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Ecosystem Health in Walpole Island: Exposure to POPs and Heavy Metals in the WIFN Community

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Ecosystem Health in Walpole Island:

Exposure to POPs and Heavy Metals in the WIFN Community

(Spine Title: Ecosystem Health in Walpole Island)

(Thesis Format: Monograph)

By:

Julie D. Hill

A thesis submitted in partial fulfillment

of the requirements for the degree of

Master of Science

Society of Graduate and Postdoctoral Studies

The University of Western Ontario

London, Ontario, Canada

Abstract

Walpole Island First Nation (WIFN) community is located on the St. Clair River downstream from the large industrial area, Chemical Valley. Members of the WIFN have historically been exposed to persistent organic pollutants (POPs) and heavy metals which could contribute to adverse health, such as type 2 diabetes and chemophobia. We conducted a collaborative community-based biomonitoring study with volunteers from the WIFN. Hair and blood samples were used to assess exposure to POPs and heavy metals and to determine levels of stress within the community by the assay of cortisol on hair. Concentrations of POPs and heavy metals in volunteers were within ranges reported in the scientific literature for other populations, and were either lower or no different from 2 referent groups living in Ontario. Cortisol concentrations in hair of volunteers were higher compared to a referent group, indicating the possibility of enhanced stress from which chemophobia contribution is unknown.

Key Words: Persistent Organic Pollutants; Heavy Metals; Cortisol; Walpole Island First Nation; Native American Health; Chemophobia; Diabetes

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List of Abbreviations

| Abbreviation | Meaning |
|--------------------------------------|--|
| 1,2,3,4-TCB | 1,2,3,4-tetrachlorobenzene |
| 1,2,4,5-TCB | 1,2,4,5-tetrachlorobenzene |
| 2,4-D | 2,4-dichlorophenoxyacetic acid |
| 2,4,5-T | 2,4,5-trichlorophenoxyacetic acid |
| ADHD | Attention Deficit Hyperactivity Disorder |
| α -HCH | α -hexachlorocyclohexane |
| β -HCH/ β -BHC | β -hexachlorocyclohexane |
| γ -HCH | γ -hexachlorocyclohexane |
| δ -HCH | δ -hexachlorocyclohexane |
| ACTH | Adrenocorticotrophic Hormone |
| ADR | Adverse Drug Reaction |
| AHH | Aryl Hydrocarbon Hydroxylation |
| AHR | Aryl Hydrocarbon Receptor |
| Al | Aluminum |
| ALHD3A1 | Aldehyde Dehydrogenase 3A1 |
| AR | Androgen Receptor |
| ARNT | AHR Nuclear Translocator Protein |
| As | Arsenic |
| As ³⁺ | Trivalent Arsenic |
| As ⁵⁺ | Pentavalent Arsenic |
| As ₂ O ₃ | Arsenic Oxide |
| b-TSH | Blood Thyroid Stimulating Hormone |

| | |
|-----------|--|
| Ba | Barium |
| BD | Becton-Dickinson |
| Be | Beryllium |
| Bi | Bismuth |
| BW | Body Weight |
| Ca | Calcium |
| Cd | Cadmium |
| CI | Confidence Interval |
| CKD | Chronic Kidney Disease |
| CNS | Central Nervous System |
| CRH | Corticotrophin-Releasing Hormone |
| CYP | Cytochrome |
| CVD | Cardiovascular Disease |
| DDD | 1,1- <i>bis</i> (<i>p</i> -chlorophenyl)-2,2-dichloroethane |
| DDE | 1,1- <i>bis</i> -(4-chlorophenyl)-2,2-dichloroethene |
| DDT | 1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene |
| DMA | Dimethylarsinic Acid |
| DMT | Divalent Metal Ion Transporter |
| DPC..... | Dioxin-like Polychlorinated Biphenyls |
| DRE..... | Dioxin-Response Element |
| E1 | Estrone |
| E2 | Estradiol or 17 β -Estradiol |
| E3 | Estriol |
| EC | Environment Canada |

| | |
|-------------------|---|
| EDC | Endocrine Disrupting Compounds |
| EEDC | Estrogen-like Endocrine Disrupting Chemicals |
| EIA | Enzyme Immunoassay |
| ER | Estrogen Receptor |
| ER α | Estrogen Receptor α |
| ER β | Estrogen Receptor β |
| ETC | Escambia Treating Company |
| Fe | Iron |
| GABA | γ -Aminobutyric Acid |
| GLIER | Great Lakes Institute of Environmental Research |
| GLWQA | Great Lakes Water Quality Agreement |
| GSH | Glutathione Conjugation |
| GSTA1 | Glutathione-S-transferase A1 |
| HCB | Hexachlorobenzene |
| HCH | Hexachlorocyclohexane |
| HPA | Hypothalamic-Pituitary-Adrenal |
| HpCDD | Heptachlorodibenzo- <i>p</i> -dioxin |
| hsp | heat shock protein |
| HxCDD | Hexachlorodibenzo- <i>p</i> -dioxin |
| IARC | International Agency for Research in Cancer |
| ICD-9 | International Statistical Classification of Disease - 9 |
| ICP-MS | Inductively Coupled Plasma Mass Spectrometry |
| IgA | Immunoglobulin A |
| IGF | Insulin-Like Growth Factors |

| | |
|------------------------|--|
| IGFBP-1 | Insulin-Like Growth Factor Binding Protein 1 |
| IgM | Immunoglobulin M |
| IGT | Impaired Glucose Tolerance |
| LHSC | London Health Science Centre |
| LOAEL | Lowest Observable Adverse Effect Level |
| LPL | Lipoprotein Lipase |
| MINORS | ethodological Index for Non-Randomized Studies |
| MMA | Monomethylarsonic Acid |
| MMR | Mismatch Repair |
| NHANES | National Health and Nutrition Examination Survey |
| Ni | Nickel |
| NO | Nitric Oxide |
| NPCB | Non-Dioxin-Like Polychlorinated Biphenyls |
| NPRI | National Pollution Release Inventory |
| NRS | Nitrogen Reactive Species |
| NQO1 | NADP (H)-Quinone Oxidoreductase-1 |
| NYDEC | New York Department of Environmental Conservation |
| OC | Organochlorine |
| OCB | Octachlorobiphenyl |
| OCDD | Octachlorodibenzo- <i>p</i> -dioxin |
| OCS | Octachlorostyrene |
| OGTT | Oral Glucose Tolerance Test |
| <i>o,p'</i> -DDT | 1,1,1-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)ethane |
| P | Phosphate |

| | |
|------------------------|--|
| PAHs | Polycyclic Aromatic Hydrocarbons |
| Pb | Lead |
| PBBs | Polybrominated Biphenyls |
| PBS | Phosphate Buffered Solution |
| PBDEs | Polybrominated Diphenylethers |
| PCBs | Polychlorinated Biphenyls |
| PCDDs | Polychlorinated Dibenzodioxins |
| PCDFs | Polychlorinated Dibenzofurans |
| PCNs | Polychlorinated Naphthalenes |
| PCQs | Polychlorinated Quarterphenyls |
| Pd | Palladium |
| PHAH | Polyhalogenated Aromatic Hydrocarbon |
| <i>p,p'</i> -DDE | 1,1-bis-(4-chlorophenyl)-2,2-dichloroethylene |
| <i>p,p'</i> -DDD | 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene |
| <i>p,p'</i> -DDT | 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane |
| ppb | Parts per Billion |
| ppm | Parts per Million |
| ppt | Parts per Trillion |
| POMC | Proopiomelanocortin |
| POPs | Persistent Organic Pollutants |
| PSS | Perceived Stress Scale |
| PSTD | Post Traumatic Stress Disorder |
| Pt | Platinum |
| PTWI | Probable Tolerable Weekly Intake |

| | |
|------------------------|--|
| QCB | Pentachlorobenzene |
| RAP | Remedial Action Plans |
| RBCs | Red Blood Cells |
| ROS | Reactive Oxygen Species |
| Sb | Antimony |
| T _{1/2} | Elimination Half-life |
| T2D | Type 2 Diabetes |
| T ₄ | Thyroxine |
| TB | Tuberculosis |
| TCDD | 2,4,7,8-tetrachlorodibenzo- <i>p</i> -dioxin |
| TDI | Tolerable Daily Intake |
| TEQ | Toxic Equivalent Quotient |
| TH | Thyroid Hormones |
| TT ₃ | Triiodothyronine |
| TTR | Transthyretin |
| U | Uranium |
| UGT1A2 | UDP-glucuronoxyltransferase 1A2 |
| US | United States |
| VP | Vasopressin |
| WHO | World Health Organization |
| WIFN | Walpole Island First Nation |
| XAP2 | X-associated Protein 2 |
| XRE | Xenobiotic Response Element |
| Zn | Zinc |

Chapter: 1 – *Introduction*

Preamble

This thesis deals with interrelated issues about environmental contaminants and health in a specific population from Walpole Island. This First Nation population lives in close proximity to major sources of pollution in Canada with an unknown level of exposure to these pollutants. There is a fear within the community regarding the possible adverse effects the unknown exposure is having on their health, the health of their children and the health of the environment. This thesis discusses these issues and evaluates current levels of exposure within volunteer members of the community.

1.1 Persistent Organic Pollutants

1.1.1 General Overview

Persistent organic pollutants (POPs) are organic chemicals that have specific characteristics that distinguish them from other chemicals. They resist enzymatic and non-enzymatic degradation which results in their persistence in the environment due to their long half-lives, frequently longer than 1 year, in soil, sediment, air, biota or animal tissue (K. C. Jones & de Voogt, 1999). Similarly, these very stable chemicals are lipophilic, which allows them to be stored in fat tissue of animals (including humans) and to bioaccumulate and biomagnify in the food web, with predators possessing the highest concentrations. Concentrations of POPs increase as species ascend the food web. The highest concentrations of POPs tend to be found in top predators of the food chain, including fish and meat consuming humans.

POP chemicals also have the ability to enter the gas phase, particularly under elevated environmental temperatures (K. C. Jones & de Voogt, 1999). POPs can be volatilized from soil, vegetation and water into the atmosphere, providing a mechanism for their long distance atmospheric transport, typically from equatorial and temperate areas of the Earth towards the poles.

There have been a few well known cases of poisoning with POPs. One important example of occupational and environmental exposure occurred during the Vietnam War. In 1965 the United States (US) Army implemented Operation Ranch Hand, which involved spraying approximately 77 million litres of herbicides over approximately 2.6 million hectares of land in south and central Vietnam (Stone, 2007). Agent Orange, the herbicide used, consisted of 2,4-dichlorophenoxyacetic acid (2,4-D) and two forms of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which was contaminated with the POP, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), as an impurity formed during synthesis (Stone, 2007). Health problems have developed in American and Vietnamese veterans as well as in allies that were supporting the US in the war in Vietnam, including servicemen from Korea. Amongst the most important potential impacts on human health are reports that showed that Vietnam veterans from all relevant countries tend to have a significantly increased incidence of type 2 diabetes (T2D) when compared with veterans who did not serve in Vietnam (Henriksen *et al.*, 1997; Kim *et al.*, 2003; Kang *et al.*, 2006). Other health problems have been reported in Ranch Hand veterans including an increased risk for developing peripheral neuropathy (J. Michalek *et al.*, 2001), and increased risk for developing cancer (J. E. Michalek & Pavuk, 2008).

