR and T2D.

## 2. The Association of POPs with IR and T2D

This chapter begins with a review of uses and environmental sources of each POP compound included in the analyses followed by a review of the epidemiological evidence of an association between impaired glucose tolerance and exposure to POP. Later in the chapter is a review of experimental studies to assess the biological plausibility of this association.

POPs include organochlorine pesticides (OCP), polycarbonated biphenyls (PCB) and polybrominated diphenyl ether (PBDE) flame retardants. These chemical compounds are lipophilic which leads to their concentration in the lipid fraction of cells. These compounds are also chemically resistant to environmental and metabolic degradation and therefore persist in the environment and cells, and consequently bioaccumulate with age.<sup>19</sup> Figure 1 provides an indication of the bioaccumulation of a POP compound. Polychlorinated biphenyl congener #153 is one of the PCB congeners often analyzed in epidemiological studies of POPs and population health outcomes. The lipid-adjusted plasma concentration of PCB 153 is positively associated with age as shown in Figure 1. The pattern of increased plasma concentration of PCB 153 among older adults is similar for other POP compounds. The increase of plasma concentration with age is indicative of greater lifetime exposure to POPs among older individuals. Based upon our knowledge of the kinetics of POPs bioaccumulation, metabolism and excretion, it also may indicate that exposure exceeds excretion in the general population.<sup>20</sup> However, it is important to note the data shown in Figure 1 from the Canadian Health Measures Survey are cross-sectional. Thus, older age groups may have experienced higher exposures to POPs earlier in their lives than is currently experienced by younger adults.



**Figure 1. Population Percentiles of Lipid-Adjusted Plasma Concentration of PCB 153**

Dietary consumption of animal and plant products containing POPs is estimated to account for more than 90% of total human exposure.<sup>21</sup> Exposure through dermis contact and inhalation comprises the remainder of human exposure to POPs. However, diet likely accounts for relatively more exposure in North America and Europe than in Asia and Africa where organochlorine pesticides are still used in some regions for malarial and agricultural pest control.<sup>21,22</sup> Exposures have also occurred through certain work environments, industrial accidents and higher-dose contaminations of specific environments or food products. For example, occupational exposure to organochlorines was common through the manufacture and use of organochlorine and dioxin-like herbicides.<sup>23</sup> Accidental contamination results from improper use, storage or disposal of POPs. Occupational and accidental exposures have decreased since the 1990's when many governments began prohibiting the production and use of these chemicals through the Stockholm Convention on Persistent Organic Pollutants.<sup>24,25</sup>

## **2.1. What Are Persistent Organic Pollutants?**

POPs are synthetically produced chemicals that were widely used in industrial equipment, agricultural and domestic pesticides and flame retardant materials. These compounds are classified as organic because of their carbon ring molecular structures. POP compounds are resilient to environmental degradation through chemical, biological, and photolytic processes.<sup>26</sup> POPs have relatively long-half lives. Given their long-half lives, lipophilicity, and resilience to degradation by environmental or biological processes, POPs have been observed to persist in the environment, to be capable of long-range transport, bioaccumulate in human and animal tissue, and biomagnify in food chains.<sup>26</sup>

Contaminants, such as organochlorine pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers have been detected in fish, eggs, meat and rice in a number of countries including the USA, Germany, Belgium and Spain.<sup>27-30</sup> In Hong Kong higher concentrations of PAHs, DDTs, PCBs, PBDEs and PCDD/Fs have been observed in fish, meat, egg, milk, cooking oil, nuts, vegetable and rice sold in local markets.<sup>31-35</sup> These measured exposures may be related to both local and distant contamination events. For example, contamination of food chains in the Arctic and Antarctic regions can only be from transcontinental transport of these compounds and migration of marine and bird species. POPs can be assumed to be ubiquitous in the environment.

The analysis in this study examines three broad groupings of POPs including organochlorine pesticides, polycarbonated biphenyls for industrial applications and polybrominated diphenyl ether flame retardants. There are other classes of chemicals identified by the Stockholm Convention as POPs. However the data used for the analysis in this study is limited to the chemicals described below.

### **2.1.1. Organochlorine Pesticides**

Organochlorine insecticides and herbicides are synthetically produced chemical compounds used mostly for agricultural insect and weed control. Many of these compounds are designated as a Track 1 substance under the Government of Canada's

Toxic Substance Management Policy with the objective of virtual elimination (i.e., no measurable releases into the environment in Canada).<sup>36</sup> Appendix B describes the criteria for inclusion as a Track 1 substance and the list of designated substances.

These compounds are commonly known by their commercial names or associated product names. A technically more precise system for uniquely identifying each distinct compound is the Chemical Abstract Service Registry Number (CASRN) system of the American Chemistry Society. The organochlorine pesticides included in the analysis are described below.

#### **2.1.1.1. *p,p'*-Dichlorodiphenyldichloroethylene (CASRN 72-55-9)**

Dichlorodiphenyltrichloroethane (DDT) has never been manufactured in Canada, but was imported and widely used for several decades across the country. It was first registered for use in Canada in 1946. As a broad-spectrum insecticide, DDT was popular due to its effectiveness, persistence, and relatively low cost. Initially it was used to control vectors of insect-borne human disease mainly mosquitoes to prevent malaria, and midge flies and lice to prevent typhus.<sup>37</sup> The use of DDT greatly expanded when it was later applied for agricultural pest control through widespread spraying and dusting of trees, fruit and other food crops. This insecticide was imported into Canada until the mid-1970s.<sup>38</sup> In the 1970s and 1980s, Canada and other countries began prohibiting the use of DDT because of the increasing awareness of the ecological and human health effects associated with exposure to DDT.<sup>37</sup> The Pest Control Products Act required all remaining stocks of DDT to be disposed by December 31, 1990. DDT continues to be used in some African countries primarily to control malaria.<sup>39</sup>

The application of DDT directly to soil has led to residues entering irrigation systems which are usually drained into surface water bodies.<sup>40</sup> These compounds are transported through and accumulate in river systems and are absorbed by animals and plants. In addition, food imported into Canada from other countries that still use DDT may contain residues of DDT or its metabolites, and therefore be a continued source of exposure.

Since DDT and its metabolites are lipophilic, these compounds bind with animal fats, particularly in fish, meat, poultry and dairy. Thus, consumption of these foods is the

primary source of exposure to DDT and its metabolites in the Canadian population. Foods with relatively higher levels of these compounds are in fish caught in the Great Lakes and St. Lawrence River.<sup>37,41,42</sup>

The transport characteristics of DDT and its metabolites lead to relatively higher exposures in northern populations, especially the Inuit who eat more wild foods such as fish, seals, and whales. These foods have higher trophic levels and therefore this population is relatively more exposed than other populations in Canada. Concentrations of DDT and DDE are relatively higher in the polar region because the semi-volatile properties of DDT enable its long range atmospheric transport to these regions. DDT is introduced into the Arctic marine ecosystem through the food web from microbial–protozoa to fish to seal to polar bears and humans.<sup>43,44</sup>

DDT is a dichlorodiphenylethane compound. The technical grade of DDT is mainly a mixture of three DDT isomers including *p,p'*-DDT (65-80%), *o,p'*-DDT (15-21%), and trace amounts of *o,o'*-DDT.<sup>45</sup> Up to fourteen different compounds usually comprised DDT.<sup>37</sup> In many studies, the term DDT refers to *p,p'*-DDT.<sup>37</sup>

When DDT breaks down in the environment, its more stable chemical forms, include:

- DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) (CASRN 72-55-9)
- DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane) (CASRN 72-54-8)

DDT is relatively unstable and therefore does not persist for long in its original chemical form in the environment and organisms. However, once broken down into its more stable forms of DDE and DDD, these compounds are highly resilient to degradation in the environment and to metabolism in organisms. The percentage of the Canadian population with detectable levels of DDT and DDE reflects the difference in persistence. The 2007-2009 Canadian Health Measures Survey found that among 20 to 79 year-olds only 9% have detectable levels of DDT while close to 99% have measurable levels of DDE.<sup>46</sup> Detectable levels of DDT indicate more recent exposure to this organochlorine insecticide in its original chemical form.

The Canadian Total Diet Study indicates that average DDT and DDE residue levels in food are generally below 1 µg/kg.<sup>47</sup> In foods where a Maximum Residue Limit (MRL) has been established by the Canadian Food and Drug Regulations of Health Canada, the detectable levels of DDT and DDE were typically less than 1% of the MRL.<sup>46</sup> Health Canada (2007) has a provisional tolerable daily intake (pTDI) for DDT of 10 µg/kg body weight/day.<sup>48</sup> DDT is considered to be of concern to the environment and to human health and is managed under Track 1 of the Government of Canada's Toxic Substance Management Policy.<sup>49</sup>

Following intake of DDT or its metabolites, the compounds are absorbed mainly through the intestinal lymphatic system. To a lesser extent, these compounds are absorbed directly into the portal blood system. DDT and its metabolites are circulated via blood to all organs. These compounds are stored in proportion to the lipid content of each organ. DDT is generally metabolized into DDE and its conjugates before excretion mainly through urine and to a lesser extent through feces, semen, and breast milk.<sup>37</sup> Human breast milk concentrations of DDT and DDE have been declining in Canada from an average concentration of 134 ng/g whole milk in 1967 to 7.5 ng/g whole milk in 1992.<sup>50</sup> In specific populations surveyed in the Canadian Arctic, DDT has been detected in both maternal blood plasma and umbilical cord plasma. The sum concentrations of DDT and its metabolites in maternal blood plasma were found to be highest in the Inuit population of the Baffin region with an average of 2.2 µg/L and ranging from 0.59 to 6.4 µg/L. In comparison, the Caucasian mothers in this region had an average sum concentration of 0.96 µg/L ranging from 0.22 to 11.3 µg/L.<sup>51</sup>

A study in 1992 of two areas in the Great Lakes region of Ontario monitored 232 anglers aged 18 to 64 years-old and their families for levels of *p,p'*-DDE in blood. Among males who ate fish from the Great Lakes, the median concentration of *p,p'*-DDE in blood plasma was 383.1 µg/kg lipid and was 292.8 µg/kg lipid among males who did not eat fish from the Great Lakes.<sup>52</sup> However, there was no difference between females who ate Great Lakes fish and females who did not. The median blood plasma level was 364.1 µg/kg lipid among females who ate Great Lakes fish and 359.8 µg/kg lipid for females who did not eat fish from the Great Lakes.

A well-documented effect of DDT and its metabolites is the impairment of nerve impulse conduction, which has been observed in both humans and animals, resulting in altered sensations, tremors, and convulsions.<sup>37</sup> Other documented toxic effects of exposure to DDE include hormone alterations during reproduction and development in animals.<sup>37,53</sup> The International Agency for Research on Cancer classifies DDT, DDE, and DDD as possible human carcinogens (Group 2B), based on sufficient evidence of carcinogenicity in animals.<sup>54</sup>

Organochlorine pesticides such as DDT continue to be used in regions of Africa and south Asia for control of malaria and related infections. The continued use of DDT is permitted by the Stockholm Convention for indoor spraying for control of vectors of malaria, dengue, visceral leishmaniasis, and Chagas disease. The Stockholm Convention exemption for DDT complies with recommendations and guidelines of the World Health Organization's Position Statement on the use of pesticides.<sup>55</sup> In 2005, the global production of DDT used for disease vector control was estimated to reach 5,000 metric tons.<sup>56</sup> An additional 160 metric tons of DDT were produced for agriculture purposes.<sup>56</sup> DDT continues to be produced in the manufacture of organochlorine pesticides including aldrin and dieldrin in developing countries.<sup>57</sup>

A prohibition of the use of DDT could have significant consequences for the spread of malaria due to the absence of equally effective and efficient alternatives.<sup>55</sup> The use of DDT in malaria prevention has led to debates over its impact on population health. DDT has been the principal method for malarial vector control and thus is important for public health in many African and south Asian countries. However, it may also induce adverse human health effects.<sup>58-60</sup>

#### **2.1.1.2. Chlordane (CASRN 57-74-9)**

Chlordane is an organochlorine pesticide with a base chemical formula of  $C_{10}H_6Cl_8$ . More than 140 structurally related compounds comprise Technical chlordane (CASRN 12789-03-6). The major components include:

- $\alpha$ -chlordane, or (1 $\alpha$ ,2 $\alpha$ ,3 $\alpha\alpha$ ,4 $\beta$ ,7 $\beta$ ,7 $\alpha\alpha$ )-1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene (CASRN 5103-71-9)
- $\gamma$ -chlordane, or (1 $\alpha$ ,2 $\beta$ ,3 $\alpha\alpha$ ,4 $\beta$ ,7 $\beta$ ,7 $\alpha\alpha$ )-1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene (CASRN 5103-74-2)

- cis-nonachlor, or (1 $\alpha$ ,2 $\alpha$ ,3 $\alpha$ ,3 $\alpha$ ,4 $\beta$ ,7 $\beta$ ,7 $\alpha$ )-1,2,3,4,5,6,7,8,8-nonachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene (CASRN 5103-73-1)
- trans-nonachlor, or (1 $\alpha$ ,2 $\beta$ ,3 $\alpha$ ,3 $\alpha$ ,4 $\beta$ ,7 $\beta$ ,7 $\alpha$ )-1,2,3,4,5,6,7,8,8-nonachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene (CASRN 39765-80-5)

Oxychlordanes are the most important of the metabolites from exposure to chlordane. The chemical compound of this metabolite is 2,3,4,5,6,6a,7,7-octachloro-1a,1b,5,5a,6,6a-hexahydro-2,5-methano-2H-indeno(1,2-b)oxirene, and its CASRN number is 27304-13-8.

Chlordane is a synthetic chemical mixture with no natural sources. Its presence in the environment is the consequence of human activities. In Canada, the use of chlordane began in the 1940's as a broad-based insecticide for a variety of agricultural crops. It was also used for residential applications such as lawns and gardens. Inside homes and other buildings, it was used for fumigation and for prevention of termite infestations.<sup>61,62</sup> Chlordane was never manufactured in Canada. Its use in Canada was discontinued in 1998. It is no longer registered for use as a pesticide in Canada and cannot be imported to or exported from Canada.<sup>25</sup> Production, sale, and use of chlordane in the United States was prohibited a decade earlier in 1988.<sup>61</sup>

Chlordane has been detected in all environmental media. While its use has been discontinued in Canada, chlordane compounds are very resistant to degradation.<sup>61</sup> These chemicals are reported to have long-range transport possibly through river flows, atmospheric winds and migrating marine species and birds.<sup>61</sup> Chlordane can remain for decades in soils where it was previously applied. These residues can persist in soils for more than twenty years due to their estimated one-year half-life in soil,<sup>63</sup> strong adsorption onto organic substrates, and very low solubility in water.<sup>61</sup> Chlordane may enter water bodies through leaching from soils and subsequent transport through groundwater, or by deposition from the atmosphere. Due to its low solubility, once in the water, chlordane tends to bind to sediments<sup>63</sup>, and therefore chlordane compounds are not normally detected in drinking water.<sup>39</sup>

Food crops grown in these soils continue to show detectable concentrations long after use was discontinued.<sup>61</sup> Consequently, occupational exposure may occur with individuals working in agricultural areas where chlordane has previously been applied.

Due to the prohibition on use in Canada and the United States, environmental concentrations are expected to continue to gradually decrease over time, and the potential for new industrial releases is low in Canada and the United States.<sup>61</sup>

Chlordane can be absorbed through oral, dermal, and inhalation exposure. The principal source of exposure to the public is through ingestion of foods containing traces of chlordane.<sup>61</sup> Once absorbed, chlordane isomers are preferentially metabolized into oxychlordane, and to a lesser extent, to heptachlor. Biological samples tend to contain primarily oxychlordane and nonachlor compounds.<sup>53</sup> Excretion of these chemicals from the body occurs following months to years of retention. Breast lactation is a major excretion route in females who are breast feeding a child. Thus, breast milk consumption is a major exposure source for infants who are breast fed.<sup>53</sup> Plasma samples from mothers in the Canadian Arctic in 1994–1999 consistently had detectable levels of chlordane compounds such as trans-nonachlor in 98.18% of samples, and oxychlordane in 95.58% of samples.<sup>51</sup> In a pilot study carried out in 1992 in two regions of the Great Lakes area of Ontario, 232 anglers were assessed for the levels of a number of chlordane metabolites in blood plasma. The geometric mean and maximum concentrations were respectively: 17.1 µg/kg plasma lipid and 48.4 µg/kg plasma lipid for oxychlordane, and 21.0 µg/kg plasma lipid and 116.2 µg/kg plasma lipid for trans-nonachlor.<sup>64</sup>

High dose exposures to chlordane compounds are associated with negative effects on the nervous system, digestive system, and liver.<sup>61</sup> The toxicity of chlordane contamination is significantly influenced by environmental and biological degradation processes that have taken place, which are often isomer specific.<sup>61</sup>

The International Agency for Research on Cancer classifies chlordane as a possible human carcinogen (Group 2B) based on evidence from rodent studies on liver cancer.<sup>65</sup> The United States Environmental Protection Agency classifies chlordane as Group B2, a probable human carcinogen.<sup>66</sup>

Chlordane pesticides are not registered for use or sale in Canada by the Pest Management Regulatory Agency.<sup>67</sup> Chlordane is classified as a POP and as a severe marine pollutant by the Stockholm Convention, Health Canada and Environment

Canada. The Government of Canada conducted a screening assessment that concluded technical grade chlordane is of concern for the health of non-human organisms. However, no further action is deemed necessary for chlordane compounds since Canadian industries no longer import, export or manufacture.<sup>68</sup>

#### **2.1.1.3. Hexachlorobenzene (CASRN 118-74-1)**

Another synthetic organochlorine pesticide included in the analysis is hexachlorobenzene (HCB) which is also known as pentachlorophenyl chloride. HCB has the molecular formula  $C_6Cl_6$ . This compound does not occur naturally in the environment. Previously, HCB was used in pesticides, fireworks, ammunition, synthetic rubber, wood preservative, dielectric fluids, and aluminum fluxing agents.<sup>69-71</sup> In Canada from the 1940s through 1970s, the primary application was as a fungicide treatment for grain seeds.<sup>69</sup> Commercial use of HCB has been prohibited in Canada since 1976.<sup>25</sup> Production of HCB in North America ended in the late 1970s.<sup>69</sup>

Up until the late 1970s, HCB entered the environment through agricultural applications and disposal of industrial and commercial waste.<sup>69</sup> Since the prohibition of the production and use of HCB in North America, environmental contamination has occurred from the manufacture and use of chlorinated solvents and pesticides that contain HCB impurities, and from industrial and incineration emissions that produce HCB due to incomplete combustion.<sup>72</sup> Once in the environment, the HCB compound is very resistant to environmental and metabolic degradation making it highly persistent in all environmental media.

The Canadian population is primarily exposed to HCB through the consumption of animal fat, particularly from animals fed crops grown in soil contaminated by HCB. Canadians are also exposed to HCB through the consumption of crops grown in contaminated soils. The 1998 Canadian Total Diet Study found concentrations of HCB in food ranging from 0.29 parts per billion (ppb) in fish to 1.00 ppb in butter.<sup>73</sup>

After ingestion, the concentration of HCB varies with the fat content of various body tissues.<sup>74</sup> The slow metabolic breakdown of this compound leads to bioaccumulation in adipose tissue.<sup>75</sup> The whole body half-life of HCB is estimated to be

six years.<sup>76</sup> The HCB parent compound is primarily eliminated through feces. Only a smaller portion of HCB is eliminated as metabolites through urine and feces.<sup>77</sup>

Following the prohibition on use of HCB in Canada and the United States, the concentrations of HCB in human breast milk declined in southern Canada from an average concentration of 2 µg/L in 1975 to 0.44 µg/L in 1992.<sup>78</sup> Concentration levels vary across region and population groups reflecting differences in exposure and in diet. Inuit and other northern Aboriginal populations rely substantially on animal fat in their diet from fish, marine mammals, and other animals. In the Canadian Arctic from 1995 to 1999, concentrations of HCB in maternal blood plasma were highest in the Inuit population of the Northwest Territories and Nunavut with a geometric mean of 0.47 µg/L from a minimum level of 0.05 to a maximum of 4.51 µg/L, compared to the northern Caucasian population with a geometric mean of 0.12 µg/L ranging from 0.04–0.61 µg/L.<sup>51</sup> The higher geometric mean for Inuit mothers reflects their higher proportion of animal fat, particularly from marine mammals, in the diet of their communities.

Animal studies of long-term consumption of large amounts of HCB have reported damage to the liver, kidney, and thyroid including tumours in each of these organs, and damage to the nervous system.<sup>69,77,79,80</sup> Evidence of the health conditions associated with high-dose dietary exposure to HCB over several years among humans is from a food contamination situation in Turkey. HCB-treated seeds intended for use in agriculture were instead sold for human consumption over a period of several years. This prolonged high-dose exposure to HCB was associated with increased OR of liver disease, higher death rates in young children, skin lesions, hyperpigmentation, hirsutism, colic, weakness, enlarged thyroid, and porphyrinuria.<sup>69,81</sup>

Environment Canada and Health Canada concluded that HCB is of concern to the environment and to human health.<sup>72</sup> Health Canada has classified HCB as a probable human carcinogen (Group II), based on inadequate data on humans, and sufficient evidence of carcinogenicity in several rodent studies of high dose exposures over the animals' lifetimes.<sup>72</sup> Health Canada has established a provisional tolerable daily intake (pTDI) for HCB of 0.27 µg/kg body weight/day.<sup>48</sup>

#### 2.1.1.4. Hexachlorocyclohexane

Hexachlorocyclohexane (HCH) is also known as benzenehexachloride. HCH has the chemical formula  $C_6H_6Cl_6$ . HCH is an organochlorine compound with eight isomers including  $\alpha$ -HCH [CASRN 319-84-6],  $\beta$ -HCH [CASRN 319-85-7],  $\gamma$ -HCH [CASRN 58-89-9], and others. The isomers are distinguished by the position of chlorine atoms on a six-carbon ring. HCH is a synthetic chemical with no natural sources.

The first pesticides produced with HCH were in the form of a technical mixture of several HCH isomers. Currently, only the  $\gamma$ -HCH isomer continues to be used in pesticides. Most of the pesticide potency is found in the  $\gamma$  HCH isomer. The pure  $\gamma$ -HCH isomer compound is commonly known as lindane.<sup>82,83</sup>

The release of HCH into the environment began in Canada with its application as a pesticide for agricultural crops. The most extensive application of HCH occurred on canola crops on the Prairies.<sup>82</sup> Another important source of exposure, particularly in northern regions, is the atmospheric transport of HCH isomers around the globe.<sup>84</sup> Environmental levels of  $\gamma$ -HCH could be expected to be more widely detectable and relatively higher than other HCH isomers since lindane remains the only approved product containing HCH in Canada. However, the world-wide extensive use of the HCH technical mixture as an agricultural pesticide for several decades has led to many HCH isomers being detected in environments throughout Canada and around the world. The very high persistence of  $\beta$ -HCH and its sub-compound of  $\alpha$ -HCH as compared to  $\gamma$ -HCH results in  $\beta$ -HCH being the most widely detected HCH isomer with the highest environmental concentrations of the isomers.<sup>82</sup>

Canadians are primarily exposed to HCH isomers through consumption of food with HCH residues. Other sources of exposure to HCH isomers include drinking water and inhalation of ambient air. The use of prescription treatments for head lice and scabies can result in short-term exposure to  $\gamma$ -HCH.<sup>82</sup>

HCH isomers have been detected in a wide range of foods including dairy products, meat, fish, poultry, fruits, vegetables, peanuts, seeds, sugars, oils, and fats.<sup>85,86</sup> The Canadian Total Diet Study found the average concentrations of HCH residue in food are generally below 1  $\mu\text{g}/\text{kg}$ , although in some years, concentrations up

to 8 µg/kg were found in peanuts, peanut butter, and chocolate bars.<sup>86</sup> The detectable levels of HCH residues in foods were typically found to be less than 1% of the Maximum Residue Limit (MRL) as established by Health Canada's Food and Drug Regulations.

When consumed and absorbed by humans, HCH is metabolized in the liver prior to elimination in urine. However, many of these metabolites excreted in urine are not solely produced from HCH. Therefore, the blood concentrations of HCH isomers are a better indicator of tissue levels of HCH than are metabolites in urine. Blood plasma concentrations are much higher for β-HCH than for other isomers because of the very long lasting persistence of β-HCH. The blood elimination half-life of the β-HCH isomer is approximately seven years, whereas the blood elimination half-life of the γ-HCH isomer is only 20 hours due mostly to excretion.<sup>53,82</sup>

Following the restrictions on the use of HCH for agricultural applications, the concentrations of β-HCH in human breast milk in Canada decreased from 8 ng/g whole milk in 1982 to 0.71 ng/g whole milk in 1992.<sup>78</sup> Unlike other organochlorine pesticides, the maternal blood and umbilical cord blood concentrations of β-HCH in the Canadian Arctic from 1995 to 1999 were similar between Inuit mothers and Caucasian mothers both with a geometric mean of 0.09 µg/L. There were non-detectable levels in maternal blood and umbilical cord blood for some Inuit and some Caucasian mothers. The maximum maternal blood concentrations observed were similar for Inuit mothers at 0.44 µg/L and for Caucasian mothers at 0.55 µg/L. The maximum concentrations observed for umbilical cord blood were higher among Inuit mothers at 0.31 µg/L as compared to northern Caucasian mothers at 0.14 µg/L.<sup>51</sup> The detectable levels of HCH residues in foods were typically found to be less than 1% of the Maximum Residue Limit (MRL) as established by the Health Canada's Food and Drug Regulations. Health Canada has established a provisional tolerable daily intake (pTDI) for total HCH of 0.3 mg/kg body weight per day.<sup>48</sup>

Exposure to high doses of the HCH technical mixture is associated with nervous system damage, malaise, vomiting, tremors, apprehension, confusion, loss of sleep, impaired memory, and loss of libido.<sup>82</sup> Exposure to lindane and other HCH isomers is associated with damage to the liver, kidneys, and endocrine system. An immunotoxic potential may also be associated with high-dose exposure to lindane or other HCH

isomers.<sup>82,83</sup> HCH is classified as a possible human carcinogen (Group 2B) by the International Agency for Research on Cancer based on inadequate human data but sufficient evidence in animals for the HCH technical mixture and the  $\alpha$ -HCH isomer.<sup>87</sup> The IARC reports there is comparatively less evidence of human carcinogenicity for the  $\beta$ -HCH isomer.

The Pest Management Regulatory Agency regulates the sale and use of organochlorine pesticides such as lindane in Canada. A comprehensive review of lindane was completed by the PMRA in 2002. Following from this review, the registrations of products containing  $\gamma$ -HCH were cancelled by December 31, 2004. Lindane products are no longer registered for sale and use under the Pest Control Products Act. However, lindane remains in use as a therapeutic product under the Food and Drugs Act to control lice and scabies infestations among groups of people as in a school.<sup>88,89</sup> The Food and Drug Act registration allowing for the therapeutic use of lindane is expected to be cancelled by August 2015 in compliance with an amendment to the Stockholm Convention which proposes the prohibition of the sale and use of  $\gamma$ -HCH.<sup>90</sup>

### **2.1.2. Polychlorinated Biphenyls (PCBs)**

Polychlorinated biphenyls (PCBs) are a group of synthetic chlorinated organic compounds that do not occur naturally in the environment. These compounds were produced synthetically as mixtures for a diverse range of applications until the late 1970s mainly in the United States. PCBs were also produced in West Germany, Italy, France, and Japan.<sup>91</sup> Products containing PCBs include electrical capacitors and transformers, heat transfer and hydraulic fluids, flame retardants, inks, adhesives, lubricants, surface coatings, anti-fouling agents, and plasticizers.<sup>91</sup> PCBs were never manufactured in Canada, and have not been produced in the United States since 1979.<sup>91,92</sup> Use of PCBs has been restricted in Canada since 1977 and is currently limited to products in use prior to 1977.<sup>93</sup> Since the invention of PCBs, between 1.3 to 2 million tonnes of PCBs have been produced as industrial products throughout the world.<sup>94-96</sup>

There are 209 possible chemicals called congeners within the PCB group. The basic chemical structure consists of a biphenyl molecule with up to ten chlorine atoms

substituted for hydrogen atoms at different positions around the biphenyl molecule. The PCB congeners are named based on their structure (e.g., 2,4,4'-trichlorobiphenyl), but are more commonly referred to by classification number assigned by the International Union of Pure and Applied Chemistry (IUPAC). The IUPAC classification assigns a number to each of the congeners. The IUPAC system progressively assigns higher classification numbers as the number of chlorine atoms in the congener molecule increases. Commercial mixtures of PCBs are also known by their respective trade names, including Aroclor, Chloretol, Dyknol, Inerteem, Kanechlor, Noflamol, Phenoclor, and Pyranol.<sup>91</sup>

PCBs enter the environment through their use, ineffective containment, and improper disposal. PCBs have been detected in air, water, soil, and dust. Once in the environment, PCBs are persistent and the congeners with higher numbers of chlorine atoms have longer half-lives. As a result of this persistence, PCBs in the environment are available for uptake by plants and animals, and can be magnified many fold within the food chain. Consequently, while exposure to PCBs in air, water, soil and dust may contribute to an individual's total body burden, the predominant source of exposure is through diet. Since PCBs are lipophilic and bioaccumulate in lipids, the dietary exposure to PCBs is largely through consumption of animal fats including meat, fish and dairy, as well as human breast milk.<sup>20,97</sup>

Estimated dietary intake of PCBs in the United States and Canada have decreased since at least 1978,<sup>91,97</sup> and PCB concentrations decreased substantially in Canadian mothers' breast milk between 1982 and 1992.<sup>78</sup> The 1993-1998 Total Diet Study found variation in concentrations in foods from 4.97–9.65 ng/kg body weight/day for infants, 7.22 ng/kg body weight/day for children 1–4 years, and 1.16–2.67 ng/kg body weight/day for adults 20+ years.<sup>47</sup>

PCBs are metabolized by various cytochrome P-450 enzymes to polar metabolites that can undergo conjugation with glutathione and glucuronic acid. The major routes of excretion of PCBs are feces and, especially for its metabolites, urine.<sup>91</sup>

Exposure to PCBs usually involves mixtures of individual PCB congeners. For example, PCBs 138, 153, and 180 are the most commonly found PCBs in human

tissues.<sup>91</sup> However, concentrations found in humans and other animals may not reflect the exposure profile in the environment. PCB profiles in human serum immediately following exposure reflects the profiles in the exposure sources. Yet, the selective metabolism, excretion, and deposition of PCBs in animals alter the congener profile within four to twenty-four hours following exposure. Thus, in most cases, the PCB profile in adults represents a steady state body burden that does not necessarily match the profile of environmental mixtures of commercial PCB formulations.<sup>91</sup>

The estimates to compare changes in lipid-adjusted plasma concentrations of PCBs over time in Canada are very limited. The first nationally representative survey which analyzed blood samples for PCB congener concentrations was the 2007-2009 Canadian Health Measures Survey (CHMS). The CHMS reported on 1,668 respondents, the geometric mean and 95<sup>th</sup> percentile for PCB 138 were 10.13 µg/kg lipid and 44.79 µg/kg lipid respectively.<sup>46</sup> The geometric means and 95<sup>th</sup> percentiles for plasma concentrations were 18.31 µg/kg lipid and 85.64 µg/kg lipid respectively for PCB 153, and were 15.21 µg/kg lipid and 77.33 µg/kg lipid respectively for PCB 180. Much higher levels were reported in an exposure assessment study carried out in 1992 in two separate regions in the Great Lakes area of Ontario, 232 participants were assessed for the levels of environmental contaminants in blood plasma samples. The measured respective geometric mean and maximum concentrations were 43.4 and 242.9 µg/kg lipid for PCB 138; 57.2 and 259.0 µg/kg plasma lipid for PCB 153; and 41.5 and 235.0 µg/kg lipid for PCB 180.<sup>52</sup> The Great Lakes study is much more limited in terms of region covered, sample size and representativeness. The differences in the reported plasma concentrations of PCBs indicate that plasma concentrations may have decreased over time.

PCBs are considered to be of concern to the environment and to human health and are managed under Track 1 of the Government of Canada's Toxic Substances Management Policy.<sup>93</sup> Health Canada considers PCBs as posing a high risk to human health.<sup>98</sup> PCBs are on the Export Control List in Part 2, Schedule 3 of the Canadian Environmental Protection Act, 1999 as "substances subject to notification and consent", and Environment Canada maintains an inventory of PCB use and stored PCB waste within Canada.<sup>99</sup> The International Agency for Research on Cancer classified PCBs as Group 2A (a probable human carcinogen).<sup>100</sup>

### **2.1.3. Polybrominated Diphenyl Ethers**

Two structurally closely related classes of POPs are polybrominated diphenyl ethers (PBDE) and polybrominated biphenyls (PBB). These compounds are used extensively as fire retardants. PBDEs and PBBs each have 209 different congeners. The congeners are distinguished by the number of bromine atoms and their location on the two benzene rings.

