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The Association Between Persistent Organic Pollutants, Type 2 Diabetes, and Insulin Resistance in Two First Nations Communities in Northern Ontario

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THE ASSOCIATION BETWEEN PERSISTENT ORGANIC POLLUTANTS, TYPE 2 DIABETES, AND INSULIN RESISTANCE IN TWO FIRST NATIONS COMMUNITIES IN NORTHERN ONTARIO

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Abstract

Recent evidence suggests an association between persistent organic pollutants (POPs) and type 2 diabetes. In two First Nations communities of high wild food consumption, specific objectives were formulated to: 1) compare POP levels between diabetics and non-diabetics; 2) investigate the association between POPs and insulin resistance in non-diabetics; and 3) determine the effects of POPs on certain inflammatory markers.

Results indicated significantly higher age-adjusted plasma concentrations of some POPs in diabetics as compared to non-diabetics. Body mass index was found to be the most significant predictor of insulin resistance. There was a positive association of Tumour Necrosis Factor (TNF)-α with oxychlordane and mono/di-ortho substituted polychlorinated biphenyls (PCBs). Positive associations were detected between adiponectin and mono/di-ortho substituted PCBs as well as some pesticides. Results indicate that diabetics have higher levels of some POPs than non-diabetics. In non-diabetics, POPs are unrelated to insulin resistance although some are positively associated with inflammatory markers.

Résumé

De récentes évidences suggèrent une association entre les polluants organiques persistents (POPs) et l'incidence du diabète de type 2. Au sein de deux communautés de Premières Nations caractérisée par une consommation élevée de nourriture sauvage, les objectifs spécifiques de cette thèse étaient de : 1) comparer les concentrations de POPs entre sujets non-diabétiques et diabétiques; 2) examiner les associations entre les concentrations plasmatiques de POPs et la sensibilité à l'insuline d'individus non-diabétiques; et 3) déterminer les effets des POPs au niveau de marqueurs inflammatoires plasmatiques.

Nos travaux démontrent qu'en comparaison aux sujets non-diabétiques, les diabétiques présentent des concentrations plasmatiques de certains POPs statistiquement élevées, même après ajustement de l'âge des participants. Les concentrations de facteur de nécrose de tumeur (FNT)-α sont associées positivement aux concentrations d'oxychlordane et les biphényle polychloré (BPC) substitués en positions mono/di-ortho. Les concentrations d'adiponectine sont associées positivement à certains BPC et pesticides. Dans l'ensemble, nos travaux ont montré que les diabétiques présentent des concentrations plasmatiques de POPs plus élevées que les individus non-diabétiques. Chez les individus non-diabétiques, les concentrations de POPs ne sont pas associées à la résistance à l'insuline malgré que ces dernières soient liées à certains marqueurs inflammatoires.

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List of Acronyms and Abbreviations

AhR	aryl hydrocarbon receptor
AdipoR1	adiponectin receptor 1
AdipoR2	adiponectin receptor 2
ANCOVA	analysis of covariance
ARNT	aryl hydrocarbon receptor nuclear translocator
AUC	area under the curve
β-HCH	β-hexachlorocyclohexane
BMI	body mass index
CVD	cardiovascular disease
CYP	Cytochrome P450
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DL	detection limit
DLC	dioxin-like compound
DRE	dioxin response element
ECD	electron capture detector
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FPG	fasting plasma glucose
FPI	fasting plasma insulin
GC-MS	gas chromatography-mass spectrometry
GLUT	glucose transporter

HCB	hexachlorobenzene
HOMA-IR	homeostasis model assessment of insulin resistance
HOMA-β	homeostasis model assessment of beta cell function
HSP	heat shock protein
HWF	high wild food
IL	interleukin
IRS	insulin receptor substrate
LWF	low wild food
NF-κB	nuclear factor kappaB
NHANES	national health and nutrition examination survey
OC	organochlorine
OGTT	oral glucose tolerance test
PBB	polybrominated byphenyl
PBDE	polybrominated diphenyl ether
PCA	principal component analysis
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
POP	persistent organic pollutant
PPAR	peroxisome proliferator-activated receptor
PPRE	peroxisome proliferator-activated receptor responsive element
RXR	retinoid X receptor
SOCS-3	suppressor of cytokine signaling-3

TCDD	tetrachlorodibenzo-dioxin
TNF	tumour necrosis factor
TNFR	tumour necrosis factor receptor
TZD	thiazolidinedione
WHO	world health organization

Chapter 1: Introduction

1.1 *Nutrition Transition and Type 2 Diabetes*

In First Nations communities, a decreased portion of the diet is now coming from traditional foods. This shift from traditional practices to eating store-bought or market foods is referred to as a "nutrition transition." The transition comprises a change in food availability and an increase in the use of processed foods high in starch, fat, and sugar (Popkin *et al.* 2002). There is also an association between the nutrition transition and level of poverty (Popkin 2004). First Nations communities tend to be impoverished and therefore community members often purchase cheap, filling foods low in vitamin and mineral content and high in saturated fats and refined sugars (Damman *et al.* 2008).

The loss of culture and identity that are represented by traditional foods also stems from the nutrition transition (Damman 2008). Traditional knowledge of food preparation and hunting could start to disappear with more people eating modern foods. The other loss is the nutritional benefits many traditional foods provide. These foods are high in polyunsaturated fatty acids known to be protective against heart disease (Dewailly *et al.* 2001) and possibly insulin resistance (Kusunoki *et al.* 2003). Traditional foods are also high in important dietary minerals and are a good source of protein (Kuhnlein *et al.* 2002).

Nutrition transition is not unique to First Nations communities. Many societies around the globe are shifting to a Westernized diet. In Korea, a large increase in animal food product consumption was concurrently observed with a fall in total cereal intake

(Kim 2001). In Iran, the usage of sugars, fats, and oils has increased along with large declines in the consumption of meats, fruits, and vegetables (Ghassemi *et al.* 2002). In India, higher fat consumption as well as reduced production and use of pulses and legumes have been noted (Shetty 2002). One of the global consequences of the nutrition transition is an increase in non-communicable diseases (Popkin *et al.* 2001). A study in Chile (Albala *et al.* 2001) determined an increased sedentary behaviour as one of the contributing factors to the increase in non-communicable diseases. Traditionally, members of Canadian First Nations communities are hunters, anglers, and gatherers, contributing positively to physical activity. Less reliance on traditional foods could mean less hunting and fishing, thus a reduction in physical activity levels.

The prevalence of type 2 diabetes is increasing worldwide (WHO 2009). In Canadian First Nations communities, the prevalence of type 2 diabetes has been reported to be 3 to 5 times higher than the general Canadian population (Young *et al.* 2000; Green *et al.* 2003). In the Sioux Lookout area of northern Ontario, the incidence of diabetes increased by 45% over a 10 year span (Fox *et al.* 1994). Reasons for the higher rate include elevated levels of obesity (Kaler *et al.* 2006), a low amount of physical activity (Dyck and Cassidy 1998), and poor food availability and choices (Popkin *et al.* 2002). These issues are all related to the nutrition transition mentioned above. Communities that have undergone or are presently going through a nutrition transition are prone to increased rates of diabetes.

The high prevalence of diabetes and obesity in northern Ontario could be considered as reason to encourage more traditional food consumption due to its health benefits. However, there is a possible trade-off between the benefits of traditional food

consumption and the possibility that some of these foods may be contaminated with pesticides and other organic compounds. In northern Ontario, there is a high prevalence of diabetes despite the reliance of many community members on traditional foods. Therefore we investigated a possible association between chemicals present in these foods and diabetes.

1.2 *Persistent Organic Pollutants in Northern Communities*

Persistent Organic Pollutants (POPs) are either produced as byproducts to chemical processes or intentionally, such as pesticides. The POPs have the ability to resist degradation in the environment through light, chemical, or biological processes, making them an ecological risk (Gramatica and Papa 2007). They are a potential risk for human health due to their tendency to accumulate in fatty tissues. The POPs are lipophilic, meaning they accumulate in lipid tissues of organisms (Li *et al.* 2006). Because they are retained by each organism exposed to them, animals at high trophic levels end up accumulating even more POPs in their tissues through a process known as biomagnification (Mackay and Fraser, 2000). Since humans are at the top of the food chain, this is of particular concern to human health, especially those relying heavily on foods derived from the land. Many POPs are volatile, and thus able to move through the atmosphere away from their production source. The "grasshopper effect" describes a progressive evaporation of semi-volatile POPs towards colder northern climates, which is believed to contribute to enrichment of some POPs in northern latitudes (Figure 1.1; Fisher, 1999). Therefore POPs can affect ecosystems and communities far away from

their point of release, including First Nations communities that are located in northern areas of Canada.

There are a variety of different POPs. The focus here will be on polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, and poly-brominated diphenyl ethers (PBDEs). The PCBs were produced until the 1970s in various industries for use in capacitors and dielectric fluids. There are a host of toxic effects that have been associated with exposure to PCBs. Much of this work has been done by observing human populations that have been exposed to acute levels of PCBs occupationally or because of residential proximity (Fitzgerald *et al.* 2007). A study conducted on children exposed to PCBs *in utero* found that PCB exposure led to long-term consequences on IQ level and reading comprehension (Jacobson and Jacobson, 1996). Certain cancers, such as breast, stomach, and lung cancer (Lucena *et al.* 2001; Pavuk *et al.* 2004), have also been linked to PCB levels. Exposure to PCBs may result in fertility issues as well as Spanò *et al.* (2005) showed an adverse effect of PCB exposure on the integrity of sperm chromatin.

There are a variety of OC pesticides but many have been banned from use in North America since the 1970s. Some tropical countries still use dichlorodiphenyltrichloroethane (DDT) for malaria vector control (WHO, 2007). Due to atmospheric transport, as mentioned earlier, OC pesticides still persist in areas where they are no longer in use (Hoff *et al.* 1992). The extent to which a compound is affected by atmospheric transport depends on its volatility. For example, hexachlorocyclohexane (HCH) is more volatile than DDT meaning that DDTs remain closer to their source (Bard 1999). Oceanic transport can also move these pesticides around, on a global scale (Barrie

et al. 1992). The OCs can make their way into water systems through soil erosion (Zhou *et al.* 2008) and surface runoff (Hunt *et al.* 1999).

Many studies have been performed to investigate possible roles for OC pesticides in the development of health defects, such as cancer, in humans. In Egypt, incidence of colorectal cancer has been associated with serum concentrations of DDT, HCH, and hexachlorobenzene (HCB; Soliman *et al.* 1997). Prevalence of pancreatic cancer has been associated with exposure to OC pesticides in California (Clary and Ritz, 2003). Dieldrin, another OC pesticide, was positively correlated with hypothyroidism, suggesting a possible influence of OC pesticides on the thyroid endocrine axis (Rathore *et al.* 2002). Dieldrin has also been associated with Parkinson's disease (Fleming *et al.* 1994).

The PBDEs are brominated fire retardants used in a number of household products such as textiles, cabinets, and televisions (IPCS, 1997). The PBDEs have been used since the 1980s to prevent flash fires, meaning that they slow down the spread of fire in homes, which is the reason behind their widespread use (Rahman *et al.* 2001) and unlike PCBs, are still being produced. Although they are useful, studies conducted on mice have shown associated toxic effects including neurotoxicity, developmental abnormalities (Darnerud *et al.* 2001), and disruption of hormones (Kuriyama *et al.* 2007). Exposure to these chemicals may be related to the consumption of certain foods that have high levels of PBDEs such as certain fish and meat (Voorspoels *et al.* 2007). Also, given that PBDEs are found throughout the home, accumulation in house dust poses another intake route (Tan *et al.* 2007).

Although not a POP, mercury (Hg) exposure is frequently related to wild food consumption due to its ability to biomagnify through aquatic-based food chains (Campbell *et al.* 2008) and its prevalence in the environment. Mercury ingestion is of particular concern for individuals whose diets are comprised of high amounts of fish (Da Silva Brabo 2000; Hughner *et al.* 2008) as fish bioaccumulate methyl-Hg out of the water (Dórea 2008).

Mercury makes its way into the environment from both natural and anthropogenic sources. The main anthropogenic source of Hg is emissions from coal smoke (Streets *et al.* 2009). The degassing of the Earth's crust is the natural source of Hg (Magos and Clarkson 2006). Studies have linked low-level Hg exposure in children to autism (Lee *et al.* 2003) and to various neurophysiological anomalies (Counter 2003). Adults chronically exposed to low amounts of Hg score lower than average during neurobehavioural tests (Ngim *et al.* 1992).

1.3 *The POPs and incidence of type 2 diabetes*

Recent evidence suggests that POPs may play a part in the development of diabetes (Carpenter 2008). The idea that diabetes may be associated to POP levels in the body was initially established through studies on dioxin levels. People working or living in proximity to accidental dioxin releases have suffered high exposure levels. There is an infamous case of a group of Vietnam War veterans with high plasma levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; the most potent dioxin). The TCDD was a

component of Agent Orange, a herbicide used during the Vietnam War (Pirkle *et al.* 1989).

Henriksen *et al.* (1997) studied incidence of diabetes, time to onset of diabetes (post-exposure), and fasting and post-prandial glucose abnormalities in veterans of the Vietnam War. The study involved a comparison of veterans of Operation Ranch Hand (involved in spraying Agent Orange) to other veterans. Veterans of Operation Ranch Hand were divided into Background, Low, and High exposure groups depending on the current and initial dioxin levels. They found that the incidence of diabetes and glucose abnormalities were higher in veterans of Operation Ranch Hand than other veterans. The time of onset to the disease was decreased in veterans exposed to both low and high concentrations of Agent Orange (compared to those not exposed at all). An interesting point is that in non-diabetic Ranch Hand veterans, insulin levels were elevated in the high-exposure category putting forth the idea that the dioxin actually may be causal in terms of insulin abnormalities. One difficulty present with this study was the probable exposure of these veterans to other herbicides, which may have adverse effects for diabetes and could thus confound results.

In 1976 an industrial accident at a chemical manufacturing plant near Seveso, Italy released TCDD into the environment, leading to the highest known TCDD exposure to a residential population (Eskenazi *et al.* 2004). A study performed by Bertazzi *et al.* (1998) showed diabetes-related deaths to be slightly higher for people within the exposure range of the accident, especially for females. Data concerning incidence of diabetes was not included. A study conducted in the USA (Sweeney *et al.* 1997/98) compared males occupationally exposed to dioxins to age-matched males with no known

dioxin exposure. Sweeney *et al.* found a positive correlation between risk of diabetes, fasting glucose levels and serum concentrations of TCDD. They noted however, that age and weight (i.e. traditional risk factors of diabetes) were more prominent predictors for the development of diabetes.

More recently, several studies have found a positive correlation between the incidence of diabetes and plasma levels of PCBs, OC pesticides, and some other POPs. Generally most PCB and OC pesticide studies link exposure to diet and occupation or location, rather than acute accidental exposure. Therefore, exposure is chronic and the resulting pattern may differ from what is seen from studies linking dioxins to diabetes, mostly dealing with acute accidental exposure.

Lee *et al.* (2006) found that the OC pesticides oxychlordane, DDE, and *trans-nonachlor* are strongly positively associated with diabetes as well as two dioxins and PCB-153. The PCB-153 is a non-coplanar PCB congener generally dominant among other PCB congeners in the environment and in human plasma (Rignell-Hydbom *et al.* 2007). Lee *et al.* (2006) conducted their analysis using lipid-adjusted data from a National Health and Nutrition Examination Survey (NHANES) conducted from 1999 to 2002 and was based on 2016 participants, representative of the general population of the USA. Given the large sample size, they were able to adjust for age, sex, ethnicity, and obesity. However, they were unable to separate the type 1 diabetic participants from the type 2 diabetics, making their results a possible over-estimation. Using the same data set, Everett *et al.* (2007) also found significant positive associations of PCB126 (a coplanar congener) and DDT with the prevalence of diabetes.

A study of 257 participants recruited around Belgium found a positive trend for PCBs, polychlorinated dibenzodioxins (PCDDs), and polychlorinated difurans (PCDFs; Fierens *et al.* 2003) with respect to incidence of diabetes. Specifically, the researchers studied four coplanar PCBs, 12 non-coplanar PCBs, and seventeen PCDD/Fs. Their results show significant differences between lipid-adjusted plasma concentration levels for all types of contaminants between diabetics and non-diabetics. In their sample size of 257, only 9 self-reported to be diabetic (i.e. this was not verified as a part of the study), which seems to be a low ratio given that the results are based on comparisons between their diabetic and non-diabetic subjects.

In eastern Slovakia, water and food chain pollution occurred from the release of PCBs from the Chemko chemical factory during the period of 1959-1984 (Kocan *et al.* 2001). Radikova *et al.* (2004) examined relations between levels of several OC pesticides, 15 PCB congeners, and diabetes in 2050 individuals. Once the subjects were divided into tertiles (low, medium, and high) depending on the POP concentrations, the authors found a higher frequency of diabetes in the high and medium tertiles. One key aspect of this study is their method for establishing diabetic status within their population. Oral Glucose Tolerance Tests (OGTTs) were administered to quantify the insulin and glucose responses of subjects to a 75-g sugar load. The OGTT is used to establish levels of impaired glucose homeostasis, insulin resistance, and whether an individual is considered diabetic or not (Matsuda and DeFronzo, 1999). It is also more reliable than self-reporting of diabetes. Using the OGTT allowed Radikova *et al.* to quantify impaired glucose tolerance. They show a significantly higher frequency of impaired glucose tolerance for individuals of the high and medium tertiles, which follows the same trend as

seen for diabetic individuals compared to non-diabetics. The impaired glucose tolerance data attempts to strengthen the correlation between POPs and diabetes by showing the development of diabetes, which is missing from studies where the only possible comparison is between diabetics and non-diabetics.

In Sweden, consumption of fatty fish from the Baltic Sea is the major source of POP exposure (Svensson *et al.* 1991) and is thus a particularly important source for fishermen and their families, who are generally high fish consumers. Rylander *et al.* (2005) found significant associations between lipid-adjusted plasma concentrations of PCB-153, DDE, and diabetes prevalence with a study population comprised of 196 men and 184 women. This pattern was observed for DDE but not PCB-153 when only the female participants were considered. Among the men, positive trends existed between both POPs and diabetes prevalence. PCB-153 was used as a proxy of PCB exposure. According to the authors, it correlates well with total PCB concentrations in plasma. The pesticide DDE has been shown to be a relevant proxy of POP exposure for fatty fish consumers in this area

The same research group in Sweden conducted a follow-up study on 1439 Swedish women, all wives of fishermen from the east and west coasts of Sweden. They found higher serum levels of PCB-153 and DDE linked to a significantly increased risk of diabetes (Rignell-Hydbom *et al.* 2007), based on lipid-standardized plasma levels. The results of this study confirmed results produced earlier (Rylander *et al.* 2005).