A second incident that involved a significant release of TCDD into the environment occurred outside Milan, Italy in 1976, near the small town of Seveso. A factory that produced trichlorophenol, which is an intermediate for the synthesis of the antiseptic hexachlorophenol, accidentally leaked a mixture of trichlorophenol sodium hydroxide, sodium salt of ethylene glycol and other by-products including TCDD (Reggiani, 1978). The chemicals precipitated in the form of a cloud of droplets on meadows, trees, houses and roads southeast of the factory, directly into a populated urban area. Samples of vegetation and soil collected from contaminated areas showed TCDD concentrations from less than 0.09 µg/g to greater than 100 µg/g in grass, and this helped to determine areas of high exposure compared to those of low exposure (Reggiani, 1978). Acute symptoms included nausea and skin irritation (mainly redness and swelling), generally from inhalation of airborne TCDD dust and mainly resulted in hospitalization (Reggiani, 1978). In the weeks following the accident, chloracne, a skin disease similar to acne but caused by high concentrations of TCDD or polychlorinated biphenyl (PCB) exposure, developed mainly in children (Reggiani, 1978). In the years following the accident other health problems have developed including increased incidence of T2D in females, increased mortality from cardiovascular disease in males and increased mortality from cancer in both males and females (Bertazzi, 1997).

A third example is of a mass PCB poisoning in Taiwan. In 1979, students and factory workers in the Taichung County in Taiwan began to develop similar symptoms of chloracne, follicular accentuations and pigmentation of the skin and nails (Hsu *et al.*, 1985). Eventually it was discovered that both the students and factory workers had consumed the same brand of cooking rice oil (Hsu *et al.*, 1985). By the end of February

1983, the rice oil was found to contain Kanechlor - 400, 500 mixture (mixture of PCBs) at concentrations of 65 – 108 ppm and at this time there were 2 061 cases of poisonings reported, a cohort that is now known as the Yucheng (oil-disease) cohort (Hsu *et al.*, 1985; Wang *et al.*, 2008). Many adverse health effects occurred; including 39 babies born from PCB intoxicated mothers with hyperpigmentation, pneumonia, bronchitis, sepsis, and premature and congenital weakness (Hsu *et al.*, 1985). Other people died from hepatoma, liver cirrhosis or liver disease with hepatomegaly and those who survived have a higher prevalence of T2D when compared with populations who were not a part of the Yucheng cohort (Hsu *et al.*, 1985; Wang *et al.*, 2008).

This was not the first recorded case of PCB poisoning resulting in chloracne symptoms. In 1968, almost 10 years before the first symptoms were reported in Taiwan, 1800 people in western Japan developed a skin disease, later named the Yusho disease (Todaka *et al.*, 2009). The cause of the disease was determined to be ingestion of rice bran oil contaminated with PCBs, polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Todaka *et al.*, 2009). The contamination occurred when commercial PCB preparations were used for heat exchange, and the pyrolysis of PCBs and benzenes at high temperatures produced the PCDDs and PCDFs (Tsukimori *et al.*, 2008). More than 1 900 people developed acne-like eruptions, pigmentation of the skin and nails, and conjunctivae, increased discharge from the eyes, and paresthesias of the extremities (Tsukimori *et al.*, 2008). Approximately 500 people died, and the estimated ingested dose of PCBs was 633 mg, of PCDFs was 3.4 mg and of polychlorinated quarterphenyls (PCQs) was 0.62 mg on average (Tsukimori *et al.*, 2008). Long-term health effects include adverse pregnancy outcomes from exposed mothers

such as increased risk of spontaneous abortion, increased risk of pregnancy loss, still birth, preterm delivery, fetal growth restriction and low birth weight; lung toxicity such as pulmonary edema, pleural effusions, vascular congestion, and haemorrhage; alterations in immune status such as reduced immunoglobulin A (IgA) and immunoglobulin M (IgM) in serum; as well as many other health problems (Nakanishi *et al.*, 1985; Tsukimori *et al.*, 2008).

In 2001, the Stockholm Convention identified 12 POPs (Fig. 1) for specific reduction/elimination of release. These chemicals are a mix of pesticides, herbicides, insecticides and fungicides: aldrin; chlordane; 1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene (DDT) and metabolites, 1,1-*bis*(*p*-chlorophenyl)-2,2-dichloroethane (DDD) and 1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene (DDE); dieldrin; endrin; heptachlor; hexachlorobenzene (HCB); Mirex; PCBs; PCDDs; PCDFs; and toxaphene. As there are 209 different PCB congeners, depending on the number and placement of chlorine atoms and diverse PCDDs and PCDFs, these 12 groups of chemicals encompass many individual compounds.

There are also many other POP chemicals that have not been included in the 'Stockholm 12', but that are still considered detrimental to the environment and ecosystem health. Other chemicals of particular concern in this thesis are those that are prevalent in the sediment and wildlife on Walpole Island. These chemicals (Fig. 2) are: α , β , γ and δ - hexachlorocyclohexane (α -HCH), (β -HCH), (γ -HCH) and (δ -HCH); octachlorostyrene (OCS); pentachlorobenzene (QCB); *cis*- and *trans*-nonachlor; 1,2,3,4-tetrachlorobenzene (1,2,3,4-TCB) and 1,2,4,5-tetrachlorodbenzene (1,2,4,5-TCB) (Great

Lakes Institute for Environmental Research & Department of Biological Science,
University of Windsor, 2006).

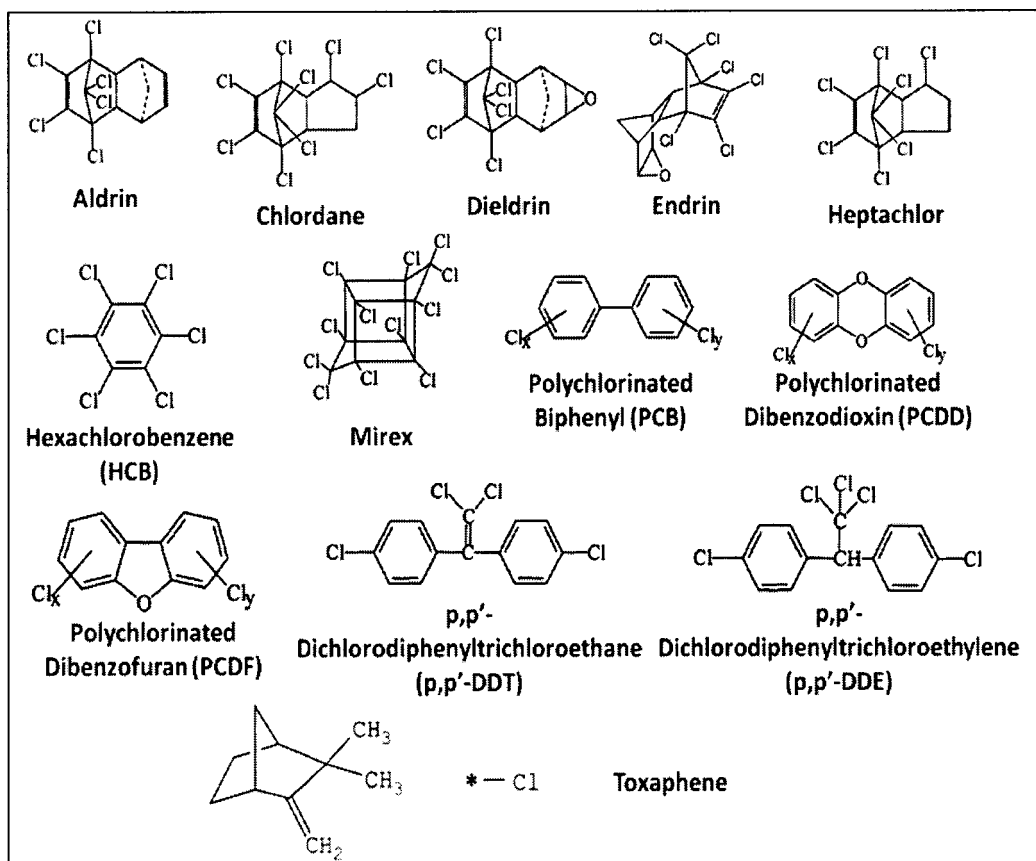


Figure 1: Chemical structures of POPs included in the Stockholm 12.

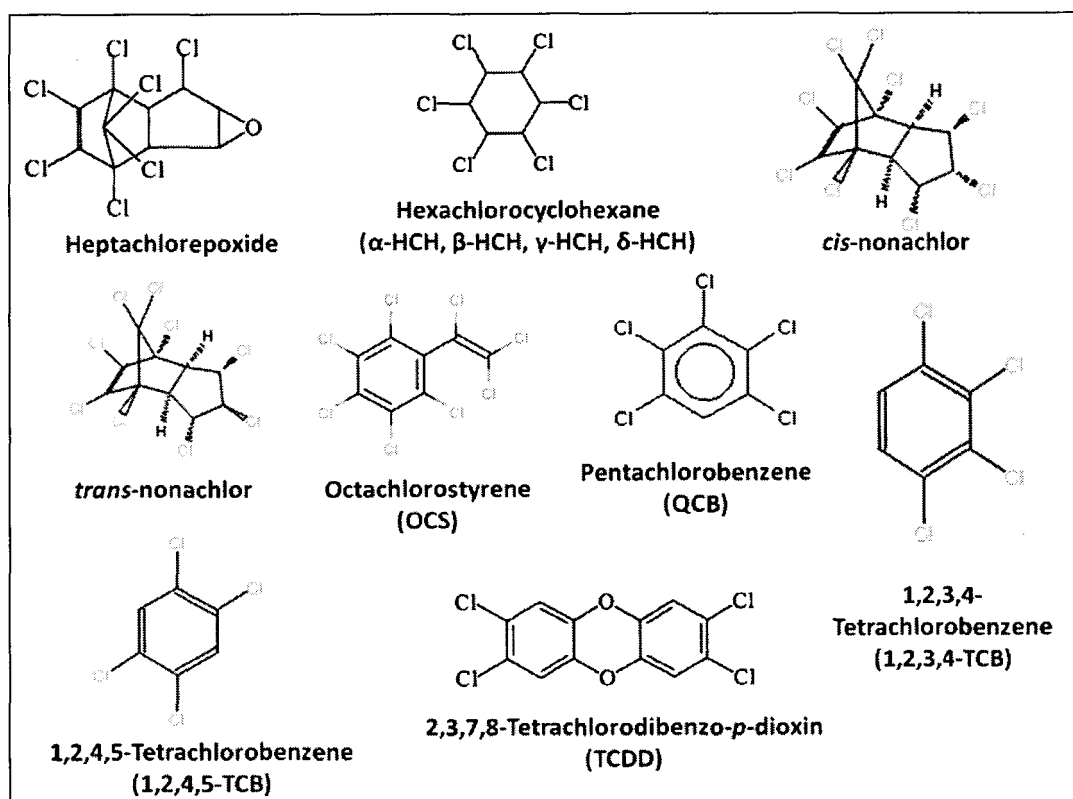


Figure 2: Chemical structures of other POPs of particular interest in the Great Lakes.