PBDEs and PBBs are synthetically manufactured and do not occur naturally in the environment. These compounds are primarily used as fire retardants in a wide range of products.<sup>101</sup> In 1999, the Government of Canada prohibited the manufacture of seven congener groupings of PBDEs through the Canadian Environmental Protection Act (CEPA).<sup>102</sup> CEPA regulates the manufacturing, sale and use of tetra-, penta-, hexa-, hepta-, octa-, nona-, and deca-BDEs. Although these compounds are prohibited from being produced in Canada, chemical formulations including resins, polymers or substrates which contain PBDEs are permitted to be imported into Canada. Semi-finished and finished products containing PBDEs are also permitted to be imported into Canada.<sup>103</sup>

CEPA also prohibits the use, sale, and import of commercial mixtures, resins, and polymers containing PBDEs that meet the criteria for virtual elimination including congeners groupings of tetra-BDE, penta-BDE, and hexa-BDE.<sup>104</sup> In 2009, the Government of Canada proposed Performance Agreements with industry to manage and reduce environmental releases of deca-PBDEs during manufacturing of plastics and textiles.<sup>105</sup> The Government of Canada also proposed regulations to control use of PBDEs in domestic and imported manufactured products.<sup>105</sup> Finally, the Canadian government also proposed a strategy to manage the disposal of products containing PBDEs at the end of the product's life.<sup>105</sup>

The penta-, octa-, and deca-BDE congeners are classified as high production volume (HPV) chemicals by the Organisation for Economic Co-operation and Development.<sup>106</sup> The deca-PBDEs are also deemed as high production volume chemicals by the United States Environmental Protection Agency.<sup>107</sup> In the United States, the use of penta- and octa-PBDEs in manufacturing was voluntarily ended in 2004.<sup>108</sup> Deca-PBDEs are the only remaining congeners still widely used in

manufacturing products.<sup>109</sup> Deca-PBDE compounds are used primarily as flame retardants in the manufacture of thermoplastics and polymer resins with broad application in many consumer products.<sup>104</sup> Prior to 2004, manufacturers used penta-BDE compounds as flame retardants in polyurethane foam used in furniture and foam padding under carpets.<sup>110</sup> Manufacturers used octa-BDEs in plastic housings, office equipment, and in electronic and electrical products.<sup>110</sup>

The higher brominated PBDE congeners have substantially shorter half-lives compared to the highly persistent lower brominated congeners.<sup>111</sup> The higher brominated congeners such as hepta-BDEs through deca-BDEs experience debromination to form lower brominated congeners from tetra-BDEs to hepta-BDEs.<sup>112</sup> Since higher brominated congeners experience debromination over time and the lower brominated congeners are much more persistent, it is principally the lower brominated congeners which bioaccumulate in adipose tissue, serum, and breast milk.<sup>111</sup> Concentrations of lower brominated congeners may indicate either environmental exposure to lower brominated PBDEs or intake of higher brominated PBDEs which have experienced debromination to form highly persistent lower brominated congeners.

Human exposure to PBDEs and PBBs is through food, drinking water, soil, dust, and air. The principal source of exposure for most age groups is through food including breast milk.<sup>101</sup> People may also be exposed to PBDEs and PBBs through contact with consumer products containing the compounds. The evidence is not consistent on the importance of contact with consumer products as a source of exposure. Two studies have reported that house dust from degraded flame retardant material in furniture, carpets and other household products comprises more than 80% of overall adult intake of PBDEs and PBBs.<sup>113,114</sup> In contrast, Health Canada has assessed the exposure to PBDEs through contact with consumer products to be negligible in comparison with exposure through food.<sup>101</sup>

Concentrations of PBDEs have been detected in a wide variety of foods with higher concentrations in freshwater fish at 1461.9 parts per trillion (ppt) and marine fish at 1164.9 ppt. Lower concentrations of PBDEs were found in butter at 264.5 ppt and in canned luncheon meats at 248.4 ppt.<sup>115</sup> A study by Fraser et al in 2009 found an association between an individual's consumption of different diets and food groups with

his or her serum concentrations POPs.<sup>116</sup> This study used data from two dietary instruments in the 2003–2004 National Health and Nutrition Examination Survey (NHANES). These instruments are a 24-hr food recall (24FR) and a 1-year food frequency questionnaire (FFQ). Fraser et al examined food intake among NHANES respondents and regressed serum concentrations of five PBDEs (BDE congeners 28, 47, 99, 100, and 153) and their sum against reported food group consumption while adjusting for age, sex, race/ethnicity, income, and body mass index. Their analyses found the serum concentration of the group sum among vegetarians was 23% ( $p = 0.006$ ) and 27% ( $p = 0.009$ ) lower than among omnivores for 24FR and 1-year FFQ, respectively.<sup>116</sup> The serum concentrations of five PBDE congeners were associated with consumption of poultry fat. The consumption of red meat fat was associated with serum levels of PBDE 100 and 153. An association was not observed between serum PBDEs and consumption of dairy or fish. The results were similar for both dietary instruments but were more robust when using the 24FR. These findings indicate that consumption of poultry and red meat could contribute to increasing the serum concentrations of PBDEs and possibly other POPs.

Among infants younger than seven months who are not breast fed, dust is the main source of exposure.<sup>101</sup> Breast-fed infants potentially have the greatest exposure to PBDEs and PBBs of any age group since breast milk has higher concentrations of these compounds than do other foods.<sup>101</sup> A Health Canada report on the state of science on tetra- through to deca-PBDEs found congeners 47, 99, 100 and 153 to have the highest concentrations of PBDEs in breast milk.<sup>101</sup> This report compared the minimal exposure level associated with health effects for each congener to the upper bound estimate of exposure among breast-fed infants who are potentially the population with the greatest exposure to these congeners. The minimal exposure level associated with health effects, specifically neurobehavioural effects in mice, is 0.8 mg/kg of body weight. The upper bound of the 95% confidence interval for estimated average exposure through breast milk is 2.6  $\mu\text{g}/\text{kg}$ -of body weight per day in breast-fed infants. The reported minimal level for health effects has a margin of 300 times higher than the reported upper bound for estimated average daily exposure. However a limitation of this type of comparison is that the lower brominated congeners bioaccumulate in adipocytes and

other cells with lipids, and therefore concentrations in tissues may be considerably higher than indicated by estimates of daily exposure.

## **2.2. Evidence of T2D Associated with Higher-Dose Exposures to POPs in Human Populations**

### **2.2.1. *Higher-Dose Exposures From Severe Occupational and Environmental Contamination***

Early studies linking POPs to IR and T2D focused on populations with higher-dose exposures related to severe occupational<sup>117–119</sup> and environmental contaminations.<sup>120</sup> “Higher-dose,” as used here refers to exposures that were higher than those occurring in the general populations today. The most extensively studied occupational exposures to POPs are among American veterans of the war in Vietnam.<sup>119,121–123</sup> Some American military personnel were exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) through the unsafe transport, storage and extensive spraying of the Agent Orange herbicide and defoliant. This pollutant is known to have a long retention in body fat. TCDD has a long half-life, which is estimated to be 7.6 years (95% confidence interval (CI) 7.0–8.2 years).<sup>124</sup> TCDD is considered to be the most toxic of the dioxin and dioxin-like compounds.<sup>125,126</sup>

In 2001, a review of the evidence in the scientific literature led the Institute of Medicine (IOM), National Academy of Sciences to conclude there was limited and suggestive evidence of an association between exposure to the herbicides used in Vietnam or the contaminant TCDD and T2D.<sup>127</sup> Henriksen and colleagues found an increased risk of diabetes among Operation Ranch Hand veterans with the highest serum concentrations of TCDD.<sup>123</sup> Furthermore, in a prospective study of these veterans with repeated measurements over fifteen years, there was no relationship observed between the rate of dioxin elimination and the occurrence or time of onset of diabetes.<sup>128</sup> Based on this evidence the authors concluded that the association between dioxin exposure and T2D was not due to slower metabolism among those with T2D, which might have led to a spurious conclusion about the direction of causation in cross-sectional studies.

Studies of high-dose exposures have also included epidemiological studies following industrial accidents. One of the most extensively studied industrial accidents and its related health outcomes occurred in Seveso, Italy.<sup>129-132</sup> In 1976, an accident occurred in a chemical plant in Seveso resulting in high exposures to the dioxin, TCDD, among surrounding residents as far as 6 km from the plant. The areas surrounding the plant were classified as high, intermediate and low exposure zones according to results of soil sampling. Most residents living in the high exposure zone were ordered to leave their houses after a few weeks while those in the other zones remained in their homes, but regulations were implemented to limit their exposure. Among males, risk of cardiovascular mortality was increased in all areas, especially the high zone over the 15 years after the accident. Among females, the risk of death from T2D was elevated in the high and intermediate zones, especially in the second decade after the accident. However, this association only reached statistical significance in the intermediate zone (RR 1.9, 95% CI 1.1-3.2), which had a larger sample size. Among males, no deaths from diabetes were found in the high zone, and the elevation of risk in the other zones was only suggestive.<sup>129,132</sup>

### **2.2.2. Consumer Product Higher-Dose Exposure to POPs**

Two prospective studies in Michigan and Taiwan have examined the relationship of POPs and diabetes resulting from consumer exposure to contaminated food products. The longest duration study was the Michigan PBB cohort established in 1976 with follow-ups in 1991-1993 and 2001.<sup>133</sup> In 1973, a fire-retardant product containing polybrominated biphenyls (PBBs) was accidentally added to animal feed and distributed to farms throughout Michigan. As a result, approximately 295 kilograms of PBBs entered the consumer food market. The first effects became apparent in late 1973, when animals that ingested the contaminated feed began to exhibit symptoms of a previously unreported consumptive disease. The source of the exposure was not identified until April 1974. Michigan residents, especially farm families, consumed contaminated meat, eggs, and dairy products containing high levels of PBB for approximately 8 months before any preventive measure was taken. To monitor for potential health effects in consumers, the Michigan Department of Public Health invited people from exposed farms and households to participate in a longitudinal study. In total, 5,076 individuals enrolled in 1976.

Vasiliu and colleagues<sup>133</sup> analysed the Michigan PBB cohort data from 1,384 individuals who in 1976 were at least twenty years-old, did not have diabetes, provided serum levels for polybrominated biphenyls (PBB) and polychlorinated biphenyls (PCB), and participated in at least one follow-up survey. The study reported that higher PBB serum levels were not a risk factor for the incidence of T2D. However, in females, but not in males, higher PCB serum levels were associated with increased incidence of type 2 diabetes (incidence density ratio (IDR) = 2.33; 95% CI = 1.25 – 4.34 in the highest PCB group compared with the lowest). In both males and females, overweight and obesity increased the incidence of T2D.

Another studied incident of higher-dose exposure through a consumer food product occurred in central Taiwan. In 1979, approximately 2,000 Taiwanese consumers were exposed to polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) through contaminated rice-bran cooking oil. A prospective sample of exposed consumers was matched with neighbours by age and sex who were not exposed to study the long-term effects of the exposure. A twenty-four year follow-up study reported the exposed females had higher OR (OR 2.1 CI: 1.1-4.5) of incident T2D during the 24 years following the exposure after controlling for diabetic risk factors and other health behaviours.<sup>134</sup> Females with chloracne (a skin condition symptomatic of high-dose dioxin exposure) had even higher OR of developing T2D (OR: 5.5 CI: 2.3-13.4). There was no association between exposure to the contaminated cooking oil and incident T2D among males in the cohort. Recently, a thirty-year follow-up study examined the standardized mortality ratios (SMR) by cause of death to further compare the differences in health outcomes between the exposed and unexposed individuals.<sup>135</sup> The standardized mortality ratio for all causes of death was 1.2 (95% CI: 1.1–1.3) when comparing the exposed to unexposed groups. The SMR for diseases of the circulatory system was 1.3 (95% CI: 1.0–1.6). For diseases of the musculoskeletal system and connective tissue the SMR of 6.4 (95% CI: 2.8–12.7) was substantially elevated in the exposed group. Among exposed males, the SMRs were also increased for diseases of the digestive system (SMR of 1.9, 95% CI: 1.2–2.8), malignant neoplasm of the stomach (SMR of 3.5, 95% CI: 1.5–7.0), and malignant neoplasm of lymphatic and hematopoietic tissue (SMR of 3.0, 95% CI: 1.1–6.6). The SMR for total neoplasms was increased but not significant for the exposed group with a SMR of 1.3 and 95% CI: 0.9–1.7.

## **2.3. Evidence of T2D Associated with Lower-Dose Exposure to POPs**

Following from the earlier epidemiological studies of specific occupational and industrial accident exposures, more recent research has examined the health conditions associated with lower-dose environmental exposure in general populations. This research extended the analysis to include IR and reduced beta cell function, which are important components of T2D and might provide early markers of elevated risk for T2D.<sup>136</sup> These studies have utilized biomonitoring to improve exposure classification compared to the previous studies in higher-exposed populations cited above. In this section I discuss, these population-based studies and their findings of any associations between elevated blood concentrations of a variety of POPs compounds and increased OR of IR, reduced beta cell function or T2D. These studies have included a variety of research designs testing a range of specific pollutants across different populations.

### **2.3.1. Evidence from the U.S. NHANES**

Several studies showing associations between POPs and IR or T2D have used data from the U.S. National Health and Nutrition Examination Survey (NHANES), which is similar in design to the Canadian Health Measures Survey (CHMS) which was used for the analysis here. Both NHANES and CHMS are large, cross-sectional studies that were designed to provide nationally representative estimates of exposure to environmental pollutants using biomonitoring techniques. Whereas NHANES has been repeated four times since 1999 in the US, the first implementation of the Canadian Health Measures Survey was recently completed in 2009.

Lee and colleagues examined the six POPs which were detected in at least 80% of respondents in the 1999-2002 NHANES including PCB 153, two dioxin congeners (HpCDD and OCDD) and three organochlorine pesticides (oxychlorane, *p,p'*-DDE and trans-nonachlor).<sup>137</sup> They found increasing ORs of diabetes (the dataset did not allow distinction of type 1 or 2) with increasing serum concentration of all compounds, although the association was less linear for OCDD than the other compounds. They also found an association between the OR of diabetes and the sum of all six compounds. The lowest category of exposure (<25th percentile) only had 2 diabetic

cases and so the second category (25th to <50th percentile) was used as the reference group. The ORs of diabetes increased with exposure. Compared to the second category of exposure, there were decreased OR of diabetes in the lowest (OR 0.1, 95%CI 0.0-0.3), similar OR in the third (50th to <75th percentile; OR 1.1, 95%CI 0.6-1.7) and higher OR in the fourth (75th to <90th percentile; OR 2.7, 95%CI 1.5-4.9) and fifth ( $\geq$ 90th percentile; OR 2.7, 95%CI 1.5-4.8) categories after adjustment for age, sex, race/ethnicity, poverty income ratio, BMI, or waist circumference.

There was a significant interaction ( $P < 0.001$ ) between the summed serum concentration of the six POP compounds and age in the OR of having diabetes, but no interactions with sex, poverty income ratio and BMI. However, because the authors used the lowest exposure group as the reference in the tests of interaction, these estimates may be affected by the sparse number of diabetics in the reference group. The association between the sum of the six POP compounds and diabetes appeared to be stronger among the 20-39 and 40-59-year-olds compared to those  $\geq 60$  years. The authors reported that these results appeared to be due primarily to increased prevalence of diabetes among the lower exposure groups among those  $\geq 60$  years rather than lower prevalence of diabetes among those aged  $\geq 60$  years who had high exposure. Although time trends cannot be directly observed with these cross-sectional data, these results are consistent with a hypothesis that POPs may accelerate the progression towards diabetes in combination with other risk factors for the disease.

The prevalence of diabetes among those in the lowest exposure category ( $\leq 25$ th percentile) was very low (0.4%) even among those with high BMI suggesting the possibility of POPs being involved in the link between obesity and diabetes.<sup>137</sup> In a subsequent study of non-diabetics from NHANES, Lee and colleagues found there was no association between waist circumference and IR for respondents in the lowest quartile of POP serum concentrations.<sup>138</sup> The association between waist circumference and HOMA-IR became stronger with each higher quartile of serum concentration of organochlorine pesticides. Lee and colleagues concluded the evidence from their 2006 and 2007 studies was consistent with their hypothesis that POPs particularly OC compounds and metabolites interact with obesity to increase the risk of IR and T2D.

In a subsequent analysis of NHANES 1999-2002, Lee et al <sup>139</sup> examined the cross-sectional associations of diabetes with 19 POPs with observable concentrations in at least 60% of participants across five subclasses including polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), dioxin-like PCBs (dl-PCB), non-dioxin-like PCBs (ndl-PCB), and OCPs. When modeled individually, most were positively associated with diabetes after adjustment for age, sex, race, poverty income ratio, BMI, and waist circumference. The associations were stronger for PCBs and OC pesticides than for PCDDs and PCDFs. When the five subclasses (sums of compounds within each subclass) were modeled simultaneously, only dioxin-like PCBs and OC pesticides were significantly associated with diabetes. A weaker association between PCDFs and diabetes was observed. There were no observed associations for dioxins or non-dioxin PCBs. This analysis shows that because there are often strong correlations between the concentrations of different POPs in biological samples, it is important to model compounds together when not impossible due to collinearity. It also points out that it can be difficult to disentangle which POPs compounds are causally associated with health outcomes due to high correlations in concentrations between them. The correlations between the POP measures selected for this current study are shown in chapter 4 below. The Pearson correlations among many of the POP compounds were high (~0.9) and when modeled together the summed group measures for the POP subclasses had very substantially increased p-values indicating collinearity among these measures.

### **2.3.2. Findings from CARDIA**

A relevant longitudinal survey is the Coronary Artery Risk Development in Young Adults (CARDIA) study. This study was used for a small prospective case-control analysis (90 cases and 90 controls) of young adults followed from 1987-88 through 2005-06.<sup>140</sup> Cases were those who had incident diabetes (ever taking diabetic medication or fasting glucose  $\geq 126$  mg/dL observed on at least two follow-up visits) more than two years after baseline. Controls were randomly selected from among those who had observed glucose  $< 100$  mg/dL at all 5 follow-up visits over 20 years. The small sample size in this study limited its power and interpretation. However, compared to the lowest exposure groups, they observed higher OR of diabetes in the second quartiles of several compounds when modeled individually including trans-nonachlor and PCBs 74,

153, 170, 178, 183, 187, 196/203 and 153. They also created ranks for each compound and then summed the ranks to create an overall measure of POPs exposure. There were no associations between quartiles of this measure and incident diabetes, but there were higher OR of diabetes in the third sextile relative to the first (OR 3.7, 95% CI 1.1-12.3) although the confidence interval was very wide. These results provide limited evidence of a non-linear association between POPs and incident diabetes. The observed inverted U-shape association between POP concentrations and diabetes is consistent with the biological response to endocrine disruptors.<sup>141,142</sup> POPs are known to be endocrine disruptors.<sup>143</sup> Lee et al (2010) conclude we should not expect a linear dose response with cellular toxicity by POPs. Chapter 5 provides a more complete discussion of debate concerning low-dose effects and nonmonotonic response to endocrine disrupting chemicals.

### **2.3.3. *Results from a cross-sectional population health survey in Belgium***

A smaller cross-sectional population study in Belgium also found an association between serum levels of POPs and type 2 diabetes.<sup>144</sup> This study of 257 individuals across Belgium found diabetics had significantly increased serum levels of dioxins, coplanar PCBs and of the twelve PCB markers. After adjustment for age and other covariates, serum total toxic equivalent<sup>a</sup> activity (sum of PCDD/Fs and coplanar PCBs) and twelve PCB marker concentrations in diabetics were 62% (p=0.0005) and 39% (p=0.0067) higher, respectively, than among non-diabetics. The OR of diabetes were significantly increased for respondents in the top 10% of serum concentrations of POPs compared to the lower 90% of respondents after adjustment for concentrations of dioxins (OR 5.1 , 95% CI 1.18 - 21.7), coplanar PCBs (OR 13.3, 95% CI 3.31 - 53.2) or twelve PCB markers (OR 7.6, 95% CI 1.58 - 36.3).

<sup>a</sup> Toxic equivalency is described and discussed in chapter 5.

#### **2.3.4. Evidence from the Inuit population of Greenland**

Since 1997, the Arctic Monitoring and Assessment Programme (AMAP) has produced integrated assessments on the status of and trends in POP levels in the Arctic ecosystem. AMAP has published several studies on biomonitoring of POPs and their health risks for Arctic populations.<sup>145–149</sup> The Arctic Council of the circumpolar nations commissioned these studies to better understand the global atmospheric and ocean currents which transport POPs into the Arctic, the biomagnification of POPs in the Arctic ecosystem, and human health effects related to exposure to POPs.

Across Arctic countries, the population health indicators are generally worse for the Indigenous populations as compared to non-Indigenous populations.<sup>150</sup> This difference is often attributed to socio-economic conditions and lifestyle.<sup>150</sup> However, contaminants in indigenous foods may have a contributing role in the lower health outcomes among Indigenous peoples.<sup>150</sup> Indigenous peoples have a much higher consumption of marine mammals and birds than do non-indigenous people in the Arctic. Exposure to POPs is higher among indigenous people in part because of their diet of fish, marine mammals and birds while non-indigenous people have a greater reliance on imported foods from the south.<sup>147,148</sup> In Greenland, the POP serum concentrations are relatively lower in communities with the most European influenced diets and lifestyles.<sup>151</sup> The AMAP studies have reported an association between exposure to environmental contaminants and immunological, cardiovascular and reproductive negative outcomes in some Arctic populations.<sup>150</sup>

During the 1950's and 1960's, the Greenland Inuit appeared to not experience the rapid rise in the incidence of T2D that was reported among many sub-Arctic North American Aboriginal peoples.<sup>152–155</sup> The traditionally low prevalence of T2D among Inuit populations has been attributed to a diet with high levels of polyunsaturated fatty acids from marine mammals and fish.<sup>156–158</sup> Since the 1970s, the prevalence of T2D has increased among Inuit populations, but remains comparable to, or lower than, the prevalence among Canadians and Americans. Some of the estimates of diabetes among the Inuit are based upon self-reported diagnosis from a health professional. The limited access to health professionals in the Arctic countries may lead to an under-estimation of diabetes based solely on self-reporting by respondents.<sup>159</sup> A 1999-2001

population health study of Greenland Inuit reported that 70% of respondents with diabetes had not been previously diagnosed, and the prevalence of diabetes was 25% higher than in Denmark.<sup>159</sup>

Two Canadian Inuit population health surveys in 1992 and in 2004 in the Nunavik region of northern Québec did use fasting glucose measurement and questions on medication use to estimate the prevalence of diabetes. The surveys found that the prevalence of T2D among the Nunavik Inuit was comparable with the prevalence for the Canadian population. In 2004 among adults 20 years of age or older, 4.8% of the Nunavik Inuit were reported to have diabetes which was the same estimated prevalence for the Canadian population.<sup>160</sup> The Nunavik study did not distinguish by type of diabetes. However, the prevalence of risk factors for diabetes such as obesity were relatively higher among the Nunavik Inuit in 2004 than in 1992.<sup>160</sup>

Bonefeld-Jørgensen asserts that differences in health outcomes between Inuit and other populations may be less than could be expected due in part to differences in the ability of environmental pollutants to induce expression in some nuclear receptors.<sup>161</sup> These differences between the Inuit and other populations such as Caucasians may potentially affect the extent of the association of exposure to POPs and the prevalence of metabolic disease in each population.<sup>161,162</sup> Bonefeld-Jørgensen has also postulated that the high consumption of n-3 polyunsaturated fats and other nutrients from marine mammals and fish by the Inuit has a protective effect countering the negative effects of POPs on metabolism.<sup>163</sup> However, animal studies have shown that POPs may suppress the benefits of omega 3 fatty acids as discussed below.<sup>10,11</sup>

The AMAP studies have commented on the relatively high dietary exposure among Inuit to POPs and the comparable prevalence of diabetes with other populations with lower dietary exposures. However, only descriptive comparisons of relative risk between low and high concentrations of POP group sums have been published. Statistical models adjusted for confounding factors such as age, obesity and physical activity have not been published using the AMAP data.<sup>164</sup>

In 2007, a study of 352 Mohawk reservation members in Akwesasne examined the association of POP serum concentrations and diabetes (not distinguished by type of

diabetes). Nearby this community, three large aluminum foundries operated for several decades.<sup>165</sup> All three foundries used PCBs (primarily Aroclor 1248) and discharged PCBs into the St. Lawrence River upstream from Akwesasne for several decades. Breast milk among Mohawk mothers<sup>166</sup> and serum among Mohawk adults<sup>167</sup> have been correlated with rates of consumption of local fish, although fish consumption declined prior to these studies due to public health advisories to limit consumption of fish from the river.

The study found elevated serum PCBs, DDE, and HCB were positively associated with diabetes after adjusting for potential confounders, whereas a negative association was observed for serum concentrations of mirex.<sup>168</sup> The researchers used a standardized questionnaire to collect demographic, medical, and lifestyle information from participants 30 years of age or older. Fasting serum samples were also collected and analyzed for 101 PCB congeners, DDE, HCB, and mirex along with fasting glucose, triglycerides, and cholesterol. Participants were identified with diabetes who had fasting-glucose values > 125 mg/dL and/or who were taking medication for diabetes. The analysis used logistic regression to assess the association between POP serum levels and diabetes, while adjusting for the potential confounding variables of age, BMI, smoking, sex, and serum lipid levels. POP serum levels were categorized into tertiles, and the lowest tertile was used as the reference category. The prevalence of diabetes for this sample was relatively high at 20.2%. The adjusted OR (OR) of having diabetes for participants in the highest tertile of total PCB concentration compared with the lowest tertile was 3.9 (95% confidence interval, 1.5-10.6). The corresponding adjusted ORs for DDE and HCB were even higher. Elevated serum mirex was not associated with diabetes. After adjustment for other POPs, the OR for HCB remained significant, whereas ORs for PCBs and DDE remained elevated but not statistically significant. In contrast, after adjustment for other POPs, the OR for mirex became statistically significant and indicated an inverse association.

## 2.4. Biological and Genetic Evidence of the Relationship Between Exposure to POPs and Metabolic Outcomes

Predicting the metabolic effects of POPs as a group of compounds is difficult since individual POPs may have different biological effects and potential.<sup>169–171</sup> For example, some PCB congeners including hydroxy-PCBs have estrogenic potential while others are anti-estrogenic such as PCB153, PCB180, and PCB138 or anti-androgenic such as PCB-138.<sup>170</sup> However, recent experimental animal and human microarray studies described below provide insight into the molecular biological mechanisms underlying the observed metabolic and physiological effects reported in epidemiological studies.<sup>10,11,172–176</sup>

In animal studies, morphologic changes have been reported in the structure of pancreatic beta cells following PCB exposure<sup>175,177</sup>, and altered expression of gluconeogenic enzymes were found in rat liver.<sup>172</sup> HCB has been reported to disrupt the gluconeogenic pathways in rats<sup>178</sup> Epidemiological studies have also investigated possible metabolic pathways. Employees at a PCB production facility were found to have impaired immune system regulation.<sup>179</sup> Michalek and colleagues<sup>180</sup> concluded the dioxin-like action on insulin regulation observed among US Veterans exposed to TCDD during Operation Ranch Hand may be mediated through sex-hormone binding globulin. PCBs have been observed to induce several different cytochrome P450 enzymes in the liver and other tissues<sup>181</sup>, this results in unique patterns of gene induction<sup>182</sup>.

Two recent experimental studies by a team of Norwegian researchers provide important insights into the cellular and metabolic effects from the bioaccumulation of POPs in tissues.<sup>10,11</sup> Both of these studies used mouse models in which the treatment group had dietary exposure to POPs through salmon oil or salmon fillet made from farmed Atlantic salmon. The use of retail purchased salmon introduces an environmentally relevant dietary exposure to POPs into the designs of these studies. Salmon and other fatty fish are important dietary sources of exposure to POPs for humans. The more recent of these two studies also introduced different diet regimes with specific fat content into the study design.<sup>11</sup> The use of different diet regimes was to determine if the association between the exposure and metabolic effects was impacted

by diet and to test whether POPs exposure modified the beneficial effects of dietary polyunsaturated fatty acids and other nutrients in fish.

#### **2.4.1. *Suppression of Protein Kinase B Phosphorylation in Mouse Muscle Tissue***

Ibrahim and colleagues' study provides an important indication of the molecular regulatory pathway through which POPs could affect cellular function and metabolic response to insulin.<sup>11</sup> This study reported decreased protein kinase B (PKB) phosphorylation in gastrocnemius muscle tissue from mice exposed to POPs through a diet including farmed Atlantic salmon fillet as compared to animals without salmon in their diet.

Protein kinase B also known as Akt Ser/Thr kinase is well conserved across a broad range of species.<sup>183</sup> PKB is involved in several diverse cellular processes. Among its many roles, PKB is a critical signalling pathway that mediates the metabolic effects of insulin in several physiologically important target tissues including adipocytes and muscle. PKB phosphorylation is activated via insulin-stimulation of phosphatidylinositol 3'-kinase (PI3K). PKB regulates glucose transport, glycolysis, protein synthesis, lipogenesis, glycogen synthesis, suppression of gluconeogenesis, cell survival, determination of cell size and cell-cycle progression.<sup>183</sup>

In an earlier mouse study, Ruzzin and colleagues found in adipocytes exposed to POPs there was reduced expression of both peroxisome proliferator activator protein- $\gamma$  co-activator-1 $\alpha$  (PGC1 $\alpha$ ) and insulin-signalling gene-1 (Insig-1).<sup>10</sup> PKB is upstream and an important regulator of PGC1 $\alpha$  and Insig-1.<sup>183</sup> The suppression of PKB phosphorylation could be expected to lead to lower expression of PGC1 $\alpha$  and Insig-1 as observed by Ruzzin and colleagues.

In a subsequent study, Ibrahim and colleagues extended the research conducted by Ruzzin et al..<sup>11</sup> Ibrahim and colleagues' study exposed mice to one of three diet regimes: a very high fat (VHF) diet regime; a western high carbohydrate/high fat (WD) diet regime; or a control (C-fed) lower fat diet with nutritionally appropriate levels of fat and carbohydrate and no exposure to POPs. Within the very high fat (VHF) diet regime, two dietary groups were compared: the VHF diet with salmon fillet (VHF/S) and the VHF

diet without salmon fillet (VHF). Similarly, the western high carbohydrate/high fat (WD) diet regime had two dietary groups: WD group with salmon fillet (WD/S) and the WD without salmon fillet (WD). The salmon fillets were from commercially purchased farmed Atlantic salmon. Farmed Atlantic salmon was selected as the dietary source of exposure to POPs. The level of protein in the purified diets was adjusted to an isonitrogenous basis at the expense of carbohydrates and corn oil was adjusted according to lipids present in salmon fillet so that total dietary fatty acid concentrations were similar in all diets. The energy content of each diet was also similar.

Ibrahim et al. found the mice with the high fat diet without exposure to POPs (VHF) had higher blood glucose levels at all the time points following the glucose load as compared to the control group (C-fed) mice on a lower fat diet. This finding confirms the diabetic phenotype associated with mice on a high fat diet as previously reported.<sup>184</sup> The dysregulation of whole-body glucose homeostasis was significantly worse in mice with a high fat diet with exposure to POPs (VHF/S diet). This dysregulation was associated with increased insulin production in response to a glucose challenge. The glucose clearance response was significantly reduced in VHF/S-fed animals following an insulin load as compared to both the VHF-fed mice and the control-fed mice. Furthermore, VHF/S-fed mice had increased blood glucose and plasma insulin relative to the VHF and C-fed animals. These findings indicate that a high fat diet contributes to the insulin resistant phenotype, and the high fat diet with exposure to POPs significantly aggravates impairment of glucose homeostasis.

To confirm that dietary exposure at an environmentally relevant concentration to POPs is associated with increased impairment of glucose homeostasis in a more conventional experimental diet, Ibrahim et al. compared mice with a more conventional high-fat/high-carbohydrate diet (western diet or WD) and mice on a Western diet with exposure to POPs (WD/S). In contrast to the previously described experiment with high fat diets, in the fasted state, there were no differences in blood glucose and plasma insulin concentrations between WD-fed mice and WD/S-fed mice. However, in the fed condition, a mild increase of blood glucose and a substantial increase in plasma insulin levels were observed in POPs exposed (WD/S-fed) mice relative to the unexposed (WD-fed) mice. Both glucose and insulin tolerance tests indicated that animals fed the WD/S had significantly greater hyperglycaemia and IR compared with the WD-fed mice.