Vasiliu *et al.* (2006) conducted an epidemiological study with 1384 participants in Michigan on PCBs, polybrominated biphenyls (PBBs), and diabetes. Vasiliu *et al.* found a positive association of diabetes incidence with concentration of PCBs in women but not

in men. This does not concur with what was published by Lee *et al.* (2006) as they found a difference in PCB-153 between diabetics and non-diabetics even once sex was accounted for. The results presented by Vasiliu *et al.* were not lipid-adjusted, which may explain the discrepancy between their results and that of Lee *et al.* Vasiliu *et al.* were also unsure how many type I diabetics were accidentally included in their study.

There is a higher prevalence of diabetes among Mexican Americans than among non-Mexican Americans (Narayan *et al.* 2003). Using results from a Hispanic Health and Nutrition Examination Survey, Cox *et al.* (2007) determined significant associations between whole-weight serum levels of OC pesticide concentrations of trans-nonachlor, oxychlordane, DDT, DDE, and self-reported diabetes in 1303 Mexican Americans. After adjustment for serum lipid concentrations, only the association between DDT and diabetes remained significant.

Codru *et al.* (2007) conducted a study on 352 volunteers from a Mohawk population residing at or near Akwesasne looking at the relation between diabetes and serum levels of 101 PCB congeners, DDE, HCB, and mirex. Akwesasne is a heavily polluted area designated an Area of Concern by the International Joint Commission in 1985. The PCBs are expressed as total PCBs, but the researchers also looked at PCB-153 and PCB-74 separately. The congener PCB-153 was present at the highest concentration among all measured congeners and PCB-74 has been shown to be the most strongly associated with fish consumption in this population (Fitzgerald *et al.* 2007). They found a significant association between diabetes and DDE and HCB. Using lipid-adjusted values, Codru *et al.* also found a significantly higher average total PCB concentration in

diabetics. In this study, both sexes were evaluated, but this variable was taken into account using adjustments during analysis.

Most recently, Turyk *et al.* (2009a) have identified an association between DDE exposure and the incidence of diabetes in the Great Lakes area on 471 sport fish consumers. For over 30 years, scientists have been concerned for the health of the Great Lakes ecosystem due to the presence of many POPs in the sediment and biota (Tremblay and Gilman 1995). Turyk *et al.* (2009a) analyzed 13 PCB congeners as total PCBs and one congener (PCB-118) on its own as a coplanar-like mono-*ortho* representative. The PCBs were not associated with incidence of diabetes once BMI, age, and sex were taken into consideration. What is of particular interest in this study is that POP data was collected longitudinally from 1994 to 2005. This was done in an effort to see whether metabolic changes related to diabetic status influenced POP metabolism. They compared annual percent changes in POPs between diabetics and non-diabetics over this time span but did not find a significant effect of diabetes on the metabolism rates of any of the measured chemicals. This makes reverse causality seem like an unlikely explanation.

The PBDEs are a relatively newer group of POPs and thus their relationship with diabetes has not been studied to such an extent as PCBs and OC pesticides. Lim *et al.* (2008) associated 5 PBDEs with diabetes in one-third of the same cohort that Lee *et al.* (2006) used to establish associations between other POPs and diabetes (mentioned earlier). Lim *et al.* did not find a clear positive association with PBDE-153 and diabetes. The other PBDE congeners were not associated with diabetes at all.

Mercury is not lipid soluble and behaves very differently in the body than the POPs reviewed above. To our knowledge, there have not been any studies that have

observed the relationship between Hg and diabetes. Some *in vitro* evidence shows that Hg^{2+} stimulates glucose transporter activity in adipocytes due to the translocation of glucose transporters to the plasma membrane, but does not modulate insulin receptor activity (Ezaki 1989). The extent to which glucose transport is increased is much lower than what occurs in the presence of insulin (Barnes and Kircher 2005). There is also *in vitro* evidence developed using adipocytes that shows insulin-mediated glucose transport is decreased in the presence of Hg (Barnes and Kircher 2005). Whether Hg accumulation via food ingestion could modulate insulin sensitivity or have an effect on insulin resistance requires further understanding.

Several issues arise when examining literature on POPs in relation to diabetes. Different research groups looked at various combinations of POPs as contributing factors to diabetes. These studies have reached the general conclusion that POPs influence the rate of diabetes and therefore it is not possible to attribute causality from correlations to particular POP(s). Some of the data sets in the literature are available in lipid-standardized form and others are available as wet-weight only. This poses a problem when attempting to compare exposure levels between populations. Also, some of the aforementioned studies were conducted to examine effects in the general population (Lee *et al.* 2006) and others were done on particular populations of people because of geographical location (Codru *et al.*2007) and dietary choices (Vasiliu *et al.* 2006).

The other problem when comparing results is that different studies used different ways of establishing diabetic status among participants. While some studies relied on self-reporting from diabetic participants (Vasiliu *et al.* 2006), others used plasma glucose levels. Even when this was done, there is likely variation from one study to another

given different criteria for establishing diabetes. Codru *et al.* (2007) defined diabetes as having a fasting glucose value greater than 125 mg/dL (6.9 mmol/L), based on the American Diabetes Association's recommendations. In addition to this criterion, Lee *et al.* (2006) included participants with a non-fasting plasma glucose level greater than 200 mg/dL (11 mmol/L) in their diabetic category.

Although there are a substantial number of studies that have established significantly higher POP levels in diabetic individuals, the reason behind this trend remains to be elucidated. In an attempt to solve this, Lee *et al.* (2007) conducted an epidemiological study on 749 non-diabetic participants using data from the NHANES. Insulin resistance was quantified using Homeostasis Model Assessment (HOMA-IR), which is a tool used to calculate the degree of insulin resistance in an individual based on the pre-prandial plasma concentrations of glucose and insulin (Matsuda and DeFronzo, 1999). Higher HOMA-IR values are associated with higher levels of insulin resistance. Lee *et al.* (2007) found that oxychlordane, trans-nonachlor and certain non-coplanar PCBs were most strongly associated with insulin resistance. The other POPs measured, which included 4 coplanar and 5 non-coplanar PCBs, 3 PCDDs, 3 PCDFs, and 2 other OC pesticides were not associated with insulin resistance. They speculated that certain POPs may reduce insulin sensitivity (Lee *et al.* 2007), eventually resulting in insulin resistance, but did not mention possible mechanisms through which this may occur. Contrarily diabetic individuals may accumulate POPs in their plasma as a result of differences in lipid metabolism.

Jørgensen *et al.* (2008) present opposing results to those of Lee *et al.* (2007). They performed a study in Greenland with 917 subjects of the Inuit population to look at

the association between diabetes, OC pesticides, and PCBs. To establish diabetic status, Jørgensen *et al.* performed standard OGTTs on individuals who were previously undiagnosed as diabetic. The HOMA-IR values from this study did not correlate to any of the measured POPs, contrary to what was observed by Lee *et al.* (2007). Jørgensen *et al.* also studied the possible association between POPs and insulin secretion by measuring the HOMA of beta cell function (HOMA-β). They found a significant inverse association between POPs and insulin secretion through HOMA-β and stimulated insulin, meaning that POPs could have an effect on insulin secretion rather than insulin resistance.

The effect of POPs on insulin secretion has been studied *in vitro*. Fischer *et al.* (1996) found that certain PCB congeners and the PCB mixture Aroclor1254 are able to stimulate insulin release from RINm5F cells. These cells are derived from a rat insulinoma and have been successfully employed for *in vitro* studies on the action of insulin-releasing chemicals and drugs. The mechanism behind this is likely related to increases in intracellular free calcium through the enzyme calcium/calmodulin-dependent kinase II. The PCB-stimulated insulin release is blocked when this enzyme is inhibited (Fischer *et al.* 1999). The effects of PCBs and other POPs on insulin resistance have also been studied *in vitro*. In the next two sections, biologically plausible explanations that have been suggested and examined to elucidate this issue will be detailed.

1.4 *Peroxisome Proliferator-Activated Receptor-γ may be influenced by POPs*

Exposure to PCBs and dioxins may trigger the activation of a pathway involving the nuclear hormone receptor called Peroxisome Proliferator-Activated Receptor (PPAR)-γ. The PPARs form heterodimers with the retinoid X receptor (RXR; Kersten *et al.* 2000). This heterodimer is able to activate transcription, once a PPAR agonist is bound to the complex (Glass *et al.* 1997). Ligand-binding induces a conformational change in the PPAR complex and co-repressor molecules become unbound, allowing the complex to bind to PPAR response elements (PPREs) in target genes (Figure 1.2; Henry 1997). There are three identified isotypes in the PPAR family; namely PPAR-α, β, and γ. The PPARα isotype is mainly expressed in brown adipose tissue and liver, PPARβ is expressed mostly in the gut, kidney, and heart whereas PPAR-γ is expressed mostly in adipose tissue (Kersten *et al.* 2000). The PPAR-γ isotype could potentially play an important role in the pathway involved in insulin resistance (Hammarstedt *et al.* 2005).

Several mechanisms have been proposed to explain the downstream anti-diabetic effect of ligand-binding to PPAR-γ (Fujita *et al.* 1983) as there are several factors that can lead to diabetes. It is possible that the beneficial effects of increased PPAR-γ activation are a combination of these factors. Liganded PPAR-γ may increase glucose transporter (GLUT) type 4 levels directly. GLUT4 is an insulin-responsive glucose transporter located in muscle and fat cells (Rieusset *et al.* 1999). Decreased expression of GLUT4 leads to less glucose removal from the plasma. The liganded PPAR-γ/RXR heterodimer may bind to the GLUT4 gene promoter, as an increase in GLUT4 mRNA is observed in cultured adipocytes following incubation with a PPAR-γ ligand (Furuta *et al.*

2002). The other proposed effect of PPAR-γ activation involves modulation of certain adipokines, which will be explored in further detail in the subsequent section.

Dioxins and some PCBs considered as dioxin-like compounds (DLCs) are able to exert molecular effects through dioxin-activated pathways. Among other functions, these POPs may modulate levels of PPAR-γ. Due to different toxic mechanisms, there are two general PCB categories. When none of the *ortho* positions on the PCB structure are substituted with a chlorine atom, the congener is considered coplanar (Figure 1.3A). Thus it has the ability to mimic the toxic effects of dioxins, due to the similarity in structure (Tanabe *et al.* 1987) and is therefore a DLC. Non-coplanar PCBs (Figure 1.3B) do not generally behave as dioxins. Although dioxins (Figure 1.3C) are known to be more toxic than any PCB (Van den Berg 1998), coplanar PCBs can activate the same pathways to a lesser degree. There are 209 possible PCB congeners, 20 of which can be coplanar (Safe *et al.* 1985) and about 12 of which function similarly to dioxins. The PBDEs are also structurally similar to dioxins (Figure 1.3D). Whether they exert toxicity through the same mechanism as coplanar PCBs and dioxins, is still under debate (Peters *et al.* 2006; Wahl *et al.* 2008).

Dioxins and DLCs bind to a receptor called the aryl hydrocarbon receptor (AhR). The activation of the AhR pathway is reviewed extensively by Hahn (1998) and summarized here. The AhR (Figure 1.4) is initially bound to heat-shock protein (hsp90) and is thus able to remain in the cytoplasm of the cell. Once it is liganded by a dioxin or DLC (Figure 1.4), hsp90 dissociates and the liganded receptor enters the nucleus, where it heterodimerizes with AhR nuclear translocator (ARNT). When the heterodimer complex is formed, it is able to bind to dioxin response elements (DRE) on certain genes.

Therefore, genes that have a DRE can be regulated by ligand-binding of the AhR. Classic genes associated with the AhR pathway are members of the cytochrome P450 family. The Cyp1 proteins for instance, are up-regulated in this manner and are responsible for activating and detoxifying various pollutants that fit as ligands for the AhR (Nebert *et al.* 2004). Similarly, the gene coding for Peroxisome Proliferator-Activated Receptor (PPAR)-γ may be influenced through activation of the AhR pathway (Shaban *et al.* 2004).

1.5 *The POPs may contribute to chronic inflammation*

The acute inflammatory response is one the body's initial way of dealing with the invasion of external agents that are able to lead to trauma or infection. The response involves the release of a number of molecular mediators that can assist in clearing the pathogen and starting the repair process (Kumar *et al.* 2004).

Low-grade chronic inflammation is similar to this response in that it is characterized by the release of certain molecules from the white adipose tissue, known as adipokines (Trayhurn and Wood 2004), and thus a higher level of circulating adipokines in the plasma. Traditional roles associated with white adipose tissue are energy storage, thermal insulation (Young 1976), and mechanical protection for internal organs (Fonseca-Alaniz *et al.* 2007). More recent evidence indicates an added important role of the adipose tissue as an endocrine organ (Trayhurn and Beattie 2001) starting with the discovery of leptin (Zhang *et al.* 1994) as an important adipose-secreted regulator.

The list of hormones involved in signalling (now known as adipokines) is still expanding. Many of these adipokines are known to be positively related to chronic inflammation. This has allowed an establishment of strong correlations and modulatory effects of certain adipokines such as Tumour Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6) and insulin resistance (Leionen *et al.* 2003; Hu *et al.* 2004). For a healthy individual with normal sensitivity to insulin, the insulin signalling cascade commences with the insulin molecule binding to its receptor on a target cell (Figure 1.5), inducing autophosphorylation of the receptor (Kasuga *et al.* 1982). This generates a signal that is perpetuated through proteins such as IRS-1 (Insulin Receptor Substrate-1; Sun *et al.* 1991).

The inhibition of the insulin signalling pathway by TNF-α is well elucidated. When TNF-α binds to its receptor, a downstream serine phosphorylation of IRS-1 occurs (Csehi *et al.* 2005). Phosphorylation of IRS-1 either results in positive or negative feedback on the insulin receptor, depending on which site becomes phosphorylated (Figure 1.5; Zick 2001). Serine phosphorylation (as opposed to tyrosine) causes an inhibition, consequently causing IRS-1 to inhibit signal transduction from the insulin receptor (Zick 2001). Hotamisligil *et al* (1994a) treated murine adipocytes *in vitro* and found that TNF-α produced a significantly lower percent of insulin receptor phosphorylation in comparison to control cell cultures. The same group found similar results using the Zucker rat model of obesity (Hotamisligil *et al.* 1994b). Intravenous injection of a TNF receptor (TNFR)-IgG fusion protein neutralized TNF-α in these rats. Once TNF-α was neutralized, a significant increase of phosphorylation of the insulin receptor and IRS-1, in both muscle and adipose tissue was observed. The effect of TNF-

α on skeletal muscle is particularly important, as it is the main site of insulin-stimulated uptake of glucose. TNF-α mRNA is expressed in fat cells, but not muscle (Hotamisligil *et al.* 1993). This points to the likelihood of endocrine signalling from the adipose tissue, where TNF-α is secreted (Hotamisligil *et al.* 1994b).

The adipokine IL-6 inhibits insulin signalling by inducing expression of SOCS-3 (suppressor of cytokine signaling-3), which is able to bind to the phosphorylated tyrosine of the insulin receptor β sub-unit, thus inhibiting the insulin signal (Peraldi *et al.* 2001). Other signalling molecules released from adipocytes are also potentially involved in insulin resistance but these are not well characterized. Visfatin and resistin, for instance are two relatively newer such adipokines (Tanaka *et al.* 2007; Ukkola 2002) and their roles with respect to insulin resistance have not been well established.

Another important adipokine in relation to insulin resistance is adiponectin. In contrast to TNF-α and IL-6, adiponectin is regarded as a beneficial cytokine for preventing diabetes. An enhanced functionality of insulin on C2C12 myotubes has been observed in the presence of adiponectin (Wang *et al.* 2007). The binding of adiponectin to its receptors AdipoR1 and AdipoR2 likely inhibits serine phosphorylation of IRS-1 by p70 S6 kinase thus allowing for proper tyrosine phosphorylation of IRS-1 and signal transduction (Wang *et al.* 2007). The AdipoR1 is expressed predominantly in the skeletal muscle and AdipoR2 is expressed mostly in the liver (Yamauchi *et al.* 2003). Both receptors are also expressed in the adipose tissue (Fasshauer *et al.* 2004). Recently a PPAR Responsive Element (PPRE) was identified on the adiponectin promoter (Iwaki *et al.* 2003). Thus, the adiponectin gene was labelled as a potential target gene for activated PPAR-γ. This was the first description of direct transcriptional control of any adipokine

by PPAR-γ activation. Adiponectin is increasingly being recognized as a crucial mediator between the activation of PPAR-γ and modulation of insulin sensitivity (Bouskila *et al.* 2005).

Some research shows a positive relationship between dioxins, dioxin-like POPs and inflammation, though this relationship is not completely understood. Lee *et al.* (2007) report a relationship between PCB levels in serum and the metabolic syndrome. The metabolic syndrome may be related to chronic inflammation (Esposito and Giugliano, 2004). A study conducted by Hennig *et al.* (2001) examined the effect of coplanar PCBs on the secretion of IL-6 *in vitro* using porcine endothelial cells. The results of this study show that IL-6 production is increased by coplanar PCBs and not non-coplanar PCBs. Only coplanar PCBs have the ability to activate the AhR (as mentioned above) and thus the authors conclude that IL-6 may be up-regulated through activation of the AhR pathway.

A recent *in vitro* study conducted by Arsenescu *et al.* (2008) used PCB-77 as a representative coplanar PCB and PCB-153 as a non-coplanar congener. Unlike previous studies that have examined the effect of PCBs on inflammatory markers, this study was performed on adipocytes. The researchers found a significant elevation of the mRNA expression of TNF-α in the presence of PCB-77 but not PCB-153. In contrast to the results of Hennig *et al.* (2001), IL-6 did not show any significant change in the presence of PCB-77. This could perhaps be due to the different cell type used in the studies.

Another possible link between POPs and inflammation involves the nuclear factor kappaB (NF-κB). The NF-κB has been shown to be important in the regulation of genes coding for inflammatory cytokines such as TNF-α and IL-6 (Jeong *et al.* 2002). Kwon *et*

al. (2002) conducted a study *in vitro*, using a human leukemia cell line, to examine the effect of PCBs on NF-κB. The expression of both TNF-α and IL-6 increased in response to exposure to PCB-153 and was shown to occur as a result of NF- κB activation.