1.1.2 Sources of POPs

Most POPs are, or more frequently now were, used for industrial and agricultural purposes because they have been replaced wherever possible in North America, Europe and Japan with less environmentally toxic chemicals. Agricultural POPs, such as aldrin, dieldrin, endrin, chlordane, heptachlor, Mirex, toxaphene, PCDFs, HCB, DDT and PCDDs, all from the Stockholm 12, as well as many more organochlorine (OC) pesticides and insecticides, are deliberately produced and directly applied to crops and soils. Some of these POPs, such as DDT, are banned from use in many developed countries. However, they are still being used in many developing countries to combat the spread of disease such as the use of DDT to help combat the spread of malaria (P. Fernandez & Grimalt, 2003). This practise was recommended by the World Health Organization (WHO) until 2007 - 2008 (World Health Organization, 2007).

Other POPs were manufactured deliberately for diverse applications, primarily because of their high chemical and thermal stabilities. PCBs have been produced since the 1920's and have been used for a wide range of applications including plastics, adhesives, paints, varnishes, carbonless copying paper, newsprint, fluorescent light ballast and caulking compounds (Ross, 2004). PCBs have excellent electrical insulating properties and were widely used as insulators and coolants in large transformers and other electrical equipment (Ross, 2004). An estimated 501 600 000 kg of PCBs had been sold in the US by the time of their ban in North America in the 1970s (Hardy, 2002). Commercial PCB mixtures are more commonly known as Aroclor, and they are often contaminated with unknown amounts of chlorinated naphthalenes and PCDFs. PCDFs

and PCDDs are produced as by-products of combustion of natural non-anthropogenic and anthropogenic compounds, especially chlorine containing plastics (Schechter *et al.*, 1983). They are also produced during the incineration of waste and the production of chlorinated herbicides.

The production of polychlorinated naphthalenes (PCNs) began in World War 1 in Germany where they were used as waxes (Brinkman & Reymer, 1976). PCNs share many properties with PCBs, like their flame-retardant properties as well as their stability. Higher chlorinated PCNs form in combustion products such as fly ash and flue gas from waste incinerators (Falandysz, 1998). Polybrominated biphenyls (PBBs) are chemically very similar to PCBs. Production of PBBs began in the 1970s and were used mainly as flame retardants in thermoplastics of housing or office equipment (Hardy, 2002). A North American ban was put on the manufacture of PBBs in 1976 due to an accidental PBB contamination of animal feed in Michigan, which resulted in adverse health effects in humans who consumed meat contaminated with PBBs. These adverse health effects were similar to those seen from poisoning by PCBs and polyhalogenated aromatic hydrocarbons (PHAHs). Table 1 lists relevant POPs and their use and status in North America and Canada today.

Table 1: List of chemicals, their uses and status in Canada/North America.

| Chemical | Uses | Status |
|--|---|--|
| Aldrin | Agricultural and soil pesticide | Most uses banned since 1975 |
| Chlordane | Agricultural insecticide Termite insecticide | Banned since 1995 (Commissions for Environmental Cooperation in North America) |
| Dieldrin | Agricultural and soil pesticide | Most uses banned since 1975 |
| DDT | Agricultural and antimalarial insecticide | Banned in North American in 1970's Still used in developing countries to combat malaria (P. Fernandez & Grimalt, 2003) |
| Endrin | Insecticide | No longer used in North America |
| Heptachlor (heptachlor epoxide) | Agricultural, soil and garden insecticide | Production ended in 1980's (Agency for Toxic Substances and Disease Registry, 2007) |

| | | |
|--|---|--|
| Hexachlorobenzene (HCB) | No commercial use | <p>Canadian commercial use discontinued in 1972</p> <p>Released as by-products in manufacture of chlorinated pesticides and solvents</p> <p>(Environmental Canada, 2002)</p> |
| Lindane (γ-HCH) | Non-prescription drug for treatment of lice and scabies | <p>Banned for use as a pesticide in Canada</p> <p>(Government of Canada, 2007a)</p> |
| Mirex | Fire ant insecticide and fire retardant | <p>Banned in United States in mid-1970's</p> <p>Never used in Canada</p> <p>(United Nations Environmental Programme, 2006)</p> |
| <i>cis/trans</i>-nonachlor | No commercial use | <p>Component of Chlordane</p> <p>(Commissions for Environmental Cooperation in North America)</p> |
| OCS | No commercial use | Emitted as a by-product in fly ash |

| | | |
|---------------------|--|--|
| | | from waste incinerators |
| PCBs | Electric capacitors and transformers | Banned in 1977 (Hardy, 2002; Ross, 2004) |
| PBBs | Flame retardants | Banned in 1976 (Hardy, 2002) |
| PCDDs (TCDD) | No commercial use | By-product of waste incineration and bleaching of wood pulp By-product of the manufacture of the herbicide: 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and the antibacterial: hexachlorophene, both now banned from production |
| PCDFs | No commercial use | Accidental by-products of natural combustion, waste incineration and manufacture of herbicides (Schechter <i>et al.</i> , 1983) |
| PCNs | Waxes, oils, fuels, mothballs, solvents in | Still in use |

| | | |
|--|---|---|
| | pharmaceutical and agricultural products | |
| QCB | Pressure and thermal treatment of railway ties, utility poles, pilings and outdoor construction material | Not produced in Canada Impurity in several fungicides, insecticides and herbicides used in Canada (Government of Canada, 2007b) |
| Toxaphene | Insecticide | Banned in the United States in 1990 (ASTDR, 2007) |
| Tetrachlorobenzene (1,2,3,4-TCB; 1,2,4,5-TCB) | No commercial use in Canada Used in the United states as intermediates in the production of herbicides, fungicides, defoliants and insecticides | Not produced in Canada (Health Canada, 2004) |

1.1.3 Toxicity of POPs

POPs are readily absorbed from the gastrointestinal tract and less readily absorbed through the skin and lungs. The main source of exposure in humans to these chemicals is through ingestion of contaminated foods, especially high fat content foods including many species of fish, meat and dairy products. However, humans can also be exposed to POPs through inhalation, and dermal contact with the chemicals. In the body, POPs accumulate in lipids, which make up approximately 70% of white adipose tissue which represents 15 - 20% of body weight in a lean individual and can represent more than 50% of body weight in obese individuals. Hence, the body can act as a major reservoir for many different POPs (Covaci *et al.*, 2002; Mullerova & Kopecky, 2007). When a person loses weight, the concentration of POPs in the reduced white adipose tissue is increased (Jandacek *et al.*, 2005). Likewise, the elimination rate of POPs is decreased significantly when a person gains weight and their body fat content increases (J. E. Michalek & Tripathi, 1999). POPs are also stored in other fatty tissue in the body such as the liver. In animal studies with rats, guinea pigs and hamsters, the liver has been confirmed as the primary site for TCDD distribution, sometimes reaching 50 times the concentrations found in other tissues (Rose *et al.*, 1976).

POPs have also been measured in breast milk of women throughout the world and can be passed on to breastfed infants (Gladden *et al.*, 1999; Hooper *et al.*, 1999; Fangstrom *et al.*, 2005). Studies have also shown that POPs can cross the placenta and enter fetal circulation. For example, Saxena *et al.* (1981) found concentrations of OC pesticides in maternal blood, the placenta and umbilical cord blood. However, they reported higher

concentrations in maternal blood than in the placenta and umbilical cord blood, which allowed them to conclude that mothers had the highest body burden of POPs, but that there is a partial transfer of POPs to the fetus (Saxena *et al.*, 1981). Another study by Whyatt *et al.*, 2003, reported a significant correlation between measured serum concentrations of OC pesticides in umbilical cord blood and mother's blood.

POPs are not readily metabolized in the body, and therefore tend to have long elimination half-lives. The elimination half-life of TCDD is approximately 7 years in humans; 22 - 42 days in the guinea pig; 23 - 31 days in the rat and 10 - 12 days in the hamster (J. E. Michalek & Tripathi, 1999; Neal *et al.*, 1982). The $T_{1/2}$ for PCBs vary for each PCB congener. The more chlorine atoms attached to chlorinated POPs such as PCDDs, PCDFs and PCBs, and the more bromine atoms attached to PBBs, the more persistent they are in the body. Toxicity for those chemicals that act via the aryl hydrocarbon receptor (AHR) signalling pathway (see below) is dependent upon the affinity of the POP for the AHR. For example PCB congeners that contain fewer chlorine atoms are more water soluble, and are more easily metabolized by the cytochrome (CYP) P450, while PCB congeners with high numbers of chlorine atoms are more resistant to biodegradation and therefore tend to bioaccumulate.

Table 2: Elimination half-life of selected POPs in humans.

| Chemical | Half-life in Humans |
|-------------|---|
| DDT | 7 – 11 years (Rogan & Chen, 2005) |
| PCBs | 5 – 17 years (Wolff <i>et al.</i> , 1992) |
| PCDDs/PCDFs | 5 – 10 years (Vanden Heuvel & Lucier, 1993) |
| TCDD | \approx 7 years (Neal <i>et al.</i> , 1982; J. E. Michalek & Tripathi, 1999) |

In most species, polar metabolites are formed over a long period of time and excreted in urine or bile and feces (Piper *et al.*, 1973). For unmetabolized TCDD, the main route of elimination is through the feces (Piper *et al.*, 1973; Rohde *et al.*, 1999). POP concentrations measured in bile lipids are in the same ranges as those concentrations measured in serum lipids. These are indicative of POP concentrations in feces since biliary excretion is included in fecal excretion because bile concentrations are secreted into the small intestine (K. Kitamura *et al.*, 2001b). PCDDs, PCDFs and PCBs have also been found to be eliminated in the sebum secretions of hair follicles (Iida *et al.*, 1999; Kitamura *et al.*, 2001a). Elimination rates of TCDD in both humans and animals vary with body concentrations; those with high body concentrations have a faster elimination rate than those with low body concentrations (Aylward *et al.*, 2005).

Many POPs form metabolites that are biologically active. For example, DDT is metabolized in living organisms to DDE which is also lipophilic and toxic (Arctic Monitoring Assessment Programme (AMAP), 2004a). PCBs are also metabolized to more polar metabolites that may be more persistent and biologically active (Connor *et al.*, 1997). Likewise, polycyclic aromatic hydrocarbons (PAHs) are metabolized to form reactive intermediates that covalently bind to macromolecules such as DNA and proteins (Arctic Monitoring Assessment Programme (AMAP), 2004a). The microsomal metabolism of CYP P450 monooxygenase system is the most important pathway for the oxidative metabolism of POPs. Exposure to, or treatment with, PCB congeners induce isoenzymes of different CYP subfamilies. Thus, PCB congeners that are co-planar can induce CYP 1A1, 1A2, and 1B1 via the AHR and non-planar PCB congeners induce CYP 2A1, 2B1 and 2B2 isozymes via other mechanisms (Spink *et al.*, 2002). Like POPs,

some PCB congeners (those that are co-planar) act through the AHR while others act as endocrine disruptors. PCB congeners with chlorine atoms in the *meta* and *para* positions tend to have a co-planar configuration and act like TCDD, the highest affinity ligand for the AHR currently known. These PCB congeners are known as dioxin-like PCBs. The majority of PCB congeners are thought to be endocrine disrupting compounds (EDCs), acting differently from TCDD. These PCBs congeners have chlorine atoms in the *ortho* position and are known as non-planar PCBs. The *ortho*-chlorinated PCB congeners are found more abundantly in the environment than polar PCBs (Soontornchat *et al.*, 1994).