There was also evidence of a dose response among mice exposed to POPs. The authors created two exposure groups by removing some POPs from the salmon and used the untreated (S) and reduced POPs (S<sub>POP</sub>) salmon to experimentally dose the mice. Relative to mice fed a high fat diet with POPs exposure (VHF/S), the animals fed a high fat diet but with reduced POPs concentrations in the salmon (VHF/S<sub>POP</sub>) had lower blood concentrations of POPs and accumulated less visceral fat. Additionally, the reduced POPs exposed (VHF/S<sub>POP</sub>) mice exhibited less insulin and hyperglycaemia than mice fed the diet with unmodified POPs exposure (VHF/S).

In summary, the studies by Ibrahim et al. and Ruzzin et al. indicate that insulin signalling is inhibited by exposure to POPs. The suppression of PKB phosphorylation is associated with several important metabolic and physiological changes because it is an important step in the transduction of insulin signalling. Mice exposed to POPs through salmon fillets compared to mice not exposed among both high fat and western diet groups had greater IR and glucose intolerance.<sup>11</sup>

#### **2.4.2. *Increased intestinal fat absorption and obesity in mice***

Ibrahim and colleagues also conducted experiments to test if mice with dietary exposure to POPs experienced greater intestinal fat absorption and weight gain compared to mice without dietary exposure to POPs.<sup>11</sup> One experiment examined mouse intestinal absorption of dietary fat by measuring fat content in feces. Measurements of excreted fat were taken for five dietary groups including VHF/S, VHF, WD/S, WD and control (C-fed) as described above. VHF/S and WD/S mice had enhanced fat absorption as compared to their respective diet group counterparts (VHF and WD) who were not exposed to POPs through salmon in their diet. Specifically how the dietary exposure to POPs leads to increased intestinal fat absorption is unknown.

A very high fat diet was previously reported to develop a diabetic phenotype before the onset of obesity in mice.<sup>184</sup> Ibrahim et al. used this dietary model to investigate the impacts of farmed salmon fillet intake in VHF-fed and WD-fed mice. After eight weeks, mice fed VHF/S gained about two times more weight than mice fed the VHF without salmon and the control diet mice despite similar energy intake. Similarly, after six weeks, WD/S mice had significantly greater body weight gain than WD or

control animals. A likely mechanism leading to the greater body weight gain was the greater intestinal fat absorption among VHF/S and WD/S mice.

### **2.4.3. Obesity is associated with systemic inflammation**

The higher body weight gain in the VHF/S and WD/S mice was exhibited through increased visceral fat, which was associated with a prominent increase of adipocyte size in the epididymal fat pad. The increase in adipocyte size can lead to cell hypertrophy and subsequently macrophage infiltration of these cells. Ibrahim et al. found the expression of Mac2- $\alpha$ , a galactose-binding lectin expressed by activated macrophages, was increased by about thirteen-fold in epididymal fat of mice fed VHF/S compared with VHF-fed mice. This finding indicates greater macrophage infiltration in adipose tissue of mice exposed to POPs through a diet including salmon fillet.

To test if the difference in activated macrophages was associated with a difference in release of inflammatory molecules, Ibrahim et al. measured expression of IL-6, iNOS, and TNF $\alpha$ . In the white adipose tissue of mice fed VHF/S, the mRNA levels of TNF $\alpha$  and iNOS were up-regulated by about five and three fold, respectively, compared with VHF-fed mice. The expression of IL-6 was unchanged between VHF/S and VHF mice. WD/S-fed mice also exhibited greater macrophage infiltration and inflammatory response than did WD-fed mice.

In summary, these experiments by Ibrahim et al. demonstrate that mice exposed to POPs through salmon fillets compared to mice not exposed among both very high fat and western diet groups had greater intestinal fat absorption and visceral obesity. The increased visceral obesity was associated with evidence of increased macrophage infiltration and systemic inflammation. Obesity and systemic inflammation are risk factors for developing T2D.<sup>185</sup>

### **2.4.4. Human microarray studies**

Human microarray studies have found reduced expression of PGC1 $\alpha$  to be associated with T2D.<sup>186-188</sup> Among a group of patients with diabetes and matched controls in southern Sweden, PGC1 $\alpha$  was the only master regulator among many genes analyzed to show consistent down-regulation among individuals with diabetes.<sup>186</sup> Patti

and colleagues extended this analysis with a study of Mexican-American subjects.<sup>187</sup> This latter study found that not only are PGC1 $\alpha$  and PGC $\beta$  down-regulated among diabetics but these master regulators also have decreased expression among non-diabetic individuals with a family history of diabetes.

These studies may indicate a link in these populations between exposure to POPs and diabetes. Down-regulation of PGC1 $\alpha$  could be an expected consequence of reduced PKB phosphorylation following from exposure to POPs. Unfortunately, these microarray studies did not test for environmental pollutants. Nonetheless, previous studies demonstrated that some groups among Mexican-American and Swedish populations have higher exposures to POPs.<sup>189,190</sup> It cannot be discounted from the data available that the observed decreased expression of PGC1 $\alpha$  among diabetics in these studies might indicate that POPs are implicated in the pathogenesis of diabetes in these populations.

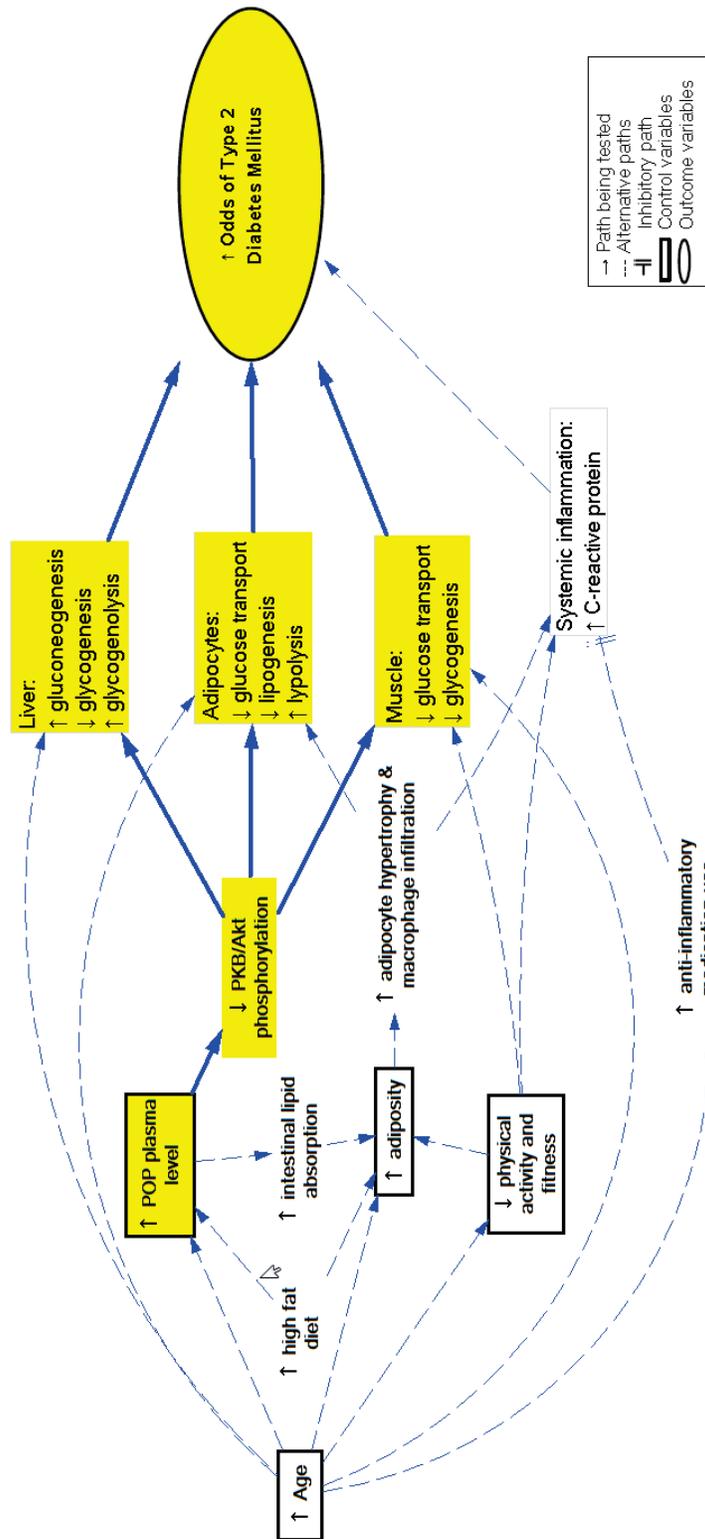
## 3. Hypothesis and Research Methods

This chapter begins with a description of the hypothesized pathways and the research questions posed for this study. Next the data source, ethics review and measures of principal interest in the analyses are described. The specification of the models is also discussed.

### 3.1. Hypotheses and Research Question

The evidence of molecular mechanisms of effect in experimental studies and supported by epidemiological studies are the basis for the hypotheses discussed here. There are two hypothesized metabolic pathways linking exposure to POPs with the odds of having T2D for the Canadian adult population. Each of these pathways is illustrated in the directed acyclic graph (DAG) in Figure 2. The outcome, exposure and control parameters were identified through *a priori* knowledge. The criteria recommended by Hernán et al. for constructing a DAG was used to confirm which parameters are appropriate to include in the model.<sup>191</sup>

The risk factors used in the models are identified in figure 2 with thick bordered boxes including age, obesity/body mass, and physical activity. The potential confounding factors of diet and anti-inflammatory medication use are not included in the models due to data limitations discussed below. Age, obesity and physical activity are each expected to have an independent effect on the odds of having type 2 diabetes after adjustment for other risk factors. Aging has direct effects on other risk factors and metabolism as indicated in figure 2.<sup>192</sup> Obesity is also known to be an important risk factor for impaired glucose homeostasis and T2D.<sup>193–195</sup> Physical activity and fitness are also important predictors of obesity, insulin resistance and T2D.<sup>196,197</sup> A description of how each of the risk factors were measured in the data, and how each was specified in the models for T2D is provided below.



**Figure 2. Directed Acyclic Graph of Type 2 Diabetes Mellitus and Risk Factors**

The first hypothesized pathway involves the suppression of PKB leading to insulin resistance which can progress to T2D. This is the principal casual pathway of interest for this analysis. In figure 2, the pathway is highlighted in yellow with solid blue arrows. The suppression of PKB phosphorylation in mouse muscle cells as observed by Ibrahim et al.<sup>11</sup> is hypothesized to also occur in human tissues. The metabolic effects from suppression of PKB in human adipose, liver and muscle cells can lead to insulin resistance and may progress to T2D as illustrated in figure 2.<sup>183</sup>

I hypothesize that plasma concentrations of POPs will be positively associated with the odds of having T2D in the adult Canadian national population. The principal research question examined in this analysis: Is the plasma concentration of POP compounds or groups associated with the odds of having T2D after adjusting for other specified risk factors in the adult Canadian population? If an association is observed between the plasma concentration of POPs and the odds of T2D, is the association stronger for a specific group of POP compounds?

The second hypothesized pathway involves the increase in intestinal fat absorption associated with exposure to POPs. It is hypothesized that adult humans have increased intestinal fat absorption and visceral obesity associated with POP levels as demonstrated in mice by Ibrahim et al.<sup>11</sup> Increased visceral obesity is associated with greater adipocyte size and macrophage infiltration of adipocytes leading to greater systemic inflammation in adult humans.<sup>185</sup> Systemic inflammation is a risk factor for insulin resistance which can progress to T2D in adult humans.<sup>185</sup>

This second hypothesis is more problematic to test with the available data. The DAG indicates that to test the association of plasma concentration of POPs with obesity, it is necessary to control for total fat and calorie consumption, physical activity and age. The available information on the respondent's diet is limited to current frequency of consumption of specific foods. The data on the respondent's diet is insufficient to adequately control for quantity of fat and calories consumed.

The analysis cannot sufficiently test for an association between POP plasma concentrations and obesity or systemic inflammation. However, if exposure to POPs is associated with increased intestinal absorption of fat and therefore obesity, we could

expect the association between obesity and T2D may become stronger among adults with relatively higher plasma concentrations of POPs. Lee and colleagues reported such an interaction in the NHANES.<sup>137</sup> The analysis here will examine if the association of obesity and T2D is dependent upon plasma levels of POPs among Canadian adults.

### **3.2. Limitation on Hypothesis Testing and Analysis**

The onset of T2D follows a prolonged pre-diabetic period characterized by insulin resistance, hyperinsulinemia and eventually pancreatic islet  $\beta$ -cell dysfunction.<sup>198</sup> Saad and colleagues proposed a two-step model for the development of T2D.<sup>199</sup> The first step is the transition from normal glucose homeostasis to prediabetes and the second step is the transition from prediabetes to T2D. Insulin resistance is characteristic of the first step, whereas the second step is largely characterized by a steep decline in pancreatic islet  $\beta$ -cell secretion of insulin. Saad et al. reported more than 90% of T2D patients are insulin resistant.<sup>199</sup> The diagnosis of T2D itself represents a good marker of IR. It could be postulated that long-term bioaccumulation of POPs could be associated with increased IR and pancreatic islet  $\beta$ -cell dysfunction. However, the cross-sectional nature of the data used here substantially limits the ability to analyze the progression of IR and  $\beta$ -cell dysfunction. Cross-sectional data is particularly limiting for the analysis of  $\beta$ -cell function since hyperinsulinemia initially increases with IR, but is followed by a period of a substantial decrease in  $\beta$ -cell secretion of insulin leading to T2D. Longitudinal data on glucose and insulin measures over time better facilitate the analysis of IR and  $\beta$ -cell function prior to the onset of T2D.<sup>198</sup>

The analysis here is limited to testing for the hypothesized association between the plasma concentrations of POPs and the odds of having T2D in the Canadian adult population. The hypothesized association dependent upon suppression of PKB phosphorylation can be examined with the available data. However, it is more problematic to investigate the hypothesis based upon increased intestinal fat absorption due to data limitations. The potential confounders of diet and anti-inflammatory medication use are not included as parameters in the models. Information on total calories consumed is important for testing if the plasma concentrations of POPs are associated with weight gain after adjustment for other factors. The available diet

information for respondents is limited to frequency of consumption for selected foods. The absence of information on quantity of food consumed substantially limits an investigation of the hypothesis that POP levels are associated with greater fat absorption leading to greater obesity. Additionally, the absence of information on the quantity of specific animal fats consumed also limits our ability to estimate the potential level of dietary exposure to POPs. Plasma concentrations of POPs are used as an indicator of body burden of these pollutants.

Other data limitations are also problematic for an analysis of the hypothesis that POP levels are associated with increased obesity and consequently greater systemic inflammation. Information on the respondent's C-reactive protein (CRP) levels is available as an indicator of systemic inflammation. However, CRP levels are affected by factors including use of anti-inflammatory medications. The data provide information on the specific medications used by each respondent in the preceding month, but information on dosage was not collected. Without information on anti-inflammatory dosage, it is difficult to assess the level of effect on CRP. Simply excluding respondents who used an anti-inflammatory medication could introduce bias into the estimates as a substantial proportion of the adult population used anti-inflammatory medications. The use of an anti-inflammatory medication could be treatment for a condition arising from systemic inflammation.

It is important to note that this analysis tests for only part of the hypothesized total effect of POPs on the odds of having T2D. Specifically, the analysis is designed to test for only the portion of the effect of POPs associated with the suppression of PKB phosphorylation. The portion of the effect of POPs associated with increased adiposity and systemic inflammation cannot be tested due to data quality limitations with the CHMS cycle 1. The analysis is not designed to test for the total effect of POPs on the odds of having T2D. Any association observed in this analysis between POP plasma concentrations and the odds of having T2D indicates just a portion of the hypothesized total effects of POPs. This is an important limitation of this analysis and other analyses which lack the data to adequately control for confounding from either one of the hypothesized pathways.

### **3.3. Data Source and Sample Selection**

The Canadian Health Measures Survey cycle 1 (CHMS) was a multi-stage complex survey conducted by Statistics Canada under the authority of the Statistics Act. The survey is designed to calculate unbiased population estimates of the Canadian non-institutionalized civilian population excluding Indian reserves, the northern territories, remote areas with low population density, institutionalized residents, and military bases. These exclusions were approximately 3.7% of the total population of Canada. Data collection for cycle 1 occurred from March 19, 2007 to February 25, 2009 at 15 collection sites selected by stratified random sampling. Using the Canadian national Labour Force Survey sampling frame, 257 potential collection sites across Canada were identified that had at least 10,000 people living within 50 km (for urban) or 100 km (for rural). The nation was divided into five regions and potential collection sites were classified according to location within a major metropolitan area (yes/no). The number of collection sites were allocated to regions proportional to population size and then final sites were randomly selected for each region within strata of major metropolitan area (yes/no) with probability of selection proportional to population size. At each collection site, dwellings were enumerated based on the 2006 Canadian Census of Population and local address registers. A random sample of dwellings was also selected to ensure newer dwellings were included. Stratified random sampling proceeded in a way to ensure roughly equal sample sizes within five age groups: 6 to 11, 12 to 19, 20 to 39, 40 to 59, and 60 to 79 years. If a child aged 6 to 11 was selected then a second participant aged 12 to 79 was selected from the same dwelling. Otherwise, only one participant aged 12 to 79 was selected from each dwelling. Sampling weights were provided by Statistics Canada to allow nationally representative estimates.

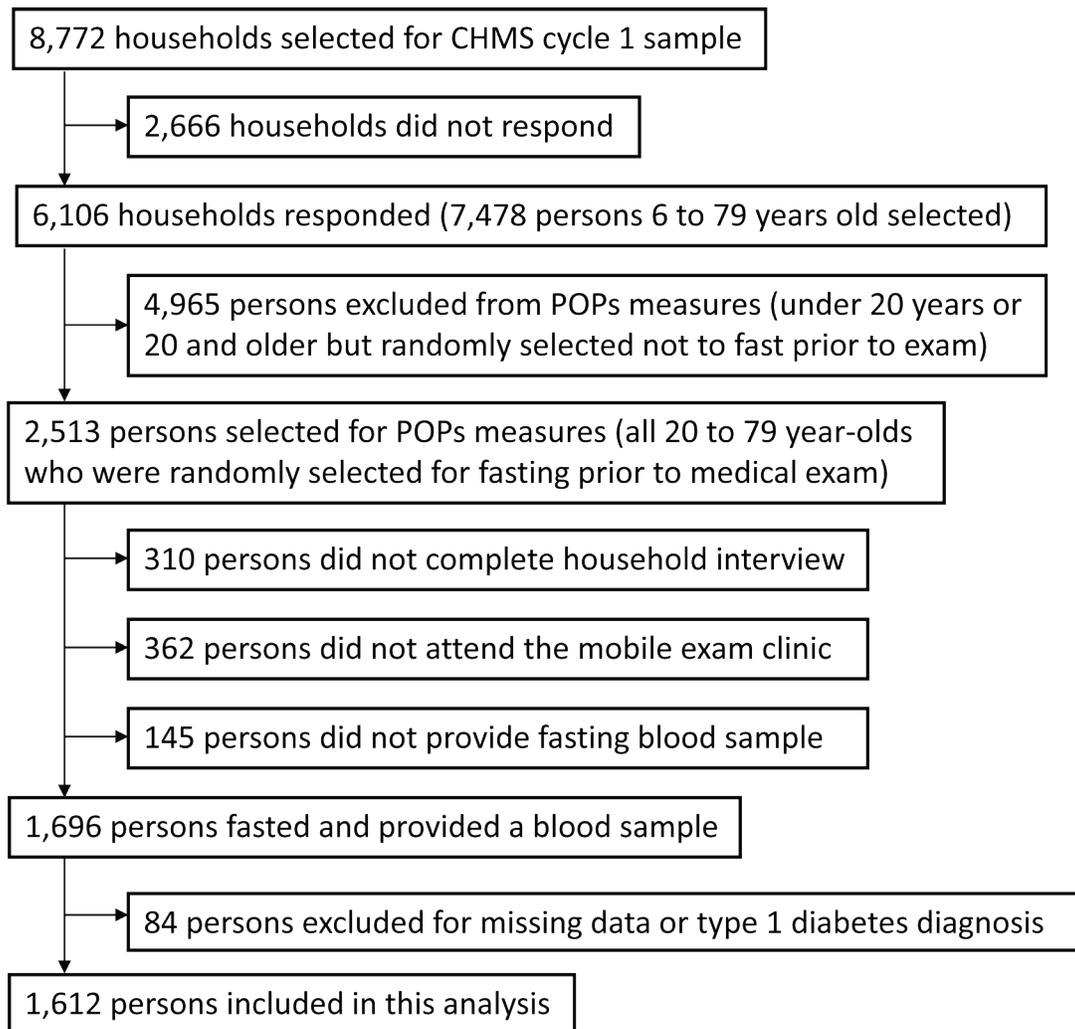
Several methods were used to minimize biases in the study. To minimize non-response, Statistics Canada sent introductory letters to each household explaining the steps of the survey and emphasizing the survey's importance by providing examples of how the CHMS would be used. All reasonable attempts were made to obtain interviews and if respondents refused procedures or to take part in the survey, they were reminded of the importance and potential benefits of the survey. Letters and CHMS brochures were available in English, French, Punjabi and Chinese and interviewers who spoke

languages other than the two official languages (English and French) were used when possible to conduct interviews with respondents who did not speak either of these languages. The MEC team used flexible hours and made all reasonable attempts to convince respondents who participated in the household interview to attend the MEC. The survey also used a carefully designed sampling strategy with precisely calculated survey design weights to ensure results were representative of the target population. Post-stratification adjustments of survey weights for non-response and calibration to official population statistics was also performed by Statistics Canada. To minimize the potential for differential misclassification of exposure, analysis of all plasma POPs were conducted by a single laboratory. The sample size was determined by Statistics Canada to yield approximately unbiased national prevalence estimates that would have coefficients of variation of 16.5% for a prevalence of 10% for each of 5 age groups (6-11, 12-19, 20-39, 40-59, and 60-79) by sex.<sup>200</sup>

The CHMS collected detailed personal information during a home interview followed several days later by a scheduled physical examination, collection of blood and urine, and physical testing at a mobile examination clinic (MEC). Among the 5,604 survey respondents, a random selection of 2,634 respondents were scheduled for a morning appointment at the MEC and were asked to fast for twelve hours prior to their scheduled appointment for the measurement of fasting glucose and insulin.

Figure 3 shows sample recruitment from initial household selection to the final number of respondents in our analysis. From the 8,772 households selected for the CHMS, 6,106 (69.6%) responded<sup>200</sup>. Within responding households, there were 2,513 persons selected to participate in the POPs sub-sample, of whom 2,203 (87.7%) responded to the questionnaire. Among these persons, 1,841 also reported to the MEC and 1,696 actually fasted and provided blood for an individual-level response rate within responding households of 67.5%. This subsample is representative of 20 to 79 years-old adults living in the target population described above at the sample selection period in 2007. At the Canadian scale (including non-response at both household and individual levels), a combined response rate of 47.0% was observed for the entire survey (including both fasting and non-fasting groups). It is important to note that the combined response rate is not necessarily obtained by multiplying the response rates at the person and household levels, since two persons were selected in some households. The

analysis excluded 84 individuals who self-reported ever being diagnosed by a health professional with type 1 diabetes or who had missing data on one or more variables giving a final sample size of 1,612 for this analysis.



**Figure 3. Sample size of the CHMS and the analysis**

### **3.4. Ethics Review and Authorization to Access Confidential Microdata**

An application for access to the CHMS microdata was approved by the Social Sciences and Humanities Research Council and Statistics Canada. Projects approved

for access are permitted to use the CHMS microdata for statistical analyses within the Research Data Centres (RDCs). As well, the approval from the SFU Research Ethics Board was obtained before conducting this research.

Statistics Canada provides access to detailed anonymous microdata within the RDCs. The CHMS microdata within the RDCs contain the detailed questionnaire responses, physical measurements and biological tests for all respondents who agreed to share their information with Health Canada and their respective provincial ministry of health. All names, addresses and other direct personal identifiers were removed from the microdata by Statistics Canada prior to beginning microdata access to ensure each record was anonymous. Survey participants provided written informed consent to Statistics Canada for the use of data for statistical research purposes. Survey procedures were approved by the Health Canada Research Ethics Board.

### **3.5. Identifying Respondents with T2D**

The outcome for the specified models discussed below was estimated as the OR of having T2D. Respondents were identified as having T2D if they met one or more of the following criteria:

- glycosylated haemoglobin (HbA1c) of 6.5% or higher
- fasting glucose of 7.0 mmol/L or higher
- diabetic medication use
- self-reported ever diagnosed with T2D by a health professional excluding a diagnosis during childhood or adolescence or a diagnosis of gestational diabetes (self-reported)

The American Diabetes Association's (American Diabetes Association, 2008) criteria for diabetes are either:

- (i) fasting blood glucose above 7.0 mmol/L with the normal range below 5.60mmol/L and (ii) has been either diagnosed with diabetes by a physician or uses diabetes medication, or
- glycosylated haemoglobin level of 6.5% or higher.

The criteria used in this analysis to identify respondents as diabetic requires respondents to meet any one of the measures for fasting glucose, HbA1c, medication use or diagnosis. This definition is preferable given that some respondents have some missing information such as the self-reported status of physician diagnosis. Nearly all respondents identified with T2D met two or more of the above listed criteria.

The Canadian Diabetes Association's 2008 Clinical Practice Guidelines are the recommended criteria for diagnosis of diabetes for Canadian physicians.<sup>201</sup> The criteria are:

- fasting plasma glucose  $\geq 7.0$  mmol/L
- 2 hour measure following 75g oral glucose tolerance test  $\geq 11.1$  mmol/L
- if fasting plasma glucose between 6.1 and 6.9 mmol/L then it is necessary to check if 2 hour measure following 75g oral glucose tolerance test  $\geq 11.1$  mmol/L
- if fasting plasma glucose between 5.6 and 6.0 mmol/L then and one or more risk factors are present then it is necessary to check if 2 hour measure following 75g oral glucose tolerance test  $\geq 11.1$  mmol/L

When a patient has an eight hour fasting glucose level between 6.1 and 6.9 mmol/L, it is necessary to measure the glucose level two hours following a 75g oral glucose tolerance test (OGTT). The OGTT was not conducted as part of the Canadian Health Measures Survey cycle 1. Without data for the OGTT, the CDA diagnostic criteria cannot be used for defining T2D in this analysis.

### **3.5.1. Self-Reported Diagnosis of Diabetes by a Health Professional**

The CHMS provides the following information on self-reported diagnosis of diabetes by a health professional. Below are the questionnaire items used by the CHMS:

- Has diabetes
- Diabetes - insulin dependent (type 1)
- Diabetes - non-insulin dependent (type 2)
- Diabetes - gestational
- Diabetes - age first diagnosed

Respondents diagnosed with diabetes during childhood/adolescence or diagnosed with gestational diabetes would have been excluded from this analysis described in this section. However, no respondents met these criteria for exclusion. Respondents with type 1 diabetes or who did not report the type of diabetes diagnosed were excluded.

### **3.5.2. Glycosylated Haemoglobin (HbA1c)**

HbA1c can identify the average plasma glucose concentration over approximately the previous three months.<sup>202</sup> It is formed in a non-enzymatic pathway by haemoglobin's normal exposure to high plasma levels of glucose.<sup>202</sup> Glycation of haemoglobin has been associated with cardiovascular disease, nephropathy, and retinopathy in diabetes.<sup>202</sup> Glycated haemoglobin testing is used for both: (i) checking blood sugar control in people who might be pre-diabetic and (ii) monitoring blood sugar control in patients with more elevated levels, such as with diabetes.<sup>202</sup> For a single blood sample, HbA1c is a better indicator of long-term glycemic behaviour than a fasting blood glucose value.<sup>203</sup>

The International Expert Committee Report, drawn from the International Diabetes Federation (IDF), the European Association for the Study of Diabetes (EASD), and the American Diabetes Association (ADA), suggests the glycosylated haemoglobin (HbA1c) level of 6.5% or greater as a diagnostic level for diabetes.<sup>204</sup> The committee's report further states that, when HbA1c testing cannot be done, the fasting and glucose tolerance tests be used.

Since HbA1c is not appropriate as an indicator of glycemic behaviour for some persons<sup>205</sup>, the HbA1c measure was changed to a missing value for respondents who donated blood within one day prior to the CHMS blood draw. As well, respondents who received erythrocytes within the month prior to the blood draw had their HbA1c measure change to a missing value. The CHMS did not collect blood from respondents with haemophilia or who received a chemotherapy treatment within the preceding four weeks.

The following conditions or characteristics which are not identified or are difficult to identify in the CHMS data affect the respondent's HbA1c level. Glycosylated haemoglobin measurement may not be appropriate where the respondent's diet has

changed substantially within 6 weeks. The standard test for HbA1c assumes a normal red blood cell aging process and mix of haemoglobin subtypes (i.e., predominantly HbA in normal adults). Therefore, people with genetic differences in the haemoglobin molecule (haemoglobinopathy) such as sickle-cell disease and other conditions may have altered HbA1c levels. Finally, HbA1c is not detectable in respondents with autoimmune haemolytic anemia with the standard method of measurement.<sup>206</sup> However, this disease is rare with a prevalence of only 1 to 3 in 100,000.

### **3.5.3. Diabetic Medication Use**

The CHMS collected extensive detail on medication use among respondents. Data were collected for up to fifteen different medications for each of prescriptions, over the counter medication and herbal remedies for a maximum of forty-five medications being used at the time of the household interview. Information was also available for up to five additional medications started between the household interview and clinical examination.

The Anatomical Therapeutic Chemical (ATC) classification system<sup>207</sup> is used by the CHMS to classify medications used by respondents. The ATC was developed by the World Health Organization's Collaborating Centre on Drug Statistics for the purpose of population estimation and analysis of medication usage. Respondents who used a medication in the ATC's A10 Medications Used in Diabetes category are identified as a diabetic medication user for the analysis presented below.

## **3.6. The Measures of POP Plasma Concentrations**

The CHMS analysed plasma, serum and urine samples for approximately eighty environmental pollutant chemicals. Lipophilic chemicals were measured in plasma, including the OCPs, PCBs and PBDEs. The plasma concentration of each POP was measured for a subsample of 1,696 respondents. POPs were measured in plasma at the Institut national de santé publique du Québec Centre de Toxicologie after enrichment with internal standards, denaturation with formic acid, automatic extraction from the aqueous matrix using solid phase separation, and cleanup on florisil columns. Analyses were done by gas chromatography (Agilent 6890) coupled to an electron capture

detector (ECD) (Agilent G2397A) and mass spectrometry detector (Agilent 5973 Network) with Agilent MSD Chem software. Ions generated were measured after negative chemical ionization. Average contamination in the laboratory blanks was subtracted from each sample for hexachlorobenzene and PBDE 47.<sup>208</sup>

POPs are lipophilic chemicals which concentrate in lipids in the body, including lipids in plasma and serum. To adjust for total lipids in serum, the POPs were analyzed in this study as weight of chemical per kilogram of total lipid ( $\mu\text{g}$  chemical/kilogram lipid). Health Canada recommends analysis of the chemical concentrations relative to lipids.<sup>46</sup> Total lipids (g/l) were estimated using the formula:

$$\text{Total lipids (g/L)} = 2.27 \times \text{total cholesterol (g/L)} + \text{triglycerides (g/L)} + 0.623$$

When the triglycerides value was below the laboratory limit of detection, then the value was imputed using the method recommended by Hornung and Reed.<sup>209</sup> There were no observations with values below the laboratory limit of detection for total cholesterol.

Preferably, the lipids and environmental chemicals should be measured in the same matrix (e.g., plasma) at the same laboratory.<sup>46</sup> Operational constraints required the environmental chemicals to be measured in plasma at INSPQ and lipids derived from total cholesterol and triglycerides were measured in serum by the Health Canada Nutrition Laboratory. The difference between lipid measurements in plasma or serum reported in several studies found plasma lipid levels to be from 1.3% to 6% lower than serum lipid levels.<sup>210-214</sup>

The INSPQ reported the use of different laboratories for testing environmental chemical concentrations in plasma and lipid concentrations in serum can introduce variability of up to 10% to 15% in lipid measurements.<sup>215</sup> Health Canada considers the measured values for the lipid adjusted environmental chemicals may be underestimated due to the plasma-serum lipid difference.<sup>46</sup> There was no adjustment to the data by the CHMS or in this study for these potential differences in measurements between lipid and serum matrices, or for potential differences in measurements between laboratories.

The measurement of POPs in plasma and lipids in serum will affect the comparability of the POPs concentrations in this population to other populations. The validity of regression coefficients in the models is not affected given that all participants in the sample had lipids measured at the same Health Canada Nutrition laboratory.

POPs were selected for inclusion in the analysis when more than 60% of the sample had detectable and measurable blood plasma concentrations: Table 1 shows the nineteen POP compounds included in the analysis and the percentage of the sample with a plasma concentration below the limit of detection (<LOD).