As mentioned above, PPAR-γ is down-regulated through activation of the AhR pathway. Down-regulation of PPAR-γ, which could occur from exposure to certain POPs, could cause a decrease in adiponectin production. Recently, Arsenescu *et al.* (2008) showed a decrease in adiponectin mRNA expression in response to PCB-77 exposure in cultured adipocytes.

Low-grade systemic inflammation is primarily associated with heart diseases such as hypertension, Alzheimer's disease (Fillit *et al.* 1991), atherosclerosis (Ross 1999), coronary heart disease (Duncan *et al.* 2003), and chronic obstructive pulmonary disease (Sin and Man, 2007). Given that similar variables are known to influence the onset of diabetes and coronary heart disease, the association between systemic inflammation and diabetes was explored and then established as well (Duncan *et al.* 2003). Factors that influence the production of inflammatory markers, could affect other diseases related to chronic inflammation as well.

1.6 *Research Objectives*

Northern communities are exposed to a high level of contaminants due to atmospheric and oceanic transport that bring POPs into otherwise pristine areas. Many traditional hunted foods are at high trophic positions on the food web (Dewailly *et al.* 1993) and hence may be enriched with certain biomagnified contaminants (Van Oostdam *et al.*

1999). Epidemiological evidence strongly suggests an association between the incidence of type 2 diabetes and plasma levels of certain POPs.

Our first objective was to compare POP levels between diabetics and non-diabetics to see if higher POP levels would be seen in diabetic subjects. Our second objective was to determine whether POPs had an influence on insulin resistance in non-diabetics, and to determine the effects of POPs on levels of the chronic inflammatory markers TNF-α, IL-6, and adiponectin.

One reason this study was conducted in northern Ontario was because of the high prevalence of diabetes in this area, providing us with many diabetic subjects. The other reason was that we wished to gather quantitative data on POP levels in an area of Canada generally neglected in POP studies, but still heavily reliant on traditional foods. This information is useful to generate quantitative evidence used to recommend healthy food choices relevant to community members in our study areas.

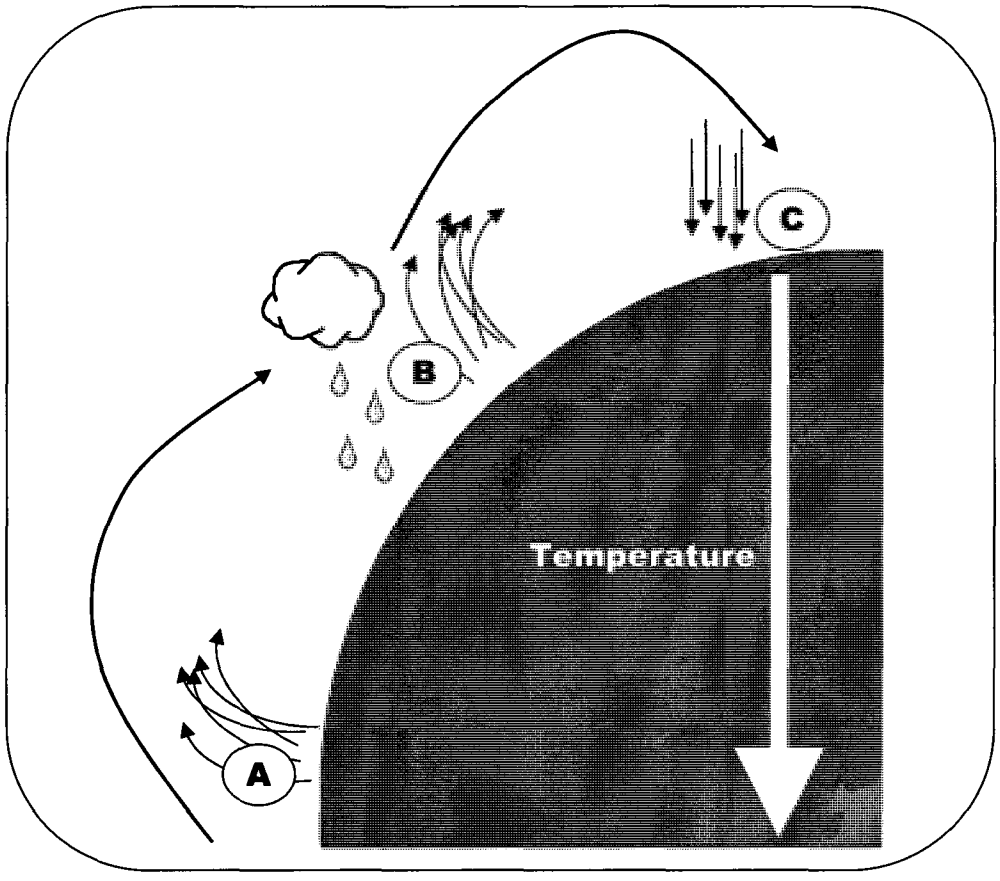

Figure 1.1 Atmospheric transport of volatile POPs. (A) The POPs evaporate in warm temperatures and travel through winds to colder regions. (B) In colder temperatures, POPs condense and fall back onto earth, but may evaporate again. (C) The temperature at which the POPs eventually settle into the environment depends on the level of volatility. This transport pattern is termed the "grasshopper effect" (Environment Canada, 2006).

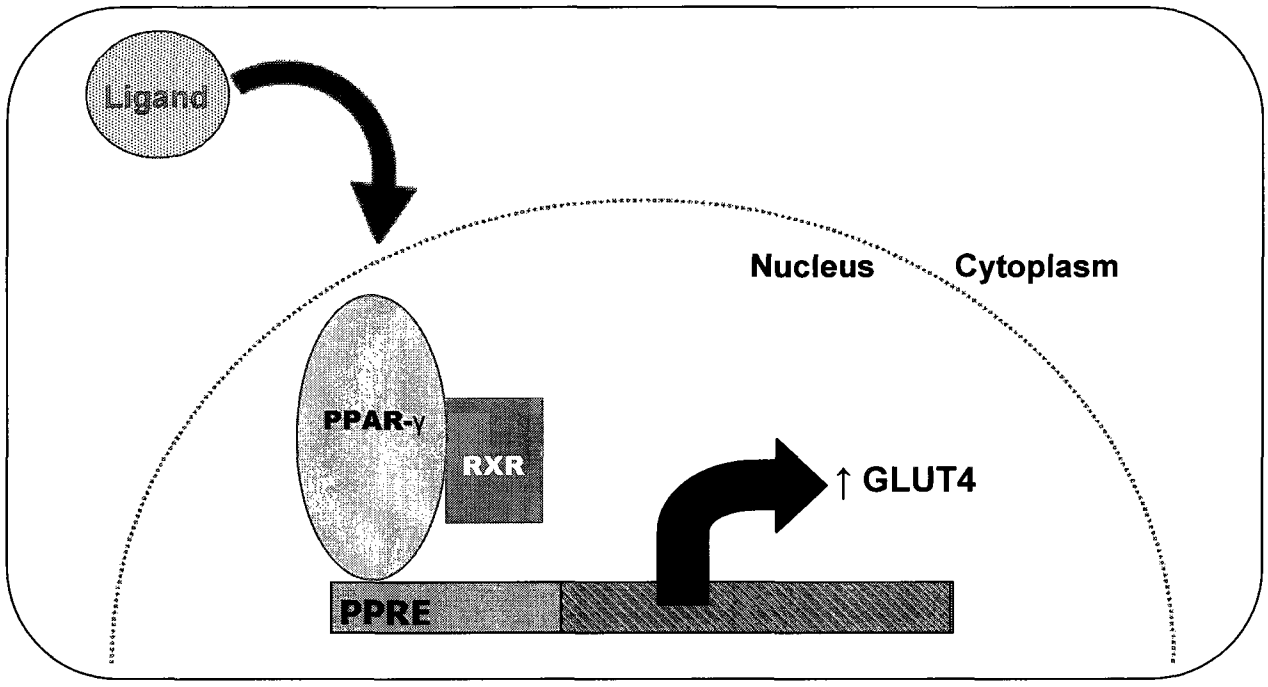

Figure 1.2 The activation of the PPAR-γ through ligand-binding. The PPAR-γ and RXR form a heterodimer capable of binding to particular ligands. Once the heterodimer is ligand-bound, it enters the nucleus of the cell and binds to response elements for the complex (PPREs) on particular genes. The production of certain proteins (such as GLUT4) can

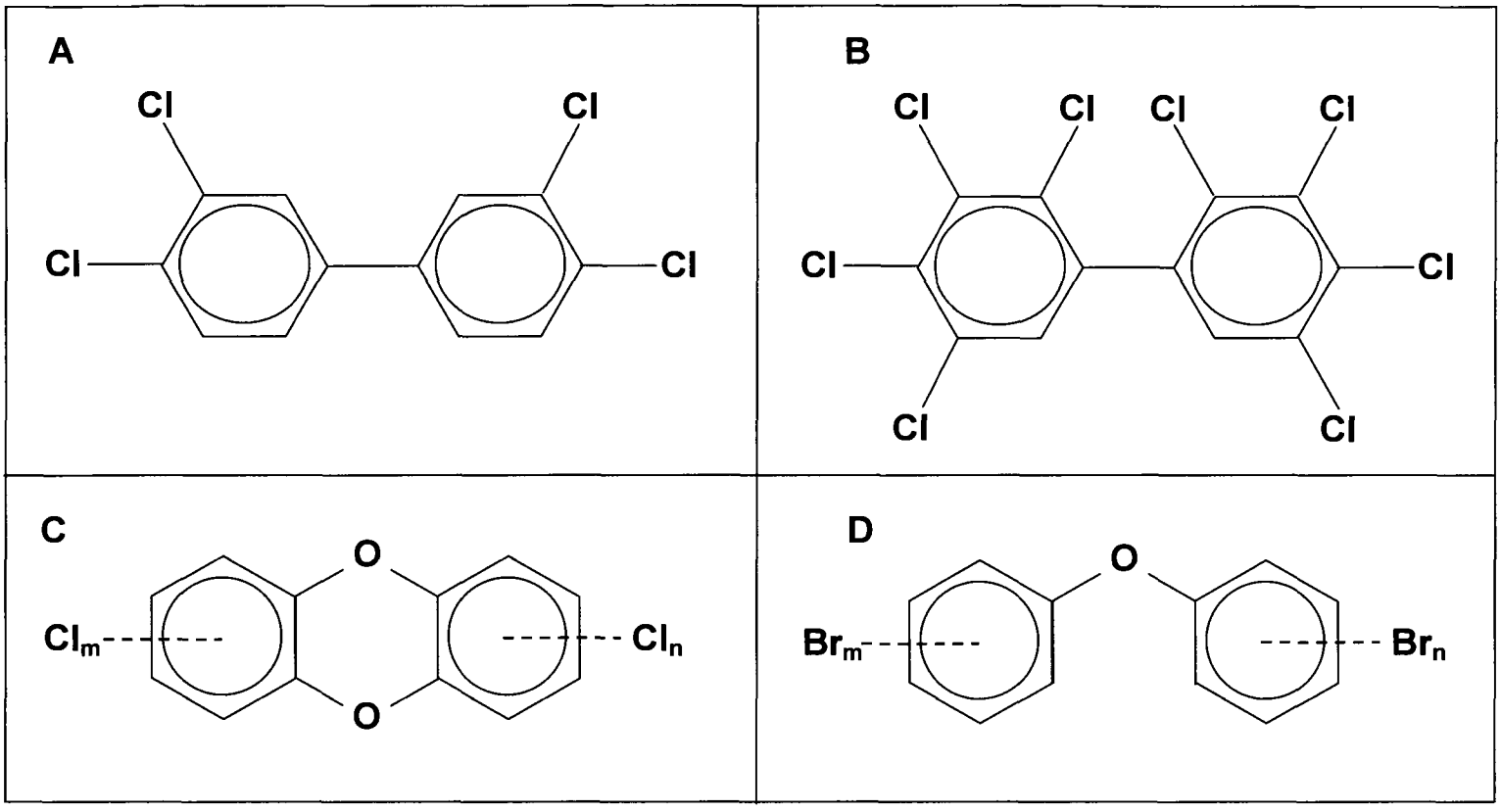

Figure 1.3 The structure of dioxin-like compounds. Coplanar poly-chlorinated biphenyls (PCBs) such as PCB-77 (A) are not substituted at the *ortho* position. The chlorine substitution at the *ortho* position of non-coplanar PCBs is exemplified by PCB-153 (B). General structures of dioxins (C) and PBDEs (D) are presented as well.

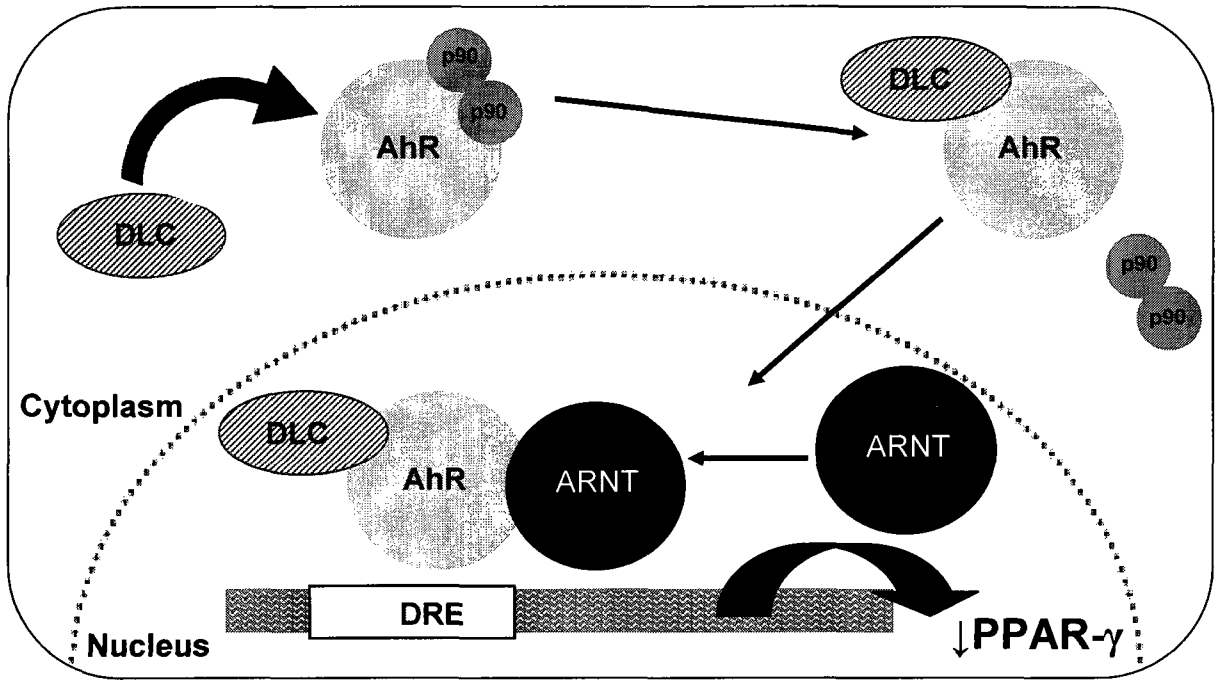

Figure 1.4 Simplified model for activation of the aryl hydrocarbon receptor pathway. A dioxin-like compound (DLC) is capable of ligand binding to the aryl hydrocarbon receptor (AhR) in the cell cytoplasm. Ligand-binding dissociates the AhR from heat shock proteins (p90) and it can subsequently enter the nucleus. The AhR heterodimerizes with AhR nuclear translocator (ARNT) inside the nucleus, forming a complex capable of binding to the dioxin response element (DRE) on certain genes. This causes the modulation of the protein the gene is responsible for.

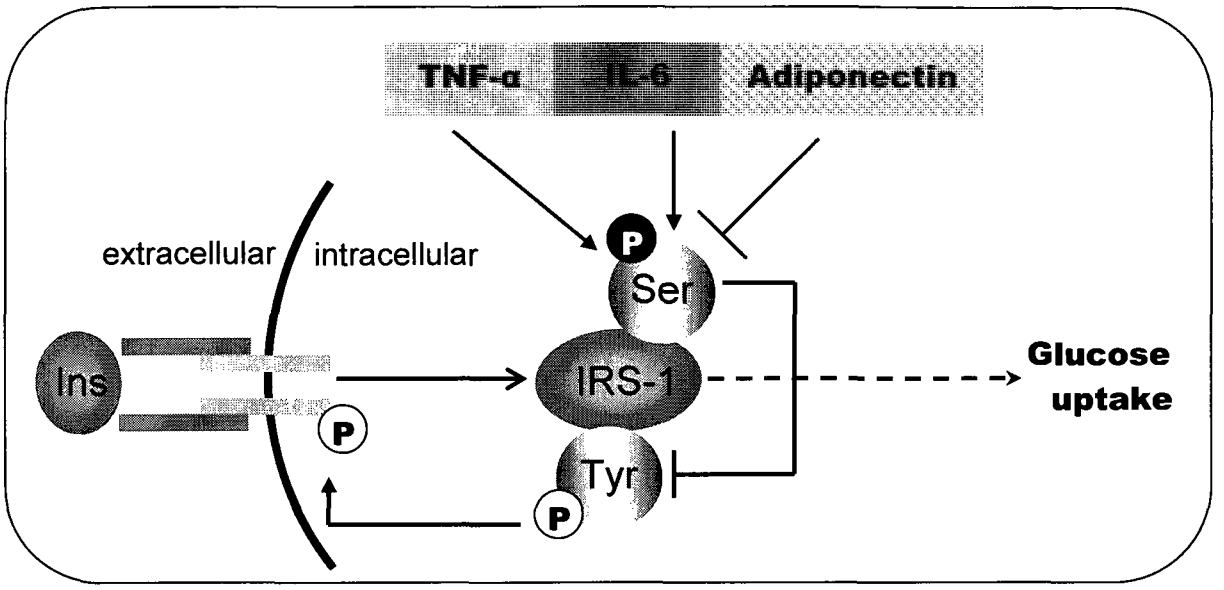

Figure 1.5 The effect of TNF-α, IL-6, and adiponectin on the insulin signal. Insulin binds to the insulin receptor (a tyrosine kinase receptor). This induces phosphorylation of the tyrosine residue of the receptor (indicated by the unfilled "P"). Receptor phosphorylation leads to tyrosine phosphorylation of the tyrosine residue of IRS-1. The downstream effect of this is glucose uptake and other insulin-induced cellular functions. If the serine residue becomes phosphorylated (indicated by the filled "P"), tyrosine (Tyr) phosphorylation is inhibited and the insulin signal cascade is terminated. In the presence of TNF-α and IL-6, serine (Ser) phosphorylation can occur. The presence of adiponectin can prevent serine phosphorylation.