1.1.4 The Aryl Hydrocarbon Receptor (AHR)

Co-planar PCBs, TCDD, PCDDs and PCDFs act through the AHR, a ligand-dependent transcription factor that regulates the expression of many different genes, causing a pleiotropic response in many different species of animals (Denison & Nagy, 2003a). Most hydrophobic aromatic hydrocarbons (e.g. PAHs, such as benzo(a)pyrene) and PHAHs, such as PCBs, PCDDs, PCDFs) are AHR ligands. The structure-activity relationships among PHAHs to bind to the cytosolic AHR receptor and induce aryl hydrocarbon hydroxylation (AHH) activity show that the affinity of the compound for the AHR accurately predicts the rank order of the toxicity of compounds in cultured cell systems. This data indicate that it is most likely the parent compounds and not resulting metabolites that produce toxicity and that their predominant toxic effects are mediated through interaction with the AHR (Poland & Knutson, 1982).

Many studies have been performed to determine how the AHR regulates gene expression. The induction of CYP-1A1 is frequently used as an end-point for these

studies because this AHR-dependent response has been observed consistently in virtually all animal species (Denison & Nagy, 2003a). PAHs and co-planar PHAHs induce CYP-1A1 (an important xenobiotic metabolizing enzyme) by activating the transcription of the CYP-1A1 gene (Mandal, 2005). TCDD is the most potent inducer of CYP-1A1, partly because it is very poorly metabolized by the CYP monooxygenases. The AHR has a finite capacity for its ligands, causing it to be saturated at low concentrations of TCDD, therefore allowing a maximum inductive response at very low doses of this chemical (Vickers *et al.*, 1985).

When TCDD enters the cell by diffusion (Fig. 3: 1.) due to its lipophilicity, it binds with extremely high affinity to the cytosolic AHR (Fig. 3: 2.). Once the ligand is bound, the AHR undergoes a conformational change, which exposes a nuclear localization sequence(s) and allows the ligand-AHR complex to translocate to the nucleus (Fig. 3: 3.) (Denison & Nagy, 2003a). The AHR is also associated with another protein known as immunophilin homolog hepatitis B virus X-associated protein 2 (XAP2), which displays structural similarity to the glucocorticoid receptor-associated immunophilin (FKBP52), except for the fact that it does appear to bind immunosuppressant drugs (Carver *et al.*, 1998; Petrulis *et al.*, 2000). XAP2 appears to act as a chaperone complex, as it is generally found bound to heat shock protein 90 (hsp90) in cells. As well, overexpression of XAP2 in cells has been shown to enhance cytoplasmic AHR levels, suggesting that the amount of XAP2 available to interact with AHR may limit the AHR steady-state levels (Petrulis *et al.*, 2000). XAP2 has also been shown to stabilize and enhance cellular levels of AHR in mice, as well as transient expression of XAP2 sequesters mouse AHR in the cytoplasm and may be involved in the regulation of intracellular movement of AHR

(Petrulis *et al.*, 2003). XAP2 is a core component of the inactive, cytosolic AHR complex with the AHR ligand-binding unit and a dimer of hsp90 (Petrulis *et al.*, 2000). The AHR is in an inactive form as a large protein complex that contains 95 kD and hsps. When the ligand binds to the AHR, the hsps dissociate from the complex in a step known as the activation step (Fig. 3: 4.) (Perdew 1988; Wilhelmsson *et al.*, 1990).

The ligand (TCDD or other PAH or PHAH) is then released from the AHR and the AHR dimerizes with the so-called AHR nuclear translocator protein (ARNT) (Fig. 3: 5a.), which converts it into an active transcription factor that binds to sites on DNA (Mandal, 2005). The AHR molecules that do not undergo dimerization or fail to bind to DNA are removed from the nucleus (Fig. 3: 5b.). Once the AHR:ARNT complex has bound to the specific DNA recognition motifs known as dioxin-response elements (DRE) (Fig. 3: 6.) or xenobiotic response elements (XRE), the transcription of CYP-1A1 (Fig. 3: 7.) and other AHR-responsive genes (Fig. 3: 8) are stimulated (Denison & Nagy, 2003a). Other genes important in drug or chemical metabolism that are induced by AHR signalling are CYP-1A2, CYP-1B1, glutathione-S-transferase A1 (GSTA1), NADP(H)-quinone oxidoreductase-1 (NQO1), UDP-glucuronosyltransferase 1A2 (UGT1A2) and aldehyde dehydrogenase 3A1 (ALHD3A1). These responses may result in important toxication and detoxication effects where CYP-1A1, 1A2, or 1A3 are involved in the metabolic activation of a chemical mutagen or carcinogen. For example, with the PAH, benzo(a)pyrene, increased toxicity will result. On the other hand, when the induced gene codes for a protein that functions in detoxification, AHR signalling can decrease the toxicity of chemicals by decreasing the amount able to react with critical macromolecules, including DNA, lipid, or protein.

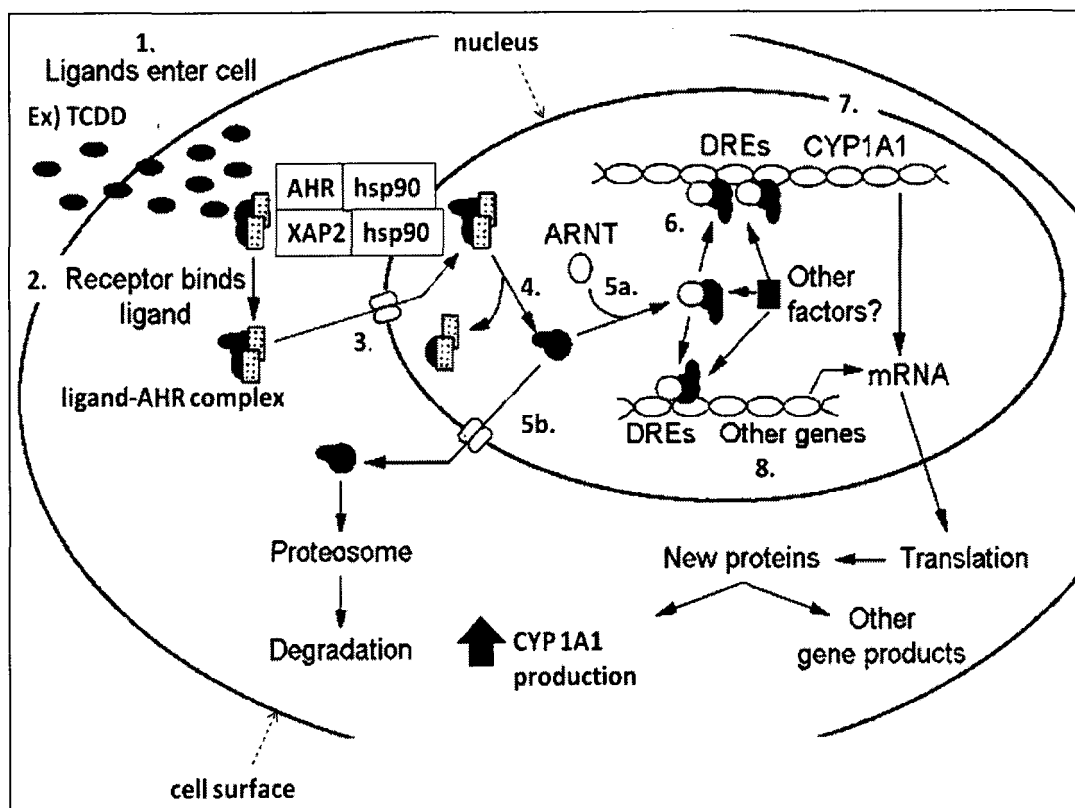


Figure 3: AHR Signalling Pathway (adapted from (Denison & Nagy, 2003b)).

1.1.5 Endocrine Disruptors

EDCs are exogenous compounds that can change endocrine function and cause adverse effects. Some PCDDs, PCDFs, and PCBs also act as EDCs. There is a similarity between endogenous hormones such as glucocorticoids and these chemicals or cross-talk between the signalling systems they activate. The ability of these POPs to act as EDCs has a lot to do with the similarity between their structures and the endogenous hormones. Figure 4 shows the structures of natural and synthetic estrogens, which are similar to many POPs structures.

PCBs that act as EDCs can do so either because the parent PCB induces enzymes via a mechanism where the AHR interacts with steroid hormone mediated transcription, for example, through shared co-activators or co-repressors (Safe *et al.*, 1995) or because the action of a metabolite mimics the action of the steroid hormone (Garner *et al.*, 1999). The most common type of EDCs are known as estrogen-like endocrine disrupting chemicals (EEDC). In short, these chemicals (PCBs, PBDEs and organic pesticides) are very structurally similar to estrogen and mimic estrogen's action in the body by binding to its receptors. In this way they can alter the synthesis, metabolism, binding, transport or any other cellular responses natural to estrogens (Cooper & Kavlock, 1997).

For example, some PCB metabolites are structurally very similar to estradiol and can bind to the estrogen receptor (ER) forming a hydroxy PCB metabolite-ER complex. This complex will translocate to the nucleus and bind to ER response elements and activate transcription or ER-regulated genes (Garner *et al.*, 1999). In female rats, PCBs have been shown to have a higher binding affinity than endogenous estrogen to estrogen

receptor β (ER β) (Salama *et al.*, 2003). EDCs that can bind to estrogen receptors not only block endogenous estrogens from binding, but also can stimulate estrogenic responses of their own (Kuiper *et al.*, 1998). Hydroxylated PCBs, DDT and its metabolites, specific herbicides and pesticides such as Kepone and methoxychlor and plastics such as bisphenol A all bind weakly to the ER α (Kuiper *et al.*, 1998). Both ER α and ER β can bind a large number of xenoestrogens. In absence of a ligand, the ER is sequestered within the nuclei of target cells in an inactive state. Once a ligand binds, a conformational change occurs within the ER which promotes homodimerization and high binding affinity to specific DNA response elements (EREs) (Hall & Korach, 2002). Even xenoestrogens with a weak binding affinity (1000 fold less than estrogen) can induce conformational changes and cause transcription to occur (Kuiper *et al.*, 1998).

Another chemical that has estrogenic activity is *o,p'*-DDT, which mimics the action of 17 β -estradiol (E2) in various species. Moreover, *p,p'*-DDT has also been shown to cause weak estrogenic activity in animals. There are 3 main estrogens produced by the body. Estradiol (E2) is the most predominant form in non-pregnant women, while estrone (E1) is produced during menopause and estriol (E3) is produced during pregnancy (Fig. 4). When compared to other DDT metabolites, *p,p'*-DDE had the strongest binding affinity to ER α , with a binding affinity similar to that of synthetic oestrogen, and diethylstilboestrol (a synthetic nonsteroidal estrogen used as a drug), but approximately 30 times weaker than natural oestrogen and 17 β -estradiol (Robison *et al.*, 1985; Kuiper *et al.*, 1998). It is hypothesized that DDT acts as an EDC in 3 ways. First by its ability to react with the ER, therefore changing intracellular signalling and causing a stimulation in estradiol synthesis (Tiemann *et al.*, 1996; Jaga 2000; Andersen *et al.*,

2002); secondly through induction of the activity of enzymes involved in estradiol biosynthesis (Crellin *et al.*, 1999; Vinggaard *et al.*, 2000); and thirdly, similar to PCBs, they can inhibit the breakdown of estradiol causing an increased bioavailability of estradiol (Garner *et al.*, 1999; Kester *et al.*, 2000; Kester *et al.*, 2002).