Each of the pollutants listed in Table 1 is a distinct chemical compound except for Aroclor 1260 (CASRN 11096-82-5), which is a technical mixture of PCBs containing approximately 60% chlorine. The CHMS calculated the concentration of Aroclor 1260 according to a commonly used formula applied in many other studies.<sup>44,51,52</sup> For each participant, the concentrations of PCBs 138 and 153 were added and this sum was then multiplied by a factor of 5.2, which corresponds to the correlation between the concentration of Aroclor 1260 and the concentrations of PCBs 138 and 153.<sup>216,217</sup> This method of calculating the plasma concentration of Aroclor 1260 allows for comparisons to older studies. Earlier studies commonly reported PCBs concentrations for commercial mixtures such as Aroclor since the analytical methods used did not have sufficient resolution to identify individual PCB congeners.

The POP measures were included as an exposure parameter into a series of separate models after the potential confounding parameters for obesity, physical activity, and age were specified and entered into the model. These confounding parameters are defined below in section 3.7. The models had the same confounding parameters for obesity, physical activity and age, but there was different exposure parameter used for each model.

**Table 1. POPs included in the analysis showing percentage of the sample below the limit of detection (<LOD)**

	% <LOD
<b>Organochlorine pesticides (OCP)</b>	
<b>Chlordane</b>	
oxychlordane (OCHL)	2.58
trans-Nonachlor (TNAC)	6.00
<i>p,p'</i> -Dichlorodiphenyldichloroethylene ( <i>p,p'</i> -DDE)	0.36
Hexachlorobenzene (HCB)	24.73
$\beta$ -Hexachlorocyclohexane ( $\beta$ HCH)	6.96
<b>Polychlorinated Biphenyls (PCB)</b>	
<b>Dioxin like PCBs</b>	
2,3',4,4',5-Pentachlorobiphenyl (PCB 118)	9.66
2,3,3',4,4',5-Hexachlorobiphenyl (PCB 156)	28.81
<b>Non-dioxin like PCBs</b>	
2,2',3,4,4',5'-Hexachlorobiphenyl (PCB 138)	1.44
2,2',3,4',5,5'-Hexachlorobiphenyl (PCB 146)	37.15
2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153)	2.52
2,3,3',4',5,6-Hexachlorobiphenyl (PCB 163)	21.37
2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB 170)	14.89
2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180)	1.38
2,2',3,4',5,5',6-Heptachlorobiphenyl (PCB 187)	19.63
2,2',3,3',4,4',5,5'-Octachlorobiphenyl (PCB 194)	29.53
2,2',3,3',4,5',6,6'-Octachlorobiphenyl (PCB 201)	30.91
2,2',3,4,4',5,5',6-Octachlorobiphenyl (PCB 203)	35.65
Aroclor 1260 (PCB 1260)	0.42
<b>Polybrominated diphenyl ethers</b>	
2,2',4,4'-Tetrabromodiphenyl ether (PBDE 47)	25.27

### **3.6.1. Imputation of values below the limit of detection**

In epidemiological studies, it is common for environmental pollutant and biomarker measurements to have values censored by the limits of detection (LOD) based on the laboratory methods used. Ignoring the observations below or above LOD can produce biased estimates as these “missing” observations are not random.<sup>218,219</sup> When the measurement for plasma concentration was below the laboratory LOD, a value was imputed based on the widely used method recommended by Hornung and Reed.<sup>209</sup> For each separate POP compound, this method imputed a single value for observations below the LOD based on the standard deviation of the geometric mean of the POP compound. When the standard deviation was less than three then the imputed value was the minimum LOD for the POP compound divided by the square root of 2. When the standard deviation was equal to or greater than 3 then the imputed value was the minimum LOD divided by 2. The Hornung and Reed method for imputation was selected as it is commonly used in epidemiological studies using pollutant measures with detection limits, and allows greater comparability with other epidemiological studies of the association between POP blood concentrations and the odds of T2D..

An alternative to Hornung and Reed method was applied by Lee and colleagues for analysis of the NHANES. Lee and colleagues used all observations below the LOD as the reference group where POP quantiles are included as exposure parameters in logistic regression models of T2D.<sup>137,138</sup> The decision of which of these two approaches to use is dependent upon how the exposure will be quantified in the model. Estimation of the odds of T2D associated with quantiles of the exposure is appropriate where the relationship between the exposure and the outcome may not be strictly linear. However, the estimation of odds for a disease can be problematic when the prevalence of the disease is very low for some of the quantiles, as is the situation with the CHMS where the prevalence of T2D is very low in the lower quantiles for the POP compounds. Therefore, the POP compounds are modeled as a continuous parameter, and the Hornung and Reed method is used for imputing the value below the LOD in this analysis.

A third basic method for imputing a value below LOD can be used if the distribution of the measurement data is known. As an example, many POP compounds are known to have lognormal distributions, and we could therefore use an alternative strategy which replaces values below the LOD with expected values of the unobserved concentrations, conditional on being less than the LOD.<sup>220,221</sup> In this approach the measurement  $Z$  and LOD can be denoted as  $E[Z|Z < \text{LOD}]$ . The calculation of the conditional expected value requires knowledge or estimation of the parameters of the measurement distribution.

The substitution methods described above are simple to apply, because one value replaces all measurements below the LOD. Except for  $E[Z|Z < \text{LOD}]$ , the distributional assumptions do not need to be known or estimated. However, because a single value represents all measurements below the LOD, parameter estimates and their variances may be biased, particularly if the proportion below LOD is high. More advanced methods have been developed for analysis of data with detection limits including: (1) “fill in” values randomly selected from an appropriate distribution; (2) extrapolation from regression on order statistics; (3) censored regression, and; (4) multiple imputation from maximum likelihood estimation; and (5) logistic regression. Evaluation of these methods have used simulation studies. Based on simulation studies of a variety of different scenarios, Uh and colleagues reported the deletion of observations below LOD and the extrapolation by regression on order statistics methods gave biased parameter estimates. Their simulations indicated that the single substitution methods such as  $\text{LOD}/2$  or  $\text{LOD}/\sqrt{2}$  underestimated variances, and logistic regression suffered from loss of power.<sup>222</sup> Tobit regression performed well when the proportion of non-detected observations was less than 30%. Overall, the multiple imputation method performed best under different scenarios of various proportions of non-detected observations, sample sizes and in the presence of heteroscedastic errors. Chen and colleagues also found multiple imputation methods to yield unbiased estimates.<sup>223</sup>

Using a more limited simulation study, Lubin and colleagues reported that traditional methods such as  $\text{LOD}/2$  can be biased unless the percentage of measurements below detection limits is small (i.e., 5–10%).<sup>224</sup> The “fill-in” method was found to produce unbiased parameter estimates when less than 30% of observations are

below the LOD. However, the fill in method may produce biased variance estimates when more than 30% of the data are below detection limits. Censored regression methods (e.g., Tobit regression) and multiple imputation were found to be unbiased methods for analyzing measurement data with detection limits. Lubin and colleagues recommended where the analysis is principally concerned with estimating regression parameters, then tobit regression should be used. Furthermore, unless the proportion of missing data is extremely high, multiple imputation was found to yield unbiased estimates and nominal confidence intervals when individualized values for measurements below detection limits were needed for additional analysis, such as relative risk regression or graphical display.

The “fill-in” method requires the form of the distribution to be specified and then estimates its parameters followed by assignment of randomly sampled values below the LOD from the estimated distribution. With appropriate estimation techniques, this approach accommodates both lower and upper LODs. The fill-in method does not require the same complex modeling as described below. Although the fill-in approach assigns random values from an appropriate distribution, it does not account for the variability of the imputation process, because the inserted values are not real data.<sup>225,226</sup>

The extrapolation of values using regression on order statistics involves computing a linear regression for data versus their normalized scores below the LOD values which are extrapolated under a specific distributional assumption. The tobit model approach supposes that there is a latent (i.e., unobservable) variable  $y_i^*$ . This variable linearly depends on  $x_i$  via a parameter (vector)  $\beta$  which determines the relationship between the independent variable (or vector)  $x_i$  and the latent variable  $y_i^*$  as in a linear model. In addition, there is a normally distributed error term to capture random influences on this relationship. The observable variable is defined to be equal to the latent variable whenever the latent variable is above zero and zero otherwise. The logistic model approach to imputation uses a dependent binary outcome of 0 for non-detected values and 1 for detected values. Finally, the multiple imputation methods estimate the mean and standard deviation by maximum-likelihood estimation from a large number of replicated samples. The results from each of these samples are then pooled.

A limitation of the analysis presented here may be the use of a simple single value imputation method for observations with POP concentrations below the laboratory LOD. The use of such an imputation method rather than censored regression or multiple imputation may result in some level of bias being introduced into the estimates. The use of the Hornung and Reed approach to imputation for measurements below the LOD is consistent with most studies using pollutant measurements with detection limits.<sup>222,224</sup>

### **3.6.2. Groupings of POP compounds and group summed measurement**

Five groupings of POPs were formed from these measures of separate POPs. Table 2 lists the POPs included in each grouping. For each group, the molar weight sum of all POPs in the group is calculated. The molar weight for each POP is calculated by converting the measurement from µg/L to µmol/L using the formula:

$$\text{mol/L} = (\mu\text{g/L}) \times \text{conversion factor}$$

where the conversion factor is equivalent to 1/mass. The mass is measured as (µg/mol). The mass for each POP is available from both Health Canada Report on Human Biomonitoring and from the Combined Chemical Dictionary.<sup>46,227</sup> The measurement using mol/L has molar weights for POPs around 1.0+E5. To shift the decimal point, I use the molar weight of µmol/L for the analysis. The measure of 1,000,000 µmol/L equals 1 mol/L.

The groupings selected for summed measures were based on commonly used groupings of POP compounds. These groupings reflect general chemical similarities as when referring to PCBs, PBDEs, and chlordane. The organochlorine pesticide compounds are grouped together based on their intended use as insecticides. The differentiation of dioxin-like PCBs from non-dioxin-like PCBs is based on the expected biological point of interference. Specifically, dioxin-like chemicals are known to bind with the aryl-hydrocarbon receptor in animal cells and result in specific biological effects.<sup>228</sup> Non-dioxin-like PCBs are expected to bind with other cellular receptors and thus have distinct biological effects. However, these groupings may not appropriately reflect the biological pathway from POPs exposure to development of insulin resistance and eventually the onset of T2D. There may be alternative groupings of POPs which better

identify the compounds most directly involved in the hypothesized pathways of suppression of insulin signalling or increased intestinal absorption of lipids. Further research is necessary to identify how specific chemical compounds are involved in each of these pathways, and other potential pathways leading to metabolic dysfunction. The use of groupings which do not reflect how these chemicals are involved in the pathogenesis of T2D would yield estimates for biologically irrelevant summed measures.

**Table 2. Groupings of Persistent Organic Pollutants Included in the Analysis**

Grouping	Molar weight sum of these POPs
POP	All POP measures listed in Table 1 excluding Aroclor 1260
Organochlorine pesticides (OCP)	BHCH, DDE, HCB, OCHL & TNAC
Chlordane	OCHL & TNAC
Dioxin like PCBs	PCBs 118 & 156
Non-dioxin like PCBs	PCBs 138, 146, 153, 163, 170, 180, 187, 194, 201 & 203

### 3.7. Parameter Selection for the T2D Model Without Adjustment for POP Plasma Concentration

Before testing for an association of POP plasma concentration with the OR of having diabetes, I specified a basic model without a pollutant parameter using established determinants of T2D. The following parameters were considered for inclusion in the basic model for predicting the OR of having T2D based on a priori knowledge and quality of the data.

#### 3.7.1. Measures of obesity

Obesity is a known risk factor for diabetes.<sup>193,195,197,229</sup> Figure 2 illustrates that obesity is expected to be a predictor of the OR of having T2D after adjusting for other predictors. The CHMS employed trained examiners for anthropometric measurements during each respondent's visit to the mobile examination clinic. Selecting the measures to adjust for obesity in the model was based on a priori knowledge and assessment of model fit. The measures of obesity selected as predictors in the models for the OR of T2D include body mass index (BMI), waist circumference (WC), and waist to hip ratio (WHR). Body Mass Index (BMI) is a comparison of "weight" relative to the "height" of

respondents. BMI was calculated by dividing weight in kilograms by height in metres squared as  $BMI = \text{weight (kilograms)} / \text{height (metres)}^2$ . Each of the measures has reported advantages and limitations as discussed in this section below.

Abdominal obesity as measured by waist circumference and waist-to-hip ratio has been reported to be a better predictor than was overall body mass for T2D,<sup>195,230</sup> metabolic syndrome,<sup>231</sup> and cardiovascular risk.<sup>232–234</sup> The American Heart Association also recommends use of WC or WHR as better predictors of health risk than BMI when a trained examiner is taking measurements.<sup>235</sup> An analysis of the Australian Risk Factor Prevalence Study found the waist to hip ratio provides a superior measure of central obesity with low measurement error, high precision, and no bias over a wide range of ethnic groups.<sup>236</sup> However, some clinicians have pointed out that ratios such as the waist-to-hip ratio can lead to misclassification particularly for some females with a relatively larger hip circumference.<sup>235</sup>

Health Canada reports that WC and WHR are most predictive of additional risk with a BMI between 18.5 and 34.9. The WC and WHR measurements are less predictive of additional health risk when BMI is greater than 35.0.<sup>237</sup> To adjust for this expected diminishing risk for BMI above 35, an interaction term between BMI and WC or WHR is included in the models as specified below.

### **3.7.2. Measures of physical activity, sedentariness and fitness**

Physical activity, sedentariness and fitness are factors known to be related to T2D.<sup>196,197</sup> There is a disease pathway through physical activity and/or fitness as shown in figure 2 after adjusting for other parameters. Therefore, physical activity/fitness is included in the model of T2D. Several measures were considered as indicators of physical activity, sedentariness and fitness. Variable selection was based on a priori knowledge and assessment of data quality and model fit.

The CHMS includes many direct measures of physical activity and fitness from an accelerometer monitor and fitness testing at the mobile examination clinic. The CHMS provides data from an accelerometer device worn by respondents for up to a week. The accelerometer data indicates general daily activity levels. Respondents also

were asked to participate in a series of fitness tests based on a modified version of the Canadian Fitness Test (mCAFT).

The direct measured data from the activity monitor and/or the fitness testing would be preferable to use in the models for their accuracy. However, non-response and missing data are significantly higher for the direct measures data than for the self-reported data. The respondents who provided direct measures of activity or fitness were substantially different in demographic and other characteristics from the respondents with missing data for the direct measures. Respondents with direct measures had significantly higher self-reported physical activity levels as compared to respondents who did not provide direct measures of activity or fitness (results are not shown).

In contrast, the self-reported measures of physical activities have a very low proportion of missing values. Therefore, a self-reported measure of activity was selected for the models over a direct measure to minimize the loss of respondents from the analysis due to missing data and to avoid introducing selection bias into the models. The daily leisure activity energy expenditure measure derived from self-reported physical activity information is the conceptually most appropriate self-reported measure. Daily leisure activity energy expenditure was calculated by the CHMS using the frequency and duration per session of the physical activity and the metabolic equivalent task (MET) value of the activity.<sup>238</sup> The MET is a value of metabolic energy cost expressed as a multiple of the resting metabolic rate. For example, an activity of 4 METs requires four times the amount of energy as compared to when the body is at rest. The measure was calculated as:

$$EE \text{ (Energy expenditure for each activity)} = (N * D * \text{MET value}) / 365$$

Where: N = the number of times a respondent engaged in an activity over a 12 month period. D = the average duration in hours of the activity. MET value = the energy cost of the activity expressed as kilocalories expended per kilogram of body weight per hour of activity (kcal/kg per hour) / 365 days. MET values are generally expressed in three intensity levels (i.e. low, medium, high). This calculation is adopted from the Canadian Fitness and Lifestyle Research Institute.

### **3.7.3. Measure of age**

Age is a potential confounder for the relationship between POPs plasma concentration and the OR of T2D. The process of aging could itself affect the onset of T2D.<sup>192</sup> Additionally, POPs are known to bioaccumulate in tissues with age and are therefore expected to have a strong positive correlation with age. To assess if there was a potential for collinearity between age and each exposure measure in the models, the correlation between age and each exposure measure was estimated. High correlations, for example above 0.9, indicate a potential for collinearity between variables when included together in a regression model. Another indicator of collinearity in a model is the change in the standard error of the estimate of the exposure parameter after adding age to the model. If the parameter estimate's standard error is substantially inflated by adding age then there may be a problem of collinearity.

The date of birth and date of data collection were included in the CHMS data. The age in years at the date of both the questionnaire interview and the date of physical examination were derived from these dates. Age in years at the time of the physical examination was used as a covariate in the models.

## **3.8. Model Specification**

The following series of models were specified to determine if an association exists between plasma concentrations of POPs and the OR of having T2D. Given that there are some conflicting reports and recommendations around the preference of measuring abdominal obesity by waist circumference or by waist-to-hip ratio<sup>235,236</sup>, I conducted a sensitivity analysis replacing waist circumference with waist-to-hip ratio in the logistic regression models. Two models were specified without a POP parameter to determine the base models into which a POP measure was added. Models with a POP measure were tested with both measures of individual POP compounds and summed measures of groups of POP compounds.

### 3.8.1. Models Without a Pollutant Parameter

The parameters described above for obesity, physical activity and age were included in the basic model for predicting the OR of having T2D. The measure selected as the physical activity parameter for the model was the daily energy expenditure during leisure activities. Years of age at the time of the physical examination was the measure selected for the parameter of age.

The obesity parameters selected were body mass index (BMI), waist circumference (WC) and waist to hip ratio (WHR). WC and WHR are closely associated measures of abdominal obesity. Including both in the same model is anticipated to introduce a problem of collinearity into the model. Separate models were tested with WC (model 1) and with WHR (model 2) as a sensitivity analysis. The testing of two models with different measures of abdominal obesity demonstrates the robustness of the results across two different measures.

(1)

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1(WC) + \beta_2(BMI) + \beta_3(WC * BMI) + \beta_4(ENERGYX) + \beta_5(AGE)$$

(2)

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1(WHR) + \beta_2(BMI) + \beta_3(WHR * BMI) + \beta_4(ENERGYX) + \beta_5(AGE)$$

Where  $\ln(p/(1-p))$  is the natural logarithmic OR of having T2D; WC is waist circumference; WHR is the waist to hip ratio; BMI is body mass index; ENERGYX is daily energy expenditure during leisure activities; and AGE is years of age at the time of the physical examination. The model fit and diagnostics for each model were assessed as described in the following chapter.

An interaction term between BMI and the abdominal obesity parameter (WC or WHR) was included in each of the above models. Health Canada has reported that increased waist circumference was associated with no increase in risk for many health conditions when BMI is above 35.<sup>239</sup> It was expected that an interaction term could be significant in both model 1 and 2. Several cut-points were tested for BMI to assess if a

binary BMI term was suitable in specifying the interaction term. The cut-points tested were based on previous studies examining increased health risk associated with BMI and abdominal obesity.<sup>240</sup> However, a cut point with a statistically significant coefficient could not be identified. Therefore, the interaction term is the product of BMI and the abdominal obesity parameter.

### 3.8.2. *Models Including a Pollutant Parameter*

To test if POP plasma concentrations were associated with the OR of having T2D, the specifications of basic models 1 and 2 were altered to include a measure of plasma concentration for a POP compound. For example, model 1 is nested within model 3 which includes one of the POP compounds listed in Table 1 in section 3.5. Similarly, model 2 is nested within model 4. As described above, there were nineteen specific POP compounds selected for analysis with the following models 3 and 4:

(3)

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1(WC) + \beta_2(BMI) + \beta_3(WC * BMI) + \beta_4(ENERGYX) + \beta_5(AGE) + \beta_6(POP)$$

(4)

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1(WHR) + \beta_2(BMI) + \beta_3(WHR * BMI) + \beta_4(ENERGYX) + \beta_5(AGE) + \beta_6(POP)$$

Where *POP* is the log transformed lipid-adjusted plasma concentration measure for one of the nineteen selected compounds listed in Table 1. The other parameters are described above for models 1 and 2. In total nineteen variants of model 3 were tested. Each variant has a different individual POP compound. Similarly, nineteen variants of model 4 were also tested. Each of the variants for model 4 also specified a different POP compound from Table 1. The plasma concentration measures for each POP compound were log transformed to improve model fit and diagnostics as described in the section on model diagnostics in the following chapter.

The measures of summed molar weight of each POP grouping as listed in Table 2 were tested in models 5 and 6. Since the summed measures were of non-lipid-adjusted molar weights, it is necessary to include in these models a parameter for total lipids in plasma. The inclusion of total lipids into the models was necessary to adjust the model for total lipids in plasma.

(5)

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1(WC) + \beta_2(BMI) + \beta_3(WC * BMI) + \beta_4(ENERGYX) + \beta_5(AGE) + \beta_6(POP) + \beta_7(LIPIDS)$$

(6)

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1(WHR) + \beta_2(BMI) + \beta_3(WHR * BMI) + \beta_4(ENERGYX) + \beta_5(AGE) + \beta_6(POP) + \beta_7(LIPIDS)$$

Where *LIPIDS* was the measure of total lipids (g/L) in plasma. The calculation for estimating total lipids is discussed above in section 3.6. The other parameters are described above for models 1 and 2.

The above models estimate the coefficient for each of the POP measures listed in Tables 1 and 2. These coefficients will vary considerable between different POP measures. The variation in coefficients among different POP measures is related in part to the range of values for each POP measure. The coefficients can be standardized to estimate the change in the log OR of having T2D given an interquartile change in the POP measure. The POP measures were standardized by first calculating the interquartile range between the 25<sup>th</sup> and 75<sup>th</sup> percentiles of each POP measure. Next each POP was then divided by its own interquartile range. The interquartile scaled POP measures were then tested in models 7 to 10. Models 7 to 10 were similar to models 3 to 6 except for the use of the interquartile scaled POP measures ( $POP_{iq}$ ). As with models 3 and 4, there were nineteen variants for each of models 7 and 8 for each of the individual interquartile scaled POP measures.

(7)

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1(WC) + \beta_2(BMI) + \beta_3(WC * BMI) + \beta_4(ENERGYX) + \beta_5(AGE) + \beta_6(POP_{IQ})$$

(8)

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1(WHR) + \beta_2(BMI) + \beta_3(WHR * BMI) + \beta_4(ENERGYX) + \beta_5(AGE) + \beta_6(POP_{IQ})$$

Models 9 and 10 had five variants similar to models 5 and 6. Each variant of models 9 and 10 had one of the interquartile scaled summed POP groupings.

(9)

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1(WC) + \beta_2(BMI) + \beta_3(WC * BMI) + \beta_4(ENERGYX) + \beta_5(AGE) + \beta_6(POP_{IQ}) + \beta_7(LIPIDS)$$

(10)

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1(WHR) + \beta_2(BMI) + \beta_3(WHR * BMI) + \beta_4(ENERGYX) + \beta_5(AGE) + \beta_6(POP_{IQ}) + \beta_7(LIPIDS)$$

### 3.9. Other Potential Confounders

Sensitivity analyses for models 1 and 2 were performed with selected other potential confounding characteristics. Sex, smoking history, racial background, low-income status, household income and height were each separately included with models 1 and 2. Each of these potential confounders was found to be not significant and did not meaningfully change the findings for the other covariates. These results are not shown.

The lipid-adjusted plasma concentrations of POPs generally increase with age. However, these levels may be lowered by pregnancy and breast feeding. Therefore, we could expect some differences in the bioaccumulation of POPs in females and males with age. However, being female was not a significant predictor of the OR of having

T2D after adjustment for obesity, daily energy expenditure on leisure activities and age. This finding did not change when any of the POP measures were included as in models 3, 4, 5 and 6. The findings for the POP measures did not change with the inclusion of the parameter for sex to any of the models.

Smoking history was defined as never a daily smoker, a former daily smoker or a current daily smoker. Smoking history was not statistically significant when added with models 1 or 2. Respondents were asked if they self-identified with one or more of the following cultural or racial backgrounds: white, Chinese, South Asian, Southeast Asian, Filipino, Japanese, Korean, West Asian, Arab, Latin American, black or another background classified as other. Racial background was not statistically significant in models 1 and 2.

Socio-economic status is known to be a determinant of health. Household income is a general indicator of the household's socio-economic status. I tested both household low-income status and household income in models 1 and 2 to determine if socio-economic status is a potential confounder. Neither of these parameters of socio-economic status was significant for either models 1 or 2. Each of these measures was tested separately in the models.

Height has been reported as a risk factor for metabolic syndrome. Height can be an indicator of factors such as mother's health and nutrition during pregnancy and following birth. These factors can influence general health and the prevalence of chronic disease during adulthood. When included in models 1 and 2, height was not statistically significant. The other covariates in the models remained statistically significant.

### **3.10. Design-based analysis**

Since the data for analysis are from a survey with a complex multi-stage sampling design, the standard model-based methods for estimating variances were not appropriate for the proposed analyses. A design-based method is more accurate. The balanced repeated resampling method as recommended by Statistics Canada was used with the bootstrap weights from Statistics Canada for calculating standard errors, confidence intervals and other measures of variance for the specified models.<sup>200</sup> This is

a superior test for variance estimation with data from a complex survey design such as the CHMS <sup>241</sup>. Furthermore, as recommended by Statistics Canada through unpublished correspondence, the adjusted Wald  $F$ -test for variance estimation was used in models due to the small number of primary sampling units in the CHMS.

## 4. Findings from the analyses

This chapter presents the findings from the analyses as specified in the preceding chapter. The estimates presented here are from the 2007-2009 Canadian Health Measures Survey conducted by Statistics Canada. The subsample used for the analysis is representative of the adult population from 20 to 79 years of age living in a private household in a province of Canada. The survey excluded residents of the northern territories, Indian Reserves, military bases, and institutions for long term care or correctional services.

The significance level used for test statistics in this analysis was  $\alpha = 0.05$ . Data preparation and analyses were performed with SAS version 9.3. The confidence intervals and p-values for the ORs were estimated using the adjusted Wald *F*-test in WesVar version 5.1 (Westat, Rockville, MD, USA). The R software version 2.14 was used for the non-parametric generalized additive modeling analyses.

### 4.1. Population Characteristics

The sample used in this analysis was representative of the approximately 22,745,000 adults aged 20 to 79 years in the target population described above in section 3.3. About 6.4% or 1,449,000 people in this population were identified with T2D based on the four criteria described above in section 3.5 (Table 3 below). The most common criterion identifying a person with T2D was having 6.5% or more of glycosylated haemoglobin (HbA1c) of total haemoglobin. An HbA1c level of 6.5% or higher indicates the individual had a generally elevated level of glucose in blood (hyperglycaemic) over the three months preceding the phlebotomy collection. Nearly two-thirds (62.9%) of type 2 diabetics were estimated to have at least 6.5% of HbA1c in total haemoglobin. Hyperglycaemia appeared to be a problem for many people with T2D indicating poor control of the disease.

The second criterion used to identify respondents with T2D was fasting blood glucose level of 7.0mmol/L or higher. Nearly half of the type 2 diabetics (46.1%) had fasting blood glucose level of 7.0 mmol/L or higher. These individuals were hyperglycaemic at the time of the fasting phlebotomy collection.

The third criterion for identifying respondents with T2D was use of a diabetic medication. Just over one-third (38%) of those with T2D were using a diabetic medication at the time of the household interview or the physical examination. The other type 2 diabetics were either able to control their blood glucose levels without medication, choose not to use any diabetic medication, or may be in poor control and unaware that they may need medication to control their level of blood glucose.

The final criterion for identifying respondents with T2D was the respondents self-report of ever having been diagnosed with T2D by a health professional. Just over half (54.6%) of type 2 diabetics reported having been diagnosed with T2D by a health professional. The other nearly half of people with T2D did not indicate ever having been diagnosed. Principally, those with undiagnosed T2D had either or both an elevated HbA1c measure of at least 6.5% or a high level of fasting blood glucose of at least 7.0 mmol/L.

**Table 3. Criteria Used for Identifying Type 2 Diabetics**

	% of all type 2 diabetics	standard error	% of adults	standard error
type 2 diabetes	100.0		6.4	0.9
HbA1c ≥ 6.5%	62.9	5.0	*3.8	0.7
glucose ≥ 7.0 mmol/L	46.1	6.6	2.9	0.5
diabetic medication used	38.0	5.7	2.4	0.3
diagnosed by a health professional	54.6	5.9	3.4	0.5
population size estimate	1,448,595	189,783	22,744,594	402,197

\* The coefficient of variance for this estimate is between 16% and 33%. Caution should be used with this estimate.

Table 4 presents the risk factors for T2D which are included in the models for estimating the predicted OR of having T2D. Type 2 diabetics tended to have higher body mass, greater waist circumference and waist to hip ratio, lower daily leisure energy

expenditure and were older than people without T2D. One-third (33.1%) of type 2 diabetics were very obese or severely obese as compared to only 7.6% of non-diabetics based on their body mass index. In contrast, only one-tenth (10.1%) of type 2 diabetics are underweight or normal weight as compared to 41.8% of non-diabetics. The underweight and normal weight classes are combined in Table 4 because the number of respondents with T2D in these BMI groups is below the minimum counts permitted for release by Statistics Canada. The BMI normative classification used to identify obesity groups is described above in section 3.7.1 on measuring obesity.

The population counts and percentages for waist circumference are presented in Table 4 as groups below or above the total population median. Eighty percent of type 2 diabetics had a waist circumference above the median for the total population. Similarly, approximately three-quarters (74.1%) of type 2 diabetics had a waist to hip ratio above the median for the total population. Population estimates of quartiles or tertiles of waist circumference and waist to hip ratio cannot be released because there were too few respondents with T2D in the lowest quantiles to meet Statistics Canada's minimum requirement for release. The normative obesity classification based on waist circumference is not used to describe the population because it excludes a substantial portion of type 2 diabetics. Section 3.7.1 describes the limitations of the waist circumference normative classification for obesity.

Type 2 diabetics tended to be less physically active during leisure time than non-diabetics. Nearly half (46.5%) of type 2 diabetics were in the lowest quartile for daily energy expenditure during leisure activities. In fact, nearly three-quarters of type 2 diabetics (71.9%) were below the total population median for daily energy expenditure during leisure activities.

The above descriptive differences in body mass, waist circumference, waist to hip ratio, and daily energy expenditure were related in part to differences in age distributions between the type 2 diabetic and non-diabetic populations. Among adults 20 to 79 years-old, nearly half (47%) were 60 to 79 years-old while only 19.3% of non-diabetics were in this age group. There were too few respondents with T2D in the younger age groups to meet Statistics Canada's release criteria for publishing more detailed age groups. The adjusted Wald chi-square test statistics for each cross

tabulation in Table 4 were below the 0.05 significance level indicating that the population distributions for type 2 diabetics and non-diabetics were different for these characteristics.

**Table 4. Population estimates of obesity, daily energy expenditure and age by diabetic status, 20-79 year-olds, Canada**

	Type 2 Diabetic			Not Diabetic			Wald Chi sq Pr > Adj F
	Population	S.E.	%	Population	S.E.	%	
<b>Body mass index</b>							0.0003
under & normal weight BMI < 25.0	146,444	44,924	10.1	8,845,761	677,325	41.8	
overweigh & obese 25.0 ≤ BMI < 35.0	822,908	169,895	56.8	10,711,173	514,761	50.6	
very or severely obese BMI ≥ 35.0	479,243	122,369	33.1	1,602,446	290,184	7.6	
<b>Waist circumference</b>							0.0001
below median ≤ 91.7cm	292,757	83,988	20.3	11,841,506	606,217	56.0	
above median ≥ 91.8cm	1,149,603	165,334	79.7	9,302,129	549,961	44.0	
<b>Waist to hip ratio</b>							0.0005
below median ≤ 0.88	373,807	120,200	25.9	11,880,288	627,497	56.2	
above median ≥ 0.89	1,068,553	141,341	74.1	9,256,188	499,695	43.8	
<b>Daily energy expenditure quartiles</b>							0.0125
lowest 1st	673,587	142,330	46.5	5,440,409	535,102	25.5	
2nd	368,546	103,882	25.4	5,499,646	396,564	25.8	
3rd	183,446	28,136	12.7	4,795,480	364,990	22.5	
highest 4th	223,016	67,484	15.4	5,560,464	466,762	26.1	
<b>Age</b>							<.0001
20-59	767,917	143,768	53.0	17,180,193	415,698	80.7	
60-79	680,678	71,773	47.0	4,115,805	120,776	19.3	

The prevalence of T2D was similar between males and females (Table 5). The Wald chi-square test indicates the difference in the prevalence of T2D between males and females was not statistically significant.