Chapter 2: Methodology

2.1 *Study overview and sites*

This study was done as a collaboration between the University of Ottawa research team, Shibogama First Nations Council, and Nishnawbe Aski Nation. Participants were recruited from two (Wapekeka and Kasabonika) of the five Shibogama communities in northern Ontario. Of the 5 communities of the Shibogama First Nations Council, these two communities were volunteered by their chiefs to take part in the project. Of the 83 participants recruited, 72 ended up participating in the study. Thirty-nine of the 72 were from Wapekeka and 33 were from Kasabonika. Wapekeka First Nation is located 26 km northwest of Big Trout Lake and 451 km northeast of Sioux Lookout (Figure 2.1). Of the approximately 363 band members, 328 live on reserve. Kasabonika First Nation is about 30 km south of Wapekeka (Table 2.1). Of the approximately 856 band members, 791 live on reserve. Both communities are accessible year-round by air. During the winter months, they are accessible by winter roads constructed over the snow and ice. The main languages spoken are Oji-Cree and English.

2.2 *Participant recruitment*

The bulk of participant recruitment was conducted by Michael Robidoux, in September 2007. Recruitment procedures were defined by local band councils and health officials. Community members were recruited and asked to meet with the community

research coordinator and researcher for an interview. Through these interviews, Robidoux determined the degree of wild food consumption of each study participant. The interviews helped determine what wild foods and parts of the animal are typically consumed in each community, what time of year they are available, and how they are generally prepared. The purpose of the interviews was to distinguish two groups of people within the participants. The 72 subjects that consented to participate in the study were divided into two groups consisting of "high-wild-food" (HWF) consumers and "low-wild-food" (LWF) consumers. The HWF consumers (n=41) were defined as participants who eat at least two meals of wild food per week. There were 18 HWF consumers in Kasabonika and 23 in Wapekeka. The LWF consumers (n=31) were defined as participants who eat less than one wild food meal per month. There were 15 LWF consumers in Kasabonika and 16 in Wapekeka. An effort was made to include participants at the ends of the wild food consumption range, avoiding individuals who eat a All participants were given a code to ensure anonymity through the course of the study (ex. "K-HW-00").

The study was approved by the University of Ottawa and Health Canada Research Ethic Boards. The inclusion criteria were that participants must be (1) aboriginal; (2) over 18 years of age; (3) not pregnant; and (4) not type I diabetic. Participants all gave their written informed consent to participate in the study.

2.3 *Anthropometric Measurements*

From mid September to early November of 2007 four members of our research team lived in the two communities (2 in each). We spent this time conducting clinical sessions (detailed below) and working with our community coordinators to ensure that participants arrived for their scheduled appointments as intended.

Clinical sessions including anthropometric measurements and Oral Glucose Tolerance Tests (OGTTs) were carried out in both communities. Body weight was determined with a standard beam scale after removing the participant's shoes. Height and waist circumference were measured with a tape. Height was measured with the participant's bare feet together, with heels, buttocks, back, and head against the wall, and following a normal inspiration. Waist circumference was measured directly on the skin (in duplicate then averaged) at the mid-point between the last floating rib on the top of the iliac crest following standard World Health Organization (WHO) procedure. Body mass index (BMI) was calculated using the height and weight of each participant.

2.4 *Insulin and Glucose Measurements*

Participants were asked to fast for 9 hours preceding the clinical session. All sessions were scheduled for 9:00 to 9:30 in the morning and a questionnaire was administered upon arrival to ensure that participants arrived fasted. Participants were also asked to refrain from any vigorous exercise 48 hours prior to the clinical session. They were also requested to not smoke or drink anything but water before the clinical

session. Amount and quality of sleep were noted for each volunteer as well as whether or not he/she had been physically active before the session. Any medication taken in the last twelve hours preceding the session was noted. The main part of the session consisted of a 2-hour OGTT performed using a 75 g solution of glucose. Each glucose drink was prepared from 75 g of glucose, 100 g of water, and 17 g of lemon juice (for taste) before departing to the communities. With the assistance of community nurses, a baseline blood sample was taken before administration of the glucose drink and was used to establish fasting glucose and insulin levels. Post-ingestion, blood samples were taken at 15, 30, 60, and 120 minutes to determine glucose and insulin concentrations. Blood samples were taken using 6 mL evacuated, sterile blood collection EDTA tubes (BD Vacutainer). Samples were immediately centrifuged at 3500 r/min and plasma was temporarily stored at -20°C in the clinic, before being shipped to the laboratory at Monfort Hospital in Ottawa where they were stored at -80°C.

Plasma glucose concentrations were assayed using spectrophotometric analysis after conversion of glucose to glucose 6-phosphate by hexokinase (Richterich and Dauwwalder 1971). Laboratory-grade reagents (Sigma-Aldrich Canada Ltd., Oakville, Ont; Fisher Scientific Ltd., Nepean Ont.) were used for preparing a standard hexokinase reaction, and after incubating prepared samples at room temperature for 30 minutes, spectrophotometric analysis of resultant NADH light absorbance was performed in duplicate using a Synergy HT Series Multi-Detection Reader (Bio-Tek Instruments Inc., Highland Park, Winoosi, Vt.), with absorbance readings of 340 nm wavelength emissions. All samples collected from each individual were analysed on the same plate, in duplicate. The intra assay coefficient of variation for glucose analyses was 3.4%.

Based on WHO standards, participants with a resting plasma glucose level greater than 7.0 mmol/L and/or a post-prandial level (after 2 hours) greater that 11.0 mmol/L were established as type 2 diabetic (n=26). The rest of the participants were considered non-diabetic (n=46). A 2-site ELISA immunoassay using 2 monoclonal antibodies (LINCO Research, St-Louis, Mo.) was used to measure plasma insulin levels with intra assay coefficient of variation of 3.5%.

Using the glucose and insulin results of the OGTTs, insulin resistance was quantified using Homeostasis Model Assessment (HOMA-IR) and Area Under the Curve (AUC) calculations for glucose and insulin. Insulin secretion was quantified using the HOMA-β (for beta cell function). The HOMA-IR values were established by dividing the product of the fasting plasma glucose (FPG; mmol/L) and insulin (FPI; mIU/mL) values by 22.5.

$$\text{HOMA-IR} = \text{FPG}*\text{FPI}/22.5$$

The HOMA-β values were determined by multiplying the FPI (ρmol/L) by 3.33 and dividing this product by the difference between the FPG (mmol/L) and 3.5.

$$\text{HOMA-}\beta = \text{FPI}*3.33/\text{FPG}-3.5$$

The $AUC_{glucose}$ and $AUC_{insulin}$ were calculated using the trapezoid method for the total AUCs (Potteiger *et al.* 2002).

2.5 *Inflammatory marker analysis*

Measurements of all inflammatory markers were done using the plasma samples collected in the fasted state from participants. Plasma Tumour Necrosis Factor (TNF)-α levels were determined using ELISA kits (average intra assay variability: 8.5%, sensitivity: 0.106 pg/mL, R&D Systems Inc., Minneapolis MN.). Plasma Interleukin-6 levels were determined with ELISA kit (average intra assay variability: 8.0%, sensitivity: 0.70 pg/mL, R&D Systems Inc., Minneapolis MN). Adiponectin was also measured in plasma by ELISA (average intra assay variability: 2.0%, sensitivity: 0.246 ng/mL, R&D Systems Inc., Minneapolis MN).

2.6 *Contaminant analysis*

All contaminant measurement was done by the Toxicology Centre at the National Institute of Public Health of Quebec. The measured contaminants include Aroclor 1260, PCB28, PCB52, PCB99, PCB101, PCB105, PCB118, PCB128, PCB138, PCB153, PCB156, PCB163, PCB170, PCB180, PCB183, PCB187, aldrin, α-chlordane, γ-chlordane, β-HCH, *cis*-nonachlor, *trans*-nonachlor, DDE, DDT, hexachlorobenzene, mirex, oxychlordane, PBB153, PBDE47, PBDE99, PBDE100, PBDE153, Parlar26, and Parlar50. Contaminants were measured using an E-446 GC-MS (gas chromatography-mass spectrometry), Chromatograph 6890 (Agilent) using a solid phase extraction followed by gas chromatography coupled to mass detection (Agilent 5973 network). The plasma samples were enriched with internal standards and denatured with formic acid.

The compounds were extracted from the aqueous matrix using solid phase separation and extracts were cleaned using florisil columns prior to analysis. Plasma samples were eluted from columns using methylene chloride-hexane (25:75 vol/vol) and analyzed on gas chromatograph equipped with dual capillary columns. Peaks were identified by relative retention times obtained on the two columns using a computer program developed by the Quebec Toxicology Centre. Generated ions were measured after negative chemical ionization. The concentration of each analyte measured was determined using percent recovery of labelled internal standards. The ECD (electron capture detector; Agilent G2397A) served to verify the detection limits for PCB congeners 28 and 52. To verify results, an interlaboratory comparison was made with the External Quality Assessment Scheme (G-EQUAS), Germany.

A particular POP was not considered during analysis if more than 40% of individuals had values below the instrument detection limit (DL) or zero values (Lee *et al.* 2007). These included: aldrin, α-chlordane, γ-chlordane, DDT, Parlar26, Parlar50, PBB153, PBDE100, PBDE153, PBDE99, PCBs 28, 52, 101, and 128 (Table 2.2). Random numbers were generated between 0 and the DL of each POP included in analysis using Microsoft Excel for individuals whose plasma levels of a particular POP was below the DL (Miller and Amrhein 1995). Between 1 and 28 random numbers were generated for 14 of the 20 POPs considered for analysis. The other 6 POPs had a 100% detection frequency for participants (Table 2.2). The replacement of non-detects with random numbers did not change the significance of statistical tests used during analysis.

2.7 *Statistical Analyses*

All statistical analyses were conducted using JMP version 5.1.2. An analysis of covariance (ANCOVA) was used to compare contaminant levels between diabetic and non-diabetic individuals, using age as a covariate. Variables that did not fit a normal distribution, as identified by the Shapiro-Wilk test, were normalized using the logarithmic function. Forward stepwise regression was used to determine significant contributors to insulin resistance. A Principal Component Analysis (PCA) based on a correlation matrix was used to cluster related variables together in order to reduce the large number of contaminants into component scores. Pearson's correlations were used to determine whether any significant relationships existed between inflammatory markers and POPs. For all analyses, results with a P-value of less than 0.05 were considered statistically significant.

Table 2.1 Community location and participant diet grouping according to wild food consumption.

Community	Latitude (N)	Longitude (W)	Reserve size (hectares)	Population	HWF[a] eaters	LWF[b] eaters
Wapekeka	53.8°	89.5°	5 566	363	23	16
Kasabonika	53.5°	88.6°	10 806	856	18	15

[a]HWF: High Wild Food
[b]LWF: Low Wild Food

Table 2.2 Detection limits and detection frequencies of measured POPs for all participants (N=72).

POP	Detection limit (μg/L)	Detection frequency (%)[a]
Aldrin	0.024	0
β-HCH	0.018	74
α-chlordane	0.0050	0
γ-chlordane	0.0030	3
cis-nonachlor	0.0050	82
trans-nonachlor	0.0080	96
DDT	0.035	1
DDE	0.294	100
HCB	0.018	68
Mirex	0.025	89
Oxychlordane	0.007	100
Parlar26	0.0060	39
Parlar50	0.0040	44
PBB153	0.030	17
PBDE47	0.095	74
PBDE99	0.042	10
PBDE100	0.052	3
PBDE153	0.030	22
PCB28	0.074	7
PCB52	0.928	0
PCB99	0.089	71
PCB101	0.024	0
PCB105	0.0050	61
PCB118	0.012	97
PCB128	0.0050	21
PCB138	0.016	100
PCB153	0.011	100
PCB156	0.0050	86
PCB163	0.0090	89
PCB170	0.0060	97
PCB180	0.0070	100
PCB183	0.0050	83
PCB187	0.0040	99
Aroclor1260	0.127	100

[a] The POPs with a detection frequency of less than 60% were not used for analysis

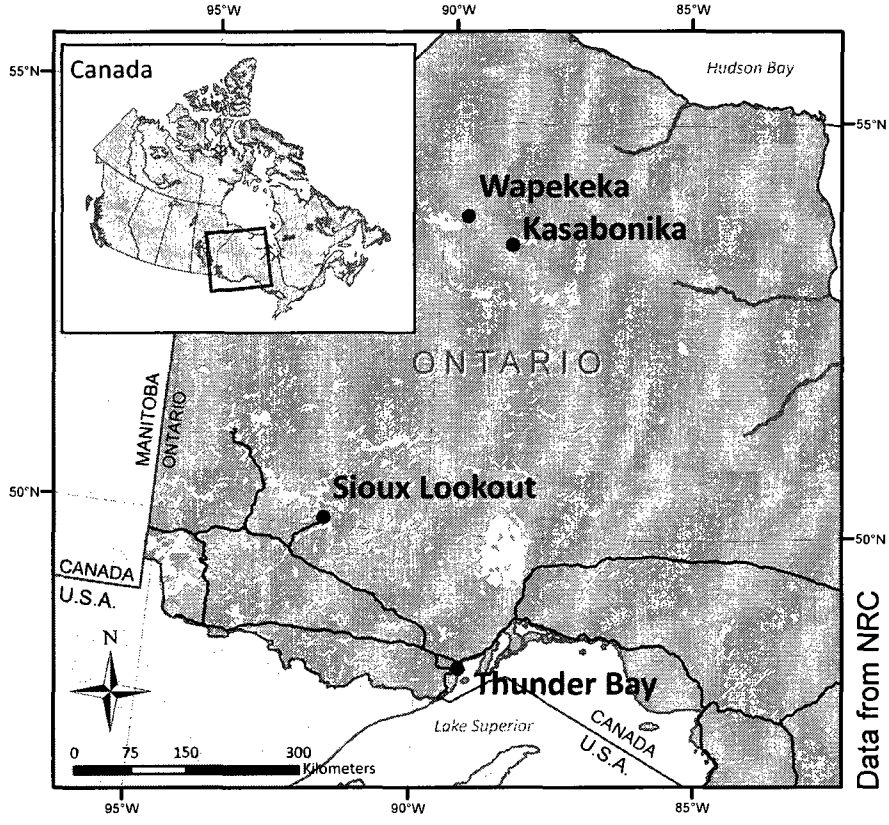

Figure 2.1 Map identifying the location of Wapekeka and Kasabonika First Nations

Chapter 3: Results

3.1 *Participant characteristics*

The participant characteristics are summarized in Table 3.1 based on diabetic status. The mean (± SE) age of diabetic individuals (50 ± 2.7) was significantly higher than non-diabetics (39 ± 1.6; $p < 0.001$). Of our non-diabetic participants, 46% were male and 38% of our diabetic participants were male. The mean (± SE) non-diabetic BMI (32 ± 0.7 kg/m^2) was the same as the diabetic BMI (32 ± 0.9 kg/m^2), and waist circumference also did not differ significantly among the two groups. The proportion of non-diabetic smokers (85%) was similar to diabetic smokers (73%). Diet was quantified using the wild food index and was not found to differ significantly with diabetic status. As a proxy of the degree of insulin resistance, the Homeostasis Model Assessment for insulin resistance (HOMA-IR) was calculated and found to be significantly higher ($p < 0.05$) for diabetic individuals. As a proxy for insulin secretion, the HOMA for beta cell function (HOMA-β) was calculated and found to be significantly higher ($p<0.0001$) for non-diabetic individuals.

The mean fasting glucose concentration was significantly higher for diabetics (9.0 ± 0.6 mmol/L) than non-diabetics (5.6 ± 0.1 mmol/L) ($p < 0.05$; Figure 3.1A). The mean glucose response following an OGTT increased in both groups but was greater in diabetic than non-diabetic individuals (group x time interaction, $p < 0.05$). This observation is reflected by a greater $AUC_{glucose}$ response for diabetics (1.0 ± 0.04 mol·min/L) as compared to non-diabetics (1.9 ± 0.10 mol·min/L) ($p < 0.05$) (Figure 3.1A).

The mean fasting plasma insulin levels did not differ between diabetic (91.6 ± 13.6 pmol/L) and non-diabetic (85.8 ± 7.4 pmol/L) participants (Figure 3.1B). Following the OGTT, insulin levels increased in both groups but to a greater extent in the non-diabetic individuals (group x time interaction, $p < 0.05$). Diabetic individuals therefore showed a statistically lower mean $AUC_{insulin}$ (33.8 ± 4.7 nmol·min/L) when compared to non-diabetics (68.4 ± 5.9 nmol·min/L; $p < 0.05$).

3.2 *Diabetic individuals have higher plasma POP levels*

Mean lipid-adjusted plasma concentrations of POPs and the mean hair Hg concentration were compiled according to diabetic status (Table 3.2). The PCBs are presented as mono- and di-ortho substituted PCBs (congeners 105, 118, 128, 138, 156, 170, and 180), other ortho-substituted PCBs (congeners 28, 99, 153, 163, 183, and 187), ΣPCBs (all 13 congeners), and Aroclor 1260. The plasma concentrations of PBDE-47, OC pesticides *cis*-nonachlor, *trans*-nonachlor, DDE, β-HCH, HCB, mirex, oxychlordane, and mercury are all presented individually. All categories of plasma PCB concentrations were higher in diabetics ($p<0.001$). With the exception of β-HCH, all the plasma OC pesticides were significantly higher in diabetics ($p<0.05$). The PBDE-47 levels were uncorrelated with diabetic status. Mercury concentrations were significantly higher ($p<0.05$) in diabetics.

Except for PBDE-47, a strong correlation was shown between all POP concentrations and participant age ($0.15 \leq R^2 \leq 0.65$; $p<0.05$). Therefore, in order to properly assess whether diabetic individuals have higher POP plasma levels, we adjusted

for the effect of age. Plasma levels of *trans*-nonachlor, oxychlordane, and DDE were significantly higher ($p<0.05$) in diabetics after age-adjustment (Figure 3.2). Age-adjusted *cis*-nonachlor was higher in diabetics (at marginal significance, $p=0.07$), as were the age-adjusted mercury levels ($p=0.07$).