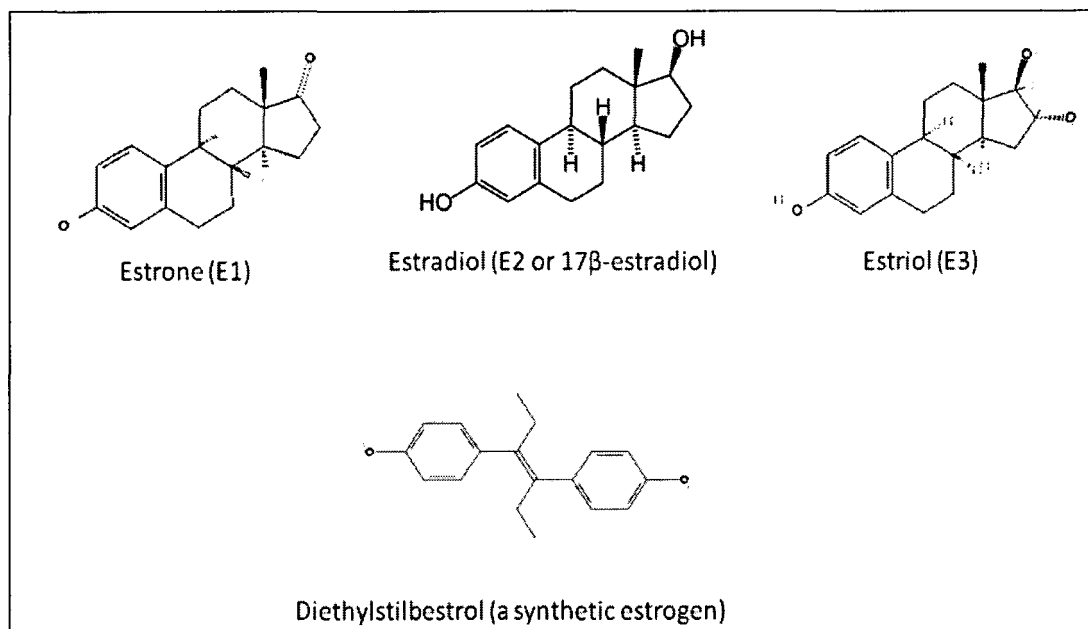


Figure 4: Chemical structures of natural and synthetic estrogen.

Both DDT isomers showed the ability to block androgen action at the level of the androgen receptor (AR) in monkey kidney CV1 cells (Cooper & Kavlock, 1997). *p,p'*-DDE was a stronger androgen inhibitor than *o,p'*-DDT. Cooper *et al.* (1997) also determined that inhibition of androgen activity was not the result of *p,p'*-DDE toxicity below treatment concentrations of 5 μ M (Cooper & Kavlock, 1997). Likewise, *o,p'*-DDT was not cytotoxic until concentrations below 5 μ M, whereas the metabolite, *p,p'*-DDD was cytotoxic at concentrations greater than 1 μ M in this cell culture system (Cooper & Kavlock, 1997). The antagonistic effects of DDT isomers on the male reproductive system appear to be mediated through inhibition of AR-androgen binding and the following inhibition of transcriptional activity (Cooper & Kavlock, 1997).

1.1.6 Adverse Health Effects of POPs

POPs have been implicated in causing a large number of adverse health effects in humans. It has been suggested that EDCs cause cryptorchidism (which is the failure of one or both testes to descend into the scrotum) in offspring of female rats exposed *in utero* to estrogenic and antiandrogenic compounds (Grocock *et al.*, 1988). Weidner *et al.* (1998) also found a significantly increased risk of cryptorchidism in male offspring of female gardeners. Fernandez *et al.* (2007) reported a significant relationship between urinary tract malformations (such as hypospadias) at birth and xenoestrogens, including *p,p'*-DDT, *o,p'*-DDT and Mirex. Exposure to POPs that are endocrine disruptors has also been associated with other reproductive disorders and reproductive cancers including testicular cancer and breast cancer. Cryptorchidism is a known risk factor for testicular

cancer and therefore, *in utero* exposure to xenoestrogens may cause cryptorchidism at birth and, later on in life, contribute to testicular cancer (Sharpe & Skakkebaek, 1993).

Cohn *et al.* (2007) reported that exposure to high concentrations of *p,p'*-DDT (a median of 17 years prior to diagnosis) predicted a 5-fold increased risk of breast cancer among women born after 1931 in California who lived during the peak period of DDT use in this area. The proposed mechanism for this association with breast cancer is the estrogenic activity and cytotoxicity of *p,p'*-DDT. Genotoxicity is one possible mechanism, because DNA damage has been reported in the literature for DDT-exposed women and children, as well as in *in vitro* experiments where cells were treated with *p,p'*-DDT or *o,p'*-DDT (Yanez *et al.*, 2004; Perez-Maldonado *et al.*, 2006).

Exposure to endocrine disruptors such as DDT, have also been associated with impaired semen quality in males exposed in the environment (Aneck-Hahn *et al.*, 2007). Guillette *et al.* (1995) reported ambiguous gonads (ovotestes) in turtles, abnormal sex hormone concentrations, poorly organized testes, and small penises in male alligators in Lake Apopka Florida in 1995 following a pesticide spill that occurred in the 1980's (Guillette *et al.*, 1995).

Exposures to PCBs are known to reduce cognitive function in both animals and humans. In the rat brain, *ortho*-substituted, non-planar PCBs inhibit the vesicular uptake of dopamine and serotonin (Mariussen *et al.*, 1999). This is postulated to lead to a decrease of dopamine concentration in nervous tissue, adversely effecting cognition, voluntary movement, motivation, sleep, mood, attention and learning. In a Dutch birth cohort of 209 breastfed infants, prenatal PCB exposure was associated with neonatal

hypotonia and decrements in psychomotor developments (Huisman *et al.*, 1995). Studies in a Michigan cohort evaluating prenatal exposure to PCBs reported a positive relation between degree of exposure and poor attention and impulse control at ages 4 and 11 (Jacobson *et al.*, 1990; Jacobson & Jacobson, 2003).

Exposure to POPs has also been associated with cardiovascular disease. Shcherbatykh *et al.*, (2005) reported an increased rate of hospitalization for stroke, particularly ischemic stroke in people living in close proximity to hazardous waste sites in New York State. Goncharov *et al.*, (2008) also found a relationship between exposure to PCBs and pesticides and self-reported cardiovascular disease in a Native American population living downstream of local aluminum foundries operated by General Motors, ALCOA and Reynold Metals. These 3 plants used PCBs as hydraulic fluids and there have been leaks into the St. Lawrence River and tributaries which are the local fishing grounds for the Mohawk Native North Americans living at Akwesasne (Goncharov *et al.*, 2008). Goncharov *et al.*, (2008) suggested that the relationship they found between exposure to POPs and cardiovascular disease could be due to an underlying mechanism by which PCB congeners and chlorinated pesticides increase lipid synthesis in the liver. Likewise, Shcherbatykh *et al.*, (2005) also proposed the mechanism linking cardiovascular disease to people exposed to POPs is through increased serum lipids, which is a known risk factor for atherosclerosis. Serum lipids and plasma triglycerides are known to be elevated in monkeys and rats after exposure to TCDD or PCBs (Rier *et al.*, 2001; Lind *et al.*, 2004b). Another possible explanation for the cardiotoxic effects of POPs is the ability of coplanar PCBs and TCDD to produce reactive oxygen species (ROS) in cells, which can damage endothelial cells, promoting the formation of foam

cells and atherosclerotic plaques and contribute to the development of cardiovascular disease and ischemic stroke (Hennig *et al.*, 2002).

High exposure to several POPs can also cause cancer and the speculated mechanisms are through the AHR, endocrine disruption, and/or the generation of ROS. For example, Mallin *et al.* (2004) reported associations between POPs exposure and female liver/biliary cancer in people who were occupationally exposed to PCNs and PCBs while working at an electrical capacitor manufacturing plant. Another epidemiological study conducted in southeast Michigan reported an association with past use of DDT-related OC pesticides in the general population and pancreatic cancer (Fryzek *et al.*, 1997).

Although POPs have been associated with different types of cancer, TCDD is the most carcinogenic of these chemicals. Kociba *et al.*, (1978) conducted a 2 year study on chronic exposure to TCDD in rats and found that continuous ingestion of 0.1 μg of TCDD/kg/day resulted in increased mortality, decreased body weight gain, slight depression of certain hematologic parameters, and morphologic changes primarily in the liver, lymph, lungs and vascular tissue (Kociba *et al.*, 1978). This TCDD treatment regiment also caused an increased incidence in hepatocellular carcinomas of the liver in female rats only and squamous cell carcinomas of the lungs, hard palate/nasal turbinates and tongue (Kociba *et al.*, 1978). This dose was considered a high dose, and Kociba *et al.*, (1978) found that ingestion of an intermediate dose (0.01 μg TCDD/kg/day; approximately 210 ppt in the diet) was less toxic, however, liver toxicity and increased alveolar hyperplasia in the lungs still occurred.

The earliest symptom of environmental poisoning with PCDDs, PCDFs or PCBs is chloracne, an acne-like eruption of blackheads, cysts and pustules most frequently found on the cheeks, behind the ears, in the armpits and groin region (Suskind, 1985). Chloracne was observed in veterans of the Vietnam War who were exposed to Agent Orange (Wolfe *et al.*, 1990); in individuals who consumed PCB and PCDF-contaminated rice oil in Yusho (Reggiani & Bruppacher, 1985); and in highly exposed individuals in Seveso (Caramaschi *et al.*, 1981). One of the most famous cases of chloracne related to TCDD poisoning occurred with the Ukraine President Viktor Yushchenko, who survived an attempted acute oral poisoning with TCDD (Sterling & Hanke, 2005).

In animals where the AHR is truncated, there seems to be increased lethality, wasting, liver damage and bilirubin increase. For TCDD toxicity across all species, the common target organ is the liver. This may be because the AHR plays a key role in normal hepatic development and differentiation and supports the adverse effect of TCDD (Fernandez-Salguero *et al.*, 1995).

POPs are also known to interfere with the thyroid. PCBs are structurally similar enough to thyroid hormones that they can bind to the thyroid hormone receptor and act as agonists or antagonists to produce their effects (McKinney 1989; McKinney & Waller, 1998). Experimental studies in rats have shown that PCBs and other OC pesticides decrease circulating thyroid hormones (THs) during development (Zoeller *et al.*, 2000; Donahue *et al.*, 2004). Hydroxylated PCBs have shown high binding affinity for the serum TH-binding protein transthyretin (TTR), therefore causing the natural ligand, thyroxine (T₄) to be displaced (Cheek *et al.*, 1999). An epidemiological study by Takser *et*

al., (2005) found a significant negative relationship between circulating triiodothyronine (TT₃) levels in pregnant women at low environmental doses of specific PCB congeners, *p,p'*-DDE and HCB. They also reported that *cis*-nonachlor was related to both increased fT₄ and decreased TT₃ in women during their pregnancy (Takser *et al.*, 2005). Depressed thyroid hormones, especially during pregnancy, can cause a multitude of health problems, such as postpartum depression syndrome (Ijuin *et al.*, 1998). A study about fetal exposure to PCBs in a Dutch cohort found hydroxylated PCBs may use TTR to cross the placenta and expose the fetus, as they showed that hydroxylated PCBs bind strongly to TTR (Soechitram *et al.*, 2004). TTR is found in serum and cerebrospinal fluid and is a carrier of thyroxine and retinol.