**Table 5. Number and Percentage of Males and Females by T2D**

	Has T2D		% T2D	95% confidence limits	
	Yes	No		lower	upper
females	758,437	10,646,264	6.65	3.62	9.42
males	690,159	10,649,734	6.09	4.72	7.90

Wald chi-square test  $p \leq 0.76$

## 4.2. Plasma Concentrations of POPs among Canadian Adults

The most prevalently detected POPs (>90% of samples as shown in Table 1) included oxychlordan and *trans*-nonachlor (components of chlordane), *p,p'*-DDE, hexachlorobenzene, beta-hexachlorocyclohexane, polychlorinated biphenyls 118, 138, 153, and 180, and Aroclor1260 (comprised of PCB's 138 and 153). Less prevalently detected (60-85% of samples) included hexachlorobenzene, polychlorinated biphenyls 156, 146, 163, 170, 187, 194, 201 and 203 and polybrominated diphenyl ether 47. Table 6 presents the arithmetic and geometric means, and their respective standard deviations, of the individual lipid-adjusted POP compounds included in the analysis. The lipid-adjusted plasma concentrations for individual POPs in this chapter are presented as  $\mu\text{g POP} / \text{kilogram lipid}$ . The estimates presented here differ slightly from those published in Health Canada's 2010 Human Biomonitoring Report because of differences in how measurements below the level of detection were imputed as described in section 3.6.

**Table 6. Arithmetic and Geometric Means and Standard Deviations of Individual POP Compounds\***

	Arithmetic		Geometric	
	Mean	Std Error	Mean	Std Error
PCB1260	227.78	14.92	150.79	1.06
PCB118	6.98	0.58	4.61	1.07
PCB138	15.12	0.93	10.17	1.06
PCB146	3.20	0.25	2.33	1.06
PCB153	28.64	1.98	18.52	1.07
PCB156	4.16	0.24	2.96	1.04
PCB163	5.13	0.39	3.50	1.05
PCB170	7.69	0.59	4.88	1.05
PCB180	25.76	1.97	15.32	1.05
PCB187	6.44	0.48	4.03	1.06
PCB194	5.39	0.47	3.28	1.05
PCB201	4.59	0.34	2.93	1.05
PCB203	3.78	0.23	2.59	1.04
βHCH	36.51	11.53	6.39	1.14
HCB	12.55	0.93	9.92	1.05
DDE	326.96	52.49	152.26	1.09
OCHL	5.61	0.25	4.25	1.05
TNAC	8.54	0.48	6.14	1.05
PBDE47	22.02	1.92	11.02	1.04

\* Each of the POP measures was lipid-adjusted ( $\mu\text{g POP/kilogram lipid}$ ).

Tables 7a-7d show the Pearson correlations for the log transformed lipid-adjusted individual POPs, and the molar weight sums of groups of POPs. Log transformations of POPs are used in the models for T2D as discussed in section 4.7 on model diagnostics. The plasma concentrations for some of the POPs were highly correlated. Oxychlordane, a metabolite of chlordane, and trans-nonachlor, a component of chlordane, were the most highly correlated organochlorine pesticides (Table 7a). The non-dioxin-like PCBs were highly correlated with one another (Table 7b). Similarly, the two dioxin-like PCBs also showed a stronger correlation (Table 7c). Among the molar weight sums of POP groupings, non dioxin-like PCBs and dioxin-like PCBs were highly

correlated (Table 7d). The log transformed molar weight of the PBDE measure was poorly correlated with the POP groupings.

**Table 7a. Pearson Coefficients and (p-values) of Organochlorine pesticides**

	<i>p,p'</i> -DDE	HCB	OCHL	TNAC
<b>β-HCH</b>	0.756 (<.0001)	0.526 (<.0001)	0.392 (<.0001)	0.381 (<.0001)
<b><i>p,p'</i>-DDE</b>		0.515 (<.0001)	0.537 (<.0001)	0.517 (<.0001)
<b>HCB</b>			0.499 (<.0001)	0.506 (<.0001)
<b>OCHL</b>				0.905 (<.0001)

\* Each of the POP measures was log transformed lipid-adjusted (µg POP/kilogram lipid).

**Table 7b. Pearson Coefficients and (p-values) of Non Dioxin-Like PCBs**

	<b>PCB146</b>	<b>PCB153</b>	<b>PCB163</b>	<b>PCB170</b>	<b>PCB180</b>
<b>PCB138</b>	0.858 ( <i>&lt;.0001</i> )	0.954 ( <i>&lt;.0001</i> )	0.903 ( <i>&lt;.0001</i> )	0.869 ( <i>&lt;.0001</i> )	0.867 ( <i>&lt;.0001</i> )
<b>PCB146</b>		0.847 ( <i>&lt;.0001</i> )	0.941 ( <i>&lt;.0001</i> )	0.844 ( <i>&lt;.0001</i> )	0.808 ( <i>&lt;.0001</i> )
<b>PCB153</b>			0.922 ( <i>&lt;.0001</i> )	0.918 ( <i>&lt;.0001</i> )	0.946 ( <i>&lt;.0001</i> )
<b>PCB163</b>				0.938 ( <i>&lt;.0001</i> )	0.913 ( <i>&lt;.0001</i> )
<b>PCB170</b>					0.978 ( <i>&lt;.0001</i> )
	<b>PCB187</b>	<b>PCB194</b>	<b>PCB201</b>	<b>PCB203</b>	<b>PCB1260</b>
<b>PCB138</b>	0.878 ( <i>&lt;.0001</i> )	0.738 ( <i>&lt;.0001</i> )	0.750 ( <i>&lt;.0001</i> )	0.774 ( <i>&lt;.0001</i> )	0.964 ( <i>&lt;.0001</i> )
<b>PCB146</b>	0.915 ( <i>&lt;.0001</i> )	0.787 ( <i>&lt;.0001</i> )	0.841 ( <i>&lt;.0001</i> )	0.829 ( <i>&lt;.0001</i> )	0.847 ( <i>&lt;.0001</i> )
<b>PCB153</b>	0.907 ( <i>&lt;.0001</i> )	0.798 ( <i>&lt;.0001</i> )	0.803 ( <i>&lt;.0001</i> )	0.807 ( <i>&lt;.0001</i> )	0.972 ( <i>&lt;.0001</i> )
<b>PCB163</b>	0.949 ( <i>&lt;.0001</i> )	0.860 ( <i>&lt;.0001</i> )	0.879 ( <i>&lt;.0001</i> )	0.872 ( <i>&lt;.0001</i> )	0.911 ( <i>&lt;.0001</i> )
<b>PCB170</b>	0.934 ( <i>&lt;.0001</i> )	0.933 ( <i>&lt;.0001</i> )	0.911 ( <i>&lt;.0001</i> )	0.908 ( <i>&lt;.0001</i> )	0.897 ( <i>&lt;.0001</i> )
<b>PCB180</b>	0.920 ( <i>&lt;.0001</i> )	0.911 ( <i>&lt;.0001</i> )	0.894 ( <i>&lt;.0001</i> )	0.886 ( <i>&lt;.0001</i> )	0.910 ( <i>&lt;.0001</i> )
<b>PCB187</b>		0.875 ( <i>&lt;.0001</i> )	0.921 ( <i>&lt;.0001</i> )	0.890 ( <i>&lt;.0001</i> )	0.892 ( <i>&lt;.0001</i> )
<b>PCB194</b>			0.970 ( <i>&lt;.0001</i> )	0.971 ( <i>&lt;.0001</i> )	0.769 ( <i>&lt;.0001</i> )
<b>PCB201</b>				0.973 ( <i>&lt;.0001</i> )	0.778 ( <i>&lt;.0001</i> )
<b>PCB203</b>					0.793 ( <i>&lt;.0001</i> )

\* Each of the POP measures was log transformed lipid-adjusted ( $\mu\text{g POP/kilogram lipid}$ ).

**Table 7c. Pearson Coefficients and (p-values) of Dioxin-Like PCBs**

	PCB156
PCB118	0.734 (<.0001)

\* Each of the POP measures was log transformed lipid-adjusted ( $\mu\text{g POP/kilogram lipid}$ ).

**Table 7d. Pearson Coefficients and (p-values) of POP Groupings <sup>a</sup>**

	Dioxin-Like PCBs	OCs	Chlordane	All chlorinated POPs <sup>b</sup>
Non Dioxin-Like PCBs	0.905 (<.0001)	0.558 (<.0001)	0.827 (<.0001)	0.681 (<.0001)
Dioxin-Like PCBs		0.607 (<.0001)	0.809 (<.0001)	0.710 (<.0001)
Organochlorine pesticides			0.597 (<.0001)	0.981 (<.0001)
Chlordane				0.672 (<.0001)

<sup>a</sup> Each of the summed molar weight POP group measures was log transformed.

<sup>b</sup> Includes all organochlorine pesticides and polychlorinated biphenyl congeners excluding PCB 1260 and PBDE 47

### 4.3. Models for T2D without Adjustment for POP Plasma Concentration

The specifications of the models without a pollutant parameter are described in section 3.8.1. The adjusted ORs, 95% confidence limits, and p-values for each model are presented in Tables 8a and 8b. The parameters in both models 1 and 2 were statistically significant at the 0.05 level. The directions of the coefficients were as expected. Waist circumference, waist to hip ratio, body mass index and age were each associated with an increase in the OR of having T2D. The negative association for the interaction terms of body mass index multiplied by waist circumference in model 1 and by waist-to-hip ratio in model 2 show the diminishing increase in the OR of having T2D as obesity increases after adjustment by the other covariates. Age was also significantly associated with increased OR of T2D after adjustment for other covariates. Daily energy

expenditure during leisure activities was significantly and negatively associated with the OR of T2D after adjustment for other covariates. Each of the parameters in these models is highly significant and remains in the models.

Tables 8a and 8b also present the unadjusted ORs, 95% confidence limits, and p-values for each risk factor. The unadjusted estimates are from univariate models of T2D. Each of the individual risk factors when not adjusted had highly significant p-values. Adjusting each risk factor by the other parameters in models 1 and 2 moves the ORs away from 1.0 with the exception of age. The OR of T2D by age lessens when adjusted by the other parameters.

**Table 8a. Model 1 OR of T2D with Selected Risk Factors**

Parameters	Crude OR (95% CI)	Adjusted <sup>a</sup> OR (95% CI)
Waist circumference(cm) (WC)	1.065 (1.046, 1.085)‡	1.154 (1.067, 1.284)†
Body mass index (kg/m <sup>2</sup> ) (BMI)	1.146 (1.085, 1.209)‡	1.604 (1.126, 2.286)*
WC x BMI	1.001 (1.000, 1.001)‡	0.996 (0.994, 0.999)†
Average daily energy expenditure	0.708 (0.570, 0.880)†	0.794 (0.666, 0.947)*
Age in years	1.065 (1.052, 1.078)‡	1.059 (1.042, 1.076)‡

‡p≤0.001 †p≤0.01 \*p≤0.05

<sup>a</sup> Adjusted by the other parameters listed.

**Table 8b. Model 2 OR of T2D with Selected Risk Factors**

Parameters	Crude OR (95% CI)	Adjusted <sup>a</sup> OR (95% CI)
Waist-to-hip ratio (WHR)	1.097 (1.065, 1.130)‡	1.365 (1.121, 1.661)†
Body mass index (kg/m <sup>2</sup> ) (BMI)	1.146 (1.085, 1.209)‡	2.542 (1.376, 4.694)†
WHR x BMI	1.001 (1.001, 1.002)‡	0.991 (0.985, 0.998)*
Average daily energy expenditure	0.708 (0.570, 0.880)†	0.788 (0.660, 0.941)*
Age in years	1.065 (1.052, 1.078)‡	1.059 (1.041, 1.078)‡

‡p≤0.001 †p≤0.01 \*p≤0.05

<sup>a</sup> Adjusted by the other parameters listed.

The unadjusted prevalence of T2D did not appear to differ between females and males (Table 5). A binary variable for female was added to models 1 and 2 to test if the

OR of T2D significantly differed between females and males after adjustment for the selected risk factors. The adjusted ORs, 95% confidence limits, and p-values for models 1 and 2 including female are presented in Tables 9a and 9b. Sex did not appear to be associated with the OR of having T2D after adjustment for the selected risk factors. The addition of female as a parameter into models 1 and 2 did not substantially change the ORs or p-values of the other parameters. Sex was not included as a parameter in the models including the plasma concentration of a POP compound.

**Table 9a. Model 1 OR of T2D with Selected Risk Factors including Sex**

Parameters	Crude OR (95% CI)	Adjusted <sup>a</sup> OR (95% CI)
Female	1.099 (0.553, 2.185)	1.229 (0.310, 4.877)
Waist circumference(cm) (WC)	1.065 (1.046, 1.085)‡	1.162 (1.038, 1.302)*
Body mass index (kg/m <sup>2</sup> ) (BMI)	1.146 (1.085, 1.209)‡	1.570 (1.042, 2.367)*
WC x BMI	1.001 (1.000, 1.001)‡	0.996 (0.994, 0.999)†
Average daily energy expenditure	0.708 (0.570, 0.880)†	0.801 (0.651, 0.986)*
Age in years	1.065 (1.052, 1.078)‡	1.057 (1.037, 1.078)‡

‡p≤0.001 †p≤0.01 \*p≤0.05

<sup>a</sup> Adjusted by the other parameters listed.

**Table 9b. Model 2 OR of T2D with Selected Risk Factors including Sex**

Parameters	Crude OR (95% CI)	Adjusted <sup>a</sup> OR (95% CI)
Female	1.099 (0.553, 2.185)	1.526 (0.399, 5.830)
Waist-to-hip ratio (WHR)	1.097 (1.065, 1.130)‡	1.390 (1.118, 1.729)†
Body mass index (kg/m <sup>2</sup> ) (BMI)	1.146 (1.085, 1.209)‡	2.534 (1.351, 4.753)†
WHR x BMI	1.001 (1.001, 1.002)‡	0.991 (0.985, 0.998)*
Average daily energy expenditure	0.708 (0.570, 0.880)†	0.803 (0.656, 0.983)*
Age in years	1.065 (1.052, 1.078)‡	1.057 (1.037, 1.076)‡

‡p≤0.001 †p≤0.01 \*p≤0.05

<sup>a</sup> Adjusted by the other parameters listed.

#### 4.4. Models for T2D Including POP Compound Plasma Concentrations

The basic models 1 and 2 were used as the foundation for fitting models including an exposure parameter for persistent organic pollutant concentrations in plasma. The specific POP compounds selected for analysis are discussed in section 3.5. A separate model was fitted for each individual POP compound selected for analysis. For each of the nineteen variants of models 3 and 4, the parameters for abdominal obesity (i.e., waist circumference, and waist to hip ratio) BMI, the interaction term of abdominal obesity and BMI, daily energy expenditure for leisure activities and age remained statistically significant. To avoid presenting nineteen tables of estimates for each of models 3 and 4, only the ORs and 95% confidence limits for the POP parameters are presented in the following tables.

Differences in the range and scale of each POP measure can make interpretation and comparisons of odd ratios difficult. When the POP measures are divided by the interquartile range from the 25<sup>th</sup> to 75<sup>th</sup> percentiles of each respective POP measure, it is easier to interpret and compare the adjusted ORs between different compounds. It should be noted that the ORs for obesity, physical activity and age were not scaled to the interquartile range, and are therefore not scaled for comparison with the POP compounds. Tables 10 and 11 present the adjusted ORs of having T2D for

interquartile scaled POP measures. These ORs are interpreted as the multiplicative change in predicted OR of T2D for an interquartile change in the POP measure.

In the crude models, all nineteen POPs except PBDE 47 were significantly associated with the OR of T2D (Tables 10a and 10b). After adjustment, all sample parameter estimates for POPs were diminished, but remained above a value of one (Tables 10a and 10b). However, only beta-hexachlorocyclohexane, and non-dioxin-like PCB's 153, 170, 180 and 1260 remained significantly associated ( $\alpha=0.05$ ) with the OR of T2D in models 3 and 4. Additionally, hexachlorobenzene also showed a statistically significant association in model 4. The precision of estimates was good and similar across POPs with the exceptions of oxychlordan and *trans*-nonachlor, which had worse precision than others.

The four statistically significant PCB non-dioxin-like measures had lower confidence bounds above 1.1. PCB 153 is a commonly used marker for exposure to PCBs and has been reported to be associated with T2D in the NHANES.<sup>137</sup> The adjusted OR of having T2D was higher than 2.0 in models 3 and 4 for PCB 153. An interquartile change in the log transformed lipid-adjusted plasma concentration of PCB 153 is associated with a doubling of the OR of having T2D. The 95% confidence limits for this OR were approximately from 1.15 to 3.85 for models 3 and 4. Even at the lower bound of this confidence interval, there was still a 15% increase in the OR of having T2D associated with an interquartile change in the level of the log transformed lipid-adjusted PCB 153. PCB 1260 (Aroclor) is a combined measure of PCB 138 and 153 congeners which had a similarly high OR of just below 2.0 for both models 3 and 4. The lower bound of the 95% confidence interval in model 3 showed an approximate 8% increase in the OR of having T2D associated with an interquartile change in the level of the log transformed lipid-adjusted PCB 1260. PCBs 170 and 180 had similar adjusted ORs of approximately 1.8 to 1.9 in models 3 and 4. The lower bounds of the 95% confidence intervals were at 1.1 for these models.

**Table 10a. Model 3 OR of T2D by inter-quartile plasma POP concentrations in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI)	Adjusted <sup>b</sup> OR (95% CI)
<b>Organochlorine Pesticides</b>		
Oxychlorane	3.842 (2.419, 6.102)‡	2.054 (0.887, 4.754)
<i>trans</i> -Nonachlor	3.606 (2.048, 6.348)‡	1.930 (0.672, 5.542)
<i>p,p'</i> -DDE	2.139 (1.364, 3.354)†	1.446 (0.865, 2.419)
Hexachlorobenzene	1.886 (1.385, 2.568)‡	1.305 (0.958, 1.777)
β-Hexachlorocyclohexane	1.666 (1.241, 2.236)†	1.501 (1.002, 2.249)*
<b>Dioxin-like PCBs</b>		
PCB118	2.638 (1.571, 4.432)†	1.516 (0.780, 2.946)
PCB156	2.721 (2.029, 3.649)‡	1.616 (0.891, 2.931)
<b>Non-dioxin-like PCBs</b>		
PCB138	2.942 (1.966, 4.401)‡	1.733 (0.949, 3.168)
PCB146	2.355 (1.662, 3.337)‡	1.651 (0.919, 2.967)
PCB153	3.106 (2.281, 4.228)‡	2.106 (1.150, 3.854)*
PCB163	3.029 (2.211, 4.148)‡	1.927 (0.949, 3.914)
PCB170	2.900 (2.371, 3.547)‡	1.897 (1.118, 3.219)*
PCB180	2.686 (2.226, 3.241)‡	1.860 (1.140, 3.035)*
PCB187	2.719 (2.043, 3.618)‡	1.769 (0.968, 3.230)
PCB194	2.256 (1.775, 2.867)‡	1.322 (0.836, 2.092)
PCB201	2.252 (1.779, 2.851)‡	1.428 (0.828, 2.463)
PCB203	2.292 (1.888, 2.782)‡	1.360 (0.894, 2.071)
PCB1260	3.055 (2.189, 4.264)‡	1.970 (1.082, 3.588)*
<b>Polybrominated diphenyl ether 47</b>	1.226 (0.841, 1.788)	1.046 (0.686, 1.596)

<sup>a</sup> Each of the individual POP measures was log transformed and divided by the inter-quartile range. Individual POP measures were lipid-normalized.

<sup>b</sup> Adjusted for waist circumference, body mass index, the interaction of waist circumference and body mass index, daily leisure energy expenditure, and age.

‡p≤0.001 †p≤0.01 \*p≤0.05

**Table 10b. Model 4 OR of T2D by inter-quartile plasma POP concentrations in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI)	Adjusted <sup>b</sup> OR (95% CI)
<b>Organochlorine Pesticides</b>		
Oxychlorthane	3.842 (2.419, 6.102)‡	2.145 (0.913, 5.042)
<i>trans</i> -Nonachlor	3.606 (2.048, 6.348)‡	1.875 (0.654, 5.376)
<i>p,p'</i> -DDE	2.139 (1.364, 3.354)†	1.479 (0.888, 2.464)
Hexachlorobenzene	1.886 (1.385, 2.568)‡	1.379 (1.005, 1.890)*
β-Hexachlorocyclohexane	1.666 (1.241, 2.236)†	1.503 (1.035, 2.183)*
<b>Dioxin-like PCBs</b>		
PCB118	2.638 (1.571, 4.432)†	1.551 (0.822, 2.929)
PCB156	2.721 (2.029, 3.649)‡	1.572 (0.859, 2.876)
<b>Non-dioxin-like PCBs</b>		
PCB138	2.942 (1.966, 4.401)‡	1.752 (0.988, 3.107)
PCB146	2.355 (1.662, 3.337)‡	1.575 (0.906, 2.738)
PCB153	3.106 (2.281, 4.228)‡	2.079 (1.163, 3.716)*
PCB163	3.029 (2.211, 4.148)‡	1.867 (0.949, 3.676)
PCB170	2.900 (2.371, 3.547)‡	1.901 (1.119, 3.229)*
PCB180	2.686 (2.226, 3.241)‡	1.836 (1.125, 2.996)*
PCB187	2.719 (2.043, 3.618)‡	1.722 (0.967, 3.067)
PCB194	2.256 (1.775, 2.867)‡	1.268 (0.814, 1.974)
PCB201	2.252 (1.779, 2.851)‡	1.362 (0.819, 2.267)
PCB203	2.292 (1.888, 2.782)‡	1.314 (0.885, 1.952)
PCB1260	3.055 (2.189, 4.264)‡	1.961 (1.103, 3.486)*
<b>Polybrominated diphenyl ether 47</b>	1.226 (0.841, 1.788)	1.086 (0.735, 1.607)

<sup>a</sup> Each of the individual POP measures was log transformed and divided by the inter-quartile range. Individual POP measures were lipid-normalized.

<sup>b</sup> Adjusted for waist-to-hip ratio, body mass index, the interaction of waist-to-hip ratio and body mass index, daily leisure energy expenditure, and age.

‡p≤0.001 †p≤0.01 \*p≤0.05

Among the five organochlorine pesticides included in models 3 and 4, only β-hexachlorocyclohexane (βHCH) had a statistically significant OR in both models. βHCH had an OR of 1.5 in models 3 and 4. However, the lower bound of the confidence interval was only 1.002 for model 3 and 1.035 for model 4. Hexachlorobenzene (HCB) is an organochlorine pesticide with an adjusted OR of approximately 1.38 for model 4.

However, the lower bound of the 95% confidence interval was only 1.005. The OR for HCB in model 3 was not statistically significant.

The POP measures in Tables 10 and 11 are lipid adjusted to indicate the internal dose by volume of lipid. As a sensitivity analysis, the same models were estimated using POP measures without lipid adjustment. Appendix B provides the estimates for these models using POP measures without lipid adjustment. The results were generally similar, although for the model 3 ORs for both PCB 170 and beta-hexachlorocyclohexane were not statistically significant. In model 4, the OR for PCB 138 was statistically significant, and the OR for PCB 170 was not statistically significant. The untransformed POP measures were also tested with these models. A separate series of these models were also tested using the untransformed POP measure and its quadratic term for each POP measure. The coefficients for the untransformed POP measures and for the POP quadratic terms were all not statistically significant at the 0.05 level (results not shown).

These results are consistent with Lee and colleagues analyses of NHANES in that the plasma concentrations of some OC and PCB compounds were found to have a significant positive association with diabetes as discussed in section 2.3.1. It is important to note the analysis in Lee and colleagues' studies differed in at least two respects from the analysis in this present study. First, the NHANES data did not distinguish by type of diabetes. This could have resulted in misclassification of some cases. Type 1 and gestational diabetes do not result from the same causal pathway proposed from exposure to POPs to T2D. The present study excluded respondents with type 1 diabetes, gestational diabetes or who did not know the type of diabetes diagnosed. Second, the POPs were categorized based on quantiles in Lee's studies. In those studies, respondents with the POP concentration below the limit of detection were the referent category while other respondents with detectable levels were assigned to categories based on the 1<sup>st</sup> quartile, 2<sup>nd</sup> quartile, 3<sup>rd</sup> quartile, 75<sup>th</sup> to 89.9<sup>th</sup> percentile, and 90<sup>th</sup> to 100<sup>th</sup> percentile. In contrast, this present study includes POP measures as log-transformed continuous measures with imputed values for measures below the limit of detection. Third, Lee and colleagues' models were adjusted for age, sex, race, income poverty ratio, BMI, and waist circumference. The basis for selecting these covariates for the models was not discussed. However, Lee et al did discuss testing a variety of

possible confounders in the models and rejecting those covariates which were not significant. The present study based the selection of covariates to adjust the model based on *a priori* knowledge and the hypothesis of the causal relationships. Hernán et al's<sup>191</sup> method for using directed acyclic graphs for identifying potential confounders based on a priori knowledge was used to select the covariates for inclusion in the models in this present study.

The models with individual POP measures used lipid-adjusted plasma concentrations. Lipid adjustment is intended to reflect the internal dose per volume of lipid for each POP compound. An alternative approach is to use the total wet weight per volume of plasma to reflect total body burden of each POP compound. A sensitivity analysis was conducted to assess if lipid adjustment may be an over-adjustment when abdominal obesity and BMI were also included in the model. When the models were replicated with POP measures without lipid-adjustment the ORs for the POPs were highly insignificant. Many of the adjusted ORs for POPs without lipid-adjustment were lower than the adjusted ORs for lipid-adjusted POP compounds (results not shown).

#### **4.5. Models for T2D Including Plasma Concentrations of Molar Weight Sums of POP Groupings**

The plasma concentrations for some of the POP compounds selected for analysis showed a statistically significant association with the OR of having T2D. The cumulative concentrations of groups of POPs may also be associated with the OR having T2D. POP compounds were summed based on molar weights as described in section 3.5. The five summed groupings of POPs and their component compounds were listed in Table 2. The basic model 1 was expanded into model 5 to include a summed grouping of POP compounds. Similarly, the base model 2 was expanded to model 6. There was a series of five variants of each of models 5 and 6, each with a different summed grouping of POPs. Since the POP groupings were the summation of molar weights, these measures were not adjusted for total lipids in blood. The measure of total lipids was added as a parameter to the model to adjust for total lipids in blood.

Table 11a shows the ORs and 95% confidence limits for log transformed summed measures for POP classes from the variants of model 5. The ORs and 95% confidence limits for the log transformed summed measures for the POP groupings from the variants of model 6 are presented in Table 11b. The parameters for obesity, daily energy expenditure and age each remained statistically significant with the addition of the summed POP grouping and total lipids measures (results not shown). The concentration of total lipids was not statistically significant for any of the variants of models 5 and 6, but remained in these models.

The summed measures of POPs classes, all were positively and statistically significantly associated with T2D in crude models (only adjusted for total lipids), but after adjustment for the selected covariates, only the summed measure of all non-dioxin-like-PCB's (PCB's 138, 146, 153, 163, 170, 180, 187, 194, 201 and 203) remained statistically significantly associated with the OR of having T2D. However, all adjusted sample parameter estimates for POPs remained above a value of one (Tables 11a and 11b). The adjusted OR of T2D (95% CI) for an interquartile increase in non-dioxin-like PCBs was approximately 1.9 with a lower bound of the 95% confidence interval of 1.07 for models 5 and 6. The precision of the estimates was good and similar for the different summed measures except for chlordane, which had worse precision than other estimates.

The models with summed measures of POP classes were also tested with untransformed measures of the summed POP groupings. A separate series of models were also tested with the untransformed POP measure and its quadratic term for each of the summed POP group measures. The estimates for the untransformed POP group summed measures were not statistically significant (results not shown).

In D-H Lee and colleagues' study of the association of diabetes (types I and II not distinguished) and POP serum concentrations in the 1999-2002 NHANES, POPs were summed using a method intended to assign higher values for relatively higher exposure to multiple POPs.<sup>137</sup> A limitation of summing molar weights is that specific POPs with higher molar weights such as PCB 153 may dominate the summed measure. Lee and colleagues' method assigned scores to ranges for each POP: 0=below limit of detection; 1=1<sup>st</sup> quartile above LOD; 2=2<sup>nd</sup> quartile; 3=3<sup>rd</sup> quartile; 4=75<sup>th</sup> to 89.9<sup>th</sup> percentile; and

5=90<sup>th</sup> to 100<sup>th</sup> percentile. The scores for all six POPs included in the analysis were summed. These summed scores were then ranked into the following quantiles: 1<sup>st</sup> quartile; 2<sup>nd</sup> quartile; 3<sup>rd</sup> quartile; 75<sup>th</sup> to 89.9<sup>th</sup> percentile; 90<sup>th</sup> to 100<sup>th</sup> percentile. Logistic regression then estimated the adjusted ORs for each of these quantiles of the summed scores. When the NHANES respondents were classified according to their respective quartile of the summed scores, adjusted odds ratios of diabetes when compared to the 1<sup>st</sup> quartile were 14.0 (2<sup>nd</sup> quartile), 14.7 (3<sup>rd</sup> quartile), 38.3 (75<sup>th</sup> to 89.9<sup>th</sup> percentile), and 37.7 (90<sup>th</sup> to 100<sup>th</sup> percentile) (*P* for trend <0.001). This approach to modeling was replicated with the CHMS data to estimate the adjusted OR for T2D. The adjusted ORs for T2D were found to be highly insignificant for the same quantiles of summed POP scores for each of the summed POP groupings in this study (results not shown).

**Table 11a. Model 5 OR of T2D by inter-quartile summed molar weights of POP grouping in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI) <sup>b</sup>	Adjusted OR (95% CI) <sup>c</sup>
Organochlorine pesticides	2.220 (1.287, 3.829)†	1.554 (0.856, 2.820)
Chlordane pesticides	4.000 (2.208, 7.245)‡	2.078 (0.741, 5.826)
Dioxin-like PCBs	2.918 (1.841, 4.625)‡	1.631 (0.855, 3.110)
Non-dioxin-like PCBs	3.125 (2.410, 4.052)‡	1.959 (1.077, 3.534)*
All chlorinated POPs	2.550 (1.394, 4.664)†	1.698 (0.854, 3.376)

<sup>a</sup> Each of the POP group summed measures was log transformed and divided by the inter-quartile range.

<sup>b</sup> Adjusted for total lipids as a model parameter.

<sup>c</sup> Adjusted for waist circumference, body mass index, the interaction of waist circumference and body mass index, daily leisure energy expenditure, and age. Models for summed measures also adjusted for total lipids.

‡*p*≤0.001 †*p*≤0.01 \**p*≤0.05

**Table 11b. Model 6 OR of T2D by inter-quartile summed molar weights of POP grouping in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI) <sup>b</sup>	Adjusted OR (95% CI) <sup>c</sup>
Organochlorine pesticides	2.220 (1.287, 3.829)†	1.584 (0.886, 2.830)
Chlordane pesticides	4.000 (2.208, 7.245)‡	2.063 (0.729, 5.836)
Dioxin-like PCBs	2.918 (1.841, 4.625)‡	1.640 (0.870, 3.092)
Non-dioxin-like PCBs	3.125 (2.410, 4.052)‡	1.920 (1.070, 3.444)*
All chlorinated POPs	2.550 (1.394, 4.664)†	1.723 (0.887, 3.348)

<sup>a</sup> Each of the POP group summed measures was log transformed and divided by the inter-quartile range.

<sup>b</sup> Adjusted for total lipids as a model parameter.

<sup>c</sup> Adjusted for waist-to-hip ratio, body mass index, the interaction of waist-to-hip ratio and body mass index, daily leisure energy expenditure, and age. Models for summed measures also adjusted for total lipids.

‡p≤0.001 †p≤0.01 \*p≤0.05

The models with summed POP measures included total lipid plasma concentration as a model parameter. Lipid adjustment is intended to reflect the internal dose per volume of lipid for each summed POP grouping. An alternative approach is to use the total molar weight per volume of plasma to reflect total body burden of each POP grouping. A sensitivity analysis was conducted to assess if lipid adjustment may be an over-adjustment when abdominal obesity and BMI were also included in the model. When the models were replicated with POP measures without lipid-adjustment the ORs for the summed POP groupings were highly insignificant. Most of the adjusted ORs for POPs without lipid-adjustment were lower than the adjusted ORs for the models including total lipids as a parameter (results not shown).

#### **4.5.1. Multiple POP Groupings in the Models for T2D**

The correlations among the POP compounds and groups of compounds (Tables 7a-7d) makes it difficult to identify which of the POP compounds or groups was most highly associated with the OR of having T2D. When models 5 and 6 were adapted to simultaneously include the summed measures of organochlorines, non-dioxin-like PCBs and dioxin-like PCBs, the p-values for these summed measures were highly not significant. The very substantial rise in standard errors may indicate a problem of collinearity in the model. The substantial increase in standard errors was also observed in models with summed POP measures of two groups of POPs. The apparent problem

of collinearity in the models inhibits an analysis to determine if one group of POPs was more highly associated with the OR of having T2D than the other groups.