When only HW food consumers were considered ($n=42$), mean diabetic plasma levels of *trans*-nonachlor, oxychlordane, and DDE were still significantly higher in diabetic participants, after age-adjustment. In this analysis, both Aroclor1260 and ΣPCBs were higher in diabetics at marginal significance ($p=0.09$).

3.3 *Inflammatory markers were unaffected by diabetic status*

None of the inflammatory markers measured were significantly different between diabetic and non-diabetic individuals (Figure 3.3). The mean TNF-α concentrations for diabetics and non-diabetics respectively were 2.8 ± 0.5 pg/mL and 2.1 ± 0.4 pg/mL. In diabetics, the mean IL-6 concentration was 3.3 ± 0.4 pg/mL and was 4.2 ± 1.1 pg/mL in non-diabetics. The total adiponectin mean concentration was 7.8 ± 1.0 μg/mL for diabetics and 5.8 ± 0.4 μg/mL for non-diabetics. For diabetics and non-diabetics respectively, the mean HMW adiponectin concentrations were 3.3 ± 0.6 μg/mL and 2.4 ± 0.2 μg/mL. The ratio of HMW to total adiponectin concentration was a mean of 0.39 ± 0.02 for diabetics and 0.39 ± 0.02 for non-diabetics.

3.4 *The BMI was the most significant predictor of insulin resistance for non-diabetics*

A forward stepwise regression analysis (r^2=0.39) was used to identify variables that correlate with insulin resistance for non-diabetics (n=46; Table 3.3). Variables included well-known risk factors associated with type 2 diabetes including age, BMI, waist circumference, smoking status, and diet (high or low-wild food group). Smoking status was a dichotomous variable with current and past smokers classified as smokers. The inflammatory markers TNF-α, IL-6, and adiponectin were included as well. The pollutants ΣPCBs, PBDE-47, Aroclor 1260, *cis*-nonachlor, *trans*-nonachlor, DDE, β-β-HCH, HCB, mirex, oxychlordane and Hg were also entered into this analysis. Except for Hg, all contaminants were lipid-adjusted. Due to the change in insulin response known to occur in diabetics, only data from non-diabetic individuals (n=46) were used. The stepwise regression shows BMI as the most significant predictor ($p<0.05$) of insulin resistance, when quantified using $AUC_{insulin}$ and explains 17.2% of the variation in $AUC_{insulin}$. This is followed by PBDE-47 and smoking status as other significant predictors of insulin resistance. The PBDE-47 congener accounts for 14.8% and smoking accounts for 6.8% of the variation. The parameter estimates indicate that BMI is positively associated with $AUC_{insulin}$, although PBDE-47 and smoking are not.

A Pearson's correlation analysis was also performed to determine any significant correlations between POPs and insulin resistance using the HOMA-IR index and included all study participants (N=72). None of the POPs showed significant correlation to insulin resistance using the HOMA-IR index ($-0.25 \leq r \geq 0.13$). A correlation analysis was also performed to determine significant associations between POPs and insulin

secretion using the HOMA-β ($-0.07 \leq r \geq 0.20$). None of the analyzed POPs or mercury were significantly negatively correlated ($p<0.05$) to insulin secretion.

3.5 *Adiponectin and TNF-α are positively associated with some PCBs and OC pesticides*

In order to determine whether environmental pollutants are associated with proinflammatory effects, a PCA was performed to determine whether contaminant patterns in the population were related. The variables included in the PCA were those with a detection frequency of more than 60% and included HCB, Mirex, oxychlordane, DDE, HCH, *trans*-nonachlor, *cis*-nonachlor, Aroclor1260, ΣPCBs, Hg, and PBDE-47 and the factor loadings were determined for each variable (Table 3.4). The PCA showed that the first component explains most of the total variance (69.1%) and all contaminants loaded positively onto the first component (Figure 3.4). The first component axis scores were therefore used as a proxy for the degree of contamination in the study populations. The first component axis scores were correlated to inflammatory markers in diabetic and non-diabetic individuals (N=72; Table 3.5). Adiponectin was positively correlated ($p<0.05$) with the first component axis scores, but no correlations were observed for either TNF-α and IL-6 (Table 3.5).

Table 3.1 Participant characteristics based on diabetic status in Kasabonika and Wapekeka First Nations, Ontario, Canada (N=72).

	Non-diabetic (n=46)		Diabetic (n=26)	
Age	39 ± 11	(23 – 68)	50 ± 14	(26 – 73)**
Sex (F:M)	10:16	–	21:25	–
Weight (kg)	91 ± 17	(60 – 150)	87 ± 16	(57 – 125)
BMI[a] (kg/m^2)	32 ± 4.9	(21 – 43)	32 ± 4.8	(22 – 40)
Waist Circumference (cm)	114 ± 13	(88 – 153)	112 ± 14	(82 – 140)
Smoking (% smokers)	85	–	73	–
Diet (Wild food index)	47 ± 28	(5 – 85)	59 ± 29	(5 – 95)
HOMA-IR[b]	3.1 ± 1.7	(0.9 – 8.1)	5.4 ± 4.8	(1.1 – 20)*
HOMA-β[c]	182.3 ± 301.5	(53.1 – 2103.2)	71.4 ± 49.0	(7.6 – 189.0)**

Data are presented as means ± SD with ranges indicated in parentheses, except for smokers (% of current and past smokers)
[a]Body Mass Index
[b]Homeostasis Model Assessment of insulin resistance
[c]Homeostasis Model Assessment of insulin secretion
significant difference between groups at $p<0.05$ (*) and $p<0.001$ (**)

Table 3.2 Lipid-adjusted plasma concentrations of POPs (μg/kg) and mercury (ppb) based on diabetic status in Kasabonika and Wapekeka First Nations, Ontario.(N=72).

	Non-diabetic (n=46)		Diabetic (n=26)	
Mercury	1390.3 ± 980.1	(131.6 – 4148.1)	2809.3 ± 2096.3	(347.6 – 8629.7)*
ΣPCBs[a]	373.7 ± 507.0	(21.2 – 2618)	1080 ± 1030	(23.7 – 4061)**
Aroclor 1260	811.5 ± 1093	(52.7 – 5890.4)	2345 ± 2174	(69.9 – 8965.5)**
Mono/di-ortho PCBs	201.1 ± 272.1	(10.7 – 1376.2)	577.5 ± 562.3	(14.7 – 2172.1)**
Other PCBs	173.7 ± 236.0	(8.9 – 1243)	502.1 ± 470.0	(9.0 – 1888.5)**
PBDE-47	6.8 ± 4.2	(0.2 – 17.9)	6.6 ± 3.9	(1.0 – 16.9)
OC pesticides				
DDE	200.4 ± 217.4	(15.9 – 1228.6)	594.0 ± 539.0	(68.6 – 2069.0)**
cis-nonachlor	2.7 ± 3.4	(0.2 – 14.1)	9.6 ± 11.4	(0.2 – 52.9)**
trans-nonachlor	11.0 ± 12.4	(0.3 – 60.2)	34.9 ± 38.0	(3.2 – 172.4)**
β-HCH	2.3 ± 3.8	(0.01 – 26.2)	2.8 ± 1.6	(0.03 – 6.8)
HCB	7.6 ± 8.6	(0.03 – 38.4)	21.8 ± 20.2	(0.09 – 70.8)*
Mirex	23.8 ± 42.8	(0.1 – 219.2)	71.9 ± 83.4	(0.3 – 298.9)*
Oxychlordane	6.4 ± 5.9	(0.7 – 24.7)	17.4 ± 16.6	(2.0 – 69.0)**

Data are presented as the mean ± SD and ranges are indicated in parentheses. A minimum value of 0 indicates a concentration below the detection limit.
[a]ΣPCBs includes congeners PCB99, PCB105, PCB118, PCB128, PCB138, PCB153, PCB156, PCB163, PCB170, PCB180, PCB183, and PCB187.
* $p<0.05$
** $p<0.001$

Table 3.3 Forward stepwise regression on predictors of insulin resistance for non-diabetic participants (n=46). The model includes the independent variables age, BMI, waist circumference, smoking status, diet, TNF-α, IL-6, adiponectin, ΣPCBs, PBDE-47, Aroclor 1260, *cis*-nonachlor, *trans*-nonachlor, DDE, β-HCH, HCB, mirex, oxychlordane, and Hg. POP values are lipid-adjusted. Only significant variables ($p<0.05$) are presented. Total r^2 value = 0.39.

Dependent variable	**Independent variable**	**(r^2 x 100)**	**F-ratio**	***P*-value**	**Parameter estimate**
$AUC_{insulin}$	BMI	17.2	14.04	0.004	0.05 ± 0.01
	PBDE-47	14.8	10.22	0.004	-52.14 ± 16.31
	Smoking status	6.8	4.63	0.04	0.20 ± 0.09

Table 3.4 Factor loadings for the Principal Component Analysis used to establish a contaminant gradient based on component 1 scores (N=72). The eigenvectors of the first 3 components of the PCA are displayed.

	Component 1	Component 2	Component 3
Variance (%)	69.1	10.7	8.4
Mercury	0.225	-0.487	0.219
ΣPCBs	0.351	-0.056	-0.001
Aroclor1260	0.353	-0.039	0.003
PBDE-47	0.070	0.577	0.774
DDE	0.347	0.062	-0.106
cis-nonachlor	0.343	-0.095	0.027
trans-nonachlor	0.353	0.034	-0.024
β-HCH	0.129	0.628	-0.550
HCB	0.303	0.075	0.165
Mirex	0.320	-0.081	-0.090
Oxychlordane	0.352	0.070	-0.052

Table 3.5 Pearson's correlations between inflammatory markers and the contaminant gradient developed from the first scores of the PCA of contaminants[a] (N=72).

	TNF-α	IL-6	Adiponectin
Contaminant gradient	**0.19**	**0.09**	**0.24***

[a] POPs included in the PCA were HCB, Mirex, oxychlordane, DDE, HCH, *trans*-nonachlor, *cis*-nonachlor, Aroclor1260, ΣPCBs, Hg, and PBDE-47

* P <0.05

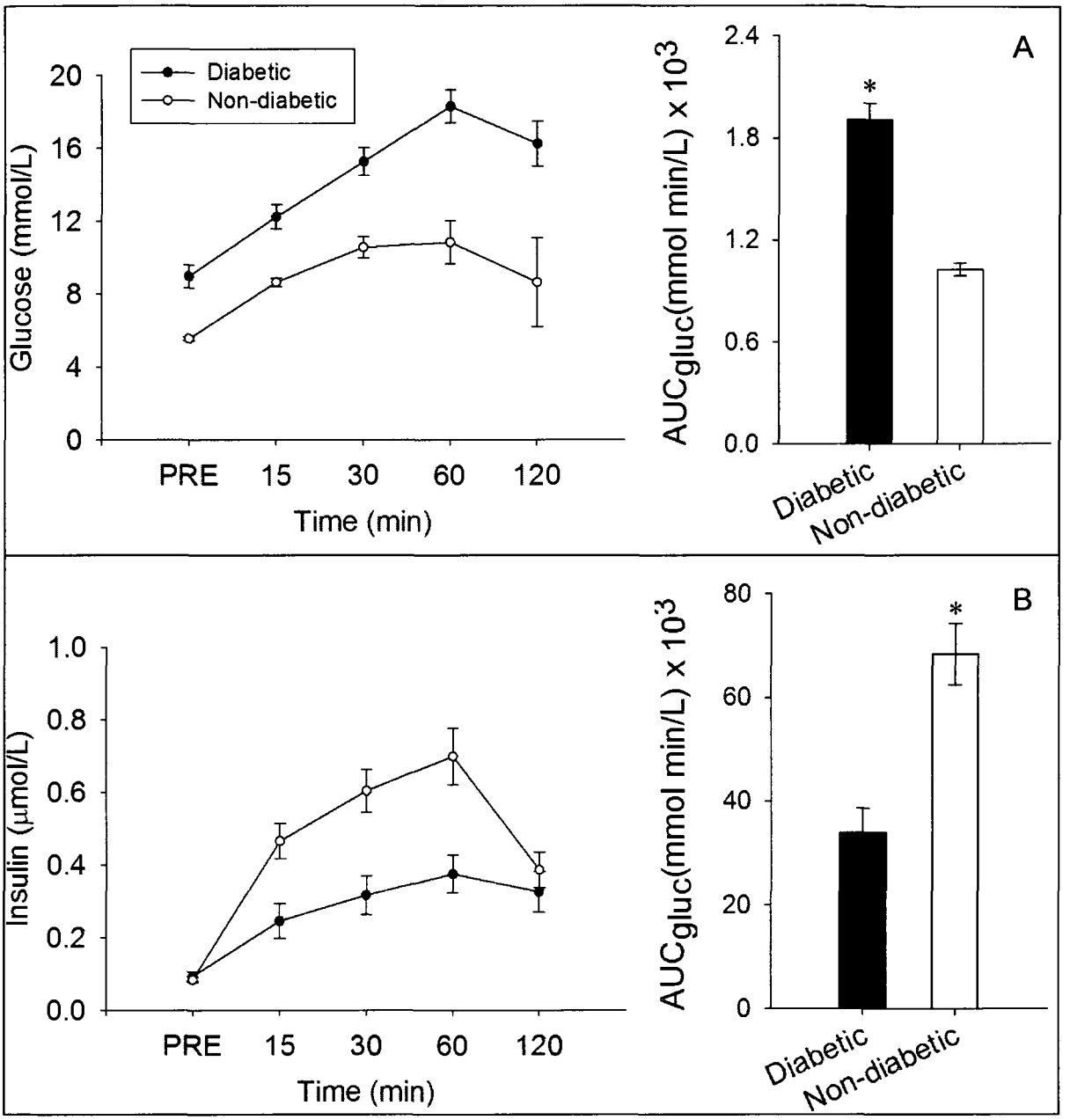

Figure 3.1 Plasma glucose and insulin levels before and after an OGTT in diabetic (n=26) and non-diabetic (n=46) individuals. Data are presented as mean ± SE. Significant group x time interaction ($p<0.05$) for glucose and insulin. See section 3.1 for specific effects. An asterisk (*) indicates a significantly higher ($p<0.05$) $AUC_{insulin}$.

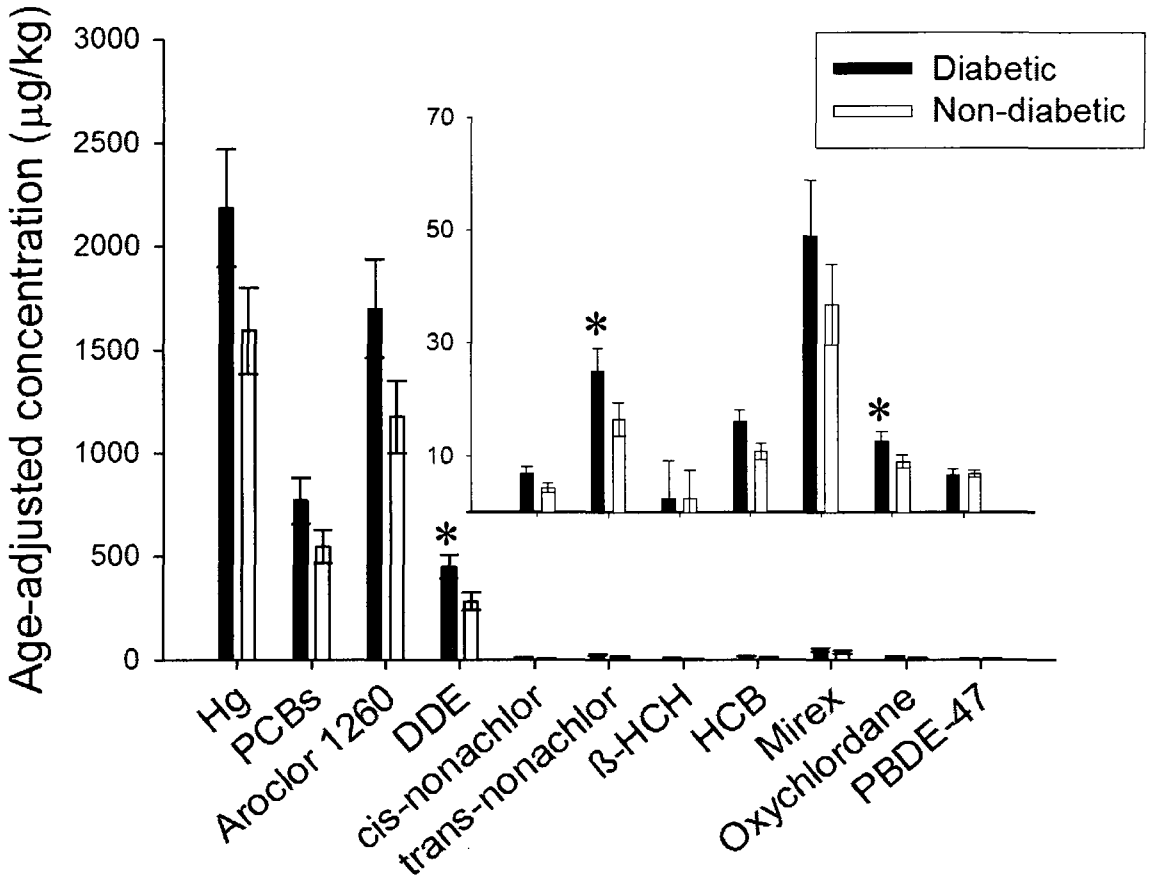

Figure 3.2 Age-adjusted plasma concentrations of POPs and mercury in diabetic (n=26) and non-diabetic (n=46) individuals. Data are presented as least square means ± SE with the exception of PBDE-47 (presented as µg/kg ± SE). Asterisks indicate significantly higher (* $p<0.05$) POP concentrations in diabetic individuals.