Another epidemiological study on a community highly exposed to HCB found a significant positive association between HCB and concentrations of thyroid-stimulating hormone (TSH) at birth (Ribas-Fito *et al.*, 2003). A study done on women, and their offspring, who were exposed to TCDD 20 - 30 years prior to giving birth during the Seveso accident, found b-TSH (which is used to screen for hypothyroidism) to be higher in newborns of women with high TCDD concentrations compared to newborns of women who were not exposed (Baccarelli *et al.*, 2008). Neonatal hypothyroidism is a disease where not enough TH is produced, and in some cases, no TH is produced, and can result in symptoms such as constipation, lack of muscle tone, failure to grow, sleepiness, choking episodes and sluggishness and if left untreated can cause severe mental and growth retardation. The over- and under-expression of THs can result in serious health effects, such as hypothyroidism and hyperthyroidism.

Positive associations between exposure to POPs and the metabolic syndrome and T2D have also appeared in the literature and these are discussed in much more detail in Chapter 3 (Lee *et al.*, 2007a; Rignell-Hydbom *et al.*, 2007; Consonni *et al.*, 2008; Lee *et al.*, 2008; J. E. Michalek & Pavuk, 2008).

In summary, there seems little doubt that environmental or occupational exposure to almost any POP will result in an adverse health effect, as long as the extent and duration of exposure are extensive enough.

Chapter 1.2 Heavy Metal Toxicity

1.2.1 General Overview of Heavy Metals

Heavy metals are naturally occurring in the environment and found as part of the Earth's crust. Depending upon chemical form, they can be absorbed and accumulate within living organisms, including humans. Low concentrations of some heavy metals, including iron (Fe) and copper (Cu), are required for homeostasis; however, as with organic chemicals, excessive concentrations of heavy metals cause toxicity. Toxic concentrations can vary for each metal and for each exposed species, frequently depending upon the efficiency of intracellular concentration mechanisms. Exposure of humans to heavy metals has increased concomitant with advancing technology, dating back to initial mining efforts that resulted in the translocation and often concentration of metals and ores from the Earth's crust. Heavy metals have been used carelessly and both the by-products of mining and the end products of manufacture, including batteries and

computers, have been unceremoniously dumped in large quantities, without a thought of the consequence to the environment or human health. Heavy metals are released at alarming concentrations into the air and water, with the potential to contaminate and poison both the water and food supply (Gossel & Bricker, 1994).

Once in the water supply, heavy metals can cause mass poisoning, such as what is currently occurring from arsenic (As) in Bangladesh. This issue arose because of a sanitary problem identified in the 1970's; people living in rural Bangladesh did not have an adequate supply of clean drinking water because of severe microbiological contamination of surface water (Nahar *et al.*, 2008). To rectify this situation, the government and international donor agencies including World Bank and UNICEF, installed millions of tube wells in attempt to access better quality groundwater for drinking (Nahar *et al.*, 2008). In 1993, it was discovered that the water from the wells was contaminated with geologically derived As (Nahar *et al.*, 2008). Natural contamination of drinking water with As is not an uncommon occurrence. It has been documented in many countries such as Mexico (Del Razo *et al.*, 1990), Argentina (Perez-Carrera & Fernandez-Cirelli, 2005), India (Ahamed *et al.*, 2006) and parts of the United States (Twarakavi & Kaluarachchi, 2006). However, the number of people at risk from As poisoning in Bangladesh makes this issue unique. In 2000, 60 million people were drinking water with As concentrations higher than the US EPA standard and many were developing symptoms of As poisoning such as dyspigmentation, keratoses and skin cancers (Sambu & Wilson, 2008). Although estimations vary, it is currently expected that more than 1 million people will experience adverse health symptoms before this As poisoning is eradicated, and that between 100 000 and 1 000 000 people will die from

compromised health effects (Sambu & Wilson, 2008). This is just one example of how heavy metals can adversely affect the health of humans.

Another example of a heavy metal poisoning occurred in Japan in the late 1940's from ingestion of rice contaminated with cadmium (Cd) (Kazantzis, 2004). Cd sludge dumped in the Jinzu river basin from zinc mines was taken up by rice plants and then entered the diet of the local Japanese through consumption of the contaminated rice (Inaba *et al.*, 2005). The water from the river was also used as an irrigation source for the rice fields, and therefore the Cd had a pathway to enter the food chain and poison approximately 400 potential victims (Nordberg, 2004). Cd concentrations found in the contaminated rice were 0.68 mg/kg (Kazantzis, 2004). The resulting disease of chronic Cd poisoning became known as Itai-Itai ("ouch-ouch") disease because of pain.

1.2.2 Sources and Uses of Heavy Metals

Heavy metals are naturally occurring throughout the environment, however, the significant quantities of heavy metals used in commerce seen in the environment today, are not natural, but are generally caused by anthropogenic sources such as industrial processes, agricultural practices, transportation and waste disposal (Arctic Monitoring Assessment Programme (AMAP), 2004b) that require concentrated metals for end products or they are generated as concentrated by-products. There appear to be 3 main anthropogenic sources of heavy metals to the atmosphere: fossil fuel combustion (by-products), non-ferrous metal production (for end products) and waste incineration (by-products). Fossil fuel production encompasses a wide range of combustion processes which include combustion of leaded, low-leaded and unleaded gasoline, the major source

of atmospheric Pb emissions and oil combustion and the major source of nickel (Ni) (Arctic Monitoring Assessment Programme (AMAP), 2004b). Non-ferrous metal production is a major source of heavy metals including As and Cd (Arctic Monitoring Assessment Programme (AMAP), 2004b) that are documented environmental toxins. However, due to more stringent regulations and improved technology to decrease emissions in Europe and North America, this source of pollution has been on the decline since the 1980s in these jurisdictions.

Table 3 shows a few chemical plants located in and around Sarnia and their release of heavy metals into air in 2008 as reported by the companies to the National Pollution Release Inventory (NPRI). Cd appears to be the most commonly released heavy metal by these plants. However, As, Ni, Pb and zinc (Zn) also are released.

Table 3: Heavy metals released into the air from factories located in and around Sarnia. (Environmental Canada, 2009).

| Company | Heavy Metal | On-Site Release | Units |
|---|---------------------|-----------------|--------|
| Imperial Oil Sarnia Chemical Plant | Cadmium | 3.2 | Kg |
| | (and its compounds) | | |
| | Lead | 8.1 | Kg |
| | (and its compounds) | | |
| | Nickel | 0.016 | Tonnes |
| | (and its compounds) | | |
| | Zinc | 0.315 | Tonnes |
| | (and its compounds) | | |
| Imperial Oil Sarnia Refinery Plant | Cadmium | 11.0 | Kg |
| | (and its compounds) | | |
| | Lead | 38.0 | Kg |
| | (and its compounds) | | |
| | Nickel | 22.0 | Tonnes |
| | (and its compounds) | | |
| | Zinc | 0.380 | Tonnes |
| | (and its compounds) | | |
| Shell Canada | Arsenic | 26.0 | Tonnes |
| | (and its compounds) | | |
| | Cadmium | 12.0 | Kg |
| | (and its compounds) | | |
| | Lead | 48.0 | Kg |
| | (and its compounds) | | |

| | | | |
|--|--------------------------------|------|--------|
| | Nickel | 6.2 | Tonnes |
| Suncor Energy Products Inc. | Cadmium (and its compounds) | 12.0 | Kg |

Some heavy metals, such as As, are used in agriculture as herbicides, although this use is declining in developed countries where heavy metal-containing products are being replaced by more selective and expensive pesticides. As is used as a herbicide in its methylarsonic and dimethylarsinic acid forms. In addition, nitro- and aminobenzenearsenic acids are commonly used as feed additives in the poultry industry (Jackson & Bertsch, 2001).

Some heavy metal salts have also historically been used as food additives. For example, in early Greco-Roman times lead (Pb) acetate was used to sweeten wine and to prevent spoiling of wine by interfering with enzymatic activity (Lessler, 1988).

Antimony (Sb) is used in metal alloys, storage batteries, solder, sheet and pipe metal, ammunition, metal bearings, castings, pewter, as well as a fire-retardant in textiles and plastics (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). Sb is used as a catalyst in the production of polyethylene terephthalate (PET) plastic bottles, common containers for storage and delivery of drinking water. Sb has been shown to leach from the plastic, contaminating the liquid being stored and exposing humans who consume this liquid, usually water (Westerhoff *et al.*, 2008). Beryllium (Be) compounds are used as metal alloys in the production of cars, computers and aircrafts, as well as in sports equipment such as golf clubs and bike frames (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). Cd is produced mainly as a by-product during the processing of zinc-containing ores and during the refining of Pb and copper (Cu) from sulphide ores however, commercially, the main use of Cd is in the manufacture of batteries (Centers for

Disease Control and Prevention & Department of Health and Human Services, July 2005). Pb has a wide variety of commercial uses, in the manufacture and storage of batteries, solders, metal alloys, plastics, leaded glass, ceramic glazes, ammunition, and shielding for protection of radiation (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). Until recently, it was used as an anti-knock additive to gasoline and became an important environmental contaminant especially for those in close proximity to roadways with heavy vehicular traffic. In this context, interstate highways that ran through urban settings were of special concern for the neurotoxicity of Pb in young children (Annest *et al.*, 1983).

Some heavy metals are used in the medical industry either as therapeutic agents or as part of medical and diagnostic equipment. More specifically, sodium stibogluconate and antimony potassium tartrate are used as antiparasitic drugs, (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). The platinum (Pt) compounds cisplatin and carboplatin are used in drugs for the treatment of ovarian and small cell lung cancer (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005; Sculier & Moro-Sibilot, 2009). Because of its extremely low solubility in water and high density, barium (Ba) sulphate is ingested and used as contrast medium for radiographs of the gastrointestinal tract (Centers for Disease Control and Prevention & Department of Health and Human Services July 2005). Be is used in medical instruments such as x-ray machines and dental bridges (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005).

Naturally occurring sources of heavy metals include: volcanic releases, soil-derived dusts, sea salt aerosols and forest fires (Arctic Monitoring Assessment Programme (AMAP), 2004b). Ba comprises approximately 0.05% of the Earth's crust and is found in certain foods, such as Brazil nuts (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). Be is a rare element on Earth and in the atmosphere but is found in trace quantities in mineral rocks, coal, soil and volcanic dust (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). Pt is also extremely rare, occurring as only 0.003 ppb in the Earth's crust (Strnad *et al.*, 2008). Of toxicological significance, organic metals and metalloids are formed in the environment through biomethylation of the heavy metal in its elemental state by microorganisms. For example, biomethylation of inorganic Sb has been shown to occur by an aerobe fungus *S. brevicaulis* (Dodd *et al.*, 1996; Craig *et al.*, 1999). Cd undergoes biomethylation and occurs naturally in its organic form in waters of the southern Atlantic Ocean (Dodd *et al.*, 1996; Pongratz & Heumann, 1996). As is also biomethylated and found as methylarsenicals in water (Cullen & Reimer, 1989; Dodd *et al.*, 1996).