#### **4.6. Do POP Plasma Concentrations Influence the Association of Obesity or Age with T2D?**

Lee and colleagues' analyses of the NHANES data as discussed in section 2.3.1 reported some evidence of an interaction of the level of summed POPs in serum and the associations of obesity and age with diabetes. To test if the association between POP plasma concentrations and the OR of having T2D increased with age, models 3 and 4 were modified. First, the models were stratified by the major age groups used in the CHMS sample design for 20 to 39 year-olds, 40 to 59 year-olds, and 60 to 79 year-olds. Next, the POP measure included in the models was the summed measure of organochlorines and PCBs. The PBDE47 compound was excluded from the summed measure based on an absence of any association with the OR of T2D (Tables 10a and 10b). The stratified models for the two younger age groups resulted in warnings that the convergence for parameter estimates in some subsamples was questionable due to the distribution of the data points. Specifically, the sample has an insufficient number of respondents with T2D in the two younger age groups to support stratification of the models for T2D into age groups.

The analysis also tested for an interaction between the summed organochlorine and PCBs measure and the association of obesity and the OR of T2D. As described above in section 3.6, the summed group measure was calculated from molar weight sums. Models were separately tested both with the lipid adjusted summed measure and with a summed pollutant measure without lipid-adjustment. The results were similar regardless of lipid adjustment.

The models for T2D were stratified by quantiles (i.e., quartiles and tertiles) of the summed OCs and PCBs measure. However, since there is a strong association between age and plasma concentrations of OCs and PCBs, the summed OCs and PCBs measure was age-adjusted. The quantiles of the summed OCs and PCBs measure were assigned within three-year age groups. A total of twenty age groups between 20

and 79 years of age were used (i.e., 20 to 22 year-olds, 23 to 25 year-olds, ..., 67 to 69 year-olds). The number of respondents with T2D was much more evenly distributed among the quantiles of the summed pollutant group measure. The even distribution of type 2 diabetics among the quantiles was important to avoid the above noted problem with sparse data when stratifying the models by age groups. The use of the age-adjusted quantiles could be interpreted as identifying respondents in body burden trajectories, although the data are cross-sectional. Young respondents in the highest quantile for plasma levels of pollutants could potentially remain in the highest quantile as they age if their exposure to, and elimination of pollutants, does not substantially change relative to other respondents over time. Similarly, respondents in each quantile of the summed pollutant measure could be understood to be within a specific trajectory for POP body burden over time.

A variety of models for T2D were stratified by the age-adjusted summed OCs and PCBs measure. The T2D models were varied by the obesity and daily expenditure parameters included. There was not a pattern of an increasing or decreasing association of obesity and the OR of T2D across quantiles of the age-adjusted summed pollutant measure. Table 12 presents the ORs from the model for T2D including body mass index and age stratified by tertiles of the summed OCs and PCBs measures divided by total lipids.

An increase in the ORs across quantiles of the summed pollutant measure would indicate the association between obesity and T2D becomes stronger as plasma concentrations of these pollutants increase. Table 12 shows no evidence of substantial change or trend in the ORs across tertiles of the age-adjusted summed OCs and PCBs measure. Similarly, there was no evidence of a trend across quartiles of the age-adjusted summed measure (results not shown). An additional test for the T2D model with BMI and age also included an interaction term between BMI and a binary (low, high) measure of the age-adjusted summed OCs and PCBs. This model was not stratified. The interaction term was not statistically significant ( $p < 0.22$ ). Unlike in Lee and colleagues NHANES studies discussed in section 2.3.1, the analysis here found no evidence of an interaction between the summed pollutant measure and the association of obesity and T2D.

**Table 12. OR of T2D Stratified by Age-Adjusted Summed OCs and PCBs Plasma Concentration**

Parameters	Crude OR (95% CI)	Adjusted <sup>a</sup> OR (95% CI)
<i>High Tertile of OCs and PCBs sum</i>		
Body mass index (kg/m <sup>2</sup> )	1.201 (1.048, 1.377)*	1.158 (1.007, 1.332)*
Age in years	1.078 (1.054, 1.102)‡	1.069 (1.045, 1.093)‡
<i>Middle Tertile of OCs and PCBs sum</i>		
Body mass index (kg/m <sup>2</sup> )	1.131 (1.037, 1.233)†	1.121 (1.025, 1.227)*
Age in years	1.051 (1.028, 1.074)‡	1.045 (1.017, 1.074)†
<i>Low Tertile of OCs and PCBs sum</i>		
Body mass index (kg/m <sup>2</sup> )	1.140 (1.039, 1.251)†	1.204 (1.060, 1.366)†
Age in years	1.060 (1.018, 1.103)†	1.083 (1.027, 1.142)†

‡p≤0.001 †p≤0.01 \*p≤0.05

<sup>a</sup> Adjusted by the other parameter listed.

## 4.7. Model diagnostics

The following model diagnostics were used to fit and adjust the logistic models and transform variables as described above. Models were assessed for goodness of fit, distribution of deviance residuals, influential observations, linearity between predicted log OR and the explanatory variables, and collinearity. The graphical plots showing individual observations such as the deviance residual plots and the GAM plots cannot be released from the Research Data Centre due to Statistics Canada's regulations to protect respondent confidentiality. Graphical displays showing individual observations on a plot cannot be released from the centre.

### 4.7.1. Goodness of fit

The Hosmer-Lemeshow goodness-of-fit test was used to assess the overall fit of the logistic models. The observations were first sorted in increasing order according to their predicted probability of outcome. The observations were then divided into groups according to the quantiles of estimated probability. The observed and expected number of events was tabulated for each outcome group. The test statistics were obtained by

calculating the chi-square statistic from the table of observed and expected frequencies and compared to a chi-square distribution. The test hypothesis is that the estimated and observed frequencies agree. Rejection of the null hypothesis indicates a lack of fit. The test statistics for the above models are not significant indicating there is not a gross lack of fit for the models. The Hosmer-Lemeshow test has some important limitations. It is sensitive to the cut-point used. It is not sensitive to misspecification of the model.<sup>242</sup>

#### **4.7.2. Likelihood ratio tests for nested models**

It is important to assess if the addition of the exposure parameter significantly contributes to the model for the OR of T2D. The likelihood ratio test was used to assess if the addition of the POP measure significantly contributed to the initial base models. These tests were performed in SAS using the GENMOD procedure to compare models 1 and 3, and similarly compare models 2 and 4. Model 1 is nested within model 3, and model 2 is nested within model 4. The addition of the POP measures as an explanatory variable to the models did significantly contribute to the log-likelihood of the models (results not shown).

#### **4.7.3. Linearity**

The linearity between the predicted log OR of having T2D and the explanatory variables was assessed using the non-parametric method of generalized additive models (GAM). The GAM plots include a smoothed non-parametric line between the outcome log OR and an explanatory variable when adjusted for other explanatory variables in the model. The log transformation of the POP measures substantially improved the linearity of these variables with the predicted log OR of T2D.

Each predictor in the models was plotted against the deviance residuals to assess if a systematic pattern could be identified in the residuals across values of the explanatory variable for each model. This is a method to identify deficiencies in specification of the parameters per the shape of the association (i.e. for example, assumptions of linearity). The plots of deviance residuals versus the predicted probabilities did not show a systematic pattern in the residuals.

#### **4.7.4. Influential observations**

Cook's D was used to test for influential points<sup>243</sup>. A further assessment for influential observations was also performed by examining graphical plots of deviance residuals, DFBETA, and DIFCHISQ. As well, the deviance residuals were plotted against the identifier number of respondents to assess if any observations appeared to be obvious outliers.

The models were assessed by graphically plotting the predicted outcome probabilities versus the difference between betas (DFBETA). A plot of DFBETAs against predicted outcome probabilities shows the standardized differences in each regression parameter estimate when a specific individual observation is excluded from the analysis. It assesses the effects of individual observations on the estimated regression parameters in the fitted model. The DFBETA statistic is calculated for each regression coefficient for each individual observation by excluding each observation in turn. Influential observations can then be identified by sorting all observations by DFBETA values and printing the observation IDs with the largest values. The DFBETAs did not indicate any observations as being highly influential.

Graphical plots were also assessed for the predicted outcome probabilities versus the difference between chi-square goodness of fit (DIFCHISQ). These plots did not indicate any observations to be highly influential. A plot of DIFCHISQ against the predicted outcome probabilities shows the change to the overall chi-square goodness-of-fit statistic by excluding each individual observation in turn. The DIFCHISQ measures the effect of individual observations on the fit in general.

#### **4.7.5. Collinearity**

Pearson correlations were produced to identify possible collinearity among the outcome and explanatory variables in the models. The only model parameters which had elevated correlations coefficients were between years of age and the plasma concentrations for some of the POP compounds but none of these correlations approached 1.0. Age and the plasma concentrations of POP compounds are expected to be correlated to some extent since these compounds bioaccumulate with age.

An indication of collinearity in a model may be when adding a possible collinear parameter substantially increases the standard error of the other possible collinear parameter. Age was included in the base models without a POP exposure parameter. When the POP parameter was added there was not a substantial change in the standard error in age.

## **5. Public Policy concerning POPs and Public Health**

This chapter begins with an examination of risk assessment, public policies, and possible implications of the findings here and in previous research. The chapter concludes with a summary of the literature discussed here, the hypothesis and findings from the analysis. Followed by a discussion of the limitations of the data and analysis in this study

### **5.1. Public Policy for the ‘Virtual Elimination’ of POPs**

In response to public concern and emerging evidence governments in Europe and North America began regulating the manufacture, use and storage of POP mixtures in the 1970’s with increasing regulation over the next thirty years.<sup>244</sup> The 1978 Great Lakes Water Quality Agreement was one of the first international agreements with the aim of ‘virtual elimination’ of environmental discharge of POPs.<sup>245</sup> Virtual elimination generally refers to reduction to levels below detection. The need for a coordinated international approach to these pollutants emerged with the acknowledgement that regulations varied considerably between jurisdictions but the environmental contamination of these pollutants and their potential health risks was global. However, implementation of this agreement was slow. The International Joint Commission of Canada and the United States (IJC) would not identify specific substances of concern for the Great Lakes basin until 1997 as discussed below. In Canada, the Government of Ontario did not identify specific substances for virtual elimination until 1992 with the release of the Candidate Substances List for Bans and Phase-outs.<sup>246</sup> Only 27 substances were identified in the Candidate Substances List following testing for persistence, bioaccumulative potential, and toxicity of more than 800 pollutants known to be in the Great Lakes basin. The Candidate List only identified substances without providing regulations to achieve the objective of virtual elimination.

The Canadian government issued its initial list of POPs for virtually elimination in 1994 with the Accelerated Reduction/Elimination of Toxics (ARET) Program. The ARET list identified 117 POP compounds based on an evaluation of more than 2,000 substances.<sup>247</sup> The ARET Program issued voluntary guidelines to Canadian industry to virtually eliminate emissions of 30 POPs on the list and to reduce releases of the other 87 substances to levels insufficient to cause 'harm'. The ARET voluntary guidelines had two short term goals: (1) to reduce emissions of POPs by 90%, and (2) reduce emissions of non-persistent toxic substances by 50% by 2000. There was participation in the ARET Program in eight industry sectors including 171 companies and government organizations and 318 facilities. The discharge of POPs into the environment was reduced by nearly 28,000 tonnes by 2000.<sup>247</sup> However, the emission reductions had largely resulted from a decline in industrial activity as production continued to be moved from Canada to other countries with lower production costs.<sup>248</sup> It is possible that total emissions did not decline as much as reported from the initial participating emitters if we include all who remained in Canada and those who moved production 'off-shore'. However, Environment Canada did not monitor or estimate the emissions from production moved to another country.

In 1995, the ARET Program was included into a broader government policy for environmental protection called the Toxic Substances Management Policy (TSMP). The TSMP has two main objectives<sup>249</sup>:

1) virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative (Track 1 substances) and;

2) management of other toxic substances and substances of concern throughout their entire lifecycle (Track 2 substances). The policy contains specific criteria for persistence and bioaccumulation.<sup>250</sup> The procedures for toxicity assessment and the provisions for implementing the TSMP for specified in the Canadian Environmental Protection Act.

The TSMP was reviewed in 1999 by the federal Commissioner of the Environment and Sustainable Development as part of a larger audit of how the Canadian

government was managing the risks from toxic substances. The commissioner reported the TSMP was not being fully implemented, nor was there a government-wide plan for implementation. Strategies for the management of specific substances, although required by the policy, had not been developed or implemented. Established government objectives had not been achieved.<sup>251</sup> Furthermore, the review found the programs were insufficient to ensure that risks could be addressed in the future. Substance-specific objectives for the protection of human health and the environment had not been adequately defined. Finally, the goals for reductions in the emissions of toxic substances had not been achieved.<sup>251</sup> A comprehensive review of the TSMP has not been published since 1999. Further details on the TSMP are provided in Appendix B.

In recognition of the international mobility of emitters, the governments of Canada, the United States and Mexico through the Commission for Environmental Cooperation of North America began work in 1995 on the Sound Management of Chemicals Initiative (SMCI) to coordinate the regulation of chemicals with an emphasis on persistent toxic substances of mutual concern to these governments.<sup>252</sup> The resolution proposed the development of North American regional action plans (NARAPs). Plans for six specific substances have been developed for DDT, chlordane, PCBs, mercury, dioxins/furans, and HCB. The SMCI coordinates NARAPs, reports on emerging issues of concern, develops partnerships and capacity, supports biomonitoring and environmental monitoring; and conducts public communication campaigns.<sup>253</sup>

In 1997, nineteen years after signing the Great Lakes Water Quality Agreement, the IJC initiated the Great Lakes Binational Toxics Strategy (GLBTS) which committed both countries to the virtual elimination of specific substances from the Great Lakes basin.<sup>254</sup> The strategy identified twelve level 1 substances as the primary focus for U.S. and Canadian government actions. The level 1 substances were referred to as the 'dirty dozen' by the United Nations Environment Program's 1997 call for virtual elimination of discharge of these substances, as discussed below. The GLBTS targeted these substances for virtual elimination from the Great Lakes basin because of their actual or potential environmental effects. The strategy only provided for voluntary guidelines for level 1 substances stated as 'challenges' for each country. The GLBTS also identifies fourteen level 2 substances that were subject to pollution prevention activities. These

substances were selected because of their potential to significantly affect the Great Lakes ecosystem.

In 2001, the IJC reviewed the GLBTS action on level 1 substances. The review concluded the strategy had achieved its principle objective to develop a 'collaborative' process among federal, state, and provincial governments, business corporations, and Aboriginal organizations to work towards virtual elimination of discharge of these substances by human activity.<sup>255</sup> However, the review noted that the collaborative partnerships themselves had not been the only reason for decreased emissions of level 1 substances. The review recognized that much of the decline in emissions could be attributed to a decrease in industrial activity as production moved outside the Great Lakes basin often to other countries.<sup>255</sup>

In 1997, the United Nations Environment Programme (UNEP) issued an international advisory to reduce the global emissions of twelve POP compounds. The "dirty dozen" included nine OC pesticides: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, HCB, mirex, toxaphene, and three industrial chemical families including dioxins, furans, and PCBs. In 2001, the Stockholm Convention on POPs was agreed upon, and the Convention came into force in 2004. The purpose of the Convention is the virtual elimination of POPs, and requires that stockpiles and waste are disposed of in such a way that the POP content is "destroyed or irreversibly transformed so that they do not exhibit the characteristics of persistent organic pollutants".<sup>256</sup> However, the 'destruction or irreversible transformation' of POPs is not required by the Convention "when destruction or irreversible transformation does not represent the environmentally preferable option" or when the "persistent organic pollutant content is low".<sup>256</sup> The 'options' considered by public and private organizations for disposal of POPs are typically restricted by the resources these organizations are willing to allocate. As discussed below, the environmentally safest methods for destroying or irreversible transformations of POPs can be very costly and require advanced technological resources. Limitations in capacity, resources and political interest may substantially limit the options from which decision-makers choose the 'environmentally preferable option'.<sup>257</sup> Currently, 152 countries have ratified the Convention. The United States has signed the Convention but has not ratified its acceptance. The Government of Canada ratified the Stockholm Convention agreement in 2001.

Three important features of the Convention include: (1) the process for reviewing new chemical compounds for inclusion as POPs for virtual elimination; (2) financial support for developing nations and nations in transition to implement reductions in the use and emissions of POPs and; (3) the exemptions allowed for disease vector control such as for malaria. Currently, China, India, many African countries, Venezuela and Yemen have exemptions for the use of DDT. This exemption means that OC pesticides continue to be manufactured and used in these countries. Many countries, including Canada, currently have exemptions for use of perfluorinated compounds. Most higher-income countries such as Canada do not permit the production of PBDEs, but do allow the import of products containing PBDEs from manufacturers in lower-income countries.<sup>24</sup>

The process for adding new substances for virtual elimination to the Convention is based in part on the precautionary principle as defined in Principle 15 of the Rio Declaration: "where there are threats of serious or irreversible damage, lack of full scientific certainty should not be used as a reason for postponing cost-effective measures to prevent environmental degradation".<sup>258</sup>

Both the reviews of the ARET Program and the GLBTS highlighted the decrease in emissions of targeted substances following implementation of their respective guidelines. Following from the North American reduction in the production and use of POPs, the concentrations of OC pesticides, dioxins, and PCBs declined in many food products over the past two decades in North America.<sup>259–261</sup> The reduced exposure levels in food were reflected in decreasing average blood concentrations of OC pesticides and PCBs over the past decade,<sup>262–265</sup> However, the average blood concentrations have remained comparable or increased over the past decade for the more recently developed POP compounds including PBDE flame retardants and some perfluorinated compounds.<sup>266–268</sup> Assessing the effectiveness of these programs for reducing production and emissions of POPs is limited by the extent of monitoring. Programs and methods for monitoring have not been consistent over time.<sup>269</sup> Only the ARET Program specified monitoring and reporting requirements.

It is important to note that both the ARET and GLBTS reviews identified that decreased production within their respective jurisdictions was responsible for much of

the decrease of emissions. If production and emissions were relocated to other countries, the global emissions of these pollutants may not have decreased or at least not to the extent indicated in these program reviews. The Stockholm Convention on POPs currently has 152 countries which have ratified the prohibition on production and use of the identified substances. However, the compliance and enforcement of these prohibitions is not consistent across all countries. Recent reports of ‘cancer villages’ in China and other countries may be indicators of one of the consequences of the movement of industrial production from higher-income nations to lower-income nations.<sup>270,271</sup> This raises concerns for the global levels of continued emissions of POPs and also for the public health inequity imposed upon poorer regions without adequate environmental regulations, enforcement, and the financial and technical resources for remediation of contaminated sites.

## **5.2. Disposal of POPs and Environmental Contamination**

The development, manufacture and use of POPs were discussed above in section 2.1. The broad application of POPs worldwide has led to environmental contamination and human exposure. Stockpiles, waste and other contaminated sites comprise large reservoirs of POPs with the potential for continued environmental contamination for decades to come. The practices used in the disposal of industrial and commercial waste contaminated by POPs and the management of contaminated sites can cause environment pollution which is a risk to human health. Governments around the world have responded to the environmental and public health risks through the development of policies, regulations and guidelines.

In this section, I describe the predominant practices of disposal of POPs and waste contaminated by POPs, and for managing contaminated sites. Next, there is a review of the policies and international agreements developed to “virtually eliminate” or manage further environmental contamination with POPs. Later the policies and guidelines to minimize public exposure and their underlying risk assessment methods are examined.

The historical and continuing production, use and disposal of POPs have led to environmental contamination at thousands of sites around the world. Remediation of these contaminated sites is an important public policy strategy for addressing the continuing exposure to POPs across populations. There are four broad types of discharge whether intentional or unintentional which result in environmental contamination requiring remediation strategies. The types of discharge discussed below include landfill disposal, discharge into aquatic systems, thermal processes and discharge from chemical stockpiles and storage sites.

### **5.2.1. Landfill Disposal of POP Contaminated Waste**

Most of the solid residues of chemical industries have been disposed into landfill depots. In industrial countries, this was a common practice until the 1970 and 1980s when hazardous waste disposal increasingly shifted to incineration. The landfills were often inadequately constructed and located to ensure long-term containment without environmental contamination. The majority of POPs disposed into landfills include HCH, HCB and PCB wastes from production sites.<sup>272–274</sup>

The manufacturing process of HCH insecticide provides a particularly stark example of the scale of hazardous waste landfill disposal practices. The process used to manufacture HCH generates a mixture of isomers of which only the gamma-isomer shows insecticidal activity. Common practice was simply to dispose of the 90% of the reaction mixture consisting of other HCH isomers after separation. As a consequence, between 4 and 7 million tonnes of waste of a variety of toxic, persistent and bioaccumulative residues are estimated to have been deposited into landfills globally.<sup>274</sup> This was the largest of the legacy POP landfill disposals, with overall quantities similar to the total of the other Stockholm Convention POP landfill disposals combined.<sup>273</sup> Adding to the complexity of the overall picture, waste HCH isomers produced during HCH production were sometimes partially recycled to produce chlorobenzenes using a thermal process. This resulted in high PCDD/F releases as waste.<sup>274</sup>

In the case of PCBs, landfill practices typically involved either no or inadequate treatment of contaminated products such as capacitors, sealants and other building residues in both municipal solid waste landfills and landfills designed for construction

wastes. Environmental contamination by PCBs from landfills is widely documented. For example, in Switzerland, PCB concentrations in fish from rivers near PCB contaminated landfills have been found to be substantially above the European Union's maximum allowable toxicity equivalency quotient for freshwater fish.<sup>275</sup> PCBs were found to be the major source of dioxin-like toxicity in these fish.<sup>275</sup> The PCBs in these rivers and fish were traced to these landfills.<sup>276,277</sup>

Many hazardous waste landfill depots will continue to contaminate environments around the world unless there is comprehensive remediation of these sites. Furthermore, hazardous chemical waste continues to be deposited into landfill sites in some developing and transitioning economies.

### **5.2.2. Discharge of POPs into Aquatic Systems**

The practice of discharging waste into aquatic systems has also been a common disposal route of waste contaminated with POPs.<sup>272,276,278</sup> There are also many cases of unintentional discharges into water systems from production facilities, waste disposal sites, and from agricultural and other locations where products containing POP's are used. Waste discharge into aquatic systems results in accumulation of POPs in various environmental matrices including sediments, fish and water as suspended particulates.

In 1962, Rachel Carson's *Silent Spring* first raised broad public awareness of the transport of pesticides through water systems and the potential environmental and health effects which could result both locally and globally.<sup>279</sup> Carson's work inspired environmental activism and much further research on the contamination of water systems by OC pesticides and other pollutants.<sup>280</sup> Public activism and scientific research pushed policy makers to develop regulations over the use of insecticides which have increasingly restricted the use of OC pesticides to disease vector control for malaria and similar diseases in countries in Africa and south Asia.<sup>24</sup>

The majority of aquatic POP discharges by production facilities are from chlorine and organochlorine manufacturers. The manufacture and use of chlorinated pesticides continues in developing countries such as Egypt,<sup>281</sup> Pakistan,<sup>282</sup> and India.<sup>283</sup> This continued production of OC pesticides results in continued contamination of environment and food chains from the waste products of the manufacturing process.

There are numerous documented investigations of aquatic discharges. The Baltic Sea has relatively higher concentrations of POPs throughout its environmental matrices due to the large number of POPs contaminated river systems flowing into this basin.<sup>284–286</sup> One of the largest documented Baltic Sea basin river discharges originated at a chlorophenol and chloralkali production facility in Finland. Approximately 28 kg TEQ was discharged into the river and mostly entered the Baltic Sea.

Long after production facilities are closed, they continue to pose a contamination risk. Well documented environmental contaminations with POPs from aquatic discharges have involved closed chemical manufacturing facilities at Laggo Maggiore in the Italian Alps<sup>287</sup>, the Venice lagoon<sup>288,289</sup> and Tittabawassee River in Michigan<sup>290,291</sup>. The harbour of Sydney, Australia was contaminated with 2,4,5-trichlorophenol and pentachlorophenol (PCP) from a closed Union Carbide pesticide facility. This contamination resulted in a ban on commercial and recreational fishing over an extensive area.<sup>292,293</sup>

### **5.2.3. Discharge of POPs through Thermal Processes**

POPs are discharged into the environment through a variety of thermal processes including waste incineration, open burning, sinter plants, and secondary metal production.<sup>294</sup> The process of incinerating of waste can itself produce PCBs which are released as atmospheric emissions.<sup>295</sup> Similarly, PCBs can also be a by-product in some metal production processes which is also emitted as atmospheric particulate.<sup>296</sup> There can be relatively widespread and rapid environmental contamination through atmospheric emissions of particulates containing POPs.

In Europe, atmospheric emissions of PCBs through thermal processes peaked in the 1990's and accounted for near to 5% of the toxic equivalency quotient (TEQ) for total PCBs in European consumed fish.<sup>274</sup> Atmospheric emissions of POPs from thermal processes are documented to have contaminated local foods such as cow's milk, eggs, and vegetables.<sup>297–304</sup>

#### **5.2.4. Contamination from Recycling of Electronic and Metal Waste**

The recycling of electronic and metal waste is increasing, particularly in developing countries. Some electronic waste and metal waste, such as vehicle shredder waste material, are contaminated with compounds such as PCBs, PBDEs, brominated dibenzodioxins and dibenzofurans and toxic heavy metals.<sup>305-313</sup> Some secondary metal industry processing, in particular recycling of aluminum, copper, iron, lead and zinc, have been identified as sources of a variety of POPs. These plants recycle scrap metals, which can be contaminated with PCBs, polyvinyl chloride (PVC), brominated flame retardant-containing plastic, and chlorinated paraffin containing oils.<sup>296,314</sup> Moreover, during the heating, melting and cooling phases of metal recycling processes, a variety POPs and PCDD/Fs can be formed and emitted.<sup>296,314</sup>

Some sites have been found to be contaminated with such an extensive spectrum of chlorinated, brominated and mixed halogenated dioxins, furans, and biphenyls that it is necessary to use a combination of instrumental- and bioassay-based assessments to adequately estimate the dioxin-like toxicity at these sites.<sup>315</sup>

The local environments surrounding these recycling sites and their associated disposal areas can become contaminated if the activities at these sites are conducted without appropriate regulation and control. For example, local drinking water has been contaminated with PCBs and PCDDs around large-scale electronic and metal recycling sites in China.<sup>316</sup> Furthermore, the breast milk from mothers living near to electronic or metal recycling sites in South East Asia have significantly elevated concentrations of PCBs and PCDDs.<sup>317,318</sup>

The disposal of electronic and metal waste from higher-income economies into lower-income developing economies raises important questions of global health inequities and the responsibility of governments and corporations in wealthier nations to protect global health and environments.

#### **5.2.5. Future Challenges from Sites Contaminated with POPs**

The future potential risk of environmental contamination by POPs would appear to be substantial for many generations to come. The safe destruction or containment of

the existing and future stockpiles of POPs and sites contaminated with POPs will be technologically challenging and very costly. The Stockholm Convention requires member states to dispose of existing stockpiles of POPs. The practice in many countries of incineration of hazardous waste could lead to substantial environmental contamination if the appropriate methods, technology, and monitoring are not utilized.<sup>319</sup>

Beyond the existing stockpiles and contaminated sites, we can expect future stockpiles of hazardous waste to increase. For example, many electrical transformers containing or contaminated with PCBs remain in use, and it is estimated that about 4 million tonnes of such equipment will need environmentally appropriate waste management as it is decommissioned in future years.<sup>320</sup> In 2007, the OECD estimated the cost of safe disposal between US\$2,000 to \$5,000 per tonne of transformers contaminated with PCBs.<sup>320</sup> This would amount to an estimated US\$8 to 20 billion to safely dispose of transformers-contaminated with PCBs alone. In contrast, from 2003 to 2010, the total Stockholm Convention's Global Environmental Facility funding was US\$550 million to support developing and transitioning economies to safely dispose of POPs. The Stockholm Convention obligates member states to have safely removed from use and disposed of all equipment containing PCBs by 2028. Electric transformers are but one of many examples of sources of potential future environmental contamination if proper containment and disposal is not implemented.

Despite the experiences in industrialized countries with high remediation costs resulting from inadequate environmental protection practices and policies, the governments of many developing and transitioning economies support the development and operation of industries based upon halogen (i.e., chlorine, bromine and fluorine) production and integrated halogenated chemical synthesis.<sup>274</sup> In many nations such industries are being established or operated without environmentally-safe waste management practices, policies and enforcement. The absence or inadequacy of safe waste management and enforcement can lead to environmental contamination requiring remediation which is often at a considerably higher cost than pollution prevention and control practices and policies.<sup>274</sup>

There is not an intergovernmental policy instrument that addresses both the identification and remediation of POP contaminated sites.<sup>274</sup> Governments that have

ratified the Stockholm Convention are required to develop strategies for identifying sites contaminated with POPs.<sup>24</sup> The Stockholm Convention further requires remediation of contaminated sites to be performed in an environmentally safe manner. A substantial obstacle to the remediation of contaminated sites is the poor quality of documentation and records.<sup>274</sup> The information on many existing POP contaminated sites is incomplete. There is also a large number of potential sites at historical and existing facilities where POPs contamination may have occurred, but the documentation and records are insufficient, and consequently requires environmental testing to determine if these sites are contaminated by POPs.

Public policies concerning the identification and remediation of contaminated sites vary substantially between countries.<sup>274</sup> Switzerland's regulations are among the most comprehensive in the world. The Swiss regulations require that landfills contaminated by POPs must be completely excavated and all contaminated matter treated to remove pollutants.<sup>321</sup> The costs of remediating Swiss landfills contaminated with POPs are very high. For example, the cost to remediate a landfill in Bonfol was approximately US\$270 million. This landfill contained 114,000 tonnes of chemical waste deposited between 1961 and 1976.<sup>322</sup> These remediation costs were paid by the Basel chemical companies including Novartis, Roche, Ciba, Clariant and Syngenta as the polluters and landfill operators.

At a landfill operated by public authorities near Kölliken, Switzerland, approximately 300,000 tonnes of chemical waste was deposited between 1977 and 1985. The Swiss government paid for most of the US\$450 million cost of remediation.<sup>322</sup> The government's policies and orders have required substantial public and industry costs to prevent further potential contamination of the environment from these chemical waste sites. It is worth considering how many governments have the financial and technical resources to safely excavate and treat contaminated sites. It is also important to consider the political capacity and prioritization for remediating sites and compelling industry to accept responsibility for remediation.

The United States government has the financial and technological resources for comprehensive remediation of POP contaminated sites. However, political interests and prioritization has limited the response of the government and industry in managing

contaminated sites. The United States Superfund for the management of abandoned contaminated sites was established by Congress in 1980 following public awareness and concern of the pollution and health effects from pollutant exposure at Love Canal, Times Beach and other hazardous waste sites.<sup>274</sup> Public awareness and activism was perhaps heightened most by exposure to toxins at the Love Canal waste site in Niagara Falls, NY. The Hooker Chemical Company disposed of more than 21,000 tonnes of industrial waste in a landfill from 1942 to 1952. The waste included significant quantities of chlorinated benzenes, phenols, pesticides, PCDDs and PCDFs. In the 1970's, non-governmental organizations effectively promoted public awareness and mobilized political activism to pressure the state and federal governments to intervene to assist local residents to relocate, to initiate a waste management program at the site, and to implement a strategy for dealing with other hazardous waste sites.

In response to public concern and activism, the U.S. Congress enacted the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) in 1980. The act established the Superfund to finance the identification of toxic waste sites and required polluters to finance waste management and pollution control activities at identified sites. Thousands of toxic waste sites have been identified through the CERCLA programs. Until 1995, the Superfund was financed through dedicated taxation of companies producing or using hazardous chemicals. In 1995, Congress did not renew the dedicated tax which has left the federal government the sole funder for the Superfund. This change in financing the Superfund has substantially limited the capacity of the fund to support remediation and pollution control activities.<sup>323</sup> Limiting the funding source to government and the high number of identified sites has led to an emphasis on containment and pollution control strategies. These strategies include site waste containment or excavation and transfer to a site better able to control contamination of the environment. The cost of a comprehensive remediation of identified sites including excavation and treatment to remove and destroy hazardous substances is prohibitive given the thousands of sites and the limited funding available.<sup>323</sup>

In the European Union, remediation of POP contaminated sites began during the 1980s. Activities around identification and remediation were principally in the more densely populated countries of The Netherlands, Denmark and Germany. The European Environment Agency has estimated there are more than 3 million sites where

industrial activities potentially may have contaminated the environment with toxins.<sup>324</sup> Priority has been given to documenting the contamination at these sites and determining which sites require remediation. In a 2007 EEA report, the investigation and documentation of existing sites was forecasted to continue until at least 2025.<sup>324</sup> This same report noted that over the previous thirty years that remediation had been completed or was in progress at 80,000 of the 3 million potentially contaminated sites. Among the investigated sites, heavy metals and mineral oil were the most frequent contaminants in soil. Mineral oil and chlorinated hydrocarbons were the most frequent contaminants found in ground water at these sites. POPs were found at many of these sites as well.<sup>324</sup>

Since 2000, the European Union Water Framework Directive has required governments and companies to prevent toxic pollution of rivers, lakes and the sea by controlling environmental contamination at the source.<sup>325,326</sup> In 2006, the European Union adopted a strategy to protect soil with the principle objectives to protect the terrestrial environment and future sustainable use of soils.<sup>327,328</sup> Despite the efforts taken, it could take decades and very substantial financial and technical resources to investigate and remediate the existing legacy of contamination in the EU countries. Until remediation is completed, many sites may potentially continue to pollute the environment with toxic substances including POPs.