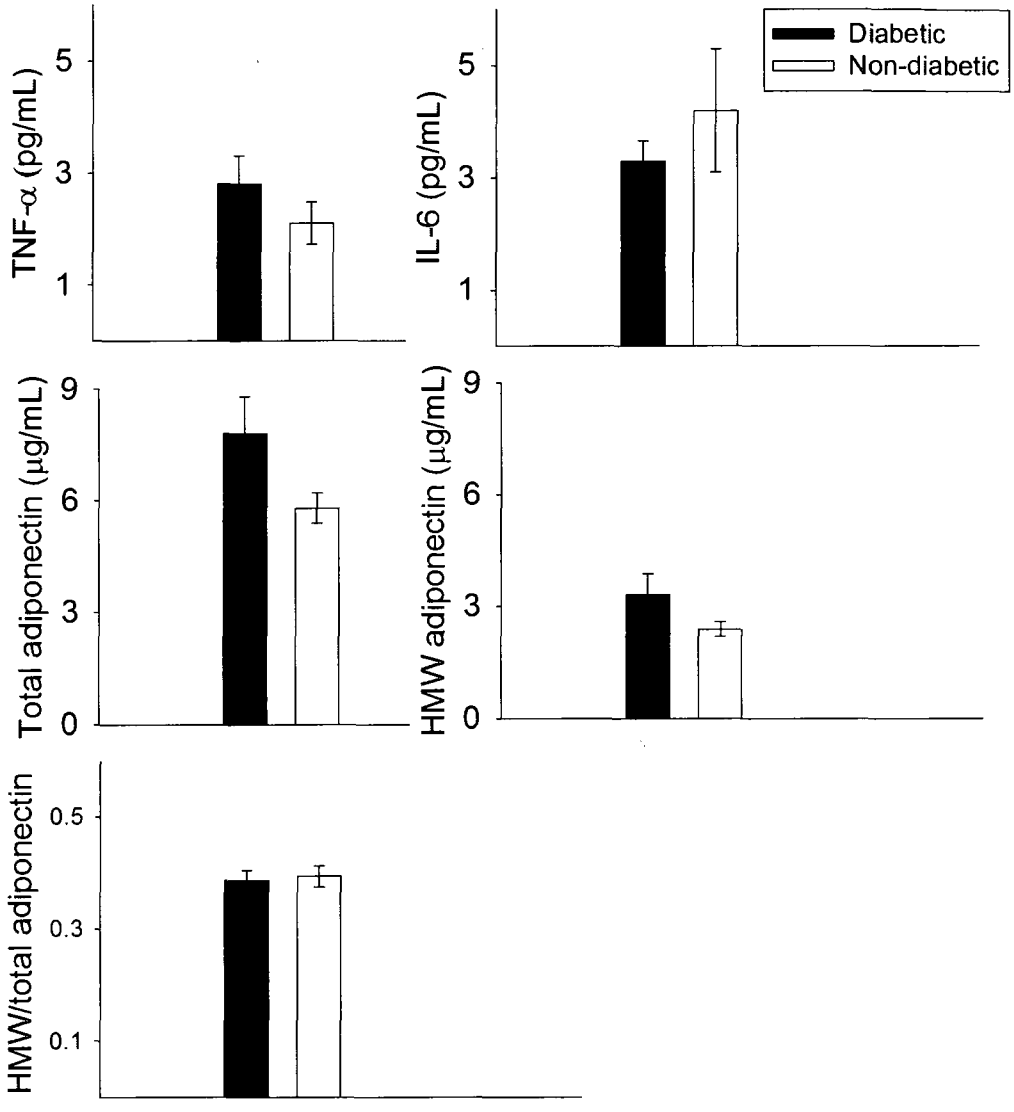

Figure 3.3 Inflammatory marker levels in diabetic (n=26) and non-diabetic (n=46) individuals. Data are presented as means ± SE.

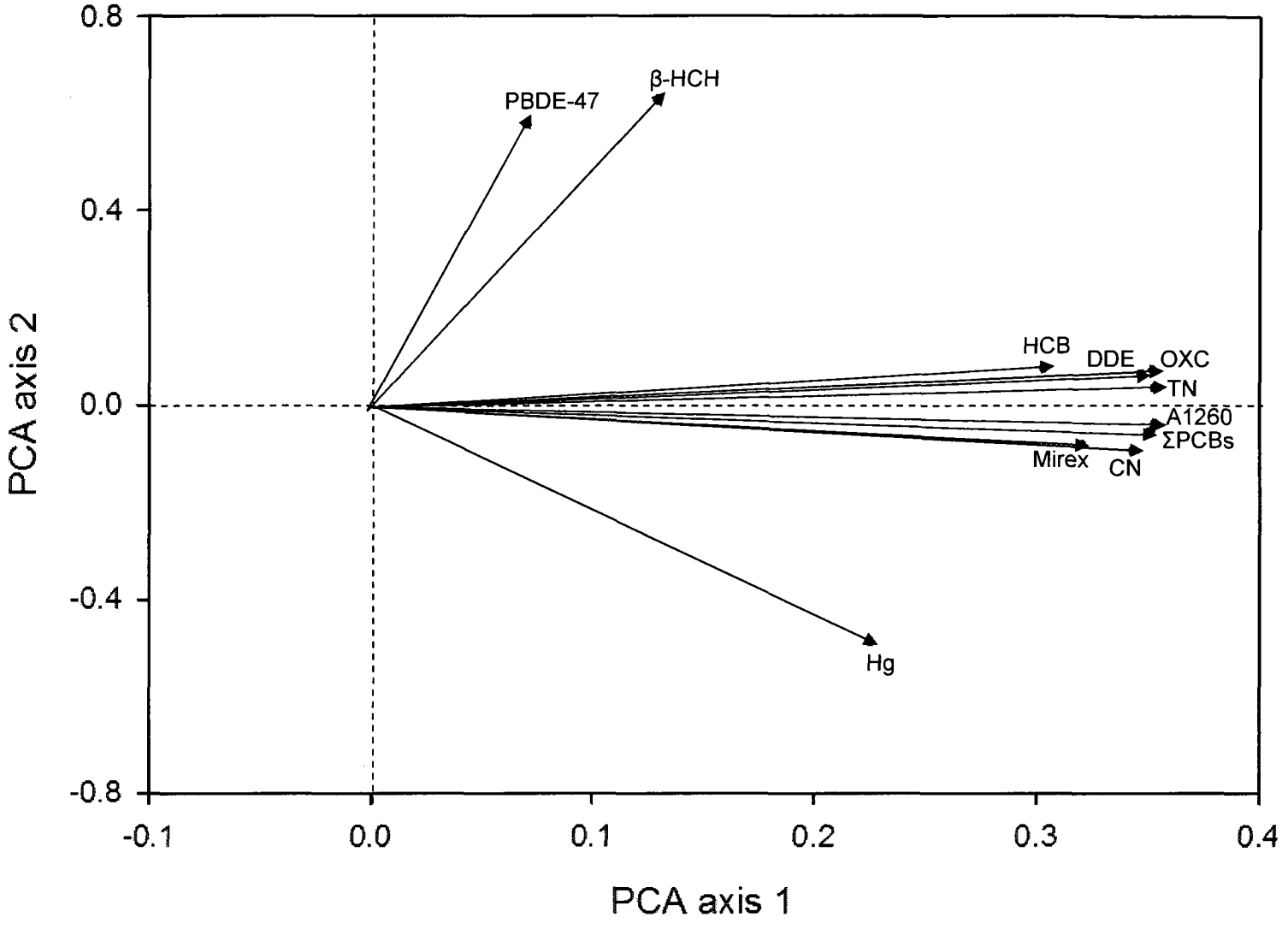

Figure 3.4 Principal component analysis including 11 POP variables measured in participants. Axis 1 scores were used as a proxy for contaminant influence. Axes 1 and 2 explain 69% and 10% of the total variation, respectively. OXC, oxychlordane; TN, *trans*-nonachlor; A1260, Aroclor1260; CN, *cis*-nonachlor.

Chapter 4: Discussion

4.1 *Some contaminants are more concentrated in diabetics than non-diabetics*

Our study was conducted in northern Ontario where there is potential for a higher exposure level to certain POPs for individuals who rely on foods derived from the land (Van Oostdam *et al.* 1999). The high-wild food consumers in our community have a mean Aroclor1260 level of 12.5 μg/L in plasma which is double the mean level seen in Great Lakes fish eaters from Cornwall and Mississauga, Ontario (Kearney *et al.* 1999), areas where ecosystem and biota contamination are of particular concern (Tremblay and Gilman 1995). The mean mirex plasma level of our population (0.38 μg/L) is more than 3 times higher than Great Lakes fish consumers. On the other hand, the mean DDE plasma levels of Great Lakes fish consumers are double those seen in our high-wild food consumers (3.1 μg/L). Fish consumers of the Great Lakes basin are generally known to have higher than average exposure levels to PCBs and certain OC pesticides such as DDT (Tremblay and Gilman 1995). The higher Aroclor1260 and mirex values in our high-wild food consumers are likely due to the higher frequency of wild food consumption compared to fish eaters in the Great Lakes area. Participants in our study were categorized as high-wild food consumers if they were eating more than 2 meals of wild food per week. In the Great Lakes area, individuals included in the study consumed fish of an amount roughly equivalent to 3 meals per month.

Our results indicate that age and lipid-adjusted plasma concentrations of some OC pesticides (i.e. oxychlordane, DDE, and *trans*-nonachlor) were higher in diabetics than

non-diabetics. These results corroborate epidemiological studies that have found higher concentrations of several pesticides in diabetic individuals. Indeed, Lee *et al.* (2006) found that diabetics had higher concentrations of oxychlordane, DDE, and *trans*-nonachlor in civilian, non-institutionalized participants from the USA, a remarkable similarity with the results described here. A similar study was conducted within the Mohawk Nation at Akwesasne (Codru *et al.* 2007), a Native American population that resides along the St. Lawrence River. Codru *et al.* 2007 measured the OC pesticides mirex, DDE, and HCB in plasma and found a significantly higher incidence of diabetes at the highest tertile of exposure to DDE and HCB. HCB was unrelated to diabetes in our study, though our mean HCB concentrations were virtually identical to those of the Akwesasne population.

The ΣPCB plasma concentrations were not significantly different between diabetics and non-diabetics in our group, after considering the effect of age. Codru *et al.* (2007) found significantly higher ΣPCB concentrations in relation to diabetes in the Akwesasne population. The mean ΣPCB concentrations (748.8 ± 635.6 ng/g) in Akwesasne resemble those in our study communities (628.7 ± 95.24 ng/g). In Belgium, people living in areas with a higher risk of POP exposure showed a significantly higher prevalence of diabetes in relation to both coplanar and non-coplanar PCBs, measured independently (Fierens *et al.* 2003). Radikova *et al.* (2004) found correlations between PCBs and diabetes, as well as other dysglycemias in Eastern Slovakia. Vasiliu *et al.* (2006) found a positive association of diabetes incidence and serum concentration of PCBs in women from Michigan, USA but not in men. Studies conducted on Swedish women and fishermen also showed an increased risk of diabetes for those with higher

concentrations of PCB-153 and DDE in serum (Rylander *et al.* 2005; Rignell-Hydbom *et al.* 2007). Lee *et al.* (2006) detected higher PCB-153 serum concentrations in diabetics than non-diabetics. This trend was not apparent in our study, although the PCB-153 serum concentrations in our population were much higher than those reported by Lee *et al.* (2006).

The previous studies examined individuals exposed to background ambient POP concentrations in the environment (Lee *et al.* 2006) or those with high occupation or residential exposures (Rignell-Hydbom *et al.* 2007). The effects of background exposure may be different from higher POP exposure thus making results difficult to compare between different studies. It also may partially explain why our results do not show a higher incidence of diabetes in individuals with high total plasma PCB levels, as seen in other studies (Table 4.1). Each study that has correlated diabetes to POPs concentrations has adjusted for different variables that influence diabetes including BMI, age, smoking status, and sex. In our study, age was the only variable that was significantly different between diabetics and non-diabetics, and therefore we did not account for other risk factors.

We did not see a difference in PBDE-47 levels between diabetic and non-diabetic individuals. This is corroborated by Turyk *et al.* (2009b) where ΣPBDEs, PBDE-47, and PBDE-153 were not significantly higher in diabetics. For our subjects, age was not significantly correlated with PBDE-47, thus there was no age-adjustment made in this case. The lack of correlation between age and PBDEs was observed by Lim *et al.* (2008) as well. The PBDEs were not in use prior to the 1980s and therefore the older subjects would not have been more exposed to them than younger subjects.

The positive tendency for Hg concentrations to be higher in diabetics has not been previously reported. To our knowledge, this is the first time Hg has been included in a study of diabetes and environmental contaminants. Studies performed *in vitro* indicate Hg stimulation of adipocyte glucose transporters (Ezaki 1989) and also decreased insulin-mediated glucose transport (Barnes and Kircher 2005) Our results do not permit us to determine whether Hg affects the development of diabetes.

4.2 *The BMI was the most significant predictor of insulin resistance*

In non-diabetic participants, BMI, PBDE-47, and smoking status are identified as predictors of insulin resistance, when quantified using the $AUC_{insulin}$ method. The BMI was the most significant predictor of insulin resistance, and is highly corroborated in the literature (Kriska *et al.* 2003). Smoking is also a known risk factor for becoming insulin resistant (Facchini *et al.* 1992). The PBDE-47 contribution was negative, meaning lower levels of PBDE-47 are indicative of a higher degree of insulin resistance. Few studies have looked at the relationship between PBDEs and diabetes. Lim *et al.* (2008) did not find an association between PBDE-47 and diabetes in US residents; however PBDE-153 was non-linearly associated with the prevalence of diabetes in their study group. An inverted U-shape was observed between PBDE-153 concentrations and diabetes. The mean PBDE-47 concentration was higher as reported by Lim *et al.* than our population. The PBDE-153 congener was below the detection limit for 78% of our study subjects and was therefore not included in our analyses.

Lim *et al.* (2008) divided their study group into quintiles based on POP exposure level. The median plasma concentration (6.2 ng/g) of PBDE-47 in our total population is approximately the same as the median concentration for the lowest quintile (6.5 ng/g) as reported by Lim *et al.* The median concentration of their highest exposure quintile (73.3 ng/g) is 4 times higher than the most highly exposed individual in our population (i.e. the exposure range of our participants is much smaller. The observed negative effect of PBDE-47 on insulin resistance could be considered part of a hormetic effect. Hormesis is a biphasic dose-response pattern characterized by a low-dose stimulation and a high-dose inhibition (Calabrese 2008). If higher PBDE-47 concentrations existed in our population, a positive effect on insulin resistance may have been apparent. Future work would have to be done to explore the possibility of hormesis involving a population with a larger range of PBDE plasma concentrations.

The exposure pathway to PBDEs is not the same as it is for PCBs and OC pesticides. There is a body of evidence indicating fish consumption and occupational exposure as primary exposure routes to PCBs and OC pesticides (Fitzgerald *et al.* 2007; Harris and Jones, 2008). Recent evidence indicates that house dust is a primary route of PBDE exposure as well as diet (Wu *et al.* 2007). The toxicity of PBDEs in relation to insulin resistance may also be different. Although PBDEs are structurally similar to PCBs, some studies indicate that they may not exert toxicity through the aryl hydrocarbon pathway, as coplanar PCBs are known to do (section 1.4; Wahl *et al.* 2008).

Jørgensen *et al.* (2008) found no association between PCBs and insulin resistance, using HOMA-IR as an index of insulin resistance, similar to what our results indicate.

They did find an inverse association of HOMA-β with both dioxin-like and non-dioxin-like PCBs which is not corroborated by the results from this study.

4.3 *Lipolysis in diabetics in relation to POP levels*

Although PBDE-47 and smoking status are predictors of insulin resistance in this population, most of the variance (17.2%) in $AUC_{insulin}$ is explained by BMI. If high plasma POP concentrations do not lead to insulin resistance, there should be an alternate explanation for why higher POP concentrations are seen in diabetics. Some have postulated that metabolic changes in diabetic individuals may alter accumulation, metabolism, and excretion of POPs (Fierens *et al.* 2003, Lee *et al.* 2006). In fact, diabetes is already established to alter the pharmacokinetics of drugs (Gwilt *et al.* 1991).

Michalek *et al.* (2003) hypothesized a higher elimination rate in diabetics, but this hypothesis was not supported by their results. Vietnam War veterans from Operation Ranch Hand were found to have a higher incidence of diabetes than other veterans (Henriksen *et al.* 1997). Michalek *et al.* (2003) assessed whether TCDD elimination rate could be related to incidence of diabetes in 343 veterans. The elimination rate for each participant was calculated from the change in TCDD concentration over time following the initial exposure. The researchers compared the TCDD elimination rate between diabetics and non-diabetics in the population but found no significant relationship between TCDD elimination rates and the incidence of diabetes.

Another explanation relates to the fate of POPs that are bound to triglycerides. There is an increased mobilization of POPs into plasma during periods of heightened

lipolysis in animals such as times of reduced food intake. Examples include seals during the post-weaning fast (Debier *et al.* 2006), sea turtles during long migration and yolking eggs (Keller *et al.* 2004), ducks during winter months (Smith *et al.* 1985), and migrating salmon that rely on lipid stores during upstream migration (Ewald *et al.* 1998). Usage of stored lipids has been shown to magnify contaminant concentrations in other tissues by releasing contaminants from lipid storage and into the bloodstream (Kelly *et al.* 2007). A study performed on subjects undergoing weight loss marked a significant increase in plasma OC levels (Imbeault *et al.* 2001a). These results corroborated with an *in vitro* study of basal lipolysis levels in adipocytes (Imbeault *et al.* 2001b). The *in vitro* analysis indicated that increases in lipolysis correspond to greater rises in OC plasma levels during weight loss.

Evidence indicates increased lipolysis occurs in diabetic individuals (Nurjhan *et al.* 1992). Nurjhan *et al.* (1992) showed that glycerol appears in the plasma of diabetics at a rate 1.5 times greater than non-diabetics, meaning a 1.5 times greater rate of lipolysis, as the appearance of glycerol was used as an index of lipolysis. It remains to be seen whether the release of POPs during lipolysis can be extrapolated to the diabetic state. When triglycerides are broken down into free fatty acids and glycerol, POPs could end up in the plasma, as evidenced in the animal studies above.

4.4 *Inflammatory marker levels are not significantly altered in diabetics*

To our knowledge, this is the first time inflammatory markers have been measured *in vivo* in relation to POPs and diabetes. The association between different POPs and inflammation has been studied *in vitro* (Hennig *et al.* 2001; Arsenescu *et al.* 2008). Mullerova *et al.* (2008) found an association between PCBs and adiponectin in humans in relation to obesity through a longitudinal intervention trial, but this was not linked to diabetes or insulin resistance.

We found no significant difference in plasma concentrations of TNF-α, IL-6, total adiponectin, or HMW-adiponectin between diabetic and non-diabetic individuals. Plasma adiponectin is generally lower in individuals with type 2 diabetes (Yaturu *et al.* 2006, Hotta *et al.* 2000). TNF-α and IL-6 are generally higher in diabetic individuals (Plomgaard *et al.* 2007). Contrarily, these markers have also been observed to be indicators of fat mass and unrelated to diabetes or insulin resistance (Carey *et al.* 2004).