1.2.3 Toxicity of Heavy Metals

This thesis focuses on 15 heavy metals: silver (Ag), aluminum (Al), As, Ba, Be, bismuth (Bi), Cd, Pb, palladium (Pd), Pt, Ni, Sb, titanium (Ti), thallium (Tl) and uranium

(U). Heavy metals identified to be of specific toxicological concern in this thesis are As, Cd and Pb.

Arsenic (As)

Humans are generally exposed to As through consumption of contaminated drinking water and food. Because of its presence in food, the Joint FAO /WHO Expert Committee on Food Additives has set a probable tolerable weekly intake (PTWI) of 0.015 mg/kg body weight (BW) (JECFA, 2001). Oral consumption of 70 – 180 mg of arsenic oxide (As_2O_3) in humans is typically fatal in less than 1 h (Jones, 2007). For chronic exposure to As, sensitive individuals will display characteristic signs of As poisoning around 20 $\mu\text{g/kg/day}$ BW, however it has been found that some individuals can ingest over 150 $\mu\text{g/kg/day}$ BW without displaying any adverse health effects (Jones, 2007).

As is found as inorganic (arsenite and arsenate) compounds or organic (arsenobetaine) compounds (Navas-Acien *et al.*, 2008). As primarily occurs in the environment as pentavalent arsenate (As^{+5}), trivalent arsenic (As^{+3}) and natural organoarsenicals. As^{+5} is the main form of As in the environment and is found in equal amounts of HsAsO_4^- and HAsO_4^{2-} . Trivalent arsenicals are the most toxic form of As (Crecelius, 1977; Gebel, 1997).

Approximately 60% of As^{+5} is absorbed from the gastrointestinal tract, compared to 80% of As^{+3} and 100% of natural organoarsenicals, which then enter the blood stream (Gebel, 1997; Caussy & Priest, 2008). After absorption, around 90% of inorganic arsenic

is cleared from the blood, with a $T_{1/2}$ of 1 - 2 h (Cohen *et al.*, 2006). The $T_{1/2}$ of As in the body is 30 – 60 h (Crecelius, 1977).

As compounds vary in their metabolism and disposition, depending on the specific compound, exposure route and affected species (Vahter & Marafante, 1983). Inorganic As compounds are metabolized to methylarsonate and dimethylarsinate and excreted in urine along with unchanged inorganic As (Navas-Acien *et al.*, 2008). In mammals, As is metabolized by reduction, glutathione (GSH) conjugation and methylation (Thompson, 1993). Methylation of As generally occurs in the liver and varies quantitatively in different species. In humans, less monomethylarsonic acid (MMA) is metabolized to dimethylarsinic acid (DMA) than in other mammals (Gebel, 1997). As that enters the blood is excreted mainly in the urine, in the form of As^{+3} , As^{+5} , MAA and DMA, as well as other organically bound arsenic compounds (Crecelius, 1977). Organic As is also excreted in the urine unchanged and is generally considered to be nontoxic.

In the body, As accumulates in vascularised organs and tissue, primarily the kidney, liver, lungs and muscle (Pomroy *et al.*, 1980; Vahter & Marafante, 1983) and to a lesser degree in hair, nails and skin (Lin *et al.*, 1998). As has also been shown to replace P and accumulate in the bone, where it can remain for long periods of time (Kimpe *et al.*, 1996).

In humans, approximately 40-70% of As exposure is absorbed, metabolized and excreted within 48 h of initial exposure (Cohen *et al.*, 2006). As can also be eliminated through the feces, sweat, skin desquamation, and by incorporation into hair and nails (Jones, 2007). The As^{+3} intermediates MMAIII and DMAIII of inorganic As were once

thought to be part of the detoxication process of As, but have now been shown to be spatially different from the As^{+3} compounds and to be highly reactive with a possible causative role in the carcinogenicity of inorganic As in humans (Cohen *et al.*, 2006).

Cadmium (Cd)

Cd is another heavy metal of toxicological concern. Although the main exposure of Cd for humans is through consumption of contaminated food, inhalation of cigarette smoke is also a significant source of exposure (World Health Organization, 2008).

Upon inhalation of Cd fumes, approximately 10 - 50% may be absorbed, the variance depending on particle size, and chemical composition (Fernandez *et al.*, 1996). Gastrointestinal absorption tends to be less than 10%, but individual absorption can vary depending on dietary habits, and is dependent on iron (Fe) status (Fernandez *et al.*, 1996; Jones, 2007). A possible reason for this dietary dependence of absorption is that Fe and Cd appear to share a common transporter (divalent metal ion transporter; DMT) in mammalian small intestine (Diamond *et al.*, 2003)

Cd is almost exclusively bound to red blood cells (RBCs) while traveling throughout the body (Apostoli, 2002). The concentration of Cd in blood reflects current exposure to Cd; while the concentration of Cd in urine reflects the concentration of Cd in the kidneys. Cd^{2+} enters cells easily through L-type voltage Ca^{2+} channels and receptor mediated Ca^{2+} channels, as both elements have similar size and charge (Henson & Chedrese, 2004). Cd elimination $T_{1/2}$ is approximately 6 weeks in blood, whereas Cd^{2+} has a biological $T_{1/2}$ of 15 – 30 y in organisms who have had chronic exposure to Cd

(Fernandez *et al.*, 1996; Henson & Chedrese, 2004), reflecting a very low rate of excretion from the body.

The main target tissue for Cd is the kidney and liver, accounting for approximately 70% of total body burden (Fernandez *et al.*, 1996; Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). Cd accumulates over time in the blood, kidneys, liver and reproductive organs such as the placenta, testis and ovaries (Henson & Chedrese, 2004). Cd accumulates slowly in the kidneys throughout the years, reaching peak concentrations at 40 - 60 years of age (Diamond *et al.*, 2003). Cd excretion occurs primarily through the kidney into the urine (Fernandez *et al.*, 1996; Apostoli, 2002).

Lead (Pb)

Pb is another heavy metal of toxic concern. Humans can be exposed to Pb through inhalation or ingestion of contaminated water or food. Although Pb is generally not found in tap water, it may dissolve into water from household plumbing systems containing Pb pipes (World Health Organization, 2008). Children are extremely sensitive to Pb, absorbing 4 – 5 times more than adults, and Pb can be transferred through the placenta to the fetus as early as the 12th week of gestation (World Health Organization, 2008). Inorganic Pb can also penetrate the blood brain barrier in children, not adults, making them the most sensitive population to Pb toxicity (Leggett, 1993). The PTWI for lead is 0.025 mg/kg BW; however, toxicity can be seen in children at ranges from 12 – 120 µg/dL in blood, with no evidence of a threshold (World Health Organization, 2008).

Adults generally absorb up to 10 - 15% of Pb from food, compared to children who absorb up to 50% of Pb from food via the gastrointestinal tract (Jarup, 2003). In the body, Pb is bound to erythrocytes during transportation and is initially distributed to soft tissues including the liver and kidneys. Systemic Pb is transported to the liver (10 - 15% of absorbed dose), where it remains for a few weeks. It also concentrates in kidney (15 - 20%) and approximately 2 - 5% of systemic Pb is excreted in urine during the first 24 h after exposure (Leggett, 1993). Pb is also classified as “calcium like” or a “bone-volume-seeking” element. This classification is in part due to the action of Pb, in that it ‘follows’ the movement of Ca in the body and regulators of Ca metabolism also affect Pb in a similar fashion (Leggett, 1993). In bone, Pb competes with Ca for deposition.

The relationship between blood and Pb is not linear, but is curvilinear. At low blood Pb concentrations there is a steady increase in blood Pb, but at high concentrations, the curve plateaus, and blood Pb concentrations change very slightly with increased exposure (Bergdahl & Skerfving, 2008). The $T_{1/2}$ of Pb in blood is approximately 1 m, whereas in bone it is approximately 20 - 30 y (Jarup, 2003). In adults, 80 - 90% of ingested Pb is excreted, while the remaining 10 - 20% is stored in bone (Lessler, 1988). Urine is the primary pathway of elimination for Pb, however minor amounts are eliminated through fecal excretion, sweat and other pathways such as hair, nails, and desquamated skin (Leggett, 1993).

1.2.4 Mechanism of Action of Heavy Metals

Each heavy metal has a novel mechanism of toxicological action in the body. The mechanism of carcinogenic action of As is not completely understood. It is thought to

vary according to tumour site and that multiple interacting mechanisms exist, including interactions with proteins by reaction with sulfhydryl groups, depletion of GSH by reaction with its sulfhydryl group, oxidative damage secondary to the generation of ROS, inhibition of DNA repair mechanisms, cytotoxicity and regeneration (Cohen *et al.*, 2006).

Cd is also thought to cause deleterious effects by deactivating DNA repair activity (McMurray & Tainer, 2003). Mismatch repair (MMR) is a system that efficiently repairs misalignments that lead to frameshift mutations and base-base mismatches in order to prevent base substitutions. Jin *et al.*, (2003) reported that exposure to Cd strongly increased the rate of frameshift mutations and the rate of base substitutions, indicating that repair of all kinds of mismatches was inhibited. They concluded that Cd mutagenesis and the mutator effects of MMR-null alleles showed such a strong similarity that Cd is a new kind of mutagen acting by inhibiting the MMR system rather than through DNA damage (Jin *et al.*, 2003).

One mechanism that some metals have in common, especially As, Cd and Pb, is their ability to create ROS, an ability that they also have in common with POPs. In human-hamster hybrid cells, As³⁺ has been shown to generate O₂⁻ at the concentration 50 µM (Liu *et al.*, 2001). Pi *et al.* (2003) reported that 18 weeks of oral exposure to As⁵⁺ in rabbits resulted in a significant decrease in plasma metabolites and a significant increase in urinary hydrogen peroxide (H₂O₂) level, indicating that exposure to inorganic arsenate in drinking water can reduce systemic nitric oxide (NO) production and potentially increase oxidative stress. As can also generate reactive nitrogen species (RNS); the exact mechanism for generating both ROS and NOS is not well understood. The mitochondria

are the suggested possible source of ROS production (Corsini *et al.*, 1999). Corsini *et al.*, (1999) confirmed that the mitochondria was an important intracellular target of As. When they treated a murine keratinocyte cell line (HEL30 cells) with As 50 μ M, it resulted in rapid and dramatic morphological changes in the mitochondria, such that the organelles lost their internal organization (Corsini *et al.*, 1999).

Cd is another metal shown to produce ROS, however it differs from As because it is not able to generate free radicals on its own. Instead, the free radicals (superoxide radical, hydroxyl radical and NO radicals) are generated indirectly (Galan *et al.*, 2001). Cd has been shown to induce stress, causing intracellular oxidation and apoptosis (Galan *et al.*, 2001). Cd has also been shown to induce anaemia and pre-treatment of vitamin E (an antioxidant) prior to Cd exposure can decrease the toxic effects of Cd on the haematological values, therefore providing a protective role in anaemia (Ognjanovic *et al.*, 2003).