The Swiss government and industry have demonstrated that comprehensive remediation can prevent continued pollution of the environment by contaminated sites. However, initiating and sustaining these policies and activities requires very substantial financial and political commitments. The experience with remediation of contaminated sites in the United States demonstrates that public awareness and activism are important for initiating and maintaining government action to manage and treat contaminated sites. In both Europe and the United States, given the high number of sites and the level of resources committed to investigation and treatment of sites, it could take decades to complete the work on existing sites. The Stockholm Convention does provide funding through the Global Environment Facility (GEF) to support investigations and pollution control in developing and transitional economies.<sup>274</sup> However, these resources are considerable less than in the developed economies. As industrial production continues to move to developing and transitioning countries with less

regulation and enforcement, the number of contaminated sites could be expected to increase. Environmental contamination by pollutants including POPs could be expected to continue well into the future.

### **5.3. Assessing Risk Associated with Exposure to POPs**

The public policies and international agreements discussed above are designed to eliminate or control the production and use of POPs. There are also regulations to manage or remediate contaminated sites. These policies, regulations and agreements are generally intended to protect public health through the virtual elimination or management of continued contamination of the environment.

The basis for assessing risk associated with POP exposure levels from specific sources such as food and water is discussed above. When reviewing the policies and guidelines for minimizing risk from exposure, it is important to understand the limitations of the standard risk assessment method. It is also important to consider the appropriateness of the standard risk assessment approach when developing policies and strategies to promote population health in the context of nearly ubiquitous lower-dose and long-term exposure to POPs across populations.

How to determine at what level of exposure to POPs there is likely to be adverse health effects is debated in several regards. The hazardous potential of POPs and other environmental pollutants are determined with risk assessment procedures as discussed above. Important questions have been raised concerning the appropriateness of contemporary methods for assessing the risk associated with long-term and ubiquitous lower-dose exposure to POPs and other pollutants identified as endocrine disrupting chemicals. This section discusses recent evidence on health outcomes associated with lower-dose exposure to EDCs, and limitations of contemporary methods used for assessing risk from exposure to EDCs.

#### **5.3.1. Lower-Dose Effects**

The evidence in recent mouse studies indicates that at least some POPs disrupt specific endocrine pathways.<sup>10,11</sup> The findings in this study and other epidemiological

investigations are consistent with the hypothesis that POPs are endocrine disruptors. An EDC is an exogenous chemical, or a mixture of chemicals, that interfere with any aspect of hormone action. The potential for negative effects of EDCs should be considered in terms of its impact on the regulation of hormone synthesis, secretion, and actions. The Endocrinology Society proposes that EDCs be identified by governments on the basis of fundamental endocrinology when managing risks to populations with even very low-dose exposures.<sup>329</sup> Such an approach stands in contrast to the commonly used rigid criteria-based static approach to toxicity when identifying EDCs and managing risks to the health of all species of life. The analysis of the health effects associated with very lower-dose exposures is limited by the LOD of laboratory methods used for measuring very low concentrations of EDCs in the environment, food and humans. For example, in this study, some of the PCB congeners with relatively higher percentages of respondents with concentrations below the laboratory LOD as shown in Table 1 had statistically not significant ORs. Better laboratory methods for measuring very low concentrations of these pollutants would have assisted in improved estimation of OR of disease and the associated confidence intervals.

The breadth of information about human exposure to a diverse spectrum of environmental chemicals is substantially increasing through large-scale biomonitoring programs in a growing number of countries.<sup>330-333</sup> However, the detection of a chemical in humans is not necessarily indicative of a negative health outcome. Assessing the extent of risk requires evidence from a broad range of experimental and epidemiological studies. An increasing number of epidemiological studies point to associations between the concentrations of EDCs in the general population and adverse health outcomes.<sup>334,335</sup> These studies suggest that lower-doses of EDCs may increase the risk of chronic disease for broad populations including groups at different levels of risk.

It is difficult to apply current risk assessment methods using general population biomonitoring data.<sup>336,337</sup> Traditionally, risk assessments examine the effects of higher doses of administered chemicals to determine the lowest observed adverse effect levels (LOAEL) and no observed adverse effect levels (NOAEL). The reference levels for safe exposure for humans are determined based on the LOAELs and NOAELs together with assigned safety factors. From this type of risk assessment perspective, the POP exposure levels for general populations in North America are deemed to be non-

negligible but safe. However, a growing body of epidemiological evidence indicates that lower-dose exposures deemed to be safe across the general population indicate an association with negative health outcomes. Lower internal doses of EDCs found in general populations are associated with obesity,<sup>338</sup> infertility,<sup>339</sup> neurobehavioral disorders,<sup>340</sup> and immune dysfunction<sup>341</sup>.

A “lower-dose hypothesis” postulates that lower doses of chemicals can have effects that would not necessarily be predicted from their effects at higher doses. In 2002, a National Toxicology Program expert panel reviewed the evidence for a lower-dose hypothesis and concluded that there was evidence for lower-dose effects for a select number of well-studied endocrine disruptors.<sup>342</sup> More recently, Vandenberg and colleagues examined the evidence and found lower-dose effects for many more chemicals used in industrial processes, plastic components, plasticizers, pesticides, phytoestrogens, preservatives, surfactants, detergents, flame retardants, and sun screen lotion.<sup>343</sup> This study selected several examples of controversial lower-dose test cases and applied an analytical weight-of-evidence approach to determine whether there was sufficient evidence to conclude that particular environmental chemicals had effects on specific biological processes. The study demonstrates how experimental design, choice of animal strain or species, study size, and inclusion of appropriate controls affect the outcome and interpretation of studies on bisphenol A (BPA), atrazine, dioxin, and perchlorate. The researchers also examined the biological pathways through which chemicals can have effects on hormonal activity at external doses that are often considered safe by the regulators.

Vandenberg and colleagues described numerous examples for many classes of environmental pollutants of non-monotonic dose–response curves observed in cultured cells, animals, and even human populations. The researchers describe how and why nonlinear responses are manifested at different levels of biological complexity. A major cause of a nonlinear dose response is competing monotonic responses such as enhanced cell proliferation and cytotoxicity. Differences in the expression of cell and tissue-specific cofactors and receptors may lead to apparently conflicting findings in dose response. Finally, receptor down-regulation, desensitization, and competition may also contribute to nonlinear dose response. The researchers call for further investigation to determine which dose–response shapes should be expected for specific chemicals

and under what specific circumstances. In support of this position, Linda Birnbaum, the director of the U.S. National Institute for Environmental Health and the National Toxicology Program, has advocated for the development of more sophisticated study designs to facilitate regulatory decisions based on a greater understanding of lower-dose effects and non-monotonic dose responses.<sup>344,345</sup>

The findings in this study and other studies discussed indicate negative health outcomes related to the ubiquitous lower-dose exposure throughout the population despite the implications from LOAEL and NOAELs that such exposure levels should be safe. It is noteworthy that this study used logistic modeling which assumes a linear association between the POP concentrations and the OR of T2D. However, some pollutants, such as *p,p'*-DDE, have a relatively very broad range of concentrations across the population and therefore may have non-linear dose-response across the full range of concentrations in the population which are not observed through these models.

Given the evidence for lower-dose effects on population health outcomes, the legacy of POPs should be a cautionary tale for the continued use of specific OC pesticides and the introduction of new persistent chemical compounds such as PBDEs. The limitations of standard risk assessment methods when calculating LOAELs and NOAELs suggests caution when assessing risk and competing risks. The regulated levels for maximum concentrations of POPs in food, water and environmental media in Canada as described below are often above current levels of exposure to these POPs.

### **5.3.2. *TEQs and Risk Assessment***

In many countries, the guidelines and regulations for concentrations of POPs in food are based on the World Health Organization's Toxic Equivalency Quotient (WHO-TEQ). Food guidelines and regulations are established to ensure toxicity levels in food are at levels expected to have negligible risk to human health. It is important to understand the limitations of the WHO-TEQ when considering the risk of metabolic diseases associated with lower-dose exposure to POPs in environments where this exposure is nearly ubiquitous and persistent.

Dioxin commonly refers to a group of 75 polychlorinated dibenzo-*p*-dioxins (PCDDs) and 135 polychlorinated dibenzofurans (PCDFs) congeners. Less than 20 of

these dioxins are believed to be highly toxic. Dioxin like-PCBs (dl-PCBs) refer to 12 non-*ortho* or mono-*ortho* PCBs exhibiting similar biological patterns to the dioxins with the highest toxicity. In 1997, the World Health Organization developed toxic equivalent factors (TEF) to facilitate risk assessment and regulation of exposure to mixtures containing PCDDs, PCDFs, and dl-PCBs.<sup>126</sup> An underlying assumption of TEF is that PCDD, PCDF, dl-PCB and TCDD congeners induce similar toxic responses. The TEF is an order of magnitude estimate of toxicity referenced to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) which is considered to be the most toxic dioxin. The toxicity equivalence factors are based on data from *in vitro* and *in vivo* experiments which allow for comparisons of the level of toxic response to each congener.<sup>125,126</sup> TEFs are applied to POP congeners with the following characteristics:

- the chemical is structurally related to PCDDs and PCDFs;
- the chemical binds with the aryl hydrocarbon receptor (AhR) and induces a AhR-mediated biochemical and toxic responses; and,
- the chemical is ubiquitous in the food chain.

The cumulative toxicity for all POP mixtures in food is calculated using TEFs to estimate the toxic equivalence quotient (TEQ) to assess total exposure to dioxins and dioxin-like compounds. TEQ measures are calculated as:

$$\text{TEQ} = \sum_{n1} (\text{PCDDs}_i \times \text{TEF}_i) + \sum_{n2} (\text{PCDFs}_i \times \text{TEF}_i) + \sum_{n3} (\text{dl-PCBs}_i \times \text{TEF}_i)$$

The TEQ measures assume the cumulative toxicity from all PCDD, PCDF and dl-PCB congeners can be calculated as an additive toxic effect. TEQ is used for developing guidelines of recommended maximum consumption of foods with known and measured concentrations these pollutants.

This approach can also describe the relative contribution of each pollutant to the total TEQ in different types of foods. For example, PCBs contribute, on average, more than 50% of the uptake of dioxin-like TEQ in the main sources of human nutrition.<sup>346</sup> The contribution of coplanar PCBs to total TEQ in fish<sup>346</sup>, dairy products<sup>347</sup> or butter<sup>348,349</sup> amount, on average, to more than 50% in the northern hemisphere, while the PCB contribution to total TEQ is less in the southern hemisphere. Similarly, dioxin-like PCBs

can account for 50% of the total TEQ in human milk, although this percentage varies significantly between countries.<sup>350</sup>

The assumption underlying the WHO-TEQ may not be valid for some POP compounds, and for risk associated with long-term lower-dose exposure. Jérôme Ruzzin identifies three main limitations of the standard risk assessment approach to food safety guidelines in relation to lower-dose POPs exposure and chronic diseases.<sup>351</sup> First, Ruzzin challenges the assumption in the WHO-TEQ of additive toxic effects. Next, Ruzzin critiques the assumption that dioxins and dioxin-like PCBs consistently activate the aryl hydrocarbon receptors (AhR) in cells with the same biological effects by toxicity. Finally, Ruzzin questions the assumption in TEQs and the related food safety guidelines that adults and children have similar exposures per weight of food and have similar absorption, metabolism and excretion of POPs.

An example of the non-monotonic response to POPs exposure is evident in Crutch and colleagues' finding of an inverted U relationship between TCDD and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) exposure and weight change in rats.<sup>352</sup> The researchers reported significant body weight gain in rats with environmentally relevant lower-dose exposure to TCDD and HxCDD. However, the researchers also found substantially higher dose exposure to these dioxins led to significant weight loss for these animals. In another rat study, adipocyte differentiation was promoted with environmentally relevant exposure to PCB-77 and TCDD, whereas there was an absence of adipocyte differentiation with a higher-dose exposure.<sup>353</sup> Additionally, the incubation of 3T3-L1 adipocytes with lower concentration of PCB 77 stimulated adipokine release, whereas this effect was absent when cells were exposed to a higher-dose of PCB 77. Ruzzin argues the dose additive assumption in the TEQ measures may not be valid, and thus extrapolating a linear decreasing toxic effect below the level of no observable adverse effects (NOAEL) may not be accurate. The process of establishing NOAELs may fail to detect environmentally relevant lower-dose effects.

The assumption that exposure to dioxins and dl-PCBs induces similar types of biological effects through the activation of aryl-hydrocarbon receptors is also questioned by Ruzzin.<sup>351</sup> The principal assumption of the TEF concept is that TCDD is the most toxic dioxin, and the potency of other dioxins, furans and dl-PCBs can be expressed as

fractions of the potency of TCDD. TCDD through AhR activation may induce cancer, and dysfunctions in reproduction and neuronal activity. However, Ruzzin argues that TEQ measures are unlikely to reflect the risk of metabolic disorders related to environmentally relevant lower-dose exposure to POPs. For example, in a recent mouse study, Ruzzin et al reported that a variety of POP compounds impaired insulin-stimulated glucose uptake in 3T3-L1 adipocytes independently of the TEQ measure.<sup>10</sup> Several epidemiological studies have found that blood concentrations of some POPs are positively associated with the OR of diabetes independent of TEQ.<sup>137,140,354</sup> The association between POP blood concentrations and the OR of diabetes independent of the TEQ indicates that risk assessments based upon the TEQ could be inappropriate for assessing risk of metabolic disorders. Given the association reported here between non-dioxin like PCBs and the OR of T2D, indicate that these metabolic effects may occur independently of AhR activation. Ruzzin and others have identified other potential modes of action by POPs including activation of constitutive androstane receptor (CAR) or steroid xenobiotic receptor (SXR), and the competitive binding to nuclear receptors.<sup>355,356</sup>

Ruzzin's final critique of risk assessments based on TEQs involves the differences in adult and early life exposures.<sup>351</sup> Regulatory policies with safe concentration levels of dioxins and dl-PCBs are usually based on a body weight. Ruzzin argues that body weight alone is not a sufficient basis for such regulations since children and adolescents require higher food intake per kilogram of body weight to maintain whole-body homeostasis and growth. Several studies have found children's exposure to dioxins and dl-PCBs exceeds the World Health Organization's recommended limit of total daily intake (TDI) of 2 pg/kg body weight.<sup>357-366</sup> Children may be more responsive than adults to environmental pollutants due to differences in absorption and excretion between children and adults.<sup>366</sup> Finally, dietary guidelines generally advise pregnant females to avoid or limit the consumption of foods reported to contain elevated levels of POPs, heavy metals and other pollutants. However, POPs bioaccumulate in the body for many years, and thus restricting or reducing exposure to these POPs only during pregnancy would not protect the fetus from exposure to these pollutants which have bioaccumulated in the mother's tissue.<sup>367</sup> The analysis presented above in chapter 4 was limited to adults from 20 to 79 years-old due to the CHMS phlebotomy design. We

cannot determine from the CHMS cycle 1 data if the model for the association between POP plasma levels and the OR of T2D for adults would be similar or different for children and adolescents.

TEQ limits are typically used for food safety advisories to moderate or reduce exposure to environmental toxicants. Such food safety advisories may not sufficiently address risks from longer-term lower-dose exposures from pollutants which bioaccumulate with age in humans. When considering the role of food safety advisories and dietary guidelines for longer-term population health protection, TEQs may not be a sufficient basis for preventing development of chronic disease related to long-term lower-dose exposure to POPs.

## **5.4. Reducing Human Exposure to POPs and Competing Risks**

The development of regulations adequate to protect people from metabolic diseases associated with long-term environmental exposure will be challenging due to competing risks and interests concerning food, economic activity and the public health implications. Below are two case examples of where the use of a complex systems approach may more effectively promote and protect public health.

### **5.4.1. *Fish consumption and T2D in Human Populations***

The consumption of fish is widely promoted as a good source of proteins and for its high levels of long chain *n*-3 polyunsaturated fatty acids (PUFA). A widely cited Dutch prospective study on cardio-vascular disease (CVD) demonstrates the benefits of a diet high in *n*-3 PUFA for lowering the risk of CVD. In Zutphen, The Netherlands, over 800 males aged 40 to 59 years-old in 1960 were interviewed on a regular basis until the 1980's.<sup>368</sup> The study found a strong inverse relationship between consumption of fish and the incidence of CVD over a 20-year period. However, fish tissues and oil have also been reported to contain detectable levels of many environmental pollutants including OC pesticides, PCBs, dioxins, PBDEs and perfluorinated compounds (PFC) which are associated with adverse health effects.<sup>369</sup> There would appear to be competing benefits and risks for diets including regular consumption of fish.

There have been mixed results from studies assessing the health risks versus benefits for reducing CVD from consuming fish. Two systematic reviews of the evidence found that a diet with regular fish consumption is associated with greater benefits to health from reduced cardio-vascular disease compared to risks from exposure to pollutants.<sup>370,371</sup> However, there is considerably less evidence comparing the benefits and risks of fish consumption in terms of T2D. Three prospective studies have reported fish consumption is related to an increase in the risk of T2D.<sup>372-374</sup> A recent systemic review of the available evidence concluded fish consumption is related to an increased risk of T2D.<sup>375</sup> Two other recent systemic reviews concluded that fish consumption does not reduce the risk of T2D.<sup>376,377</sup>

The findings from recent animal studies may help to resolve the apparently conflicting evidence on the health benefits and risks of regular consumption of fish. The findings from previous studies may be affected by the concentrations of POPs in the fish consumed by study participants. Ruzzin and colleagues found that rats exposed to salmon oil with environmental concentrations of POPs developed metabolic dysfunctions consistent with a diabetic phenotype.<sup>10</sup> However, rats exposed to salmon oil decontaminated of POPs did not develop these metabolic dysfunctions. Ibrahim et al extended this research and exposed mice to retail purchased Atlantic farmed salmon fillet with common environmental levels of POPs or to farmed salmon fillet decontaminated of POPs.<sup>11</sup> The rats exposed to the POP contaminated feed had greater IR, glucose intolerance, visceral obesity, fatty liver and chronic low-grade inflammation compared to mice exposed to decontaminated feed. An earlier study by Ruzzin and colleagues found salmon protein hydrolysate is protective against IR induced by a high-fat diet containing lard and corn oil.<sup>378</sup> Salmon protein hydrolysate contains less than 0.2% of lipids, and therefore has relative lower concentrations of POPs,

The level of POP concentrations in commercially available fish may be within safe levels according to guidelines and regulations based on NOAELS. However, these levels may be sufficient to induce metabolic dysfunctions consistent with a pre-diabetic or diabetic phenotype.

In Europe and North America, food guidelines and regulations provide maximum recommended concentrations based NOAELS for PCBs in fish. However, similar

guidelines and regulations for OC pesticides levels are typically applied only to terrestrial foods and not to seafood.<sup>351</sup> Recent evidence indicates an association between consumption of fatty fish such as salmon and T2D.<sup>372,373,379,380</sup> The European Commission's Scientific Committee on Food acknowledged the inconsistency in the application of regulations across different food products.<sup>346</sup>

Comprehensive information on levels of POPs in regional commercial fish and shellfish species would be useful for assessing population exposures from specific species, and could assist in reducing population exposure to POPs while maintaining fish consumption within regional diets. For example, predatory fish species with their high trophic position, such as tuna and salmon, have relatively higher concentrations of POP since these species consume other prey species and have relatively longer lifecycles.<sup>381,382</sup> In contrast, prey species such as capelin, herring, sardines, shrimp and mackerel may have lower levels of POPs given they consume smaller organisms which are relatively low in fats, and these prey species have relatively shorter lifecycles.<sup>383</sup> Food guidelines and other public health communication strategies to promote consumption of species with lower levels of POPs such as prey species could contribute to lowering public exposure to POPs. Many prey species are also considered to be more environmentally and economically sustainable fisheries than are fisheries for many predatory species.<sup>384</sup>

There could be substantial risks and unintended consequences arising from such population health promotion campaigns. Food safety advisories could increase the public's general anxiety over pollutants in seafood.<sup>385-387</sup> There could be substantial detrimental effects for economic output and thus public health for communities dependent on fisheries identified as a source of relatively higher exposure to POPs. The issue of trust in local foods is discussed further in the following discussion on Aboriginal community food systems and exposure to POPs.

#### **5.4.2. *Aboriginal Community Food Systems and POPs***

Aboriginal communities in Canada experience poorer health conditions than comparable non-Aboriginal communities.<sup>388-390</sup> This difference in population health indicators is particularly evident with respect to chronic diseases such as T2D.<sup>391-395</sup>

Based on the evidence of an association between POPs and T2D, it would be reasonable to investigate if Aboriginal communities with relatively high exposures to POPs may also have relatively higher prevalence of T2D. Many Aboriginal communities rely on locally harvested fish, mammals and birds for a substantial component of the communities' food resources.<sup>390,396,397</sup> These traditional diets may substantially increase the exposure to POPs for Aboriginal communities,<sup>390,396-398</sup> particularly in northern ecologies.<sup>399</sup> The global atmospheric and ocean transport of POPs<sup>400</sup> and the Arctic ecology leads to biomagnification of POPs in Arctic mammals and birds.<sup>399</sup>

Studies examining the association of POP exposure and T2D in Inuit communities and a Native American population are discussed above in section 2.3.4. The descriptive assessment in the AMAP studies of Greenland Inuit communities did not find evidence of an association between POPs and T2D nor of an elevated prevalence of T2D among Inuit as compared to non-Inuit populations. However, studies of First Nations communities have found relatively high prevalence of T2D among First Nations people as compared to the Canadian population. A 2007 study of the Akwesasne community found a statistically significant association between several POP compounds and the OR of T2D after adjustment for possible confounders.<sup>168</sup>

The conventional view of T2D and other chronic disease among Aboriginal communities has focused on the transition from traditional diets to lower-quality processed foods. Significant risk factors associated with T2D are more prevalent among Aboriginal peoples including lower socio-economic status, obesity, lower fitness, and poorer diet.<sup>389</sup> In the 1990's, Receveur and colleagues predicted that traditional locally harvested foods would continue to have a decreasing importance in northern Aboriginal communities due to decreasing stocks of these species and changing cultural norms.<sup>398</sup> Overexploitation of fisheries in some regions and climate change requires fishers and hunters to travel longer distances to find traditional food species.<sup>397</sup> These longer distances to fish and hunt requires expensive vehicles and gas which limits the frequency of trips possible during a given season.<sup>397</sup> The higher costs and reduced frequency of trips has the consequence of youth having less exposure to these activities and hunting families less able to share with the rest of the community.<sup>397</sup> Rapid cultural change among northern youth also raises questions of the relevance of traditional locally harvested foods as a significant food source for northern communities.<sup>397</sup> Therefore to

achieve good nutrition and food safety in the Arctic, Receveur and colleagues advocated for policies to ensure adequate household incomes, improved availability of good quality market food, and public education on the healthy diets with market foods.<sup>398</sup>

Where Aboriginal communities are found to have relatively higher exposures to POPs through traditional diets high in fish and animal fats, it may appear to be appropriate to provide dietary guidelines to reduce exposure to POPs through these diets.<sup>401</sup> Giles and colleagues illustrated how food advisories such as mercury contamination in fish contribute to undermining trust in traditional local foods and further the transition in Aboriginal communities from locally harvested foods towards market processed foods high in carbohydrates, sugar and salt.<sup>402</sup> The transition from traditional local food sourcing to a 'westernized' diet increasingly dependent on processed foods and beverages has been associated with decreased physical activity and an increased prevalence of diabetes, obesity and related health conditions in these communities.<sup>403,404</sup>

The comparative risks from exposure to pollutants from a traditional Arctic diet versus a westernized diet of market foods has been referred to as the 'Arctic dilemma'.<sup>163</sup> The indigenous traditional marine diet in the Arctic includes mammals' blubber which is the best source of vitamins A, D and E available in Aboriginal northern communities.<sup>396</sup> Marine and land mammal fats are important sources of trace elements and antioxidants such as selenium and polyunsaturated fatty acids. The specific organs and tissues include: beluga blubber and oil; narwhal blubber; liver from ringed seals, walrus, loche, caribou and moose; flesh from arctic char, lake trout and sculpin; eggs from cisco and loche; and muktuk (raw skin from whale and walrus).<sup>396</sup> These traditional locally harvested animals are also a source of higher exposure to pollutants due to biomagnification of pollutants such as POPs within the northern ecology.<sup>383,405</sup> However, greater reliance on market processed foods can lead to health risks, such as an increase in the prevalence of conditions associated with the metabolic syndrome.

Many Aboriginal people attribute the increasing prevalence of T2D and associated health conditions to a diminished relationship with their environment leading to a reduction in traditional land-use activities, culture and spirituality.<sup>406-410</sup> Integrating traditional knowledge systems and the biomedical scientific perspective may better inform development of public health strategies.<sup>411-413</sup> Culturally-relevant community

participation is cited as important for public health program success.<sup>414–416</sup> Interventions that do not address determinants viewed as important by Aboriginal communities are less likely to improve public health, irrespective of the empirical validity of the intervention in other contexts.<sup>406,417–420</sup>

Food advisories concerning exposure to POPs through locally harvested fish, mammals and birds may be warranted as a public health strategy to mitigate risk associated with exposure to pollutants. However, such food advisories in isolation from other public health strategies may have unintended adverse consequences for public health. For example, a strategy to improve food self-sufficiency, nutrition and physical activity through promotion of traditional land-use activities including local fisheries and hunting could be undermined by food advisories if these public health interventions are not coordinated. Food advisories of heavy metals and organochlorine exposure through consumption of fish substantially increased public anxiety and lack of trust in locally harvested foods in some regions across northern Canada resulting in decreased consumption of locally harvested fish and animals in regions where these pollutants were not reported in fish or mammals.<sup>396–398,421,422</sup>

The Canadian Platform for the Use of Real-World Evidence (CAPTURE) project worked with northern and remote Aboriginal communities to develop a tool kit for community planning, program implementation and evaluation for community food programs.<sup>423</sup> The principal elements of the recommended approach to public health and food programming in these communities include: culturally sensitivity; participatory-based planning and evaluation; community-based research; respectful relationship building throughout the community; and community-centred goals. Community strategies to maintain access to locally harvested foods have included use of community freezers for storing locally harvested fish and animals, markets in larger communities, and organized trips for youth groups to learn and participate in fishing and hunting.<sup>397</sup>

One component of a comprehensive public health approach to an Aboriginal community's food system may be to provide communities with the infrastructure and capacity to directly monitor pollutant contamination in the local foods and water, and the knowledge to use this information to make evidence-based decisions from family nutrition to the broader community food system. Replacing anxiety and loss of trust in

local foods with information on local contamination levels and the capacity to evaluate competing risks may be useful for developing community food systems and public health strategies.

## 5.5. Lessons Learned and New Chemical Products

The development and broad application of OC pesticides was widely acclaimed for improving agricultural harvests and reducing the spread of infectious disease from insects.<sup>424,425</sup> Improved agricultural harvest through the “green revolution” was seen as central to reducing hunger across populations and increasing wealth for many agricultural families.<sup>426</sup> The wide-scale control of disease-bearing insects was recognized as a principal strategy for controlling the spread of infectious disease such as malaria, typhus and dengue fever which are serious public health problems in regions of Africa and south Asia.<sup>427</sup> These economic and public health benefits were believed to substantially outweigh the potential long term negative consequences for the broader ecosystem with the efficient and appropriate application of pesticides.<sup>60,427</sup> Researchers who have questioned the use of pesticides have been criticized for failing to appreciate the vital role of pesticides for disease vector control in countries burdened by malaria.<sup>60</sup> Similarly, the introduction of PCBs was part of the chemical revolution which enabled development of expansive new industries and economic growth.<sup>428</sup>

The recent National Toxicology Program review of the literature on the effects of POPs on human health indicates POPs are endocrine disrupting chemicals with significant health effects even at environmentally relevant lower dose exposures.<sup>18</sup> The legacy of POPs controlled by the Stockholm Convention should be a cautionary tale for the introduction and use of new POPs and other chemicals without extensive evaluation for the potential for persistence, bioaccumulation, long-range transport through environments, and long term consequences of lower-dose exposure on plant and animal health.

The Stockholm Convention currently controls X POP compounds listed by the Convention. Several other compound groups are under review for addition as controlled POPs under the Convention including: hexabromocyclododecane; short-chained

chlorinated paraffins; chlorinated naphthalenes; hexachlorobutadiene; pentachlorophenol. Chlorinated naphthalene is of particular relevance in the Canadian context as it is used a diluent for pipeline transport of bitumen oil. Spills from damaged pipelines and the disposal of waste naphthalene following refinery processing of bitumen pose the risk of environmental contamination. Beyond the POPs currently under review for listing as controlled compounds under the Stockholm Convention, there are many more chemical compounds in use or development which may have long-term persistence, lipophilicity, and volatility allowing for high partitioning and global transport. It is important to evaluate the potential health effects from long-term environmentally relevant exposure to these compounds. Application of the precautionary principle for regulation of such compounds could minimize the emergence of future health effects associated with these new compounds.

## 6. Summary and Limitations

### 6.1. Limitation

The CHMS is a cross-sectional survey and as such cannot test for direct causal relationships with as much certainty as experimental and longitudinal design studies. With the CHMS cross-sectional survey design and data the analysis cannot distinguish if the exposure preceded the outcome. This limits a causal interpretation of the results because it is plausible that prediabetes and diabetes are correlated with slower metabolic clearance of POPs.<sup>429</sup> However, one previous study investigated the rate of elimination of dioxin (TCDD) in longitudinal data from US veterans of Operation Ranch Hand and found no association with the incidence or time to onset of diabetes.<sup>128</sup>

The data did not allow the analysis to distinguish between fetal, childhood and adult timing of exposures, which might be important if any effects of POPs on diabetes risk occur only during specific developmental periods. The differences in plasma concentrations of POPs may reflect bioaccumulation in tissues with age. However, the differences in age may also reflect differences in exposure levels over time. Differences in exposure levels early in life or potentially varying windows of vulnerability to exposure along the life-course may have effects which are not accounted for in the models. A further limitation of this analysis is that POPs measures were highly correlated (Tables 7a-7d). The collinearity in the models when more than one POP measure is included prevents an exploration of which particular POPs compounds were associated with T2D in Canadian adults. As such, a causal interpretation for individual POPs is not justified by this analysis.

As noted previously, due to operational constraints the POPs were measured from plasma and lipids were measured from serum in separate laboratories. It is preferable to have POPs and lipids measured in the same component of blood in the same laboratory. As a result the accuracy of the lipid-adjustment of the POP

concentrations is affected. Although this should not have limited the classification of participants accurately on a ratio scale of exposure for the purposes of building valid models, the comparisons of exposure levels and magnitudes of effects in this analysis compared to others should note this technical difference. Another limitation of the measurement of POP compounds is the laboratory LOD. To address the issue of non-detected plasma concentrations of POPs, the Hornung and Reed imputation method was used to impute a single value for all observations with a non-detectable concentration for each separate POP compound. The use of such an imputation method rather than censored regression or multiple imputation may result in some level of bias being introduced into the estimates.

The first hypothesis presented in the study is based on the action of POPs within cells to suppress the phosphorylation of protein kinase B as illustrated in figure 2. Ideally, we would want to know the concentrations of POPs in cells where suppression of PKB is hypothesized. However, the data used for the analyses provides POP concentrations in plasma. POP concentrations measured in blood are the result of the quantity entering the body through all sources of exposure, absorption rates, distribution to various tissues in the body, metabolism, and excretion of the chemical or its metabolites from the body. These processes are dependent on both the characteristics of the chemical, including lipophilicity, pH, and particle size, and the characteristics of the individual, such as age, diet, health status, and genetics.<sup>430</sup> For these reasons, the way in which a chemical will act in the body will differ among individuals and cannot be predicted with certainty. Consequently, the relationship between plasma concentrations and concentrations in cells may vary between individuals.

Diet is an important predictor of obesity and exposure to POPs through animal fats such as fish, meat and dairy products. The CHMS collected limited information on the frequency of consumption of selected types of foods. These data were found to be too limited to be useful in the analysis. Without adequate data on total calories consumed it is difficult to test the hypothesis concerning the association of exposure to POPs with increased intestinal lipid absorption. Specifically, we would want to be able to test for an association between plasma concentrations of POPs and measures of obesity while adjusting for total caloric intake, physical activity and potentially other predictors of obesity.