In our participants, the mean BMI did not differ between diabetic and non-diabetics and the majority of participants (65 %) were obese (BMI $\geq$ 30 kg/m^2). The range in BMI for our study population was also small, but likely not due to the sample size. Williams *et al.* (2006) conducted a study measuring inflammatory markers with a similar number of participants (N=73) but was able to establish a larger BMI range (18.6 - 73.1 kg/m^2) compared to our population (21.2 - 43.0 kg/m^2). Of the three inflammatory markers measured in our study, IL-6 was the only marker correlated with insulin resistance (as quantified by $AUC_{insulin}$). There may be no significant correlation between TNF-α or adiponectin with insulin resistance due to the small BMI range. Adiponectin

levels are generally lower in obese individuals (Kim *et al.* 2006), which may not be apparent here because of the high proportion of obese individuals.

Of the 72 participants, 19 are on medication for hypertension and cardiovascular disease (CVD). Some studies suggest a possible link between hypertension, IL-6, and TNF-α (Bautista *et al.* 2005). Even if no medication was taken by our subjects prior to the experimental session, some prescribed medications for hypertension and CVD could have modulated plasma levels of TNF-α, IL-6 (Andrzejczak *et al.* 2006), and adiponectin (Yilmaz *et al.* 2007). This could obscure the expected correlation between harmful inflammatory markers and insulin resistance in non-diabetic subjects. A few of the diabetic individuals have been prescribed thiazolidinediones (TZDs) which have been shown to decrease levels of TNF-α (Hofmann *et al.* 1994) and IL-6 (van Doorn *et al.* 2006) and increase levels of adiponectin (Shimizu *et al.* 2006). This drug-induced modulation of inflammatory markers may be part of the reason why diabetics have comparable inflammatory marker levels to non-diabetics in our population.

4.5 *Some associations between POPs and adiponectin*

To our knowledge, this is the first time the relationship between OC pesticides and inflammatory markers has been studied *in vivo*. Our results indicate no correlation between the POP gradient, IL-6, and TNF-α.

There is *in vitro* evidence that coplanar PCBs induce the release of TNF-α and IL-6 (Arsenescu *et al.* 2008; Hennig *et al.* 2001), but this has not been assessed in humans, however our study did not include the measurement of coplanar PCBs.

Adiponectin was positively correlated to the POP gradient. This result is the opposite of what has been observed *in vitro* on adipose cells (Arsenescu *et al.* 2008) and in humans (Mullerova *et al.* 2008). As noted above, medication for hypertension, CVD, and diabetes may modulate the levels of adiponectin, thereby altering expected patterns. The correlation of adiponectin with the POP gradient may also be due to the association of most POPs with fish consumption in our population. A fish consumption index was developed based on the number of fish meals eaten by individuals (data not shown). Beneficial fatty acids in fish are known ligands of PPAR-γ (Rossi *et al.* 2005), a receptor that can upregulate production of adiponectin (Maeda *et al.* 2001). An increase in fish consumption has been correlated with increases in adiponectin concentrations (Lara *et al.* 2007). Therefore, the positive correlation detected between several contaminants and adiponectin may be due to varying levels of fish consumption and adiponectin-modulating medications. There is no mechanistic evidence to suggest that adiponectin expression would be directly increased by exposure to PCBs or OC pesticides.

This study has a few limitations. Because of the cross-sectional nature of the study, we cannot assess whether POPs contribute to the development of diabetes. Also, the relatively small sample size may decrease the power of the statistical analyses. However, because of the relatively smaller group of participants, we were able to apply a more accurate diabetes screening process.

One of the strengths of the study is the use of the OGTTs to determine diabetic status and quantify insulin resistance. Diabetic status is often determined based solely on the fasted glucose concentration in plasma (i.e. if an individual has an impaired fasting glucose concentration, he/she is considered diabetic). Using OGTTs, the 2-hour post-

glucose load plasma concentration can be obtained and used to determine if the individual has an impaired glucose tolerance. The 2-hour value together with the fasting glucose concentration is better for determining diabetic status than using the fasting glucose concentration alone (Chen *et al.* 2002). The OGTTs are more costly and time-consuming than just using the fasting plasma glucose (Expert committee, 2003) and are therefore not generally used for larger cohorts (Codru *et al.* 2007). For larger studies, there is often a reliance on the self-reporting of diabetes (Turyk *et al.* 2009b) and individuals with type I diabetes may not be separated out of the sample (Lee *et al.* 2006). In addition, the $AUC_{insulin}$ gave us a precise way of quantifying insulin resistance (Matsuda and DeFronzo 1999) in order to predict whether any of the POPs are related to insulin resistance, and not only present at higher levels in diabetic individuals.

The novelty of this project is the quantification of insulin resistance in relation to POP concentrations. Radikova *et al.* (2004) used OGTTs in a study comparing POP levels in individuals with different types of dysglycemia, but not the development of insulin resistance. Our study gives more insight into the development of diabetes through resistance to insulin in populations highly exposed to contaminants. We are also the first to include the measurement of inflammatory markers in a study examining regarding the link between POPs and diabetes.

Future work should focus on whether any of the POPs contribute to the development of insulin resistance, given the associations seen between POPs and diabetes in this study and previous studies. One way of doing this would be to study the effect of POPs on diabetic and non-diabetic laboratory subjects by exposing subjects to POPs through diet. The rate of POP release could then be monitored, specifically targeting any differences

between diabetics and non-diabetics. This would determine whether the diabetic status alters the rate of POP release into the plasma. As migrating animals are subject to seasonal fluctuations of POPs (as mentioned above), the same trend may be seen in animals with a higher lipolysis rate due to diabetes. It is important to establish whether POPs are actually leading to insulin resistance, in order to give appropriate wild food consumption guidelines to both diabetic and non-diabetic individuals exposed to high POP levels from frequent wild food consumption.

4.6 *Conclusions*

In general, POP concentrations in serum are higher in diabetics than non-diabetics. This indicates that contaminants may not affect diabetics the same way that they affect non-diabetics. Health consumption advisories and human health risk assessments have been developed for a wide range of contaminants, but these have never treated diabetics separately from non-diabetics. If diabetics release more POPs into plasma circulation than non-diabetics, this might need to be taken into consideration when developing guidelines for tolerable POP levels in diet. There is a very strong association with BMI and the development of insulin resistance in these communities, providing a strong reason to improve diet and lifestyle practices in First Nations communities, in order to reduce BMI and the prevalence of diabetes.

There is no strong association between IL-6 or TNF-α and exposure to POPs, but there is a significant positive correlation between adiponectin and POPs. Given the confounding factors present in our study subjects that modulate plasma levels of

inflammatory markers themselves, it is possible that these factors influenced levels of inflammatory markers more than POPs and Hg. Since contaminant levels and diabetes are both prevalent in northern First Nations communities, further investigation into the causes behind this interaction (such as differences in lipid metabolism) is warranted.

Table 4.1 Compiled mean levels of PCBs and an OC pesticide (DDE) from studies of POPs and diabetes (ng/g lipid)

Group	n (diabetic); n (non-diabetic)	Medium	ΣPCBs		DDE		Reference
			Diabetic	Non-diabetic	Diabetic	Non-diabetic	
First Nations in Northern Ontario	26; 46	plasma	1080	374	594	200	Present study[a]
			397[b]	134[b]	604[b]	188[b]	
Environmentally-exposed subjects in Belgium	9; 248	serum	652[b]	402[b]	-	-	Fierens *et al.* 2003[c]
Fishermen's wives from Sweden	10; 174	serum	-	-	1600	800	Rylander *et al.* 2005
Fishermen from Sweden	12; 184	serum	-	-	1100	800	Rylander *et al.* 2005
Fish-eating women from Sweden	15; 528	serum	-	-	340	190	Rignell-Hydbom *et al.* 2007
Great Lakes sport fish consumers	61; 442	serum	2.17[b]	1.53[b]	3.01[b]	1.84[b]	Turyk *et al.* 2009b[d]

[a] ΣPCBs = sum of PCB 99, 105, 118, 138, 153, 156, 163, 170,180, 183, and 187
[b] Geometric mean
[c] ΣPCBs = sum of PCB 3, 8, 28, 52, 101. 118, 138, 153, 180, 194, 206, and 209
[d] ΣPCBs = sum of PCB 74, 99, 118, 146, 180, 194, 201, 206, 132/153/105, 138/163, 170/190, 182/187, 196/203

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Appendix 1: Anthropometric and diabetes data

Code	BMI (kg/m2)	Waist Circumference (cm)	Fasting Glucose mmol/L	Fasting Insulin pmol/L	AUC (insulin)	Diabetes (Y/N)	HOMA-IR	TNF-α (pg/mL)	IL-6 (pg/mL)	Adiponectin (ug/mL)	HMW adiponectin (ug/mL)
K HW 00	28.7	108.1	4.5	34.6	77088.6	N	1.00	1.52	1.44	3.95	1.41
K HW 01	35.7	106.9	3.9	260.4	218863.7	N	6.59	1.37	2.98	6.49	2.43
K HW 02	29.8	109.5	5.4	59.1	24754.1	Y	2.07	3.10	3.17	3.82	0.87
K HW 03	21.2	107.0	4.2	49.9	45925.3	N	1.36	2.29	32.83	4.73	1.50
K HW 04	35.5	126.2	5.1	69.7	22347.6	Y	2.28	6.26	4.19	7.88	3.23
K HW 05	35.4	117.5	5.2	153.4	82403.6	N	5.19	2.15	2.92	4.31	1.07
K HW 08	36.1	125.1	10.0	218.2	31972.2	Y	14.15	1.74	1.33	6.55	2.98
K HW 09	24.9	91.4	5.8	28.2	22116.3	Y	1.06	21.04	0.75	8.50	3.34
K HW 10	35.7	110.8	8.7	28.6	8862.5	Y	1.61	1.84	2.92	29.81	15.35
K HW 11	31.8	120.1	5.5	50.0	63258.9	N	1.77	4.44	1.65	3.48	0.81
K HW 12	21.5	81.9	14.8	25.7	4441.0	Y	2.47	1.19	2.30	8.62	4.20
K LW 15	25.5	90.1	6.0	140.7	81547.4	N	5.42	1.36	2.11	8.42	4.31
K HW 17	28.6	104.0	5.5	49.4	10974.6	Y	1.75	3.89	3.64	10.10	3.98
K HW 19	35.5	124.8	12.3	48.6	12251.5	Y	3.85	2.04	8.35	3.64	1.09
K HW 20	30.5	118.8	5.1	39.4	34740.9	N	1.30	2.76	4.74	14.64	7.52
K LW 21	28.9	93.6	5.7	105.4	22916.3	N	3.91	1.54	4.15	4.52	1.61
K LW 22	31.2	109.1	5.0	74.4	68379.1	N	2.42	2.39	1.19	5.09	2.09
K LW 24	40.3	140.2	13.2	61.6	9414.1	Y	5.23	1.19	2.64	6.40	2.77
K LW 25	22.4	88.5	5.2	26.5	60253.9	N	0.89	1.37	2.53	7.75	3.11
K LW 26	32.2	126.8	5.2	84.5	56076.4	N	2.84	1.49	2.92	3.37	1.06
K HW 27	28.3	102.0	5.7	40.8	23842.6	N	1.50	1.68	1.23	2.91	0.74
K LW 30	34.4	109.6	16.7	40.9	9196.1	Y	4.42	1.27	5.70	4.44	1.63
K LW 31	31.9	119.5	5.0	72.9	57058.5	N	2.33	2.43	3.26	3.12	3.05
K LW 32	34.9	124.3	6.1	113.9	63149.0	N	4.45	3.61	1.87	3.33	1.35
K LW 33	40.0	130.0	7.6	112.3	36571.6	Y	5.54	1.20	5.26	2.49	0.51
K HW 35	37.3	123.1	6.4	99.3	19045.0	N	4.08	2.06	1.67	4.06	1.08
K LW 36	29.1	102.9	6.0	53.4	32055.1	N	2.07	1.52	1.21	3.59	0.88
K LW 37	30.6	102.4	6.6	126.0	172649.1	N	5.36	2.36	6.27	4.50	1.27
K LW 39	42.2	130.0	6.1	103.8	58076.9	N	4.09	2.04	6.63	4.23	1.46
K LW 40	28.9	103.3	7.4	82.2	64225.7	Y	3.92	2.41	3.47	3.64	1.10
K LW 41	28.8	107.8	8.8	68.3	30762.6	Y	3.88	1.67	1.34	2.86	0.59
K HW 43	33.5	121.7	5.7	68.6	83461.9	N	2.52	0.96	1.92	3.69	1.59

***Appendix 1*: Anthropometric and diabetes data**

Code	BMI (kg/m2)	Waist Circumference (cm)	Fasting Glucose mmol/L	Fasting Insulin pmol/L	AUC (insulin)	Diabetes (Y/N)	HOMA-IR	TNF-α (pg/mL)	IL-6 (pg/mL)	Adiponectin (ug/mL)	HMW adiponectin (ug/mL)
K LW 45	29.7	152.7	5.7	37.1	36763.3	N	1.36	1.93	0.88	6.27	2.30
W HW 50	37.9	125.0	9.7	197.8	64737.7	Y	12.38	1.86	2.90	8.35	3.85
W HW 51	30.3	111.0	6.7	93.5	34674.6	Y	4.03	1.83	2.32	6.72	2.36
W HW 52	32.0	104.0	6.5	56.6	40949.6	Y	2.39	1.06	2.02	6.79	3.22
W HW 53	39.7	141.0	5.2	242.8	145784.7	N	8.13	4.92	2.42	4.60	1.58
W HW 54	28.0	102.0	10.4	19.5	7730.9	Y	1.30	1.74	1.41	10.25	4.64
W LW 55	32.8	105.5	6.7	95.2	95393.3	N	4.10	1.12	1.35	4.28	1.92
W HW 57	29.4	122.5	8.5	198.3	71502.7	Y	10.91	2.51	3.06	5.04	1.52
W HW 60	31.2	116.0	6.5	63.2	66430.5	Y	2.63	1.93	1.46	8.67	3.49
W HW 61	29.2	110.0	6.0	67.2	37991.0	N	2.59	1.89	1.60	7.90	2.99
W HW 62	33.2	108.0	10.1	40.0	12842.4	Y	2.60	1.18	4.13	9.58	4.19
W LW 63	33.5	119.0	6.0	121.5	81373.2	N	4.70	2.51	1.67	4.40	1.21
W HW 64	29.6	115.0	5.6	38.8	34928.8	N	1.40	1.70	3.22	10.57	3.90
W HW 65	36.1	126.0	5.4	55.4	62178.8	N	1.94	1.09	5.44	6.30	2.30
W LW 67	35.5	118.0	6.7	159.7	92097.0	N	6.86	1.43	3.42	6.24	2.42
W HW 68	29.8	112.5	13.4	93.3	25926.5	Y	8.06	2.89	2.15	10.04	3.92
W HW 70	36.3	132.0	4.7	147.0	110415.9	N	4.50	2.20	2.59	7.36	2.84
W HW 71	33.3	119.0	5.8	72.8	53973.7	N	2.73	1.63	2.15	8.32	3.37
W HW 72	31.8	110.0	5.5	80.8	85446.0	N	2.85	1.30	2.33	3.85	2.24
W HW 73	35.4	127.5	5.7	127.9	105177.4	N	4.70	1.38	6.21	7.01	2.90
W HW 74	32.4	114.0	5.8	54.5	36798.6	N	2.04	1.50	1.57	6.04	2.32
W HW 75	33.6	112.0	5.5	54.6	41907.7	N	1.92	1.67	1.33	9.49	4.16
W HW 76	35.4	116.0	12.5	251.7	79468.1	Y	20.30	1.54	4.54	3.53	1.03
W LW 78	27.8	98.0	6.7	62.3	27237.6	N	2.70	9.62	2.25	6.01	2.57
W HW 79	30.1	104.0	5.4	67.3	28394.0	N	2.33	1.85	1.75	2.50	0.53
W HW 80	30.6	113.0	7.5	223.8	78719.3	Y	10.82	1.58	2.35	8.19	4.01
W LW 81	22.9	86.0	5.0	35.9	29761.2	Y	1.15	3.76	7.74	14.38	7.30
W LW 82	27.4	108.0	4.7	60.0	94342.8	N	1.80	3.05	4.58	7.50	3.20
W LW 83	37.3	134.0	7.3	151.2	57923.4	Y	7.10	1.17	3.12	5.84	2.50
W LW 84	32.6	107.0	8.2	64.9	20570.2	Y	3.45	0.95	3.13	6.89	2.51
W LW 85	34.5	117.0	5.1	88.0	89859.1	N	2.87	1.15	2.29	5.93	3.60
W LW 86	31.5	109.0	4.9	51.1	50755.2	N	1.62	1.48	3.02	4.86	1.68

Appendix 1: Anthropometric and diabetes data

Code	BMI (kg/m2)	Waist Circumference (cm)	Fasting Glucose mmol/L	Fasting Insulin pmol/L	AUC (insulin)	Diabetes (Y/N)	HOMA-IR	TNF-α (pg/mL)	IL-6 (pg/mL)	Adiponectin (ug/mL)	HMW adiponectin (ug/mL)
W LW 88	25.3	97.0	5.5	33.6	23395.1	N	1.20	1.44	1.63	8.42	4.32
W LW 89	24.6	100.0	5.5	45.5	21301.5	N	1.62	1.62	1.83	4.65	1.17
W LW 90	39.0	125.0	5.8	62.5	72621.2	N	2.36	1.60	40.36	6.74	2.63
W LW 91	40.5	120.0	5.2	80.7	73503.9	N	2.73	1.53	4.18	7.14	3.93
W LW 92	25.5	97.0	6.4	88.5	46849.4	N	3.64	2.83	1.24	8.16	4.00
W HW 93	43.0	125.0	6.2	54.0	70446.6	N	2.17	1.69	3.74	6.60	2.87
W HW 94	36.5	121.5	5.6	65.2	129038.8	N	2.36	1.39	2.81	8.12	3.68
W HW 95	31.5	111.0	5.9	134.0	76656.6	N	5.08	1.29	1.62	3.05	0.75

Appendix 2: Plasma PCB concentrations (µg/L)