If as hypothesized, POP concentrations are associated with increased intestinal lipid absorption and consequently obesity, we would expect to observe increased systemic inflammation and thus increased odds of having T2D. We would want to test for an association between POP plasma concentrations and measures of systemic inflammation such as levels of C-reactive protein which is available in the CHMS data. It would also be important to adjust this model for the effect of anti-inflammatory medication dosage on CRP levels. The CHMS provides the ATC code for each prescription, over-the-counter and herbal medication used by respondents during the month preceding the physical examination. However, the lack of dosage information for medications which affect CRP makes testing this hypothesis problematic with the CHMS data.

Due to the above described data limitations on diet and medication dosage, it is important to note that this analysis tests for only part of the hypothesized total effect of POPs on the odds of having T2D. Specifically, the analysis is designed to test for only the portion of the effect of POPs associated with the suppression of PKB phosphorylation. The portion of the effect of POPs associated with increased adiposity and systemic inflammation cannot be tested due to data quality limitations with the CHMS cycle 1. The analysis is not designed to test for the total effect of POPs on the odds of having T2D. The observed association in this analysis between POP plasma concentrations and the odds of having T2D indicates just a portion of the hypothesized total effects of POPs. This is an important limitation of this analysis and other analyses which lack the data to adequately control for confounding from either one of the hypothesized pathways.

The groupings selected for summed measures were based on commonly used groupings of POP compounds. These groupings reflect general chemical similarities as when referring to PCBs, PBDEs, and chlordane. The organochlorine pesticide compounds are grouped together based on their intended use as insecticides. The differentiation of dioxin-like PCBs from non-dioxin-like PCBs is based on the expected biological point of interference. Specifically, dioxin-like chemicals are known to bind with the aryl-hydrocarbon receptor in animal cells and result in specific biological effects.<sup>228</sup> Non-dioxin-like PCBs are expected to bind with other cellular receptors and thus have distinct biological effects. The results for the summed measures of the groupings

selected for this analysis show an association between non-dioxin-like PCBs and the odds of T2D. This finding could be an indication that binding with the aryl-hydrocarbon receptor is not important for the biological pathway from exposure to T2D. However, these groupings may not appropriately reflect the biological pathway from POPs exposure to development of insulin resistance and eventually the onset of T2D. There may be alternative groupings of POPs which better identify the compounds most directly involved in the hypothesized pathways of suppression of insulin signalling or increased intestinal absorption of lipids. Further research is necessary to identify how specific chemical compounds are involved in each of these pathways, and other potential pathways leading to metabolic dysfunction. The use of groupings which do not reflect how these chemicals are involved in the pathogenesis of T2D would yield estimates for biologically irrelevant summed measures.

The first cycle of the CHMS provides plasma concentrations for selected organochlorine pesticides, PCBs, PBDEs. There are other compounds which are also identified as persistent organic pollutants by the Stockholm Convention which are not included in the CHMS data for cycle 1. Among the POP compounds measured in the CHMS plasma samples, only those POPs with less than 40% of observations below the limit of detection were selected for analysis in this study. Other POP compounds included in the CHMS data are excluded from the analysis in this study when 40% or more of the observations have plasma concentrations below the limit of detection. The laboratory's LOD also affects the analysis of lower dose effects. Imputing a plasma concentration for all respondents below the laboratory's LOD will affect the model estimates.

## **6.2. Summary**

T2D, obesity and other related chronic diseases have enormous personal, family and public costs throughout the world. The prevalence of T2D and obesity is increasing globally. Understanding the causes of these epidemics is critical for promoting and protecting health across all populations. A developing body of evidence suggests that lower-dose ubiquitous environmental exposure to POPs may be one of many determinants leading to the onset of T2D.

POPs are synthetically manufactured chemicals which do not occur naturally in the environment. The specific types of POPs examined in this study included five organochlorine pesticides, twelve polycarbonated biphenyls congeners, one aggregate measure of two PCB congeners, and one polybrominated diphenyl ether. The OC pesticides have been used for over seventy years throughout the world to protect agricultural crops and prevent the spread of disease in humans and other species. PCBs were principally used in industrial coolants and lubricants for machinery and electrical systems. PBDEs are widely present in many consumer products, vehicles and buildings as flame retardants.

These pollutants are highly persistent in the environment and in organisms due to their resilience to degradation through environmental and metabolic processes. This resilience gives these pollutants relatively long half-lives. Atmospheric and water currents together with animal migration transports these chemicals globally. Consequently, POPs are ubiquitous and detectable in water, soils and food globally.

POPs are lipophilic and therefore bind with the lipid fraction in living cells. The persistent and lipophilic characteristics of these pollutants lead to bioaccumulation of POPs in animals, particularly in higher trophic level species. Humans are exposed to POPs in food, dust, soil and water. Canadians are predominantly exposed to POPs in food particularly through fish, meat and milk.

Over the past decade, the epidemiological evidence of an association between the blood concentrations of POPs and the OR of T2D has been growing. Research on this association for the Canadian adult population is now possible with the new survey data used in this study. The 2007-2009 Canadian Health Measures Survey is a nationally representative sample of adults from 20 to 79 year-olds living in a private household in Canada. The CHMS provides a wide range of data including plasma concentrations for selected POP compounds, anthropometric measurements, and questionnaire responses. The POP compounds selected for analysis in this study had detectable plasma concentrations among at least 60% of respondents.

The results from this analysis are consistent with previous studies that showed positive associations between POPs and diabetes. A recent systematic literature review

sponsored by the US National Toxicology Program and National Institute of Environmental Health Sciences concluded that the overall evidence in studies published to December 2011 supports a positive association between some POPs compounds and T2D, especially *trans*-nonachlor, *p,p'*-DDE, PCB's, dioxins and dioxin-like chemicals.<sup>18</sup> The results are most directly comparable to those from the US NHANES, which is also a nationally-representative, North American, cross-sectional sample. The CHMS was designed with input from NHANES officials and so shares some design features that make the studies more comparable. For example, like NHANES, the CHMS utilized a mobile examination clinic with similar characteristics<sup>200</sup>. However, differences in how exposures were modeled as well as how diabetes was defined in previous studies using NHANES make precise comparisons of the results here impossible. Nonetheless, the general pattern of results is similar.

Consistent with the CHMS findings presented here, significant and positive associations with diabetes (did not distinguish by type) were observed in the 1999-2004 NHANES for beta-hexachlorocyclohexane<sup>431</sup> and PCB's 153, 170 and 180, beta-hexachlorocyclohexane and non-dioxin-like PCB's in the 1999-2002 NHANES.<sup>137,139</sup> The CHMS results here showed positive (but not significant) increased ORs of T2D with higher concentrations of oxychlorane, *trans*-nonachlor, *p,p'*-DDE, and PCB's 118, 138 and 187 whereas previous studies showed significant positive associations between diabetes and these compounds in the 1999-2002 NHANES<sup>137,139</sup> and the 1999-2004 NHANES<sup>431</sup>. Similar to the findings here, PBDE 47 was not associated with diabetes in the 2003-2004 NHANES<sup>432</sup>. PCBs 146,163, 194, 201, 203 or Arochlor 1260 were not included in previous NHANES studies, so comparisons are not possible. Several compounds that were positively and significantly associated with diabetes in previous NHANES studies but not included in our analysis were polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzo-furans, *p,p'*-DDT, heptachlor epoxide, and PCB's 74 and 126.<sup>139,431,433</sup>

The associations observed in this study are biologically plausible based on experimental research. Environmentally relevant concentrations of POPs mixtures derived from farmed Atlantic salmon have been shown to inhibit the PKB(Akt)/PI3 pathway which begins with activation of the insulin receptor and terminates in phosphorylation of Akt.<sup>11</sup> PKB is an evolutionarily well conserved pathway in mammals

important for glucose transport into cells, energy accumulation in cells, and other cellular metabolic processes.<sup>183</sup> Phosphorylated Akt is a major node in the pleiotropic effects of insulin signalling leading to enhanced uptake of glucose, glycogenesis, protein synthesis and triacylglyceral synthesis concomitant with inhibited gluconeogenesis and triacylglycerol hydrolysis.<sup>434</sup> Consistent with this mechanism, adult male mice fed POPs derived from farmed Atlantic salmon had decreased phosphorylated Akt with reduced whole-body glucose uptake and higher insulin levels after glucose challenge.<sup>11</sup> Adult rats fed POPs derived from farmed Atlantic salmon had reduced glucose uptake in hyperinsulinemic-euglycemic clamp and reduced expression of hepatic genes related to mitochondrial function.<sup>10</sup> Diminished mitochondrial function could contribute to diabetes risk by reducing the ratio of ATP production to oxygen consumption leading to increased reactive oxygen species, inflammation and insulin resistance.<sup>435</sup> This may also partially explain higher triacylglyceride levels in gastrocnemius muscles *in vivo*<sup>11</sup> and higher levels of hepatic triacylglycerol, diacylglycerol, and total cholesterol<sup>10</sup> in these animal studies. An alternate pathway may be begin with increased intestinal fat absorption from exposure to POPs as reported in mice.<sup>11</sup> This animal study found increased absorption of fats was associated with increased obesity, and potentially increased risk of inflammation and insulin resistance.

The directed acyclic graph in figure 2 was developed to illustrate the biological pathways hypothesized from POPs exposure to onset of T2D. Two hypotheses were presented in this study based on the biological pathways illustrated in figure 2. The first hypothesis is based on the predicted suppression of PKB by POPs in cells. It was hypothesized that increased concentrations of POPs is associated with increased OR of having T2D in Canadian adults. The second hypothesized biological pathway is based on the predicted greater intestinal lipid absorption from exposure to POPs as illustrated in figure 2. The second hypothesis predicts increased concentrations of POPs to be associated with increased obesity and systemic inflammation in Canadian adults.

The first hypothesis was tested by using two models of multiple logistic regressions to investigate the association of plasma concentrations of POPs with the OR of having T2D. Two models were selected to determine if the findings were consistent across different measures of abdominal obesity (e.g. waist-to-hip ratio and waist circumference). Previous research indicated that each of the measures could be valid

markers of abdominal obesity. The main findings of this study supported the hypothesis that halogenated organic substances, especially organochlorines, were positively associated with T2D in Canadians aged 20 to 79 after adjustment for important covariates. The analysis indicates statistically significant positive associations between T2D and beta-hexachlorocyclohexane, and non-dioxin-like PCB's 153, 170, 180 and 1260 (Araclor) as well as the sum of non-dioxin-like PCB's. There is roughly a doubling of the OR of T2D associated with an inter-quartile increase in the log transformed plasma concentration of these POPs measures. Among those organochlorine compounds that did not reach statistical significance in their associations with T2D, all had positive sample parameter estimates and 95% confidence intervals were predominately in the positive range. Sex, smoking history, racial background, socio-economic status, and height were not confounders in any of the models. The findings from these models are generally consistent with predicted increase in OR of T2D for the first hypothesis.

The second hypothesis was problematic to test with the CHMS data used for this study. Limitations with the diet and medication data were found to be too substantial to test for an association between POP concentrations and obesity, and also the association of POP levels with C-reactive protein which is a biomarker for systemic inflammation. It is important to note that the findings here reflect only a portion of the total hypothesized effect of POPs on the odds of having T2D. The analysis cannot test for the effect of POPs associated with pathway involving increased adiposity and systemic inflammation.

Unlike Lee and colleagues, this study did not observe an interaction between the level of summed PCBs and OCs with the association of BMI with T2D.<sup>137,138</sup> The association between BMI and the OR of T2D was examined by age-adjusted quantile levels of the sum of all PCBs and OCs. An interaction between the concentration of POPs and the association between obesity and T2D would be consistent with the hypothesized increased intestinal lipid absorption related to exposure to POPs.

There were high correlations between most of the PCBs and OC pesticides. The levels of PBDE47 were poorly correlated with the sums of PCBs and OCs. These low correlations may reflect the differences in bioaccumulation with age for PBDEs as

compared with PCBs and OCs. PBDE plasma concentrations are relatively more stable across age groups than are PCBs and OCs.<sup>46</sup> In contrast both the geometric and arithmetic averages of the PCBs and OCs rise with age. This finding is consistent with bioaccumulation of PCBs and OCs over the life-course. It is important to note the data is cross-sectional and therefore the higher concentrations of PCBs and OCs among older age groups may reflect different exposure levels among older adults as compared to younger adults.

The high correlations among most of the PCB congeners and OC pesticides makes it difficult to identify specific individual or subgroups of POPs as more highly associated with T2D. To assess if dioxin-like PCBs, non-dioxin-like PCBs or OC pesticides were more highly associated with the OR for T2D, models were tested with two or three of these sum measures included as parameters. However, adding a second or a third sum measure substantially increased the p-values of the sum measures. The substantial increase in p-values indicates a problem with collinearity within the model. From this analysis, we cannot determine if one of these subgroups of POPs is more highly associated with the OR of T2D relative to the other groups.

In summary, the results from this analysis are consistent with the conclusions of the recent review by the US National Toxicology Program and National Institute of Environmental Health Sciences that many organochlorine compounds are positively associated with T2D. This study adds one more national jurisdiction in which these associations have been observed. Given the close proximity of the US and Canada plus similarities in the designs of NHANES and the CHMS, the results presented here might be considered a consistent and independent replication of findings from NHANES in the North American context. In light of the well-described biological mechanisms in animal models, the results add to the weight of evidence that some POPs increase the risk of T2D in humans.

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## **Appendices**

## Appendix A.

### Supplementary Models for T2D Including POP Compound Plasma Concentrations without Interquartile Scaling

**Table 13a. Model 3 OR of T2D by plasma POP concentrations in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI)	Adjusted <sup>b</sup> OR (95% CI)
<b>Organochlorine Pesticides</b>		
Oxychlorane	3.544 (2.294, 5.476)‡	1.967 (0.894, 4.330)
<i>trans</i> -Nonachlor	3.086 (1.877, 5.074)‡	1.782 (0.706, 4.503)
<i>p,p'</i> -DDE	1.735 (1.252, 2.403)†	1.306 (0.900, 1.896)
Hexachlorobenzene	2.249 (1.516, 3.336)‡	1.405 (0.947, 2.085)
β-Hexachlorocyclohexane	1.499 (1.187, 1.894)†	1.381 (1.002, 1.903)*
<b>Dioxin-like PCBs</b>		
PCB118	2.288 (1.470, 3.561)†	1.462 (0.809, 2.514)
PCB156	2.206 (1.750, 2.782)‡	1.461 (0.913, 2.340)
<b>Non-dioxin-like PCBs</b>		
PCB138	2.447 (1.752, 3.418)‡	1.578 (0.957, 2.602)
PCB146	2.362 (1.665, 3.351)‡	1.654 (0.919, 2.978)
PCB153	2.373 (1.875, 3.002)‡	1.764 (1.113, 2.797)*
PCB163	2.362 (1.850, 3.014)‡	1.663 (0.960, 2.881)
PCB170	2.061 (1.797, 2.363)‡	1.545 (1.079, 2.212)*
PCB180	1.955 (1.721, 2.221)‡	1.524 (1.093, 2.124)*
PCB187	2.115 (1.707, 2.619)‡	1.532 (0.976, 2.406)
PCB194	1.753 (1.486, 2.069)‡	1.213 (0.883, 1.664)
PCB201	1.883 (1.567, 2.263)‡	1.320 (0.863, 2.020)
PCB203	1.982 (1.690, 2.363)‡	1.289 (0.911, 1.823)
PCB1260	2.433 (1.871, 3.189)‡	1.720 (1.065, 2.778)*
<b>Polybrominated diphenyl ether 47</b>	1.148 (0.889, 1.482)	1.031 (0.775, 1.372)

<sup>a</sup> Each of the individual POP measures was log transformed. Individual POP measures were lipid-normalized.

<sup>b</sup> Adjusted for waist circumference, body mass index, the interaction of waist circumference and body mass index, daily leisure energy expenditure, and age.

‡p≤0.001 †p≤0.01 \*p≤0.05

**Table 13b. Model 4 OR of T2D by plasma POP concentrations in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI)	Adjusted <sup>b</sup> OR (95% CI)
<b>Organochlorine Pesticides</b>		
Oxychlorthane	3.544 (2.294, 5.476)‡	2.050 (0.918, 4.576)
<i>trans</i> -Nonachlor	3.086 (1.877, 5.074)‡	1.738 (0.689, 4.385)
<i>p,p'</i> -DDE	1.735 (1.252, 2.403)†	1.328 (0.917, 1.922)
Hexachlorobenzene	2.249 (1.516, 3.336)‡	1.507 (1.007, 2.256)*
β-Hexachlorocyclohexane	1.499 (1.187, 1.894)†	1.382 (1.028, 1.859)*
<b>Dioxin-like PCBs</b>		
PCB118	2.288 (1.470, 3.561)†	1.454 (0.846, 2.501)
PCB156	2.206 (1.750, 2.782)‡	1.430 (0.887, 2.305)
<b>Non-dioxin-like PCBs</b>		
PCB138	2.447 (1.752, 3.418)‡	1.592 (0.990, 2.561)
PCB146	2.362 (1.665, 3.351)‡	1.577 (0.905, 2.748)
PCB153	2.373 (1.875, 3.002)‡	1.747 (1.122, 2.720)*
PCB163	2.362 (1.850, 3.014)‡	1.623 (0.960, 2.744)
PCB170	2.061 (1.797, 2.363)‡	1.547 (1.080, 2.217)*
PCB180	1.955 (1.721, 2.221)‡	1.510 (1.083, 2.106)*
PCB187	2.115 (1.707, 2.619)‡	1.502 (0.975, 2.314)
PCB194	1.753 (1.486, 2.069)‡	1.178 (0.868, 1.599)
PCB201	1.883 (1.567, 2.263)‡	1.273 (0.856, 1.893)
PCB203	1.982 (1.690, 2.363)‡	1.253 (0.904, 1.736)
PCB1260	2.433 (1.871, 3.189)‡	1.713 (1.081, 2.715)*
<b>Polybrominated diphenyl ether 47</b>	1.148 (0.889, 1.482)	1.058 (0.811, 1.379)

<sup>a</sup> Each of the individual POP measures was log transformed. Individual POP measures were lipid-normalized.

<sup>b</sup> Adjusted for waist-to-hip ratio, body mass index, the interaction of waist-to-hip ratio and body mass index, daily leisure energy expenditure, and age.

‡p≤0.001 †p≤0.01 \*p≤0.05

**Table 14a. Model 5 OR of T2D by summed molar weights of POP grouping in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI) <sup>b</sup>	Adjusted OR (95% CI) <sup>c</sup>
Organochlorine pesticides	1.791 (1.202, 2.668)†	1.380 (0.893, 2.134)
Chlordane pesticides	3.337 (1.991, 5.593)‡	1.888 (0.771, 4.627)
Dioxin-like PCBs	2.433 (1.660, 3.567)‡	1.501 (0.878, 2.566)
Non-dioxin-like PCBs	2.251 (1.871, 2.708)‡	1.609 (1.054, 2.457)*
All chlorinated POPs	1.953 (1.268, 3.008)†	1.460 (0.893, 2.387)

<sup>a</sup> Each of the POP group summed measures was log transformed.

<sup>b</sup> Adjusted for total lipids as model parameter.

<sup>c</sup> Adjusted for waist circumference, body mass index, the interaction of waist circumference and body mass index, daily leisure energy expenditure, and age. Models for summed measures also adjusted for total lipids.

‡p≤0.001 †p≤0.01 \*p≤0.05

**Table 14b. Model 6 OR of T2D by summed molar weights of POP grouping in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI) <sup>b</sup>	Adjusted OR (95% CI) <sup>c</sup>
Organochlorine pesticides	1.791 (1.202, 2.668)†	1.400 (0.916, 2.139)
Chlordane pesticides	3.337 (1.991, 5.593)‡	1.877 (0.760, 4.634)
Dioxin-like PCBs	2.433 (1.660, 3.567)‡	1.508 (0.891, 2.553)
Non-dioxin-like PCBs	2.251 (1.871, 2.708)‡	1.591 (1.049, 2.413)*
All chlorinated POPs	1.953 (1.268, 3.008)†	1.476 (0.918, 2.373)

<sup>a</sup> Each of the POP group summed measures was log transformed.

<sup>b</sup> Adjusted for total lipids as model parameter.

<sup>c</sup> Adjusted for waist-to-hip ratio, body mass index, the interaction of waist-to-hip ratio and body mass index, daily leisure energy expenditure, and age. Models for summed measures also adjusted for total lipids.

‡p≤0.001 †p≤0.01 \*p≤0.05

## Appendix B.

### Supplementary Models for T2D Including POP Compound Plasma Concentrations without Lipid Adjustment

**Table 15a. Model 3 OR of T2D by inter-quartile plasma POP concentrations without lipid adjustment in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI)	Adjusted <sup>b</sup> OR (95% CI)
<b>Organochlorine Pesticides</b>		
Oxychlorane	3.884 (2.729, 5.529)‡	1.826 (0.909, 3.666)
<i>trans</i> -Nonachlor	3.782 (2.388, 5.992)‡	1.803 (0.708, 4.590)
<i>p,p'</i> -DDE	2.253 (1.420, 3.573)†	1.406 (0.856, 2.309)
Hexachlorobenzene	2.660 (2.045, 3.460)‡	1.335 (0.973, 1.832)
β-Hexachlorocyclohexane	1.729 (1.296, 2.308)†	1.492 (0.994, 2.240)
<b>Dioxin-like PCBs</b>		
PCB118	2.804 (1.891, 4.160)‡	1.471 (0.882, 2.454)
PCB156	3.465 (2.536, 4.734)‡	1.615 (0.765, 3.410)
<b>Non-dioxin-like PCBs</b>		
PCB138	3.269 (2.394, 4.462)‡	1.712 (0.993, 2.952)
PCB146	2.749 (2.034, 3.716)‡	1.713 (0.948, 3.097)
PCB153	3.367 (2.673, 4.242)‡	2.039 (1.161, 3.581)*
PCB163	4.031 (3.019, 5.383)‡	2.085 (0.965, 4.506)
PCB170	3.085 (2.441, 3.900)‡	1.774 (0.994, 3.333)
PCB180	2.904 (2.363, 3.568)‡	1.766 (1.026, 3.039)*
PCB187	2.986 (2.309, 3.861)‡	1.734 (0.941, 3.193)
PCB194	2.775 (1.990, 3.870)‡	1.288 (0.635, 2.614)
PCB201	2.745 (2.113, 3.565)‡	1.426 (0.743, 2.740)
PCB203	2.660 (2.096, 3.374)‡	1.310 (0.734, 2.339)
PCB1260	3.298 (2.585, 4.206)‡	1.912 (1.107, 3.302)*
<b>Polybrominated diphenyl ether 47</b>	1.386 (0.883, 2.177)	1.022 (0.592, 1.765)

<sup>a</sup> Each of the individual POP measures was log transformed and divided by the inter-quartile range.

<sup>b</sup> Adjusted for waist circumference, body mass index, the interaction of waist circumference and body mass index, daily leisure energy expenditure, and age.

‡p≤0.001 †p≤0.01 \*p≤0.05

**Table 15b. Model 4 OR of T2D by inter-quartile plasma POP concentrations without lipid adjustment in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI)	Adjusted <sup>b</sup> OR (95% CI)
<b>Organochlorine Pesticides</b>		
Oxychlorthane	3.884 (2.729, 5.529)‡	1.895 (0.922, 3.892)
<i>trans</i> -Nonachlor	3.782 (2.388, 5.992)‡	1.754 (0.687, 4.482)
<i>p,p'</i> -DDE	2.253 (1.420, 3.573)†	1.437 (0.877, 2.357)
Hexachlorobenzene	2.660 (2.045, 3.460)‡	1.438 (1.035, 1.999)*
β-Hexachlorocyclohexane	1.729 (1.296, 2.308)†	1.493 (1.026, 2.172)*
<b>Dioxin-like PCBs</b>		
PCB118	2.804 (1.891, 4.160)‡	1.507 (0.912, 2.488)
PCB156	3.465 (2.536, 4.734)‡	1.569 (0.738, 3.338)
<b>Non-dioxin-like PCBs</b>		
PCB138	3.269 (2.394, 4.462)‡	1.736 (1.029, 2.928)*
PCB146	2.749 (2.034, 3.716)‡	1.623 (0.922, 2.856)
PCB153	3.367 (2.673, 4.242)‡	2.015 (1.155, 3.517)*
PCB163	4.031 (3.019, 5.383)‡	2.008 (0.955, 4.223)
PCB170	3.085 (2.441, 3.900)‡	1.784 (0.949, 3.355)
PCB180	2.904 (2.363, 3.568)‡	1.750 (1.014, 3.018)*
PCB187	2.986 (2.309, 3.861)‡	1.689 (0.937, 3.044)
PCB194	2.775 (1.990, 3.870)‡	1.231 (0.626, 2.421)
PCB201	2.745 (2.113, 3.565)‡	1.355 (0.735, 2.498)
PCB203	2.660 (2.096, 3.374)‡	1.267 (0.729, 2.203)
PCB1260	3.298 (2.585, 4.206)‡	1.905 (1.113, 3.262)*
<b>Polybrominated diphenyl ether 47</b>	1.386 (0.883, 2.177)	1.071 (0.653, 1.756)

<sup>a</sup> Each of the individual POP measures was log transformed and divided by the inter-quartile range.

<sup>b</sup> Adjusted for waist-to-hip ratio, body mass index, the interaction of waist-to-hip ratio and body mass index, daily leisure energy expenditure, and age.

‡p≤0.001 †p≤0.01 \*p≤0.05

**Table 16a. Model 5 OR of T2D by inter-quartile summed molar weights of POP groupings without lipid adjustment in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI)	Adjusted OR (95% CI) <sup>b</sup>
Organochlorine pesticides	2.298 (1.427, 3.702)†	1.496 (0.862, 2.599)
Chlordane pesticides	3.889 (2.562, 5.902)‡	1.874 (0.797, 4.410)
Dioxin-like PCBs	3.009 (2.174, 4.164)‡	1.579 (0.925, 2.694)
Non-dioxin-like PCBs	3.245 (2.656, 3.964)‡	1.868 (1.047, 3.335)*
All chlorinated POPs	2.365 (1.549, 4.484)†	1.614 (0.851, 3.062)

<sup>a</sup> Each of the POP group summed measures was log transformed and divided by the inter-quartile range.

<sup>b</sup> Adjusted for waist circumference, body mass index, the interaction of waist circumference and body mass index, daily leisure energy expenditure, and age.

‡p≤0.001 †p≤0.01 \*p≤0.05

**Table 16b. Model 6 OR of T2D by inter-quartile summed molar weights of POP groupings without lipid adjustment in individual models**

Persistent organic pollutants <sup>a</sup>	Crude OR (95% CI)	Adjusted OR (95% CI) <sup>b</sup>
Organochlorine pesticides	2.298 (1.427, 3.702)†	1.522 (0.895, 2.587)
Chlordane pesticides	3.889 (2.562, 5.902)‡	1.862 (0.788, 4.402)
Dioxin-like PCBs	3.009 (2.174, 4.164)‡	1.587 (0.936, 2.690)
Non-dioxin-like PCBs	3.245 (2.656, 3.964)‡	1.844 (1.041, 3.264)*
All chlorinated POPs	2.365 (1.549, 4.484)†	1.634 (0.888, 3.008)

<sup>a</sup> Each of the POP group summed measures was log transformed and divided by the inter-quartile range.

<sup>b</sup> Adjusted for waist-to-hip ratio, body mass index, the interaction of waist-to-hip ratio and body mass index, daily leisure energy expenditure, and age

‡p≤0.001 †p≤0.01 \*p≤0.05

## Appendix C.

### Environment Canada's Toxic Substances Management Policy: Track 1: Virtual Elimination from the Environment

A substance that meets all four criteria outlined in the table below including: persistent, bioaccumulative, toxic and primarily the result of human activity, is to be designated for virtual elimination from the environment (Track 1 substance). This objective is intended to be achieved by addressing sources of release to the environment or by removing or managing the substance if it is already in the environment.

**Table 17. Criteria for the Selection of Substances for Track 1**

Persistence <sup>1</sup>		Bioaccumulation <sup>3</sup>	Toxicity <sup>4</sup>	Predominantly anthropogenic <sup>5</sup>
Medium	Half-life			
Air	≥ 2 days <sup>2</sup>	BAF ≥ 5,000	CEPA-toxic or CEPA-toxic Equivalent	Concentration in environment largely resulting from human activity
Water	≥ 182 days	BCF ≥ 5,000		
Sediment	≥ 365 days	log Kow ≥ 5.0		
Soil	≥ 182 days			

<sup>1</sup>A substance is considered persistent when the criterion is met in any one medium.

<sup>2</sup>A substance may be considered as persistent in air if it is shown to be subject to atmospheric transport to remote regions such as the Arctic.

<sup>3</sup>Bioaccumulation Factors (BAF) are preferred over Bioconcentration Factors (BCF); in the absence of BAF or BCF data, the octanol-water partition coefficient (log Kow) may be used.

<sup>4</sup>A substance is considered toxic if it meets or is equivalent to the definition of "toxic" found in the Canadian Environmental Protection Act (CEPA), as determined through a systematic, risk-based assessment. CEPA states: "a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that (a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity; (b) constitute or may constitute a danger to the environment on which life depends; or (c) constitute or may constitute a danger in Canada to human life or health."

<sup>5</sup>On the basis of expert judgment, the concentration of the substance in any environmental medium is due largely to the quantities of the substance used or released as a result of human activity relative to contributions from natural sources. Elements and naturally occurring inorganic compounds are not candidates for virtual elimination from the environment.

The TSMP requires environmental pollution prevention strategies be used to eliminate the measurable release of a Track 1 substance from domestic sources. A Track 1 substance that cannot be managed successfully throughout its life cycle needs to be scheduled for phase-out of generation and uses. Through bilateral or multilateral agreements, the federal government will work to eliminate Track 1 substances that originate from sources outside Canada.

Remediation may be undertaken when a Track 1 substance is already in the environment. For sites under federal jurisdiction that are contaminated by a Track 1 substance, management plans need to include a strategy for the elimination of that substance, based on an analysis of risks, costs and benefits. The TSMP requires that remediation of these sites where the benefits to the ecosystem or to human health of removing the substance outweigh clean-up costs; this includes the possibility of further environmental degradation of the substance. Otherwise, the management strategies will focus on minimizing exposure and the site's potential risks.

The federal government will identify Track 1 substances proposed for virtual elimination from the environment. Stakeholders will have an opportunity to comment, with a fixed period of time to present scientific evidence objecting to or supporting a substance's selection. The federal government will render a final, public decision after reviewing all the evidence.

The onus will be on those who generate or use a Track 1 substance to demonstrate that the substance will not be released into the environment in measurable concentrations at any point in its life cycle. Measurable release limits will be developed as appropriate for a Track 1 substance to allow verification that no measurable release has been achieved and to allow enforcement of any regulations that may be developed. Limits will be based on the lowest concentration of a substance that can be accurately detected and quantified using sensitive but routine analytical methods. These limits will be established during the development of management strategies as part of consultations with stakeholders.

The objective of eliminating a Track 1 substance from the environment is set irrespective of socio-economic factors. However, the management plans such as targets and schedules to achieve that long-term objective will be based on analyses of environmental and human health risks as well as social, economic and technical considerations.

The presence of a Track 1 substance in the environment will be monitored to ensure that management plans are achieving the objective of virtual elimination and to assess the need for additional action.

The persistence and bioaccumulation criteria used to identify Track 1 substances can only be applied to chemical substances. Thus, while a chemical substance produced by organisms through biotechnology processes may be considered for Track 1 registration, the organisms themselves will not be considered for registration.

Where a Track 1 substance results from the degradation or transformation of a parent substance in the environment, the parent substance may also be considered for Track 1 registration.

Naturally occurring substances, elements or radionuclides are not candidates for Track 1 registration. However, when warranted, a natural substance that is used or released as a result of human activity may be targeted for reduction to naturally occurring levels under Track 2 registration.

This policy does not apply to pharmaceuticals when used for purposes for which they were approved under the Canada Food and Drugs Act. It does apply to those pharmaceuticals, their by-products or wastes that are of concern because of their release to the environment.

The Toxic Substances Management Policy Interdepartmental Forum, representing twelve federal government departments, co-ordinates the implementation of the TSMP. The initial list of twelve substances which met the criteria for management under Track 1 of TSMP was published in Part 1 of the Canada Gazette on July 4, 1998. They included:

- aldrin
- chlordane
- DDT
- dieldrin

- endrin
- heptachlor
- hexachlorobenzene (HCB)
- mirex
- polychlorinated biphenyls (PCBs)
- polychlorinated dibenzo-p-dioxins (PCDDs)
- polychlorinated dibenzofurans (PCDFs)
- toxaphene

Within Canada, government regulation was developed to limit or prohibit the production, use or release of these twelve POPs. Nine of these substances were active ingredients in pesticides that have been prohibited from manufacture, use or release in Canada.