Code	Aroclor 1260	PCB 99	PCB 105	PCB 118	PCB 138	PCB 153	PCB 156	PCB 163	PCB 170	PCB 180	PCB 183	PCB 187	Total Lipids (g/L)
K HW 00	3.9	0.05	0.01	0.069	0.23	0.52	0.063	0.089	0.11	0.43	0.041	0.14	5.7
K HW 01	28	0.33	0.19	1	1.6	3.7	0.41	0.55	0.67	2.7	0.32	1.1	7
K HW 02	30	0.15	0.08	0.54	1.5	4.3	0.78	0.77	1.3	5.7	0.25	1.6	8
K HW 03	11	0.05	0.01	0.076	0.46	1.6	0.28	0.35	0.52	2.3	0.1	0.63	4.2
K HW 04	17	0.12	0.062	0.42	0.83	2.4	0.32	0.42	0.49	2.2	0.14	0.75	6.4
K HW 05	11	0.18	0.093	0.43	0.68	1.4	0.14	0.19	0.25	0.89	0.13	0.36	6.8
K HW 08	78	0.68	0.46	2.6	4.6	10	1.2	1.7	2.1	7.9	0.85	3.2	8.7
K HW 09	27	0.29	0.18	0.99	1.6	3.5	0.34	0.5	0.7	2.7	0.35	1.1	6.9
K HW 10	28	0.34	0.26	1.2	1.7	3.7	0.33	0.46	0.69	2.8	0.4	1.1	4.9
K HW 11	43	0.46	0.099	0.59	2.6	5.6	0.63	0.76	1.3	4.8	0.55	1.7	7.3
K HW 12	18	0.14	0.039	0.29	1	2.5	0.29	0.34	0.47	2.2	0.17	0.74	5.2
K LW 15	12	0.17	0.041	0.26	0.76	1.6	0.13	0.23	0.24	1	0.14	0.43	6.2
K HW 17	28	0.19	0.036	0.29	1.6	3.7	0.51	0.65	0.99	3.7	0.28	1.3	5.9
K HW 19	19	0.3	0.13	0.58	1.2	2.4	0.19	0.3	0.34	1.4	0.27	0.81	7
K HW 20	2.6	0.06	0.01	0.062	0.19	0.32	0.024	0.04	0.037	0.14	0.025	0.072	6.6
K LW 21	4.4	0.04	0	0.021	0.25	0.59	0.079	0.095	0.15	0.55	0.041	0.19	6.6
K LW 22	0.38	0	0	0	0.026	0.048	0	0	0.01	0.055	0	0.019	6.3
K LW 24	5.9	0.13	0.039	0.17	0.44	0.7	0.051	0.082	0.09	0.35	0.077	0.18	8.4
K LW 25	6.1	0	0	0.042	0.24	0.94	0.18	0.17	0.38	1.8	0.055	0.42	6.6
K LW 26	7.3	0.1	0.04	0.19	0.47	0.94	0.083	0.14	0.14	0.6	0.082	0.29	8.3
K HW 27	2.2	0.04	0	0.022	0.15	0.28	0.031	0.037	0.071	0.23	0.03	0.085	8.5
K LW 30	6.9	0.06	0.01	0.08	0.38	0.95	0.13	0.16	0.2	0.86	0.064	0.32	6.8
K LW 31	2.3	0.04	0	0.044	0.16	0.28	0.026	0.036	0.048	0.17	0.025	0.074	5.3
K LW 32	2.6	0.04	0	0.023	0.17	0.33	0.033	0.053	0.065	0.24	0.027	0.1	5.5
K LW 33	0.51	0	0	0.021	0.042	0.056	0	0	0.01	0.034	0	0.01	7.3
K HW 35	1.9	0.05	0.019	0.076	0.13	0.24	0.02	0.028	0.029	0.11	0.023	0.058	5.6
K LW 36	1.3	0	0	0.01	0.062	0.19	0.03	0.036	0.048	0.21	0	0.062	6.7
K LW 37	1.5	0	0	0.029	0.097	0.2	0.021	0.027	0.041	0.19	0.022	0.075	5.8
K LW 39	3.9	0.04	0.01	0.079	0.24	0.51	0.061	0.077	0.11	0.41	0.036	0.14	5.6
K LW 40	3.4	0.04	0.01	0.064	0.22	0.44	0.046	0.054	0.083	0.34	0.04	0.12	5.8
K LW 41	13	0.07	0.01	0.11	0.7	1.8	0.19	0.28	0.38	1.5	0.12	0.64	10
K HW 43	2.1	0.04	0.01	0.048	0.15	0.26	0.024	0.035	0.05	0.17	0.024	0.076	5.9

***Appendix 2:* Plasma PCB concentrations (µg/L)**

Code	Aroclor 1260	PCB 99	PCB 105	PCB 118	PCB 138	PCB 153	PCB 156	PCB 163	PCB 170	PCB 180	PCB 183	PCB 187	Total Lipids (g/L)
K LW 45	0.38	0	0	0.01	0.023	0.051	0	0	0	0.029	0	0.01	5.8
W HW 50	5.5	0.09	0.044	0.22	0.34	0.72	0.049	0.1	0.086	0.39	0.064	0.24	7.6
W HW 51	5.5	0.08	0	0.058	0.37	0.7	0.059	0.097	0.098	0.38	0.049	0.17	6.9
W HW 52	6.1	0.11	0.049	0.21	0.38	0.78	0.047	0.1	0.11	0.47	0.097	0.27	5.5
W HW 53	0.82	0	0	0.033	0.059	0.098	0	0.01	0.01	0.041	0	0.024	8.4
W HW 54	21	0.33	0.085	0.49	1.4	2.5	0.28	0.4	0.49	1.8	0.22	0.64	5.6
W LW 55	11	0.08	0.028	0.15	0.63	1.5	0.2	0.23	0.39	1.4	0.11	0.5	7.4
W HW 57	1.3	0	0	0.018	0.057	0.2	0.038	0.035	0.062	0.26	0	0.062	7.8
W HW 60	6.6	0.09	0.047	0.23	0.49	0.78	0.078	0.11	0.15	0.58	0.075	0.24	5.4
W HW 61	4.3	0.09	0.015	0.069	0.3	0.52	0.039	0.058	0.069	0.29	0.053	0.14	7.5
W HW 62	21	0.3	0.08	0.41	1.3	2.7	0.23	0.36	0.42	1.7	0.23	0.82	5.9
W LW 63	0.52	0	0	0.027	0.041	0.059	0	0	0	0.023	0	0	4.2
W HW 64	2.8	0.05	0.017	0.069	0.21	0.33	0.036	0.039	0.086	0.31	0.052	0.12	6.8
W HW 65	0.46	0	0	0.02	0.027	0.061	0	0	0.02	0.071	0	0.022	8.3
W LW 67	3	0.03	0.01	0.055	0.2	0.38	0.052	0.053	0.11	0.37	0.037	0.12	7.8
W HW 68	34	0.24	0.17	1	2	4.7	0.75	0.86	1.3	4.9	0.35	1.6	6.5
W HW 70	0.71	0	0	0.024	0.05	0.087	0.01	0.01	0.02	0.062	0	0.025	5.8
W HW 71	0.83	0	0	0.022	0.05	0.11	0	0.01	0.025	0.092	0.01	0.031	6.2
W HW 72	4.7	0.04	0.01	0.048	0.3	0.6	0.084	0.087	0.17	0.57	0.048	0.19	8.8
W HW 73	1.1	0	0	0.038	0.067	0.14	0.02	0.021	0.032	0.11	0.02	0.042	7.6
W HW 74	2	0	0	0.02	0.12	0.26	0.039	0.038	0.077	0.28	0.017	0.075	6.2
W HW 75	3.8	0.04	0	0.034	0.26	0.46	0.058	0.067	0.11	0.39	0.041	0.13	6.3
W HW 76	13	0.12	0.045	0.26	0.96	1.6	0.21	0.2	0.42	1.5	0.16	0.47	5.7
W LW 78	6.3	0.06	0.027	0.13	0.4	0.8	0.086	0.1	0.2	0.7	0.081	0.24	8.3
W HW 79	0.48	0	0	0.01	0.031	0.061	0	0	0.02	0.063	0	0.02	9.1
W HW 80	1.9	0	0	0.029	0.11	0.26	0.04	0.048	0.07	0.24	0.02	0.073	6.2
W LW 81	13	0.23	0.06	0.33	1	1.5	0.17	0.19	0.3	1.1	0.16	0.35	7.8
W LW 82	2.7	0	0	0.034	0.17	0.36	0.048	0.053	0.087	0.29	0.024	0.088	5.4
W LW 83	0.86	0	0	0.032	0.052	0.11	0.01	0.02	0.021	0.08	0.01	0.028	5.1
W LW 84	2.3	0	0.01	0.056	0.15	0.29	0.037	0.039	0.076	0.28	0.029	0.091	5.7
W LW 85	2.7	0.04	0.016	0.079	0.17	0.35	0.037	0.051	0.084	0.27	0.034	0.094	11
W LW 86	5.5	0.03	0	0.044	0.29	0.78	0.12	0.11	0.28	1	0.058	0.26	6.7

Appendix 2: Plasma PCB concentrations (µg/L)

Code	Aroclor 1260	PCB 99	PCB 105	PCB 118	PCB 138	PCB 153	PCB 156	PCB 163	PCB 170	PCB 180	PCB 183	PCB 187	Total Lipids (g/L)
W LW 88	5.7	0.04	0.019	0.11	0.33	0.76	0.12	0.13	0.22	0.78	0.053	0.25	6.8
W LW 89	0.4	0	0	0	0.027	0.049	0	0	0.02	0.054	0	0.02	4.4
W LW 90	3.2	0.05	0.01	0.064	0.23	0.39	0.048	0.058	0.086	0.28	0.031	0.1	7.4
W LW 91	6.2	0.08	0.017	0.11	0.4	0.8	0.1	0.13	0.18	0.68	0.057	0.22	5.8
W LW 92	0.5	0	0	0.01	0.038	0.058	0	0	0.01	0.043	0	0.01	6.5
W HW 93	19	0.2	0.16	0.77	1.3	2.4	0.31	0.38	0.54	1.9	0.22	0.7	7.8
W HW 94	13	0.13	0.089	0.48	0.83	1.6	0.2	0.25	0.39	1.3	0.14	0.47	8.6
W HW 95	4	0.05	0	0.045	0.3	0.47	0.048	0.055	0.1	0.35	0.048	0.11	5.3

Appendix 3: Other POP plasma concentrations (µg/L) and Hg levels in hair (ppb)

Code	PBDE47	HCB	Mirex	DDE	ß-HCH	Oxychlordane	*cis*-Nonachlor	*trans*-Nonachlor	Hg	Total Lipids (g/L)
K HW 00	0	0.055	0.09	0.83	0.012	0.026	0.01	0.041	1637.5	5.7
K HW 01	0.04	0.21	0.67	8.6	0.024	0.17	0.099	0.34	2113.514	7
K HW 02	0.03	0.28	1.1	13	0.027	0.18	0.074	0.36	2422.766	8
K HW 03	0.04	0.091	0.52	0.93	0	0.063	0.03	0.13	2567.5	4.2
K HW 04	0.03	0.13	0.37	4.3	0.031	0.13	0.056	0.22	2938	6.4
K HW 05	0.04	0.14	0.27	2.4	0.018	0.07	0.051	0.16	2284.167	6.8
K HW 08	0	0.54	2.6	18	0.042	0.6	0.46	1.5	4621.053	8.7
K HW 09	0	0.31	0.8	4.9	0.013	0.21	0.12	0.44	5524.706	6.9
K HW 10	0	0.27	0.81	5	0.011	0.16	0.098	0.27	4250	4.9
K HW 11	0.05	0.15	1.6	5.3	0.017	0.18	0.098	0.44	3436.857	7.3
K HW 12	0.03	0.14	0.37	4.9	0.018	0.1	0.031	0.16	2476.857	5.2
K LW 15	0.05	0.059	0.24	2.9	0.014	0.075	0.01	0.12	333.7838	6.2
K HW 17	0	0.13	1.7	3.8	0.018	0.14	0.082	0.32	1751.389	5.9
K HW 19	0.04	0.2	0.7	3	0.025	0.14	0.077	0.33	2249.091	7
K HW 20	0.09	0.049	0.043	0.85	0.022	0.034	0.008	0.042	718.8235	6.6
K LW 21	0	0.055	0.13	1.3	0.018	0.036	0.01	0.06	730.6452	6.6
K LW 22	0	0	0.028	0.1	0	0.005	0	0	533.7143	6.3
K LW 24	0.13	0.087	0.065	1.7	0.023	0.044	0.027	0.098	2510.606	8.4
K LW 25	0	0.059	1	1.4	0	0.041	0.01	0.063	953.1667	6.6
K LW 26	0.03	0.076	0.12	2.1	0.018	0.067	0.022	0.1	1181.724	8.3
K HW 27	0.09	0.044	0.034	1	0.03	0.039	0.009	0.058	1919.412	8.5
K LW 30	0.07	0.06	0.15	2.2	0.019	0.038	0.01	0.067	883.8461	6.8
K LW 31	0.05	0.047	0.035	1	0.015	0.029	0.02	0.059	1239.711	5.3
K LW 32	0.05	0	0.027	0.73	0.013	0.025	0.005	0.035	436.0606	5.5
K LW 33	0.03	0	0	0.72	0.042	0.022	0	0.028	347.6136	7.3
K HW 35	0.06	0	0.02	0.7	0.017	0.032	0.01	0.045	3132.564	5.6
K LW 36	0.12	0.044	0.065	0.45	0.015	0.02	0	0.024	340.7692	6.7
K LW 37	0	0.042	0.069	0.43	0.01	0.021	0	0.025	250.9091	5.8
K LW 39	0.08	0	0.065	1.4	0.021	0.031	0.009	0.042	284.2857	5.6
K LW 40	0	0	0.1	0.89	0.01	0.022	0.008	0.033	898.3334	5.8
K LW 41	0.08	0.054	0.48	2.3	0.013	0.096	0.026	0.18	607.7143	10
K HW 43	0	0	0.046	0.53	0.011	0.019	0.007	0.03	938.1818	5.9
K LW 45	0.05	0	0	0.2	0	0.007	0	0	465	5.8

Appendix 3: Other POP plasma concentrations (µg/L) and Hg levels in hair (ppb)

Code	PBDE47	HCB	Mirex	DDE	ß-HCH	Oxychlordane	*cis*-Nonachlor	*trans*-Nonachlor	Hg	Total Lipids (g/L)
W HW 50	0.07	0.17	0.14	2	0.018	0.1	0.07	0.19	4289.687	7.6
W HW 51	0	0.058	0.067	2	0.014	0.064	0.021	0.094	2001.563	6.9
W HW 52	0.05	0.21	0.14	1.3	0	0.058	0.075	0.16	8629.697	5.5
W HW 53	0.06	0.055	0	0.48	0.011	0.026	0.01	0.039	1167.5	8.4
W HW 54	0.06	0.1	0.51	6.6	0.021	0.077	0.037	0.14		5.6
W LW 55	0.03	0.14	0.46	2.3	0.023	0.099	0.05	0.18	1855	7.4
W HW 57	0.05	0	0.02	0.92	0	0.02	0.005	0.025	1119.445	7.8
W HW 60	0.04	0.11	0.21	2.3	0.018	0.062	0.043	0.12	3039.189	5.4
W HW 61	0.11	0.06	0.053	1.3	0.012	0.037	0.018	0.072	1386.364	7.5
W HW 62	0.1	0.25	0.49	3.9	0.014	0.21	0.13	0.47	4100.541	5.9
W LW 63	0.05	0.04	0	0.5	0.11	0.029	0	0.033	66.0714	4.2
W HW 64	0.04	0.072	0.12	0.79	0	0.024	0.021	0.054	2682.059	6.8
W HW 65	0	0	0.02	0.2	0	0.006	0	0.01	553.7143	8.3
W LW 67	0.04	0	0.048	1.7	0.025	0.037	0.008	0.05	693.4211	7.8
W HW 68	0.03	0.46	1.2	11	0.044	0.37	0.17	0.68	6907.75	6.5
W HW 70	0.04	0	0	0.36	0.011	0.01	0.005	0.02	609	5.8
W HW 71	0	0	0.02	0.28	0	0.01	0	0.01	1390.286	6.2
W HW 72	0	0.11	0.081	1.4	0.014	0.055	0.026	0.098	3654.849	8.8
W HW 73	0.04	0	0.02	0.36	0	0.01	0.01	0.029	1416.875	7.6
W HW 74	0.03	0	0.058	0.61	0	0.01	0	0.01	1299.73	6.2
W HW 75	0.06	0.048	0.046	0.9	0.013	0.032	0.007	0.041	1488.235	6.3
W HW 76	0.06	0.049	0.31	2.9	0.018	0.077	0.043	0.15	2867	5.7
W LW 78	0.11	0.14	0.11	1.6	0.012	0.058	0.034	0.11	2278.378	8.3
W HW 79	0	0	0.01	0.24	0	0.009	0	0.01	698.4616	9.1
W HW 80	0	0	0.034	0.65	0	0.02	0	0.02	1218.049	6.2
W LW 81	0.06	0.07	0.12	5	0.025	0.085	0.018	0.11	29.3023	7.8
W LW 82	0	0	0.043	0.68	0	0.024	0.005	0.027	1541.563	5.4
W LW 83	0.04	0	0	0.35	0.014	0.01	0.006	0.021	544.7222	5.1
W LW 84	0.03	0.052	0.058	0.77	0	0.02	0.01	0.03	1223.243	5.7
W LW 85	0	0	0.03	0.54	0.019	0.035	0.008	0.037	135	11
W LW 86	0.05	0	0.15	1	0.02	0.033	0.009	0.044	1200.286	6.7
W LW 88	0.04	0.073	0.17	2	0.026	0.093	0.037	0.16	1414.857	6.8
W LW 89	0.04	0	0	0.25	0	0.005	0	0	523.8889	4.4

Appendix 3: Other POP plasma concentrations (µg/L) and Hg levels in hair (ppb)

Code	PBDE47	HCB	Mirex	DDE	ß-HCH	Oxychlordane	*cis* - Nonachlor	*trans* - Nonachlor	Hg	Total Lipids (g/L)
W LW 90	0.03	0.041	0.058	1.1	0.018	0.03	0.01	0.044	2356.857	7.4
W LW 91	0.06	0	0.14	1.7	0	0.032	0.01	0.055	1535.938	5.8
W LW 92	0.05	0	0	0.31	0	0.01	0	0.01	131.6216	6.5
W HW 93	0.06	0.3	0.26	5.5	0.036	0.18	0.1	0.29	4148.095	7.8
W HW 94	0.08	0.14	0.27	2.9	0.019	0.1	0.048	0.16	2053	8.6
W HW 95	0.05	0.043	0.037	1	0	0.024	0.02	0.052	847.9166	5.3