Pb toxicity has also been associated with oxidative stress and increased production of ROS. There appear to be a couple of mechanisms by which Pb induces oxidative stress, one main pathway being the heme synthesis pathway by inhibiting the heme and haemoglobin synthesis, or by changing the RBC morphology and survival (Flora *et al.*, 2008). Adegebesan *et al.*, (2005) reported that Pb exacerbates liver lipid peroxidation in protein undernourished rats which could be due to the involvement of free radicals in the pathogenesis of Pb poisoning. Another study by Bokara *et al.*, (2008) reported that Pb exposure resulted in oxidative stress, seen by the increase in lipid peroxidation products (LPPs) in the brain. As well, they found that Pb disturbed the pro-

and anti-oxidative balance in the brain causing oxidative stress and an increase in LPP and antioxidant enzymes in the hippocampus and cerebellum, compared with the frontal cortex and brain stem, which could explain the neurotoxicity of Pb (Bokara *et al.*, 2008).

1.2.5 Adverse Health outcomes of Heavy Metals

Inorganic As is acutely toxic, with exposure to large concentrations causing gastrointestinal symptoms, such as vomiting, abdominal pain and diarrhoea; severe disturbances of the cardiovascular system and central nervous systems; and death (Jarup, 2003; Jones, 2007). Chronic exposure to As is associated with hyperpigmentation, keratosis, and cancer. Initial symptoms of chronic As toxicity include skin discolourations, chronic indigestion and stomach cramps, while long-term effects include skin, lung, kidney and liver cancer, as well as gangrene-like sores (black foot disease) (Jones, 2007).

Symptoms for chronic As exposure can differ among individuals, populations, and geographic areas. A population from southern Austria in the 12th century was said to have developed immunity to chronic As exposure. They reportedly consumed As as arsenic trioxide in tonic in daily portions of up to 300 - 400 mg, a concentration that would be fatal to an individual without this tolerance (Przygoda *et al.*, 2001). There are many reasons believed for this practise, such as enhancing women's complexion, increasing ability to breathe easier, aiding digestion after heavy meals, increasing courage and increasing sexual potency (Przygoda *et al.*, 2001). In epidemiological studies, As has been shown to cause skin, bladder and lung cancer (World Health Organization, 2008).

Inhalation of Cd fumes or particles can be fatal. Acute exposure to moderate concentrations of Cd ($200 - 500 \mu\text{g}/\text{m}^3$) fumes can cause the symptoms of 'metallic fever', including metallic taste, fever, malaise, joint pains, cough, sore throat, chest tightness and fatigue and can last for a couple of days (Fernandez *et al.*, 1996). Exposure to Cd can cause kidney damage, starting out as tubular dysfunction which is reversible if caught in time, but that otherwise leads to severe kidney damage and hypertension. Cd is also associated with cancer including leukemia and cancer of the lung, bladder, pancreas, breast and prostate (Henson & Chedrese, 2004; Jones, 2007). Chronic exposure to cadmium can cause skeletal damage, commonly known as Itai-Itai disease. The disease is a form of osteomalacia and osteoporosis that starts with pain in the legs and eventually spreads throughout the entire body until the patient is bed-ridden. Bone fractures can be caused from the slightest external pressure such as coughing and some skeletal deformities can develop (Inaba *et al.*, 2005).

Cd is considered a possible human carcinogen when inhaled. Some signs of Cd toxicity include increased urinary excretion of Ca and phosphorous (P) that are associated with renal tubular damage, osteomalacia or osteoporosis (Itai-Itai disease) (Inaba *et al.*, 2005); renal dysfunction and diminished bone mineral density (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). Cd has a large range of toxic effects on reproduction. Exposure to Cd has been linked to a decline in the number of corpora lutea, decreased uterine length and increased number of implantation sites in female hamsters (Magers *et al.*, 1995). It also has stimulatory and inhibitory effects on progesterone and during pregnancy, maternal exposure is associated

with low birth weight and increased incidence of spontaneous abortion (Shiverick & Salafia, 1999; Henson & Chedrese, 2004).

Pb can interfere with Ca metabolism and signalling inducing subencephalopathic neurological and behavioural effects, and increased anaemia, kidney damage, abdominal pain, seizures and paralysis (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005; World Health Organization, 2008). As mentioned above, children and infants are the most susceptible population to Pb toxicity. When exposed to toxic concentrations of Pb during their developmental years (years 1 - 10), there is a marked reduction in growth and development in children, accompanied by diminished mental capacity frequently termed mild mental retardation (Lessler, 1988). This retardation is greatest when Pb exposure occurs during the early periods of growth, and does not seem to affect the infant/child after the growth phase has been completed (Lessler, 1988).

Many epidemiological studies have been performed on children exposed to Pb *in utero*, or shortly after birth documenting health issues in children. One population of infants exposed prenatally to relatively low concentrations of Pb (0.44 – 0.46 µg/dL) found that 3 years after birth, Mental Development Index (MDI) scores were inversely and significantly correlated with Pb cord blood concentrations (Jedrychowski *et al.*, 2009). The Bayley test for MDI, a test designed for the assessment of cognitive development of early childhood, was given at ages 12, 24 and 36 months to monitor the developmental process of the children. The authors of this study did not start seeing

statistically significant trends of MDI deficit with prenatal Pb exposure until the age of 3 years (Jedrychowski *et al.*, 2009).

Another study focussed on children ages 3, 4, 5 or 7 years (ages of intellectual functioning) from 2 cities in Yugoslavia; one located near a Pb smelter, refinery and battery factory and one having no source of Pb exposure (Wasserman *et al.*, 2000). The authors followed these children from birth for 8 years and found that prenatal elevations in blood Pb were associated with small decrements in intelligence even after adjustment for social factors (Wasserman *et al.*, 2000). Two other epidemiological studies which followed children to their early teen years found a significant relationship between exposure to environmental Pb and later childhood emotional and behavioural problems (Burns *et al.*, 1999). Pb was associated with growth in verbal comprehension deficits and early Pb exposure predicted cognitive growth patterns into adolescence in a socially disadvantaged population (Coscia *et al.*, 2003).

Acute exposure to Pb also causes proximal renal tubular damage and chronic exposure results in kidney damage (Jarup, 2003). An epidemiological study conducted by Muntner *et al.*, (2003) of the general American population found that exposure even to low concentrations of Pb is associated with an elevated serum creatinine and chronic kidney disease (CKD) in individuals with hypertension. Exposure to high concentrations of Pb (Pb intoxication) inhibits the development of RBCs in the bone marrow, therefore reducing the synthesis of haemoglobin by developing RBCs, causing anaemia (Lessler, 1988).

A study done in 1981 by Revis *et al.*, showed that both Cd and Pb can cause aortic atherosclerosis and hypertension and that Ca protects against cardiovascular effects of Cd in pigeons. They also showed that these toxic effects can occur at 0.8 ppm for Pb and 0.6 ppm for Cd (Revis *et al.*, 1981). A more recent epidemiological study done on long term Pb exposure of workers in a US factory making batteries found a small effect of exposure to lead on diastolic blood pressure (Tepper *et al.*, 2001). Likewise, a cross-sectional study conducted on a population from Seoul, Republic of Korea exposed to heavy metals, found blood Cd to be associated with heart rate variability (Jhun *et al.*, 2005).

Heavy metals can also cause cancer (Table 4). Cd, as mentioned above, has been associated with many different types of cancer and has been classified as a Group 1 carcinogen by the International Agency for Research in Cancer (IARC), indicating that it is carcinogenic to humans (IARC, 1993). In 1987, Pb was also classified by the IARC as a “possible human carcinogen”, or a Group 2B carcinogen, based on sufficient animal data and inadequate human data (IARC, 1998). As mentioned above, As is a carcinogen linked with lung and skin cancer. It has also been linked with developing tumors in the lungs, skin, liver, bladder and kidney (Waalkes *et al.*, 2004). For these 3 heavy metals, and other heavy metals not discussed here, the mechanism of carcinogenicity is generally through ROS.

Table 4: List of relevant heavy metals and their carcinogenicity. (IARC, 2009)

| Type | | | |
|------------------|-----------------------------------|---|--------------------|
| Heavy Metal | Classification for Humans | (suggestive evidence) | Date |
| Al | Al production – carcinogenic | Bladder, lung | 1987 |
| Sb | Sb trioxide - possible carcinogen | (lung) | 1989 |
| | Sb trisulfide - not carcinogenic | | 1989 |
| As and compounds | Carcinogen | (bladder, kidney, liver), lung, skin | 1987 |
| | | | 2004 (in water) |
| Be and compounds | Carcinogenic | lung | 1993 |
| Cd and compounds | Carcinogenic | Bladder, breast, leukemia, lung, pancreas, prostate | 1993 |
| Pb | Possible carcinogen | (brain, colon, kidney, | 1987 |

| | | | |
|----|---|-----------------------------------|------------------------|
| | Pb compounds (inorganic) – probable carcinogen | lung, stomach) | 2006 |
| | Pb compounds (organic) – not carcinogenic | | 2006 |
| Ni | Ni compounds - carcinogenic Ni metallic, and alloys – possible carcinogen | (kidney), lung, nose, sinuses, | 1990 |
| Ti | Not carcinogenic Ti dioxide – probable carcinogen | (lung) | 1999 In process |
| U | Depleted U – not carcinogenic | - | 1999 |

1.3 Biomarkers of Stress

Stress can be defined as a state of bodily or mental tension, resulting from factors that alter a pre-existing equilibrium (de Weerth & Buitelaar, 2005; Schneiderman *et al.*, 2005). A good physiological measure of stress is activity of the hypothalamic-pituitary-adrenal (HPA) axis (Blanchard *et al.*, 2001). To determine activity of the HPA axis as a result of acute stress, the concentration of cortisol or cortisone is typically measured in saliva, blood or feces. The analysis of cortisol in human hair is a novel and recently introduced method to assess chronic stress, as hair represents cortisol concentrations over monthly time periods, depending on how much hair is used for analysis. In 2004, Raul *et al.*, analyzed physiological concentrations of cortisol in human hair and in 2007, Kalra *et al.*, showed a positive correlation between hair cortisol content and perceived stress in healthy pregnant women. The benefits of using hair as the matrix for analysis of cortisol is that hair is solid and durable, allowing hair analysis to be conducted years after growth (Pragst & Balikova, 2006). Other advantages of hair for analysis of this type are that it can be stored at room temperature, collection is considered non-invasive for most cultures, collection of samples can be performed by non-health care workers with no risk to the donor and, most importantly from a scientific perspective, hair cortisol concentrations are not affected by acute stress (Sauve *et al.*, 2007). In this section, I discuss cortisol production as a result of stress and its incorporation into hair.

1.3.1 Cortisol

Cortisol is a glucocorticoid hormone produced by the adrenal glands. Cortisol production in response to physiological and psychological stress is mediated by the HPA axis. The HPA axis begins at the hypothalamus with the production of corticotrophin-releasing hormone (CRH) and vasopressin (VP). These hypothalamic regulators are expressed and regulated by neurotransmitters such as catecholamines, γ -aminobutyric acid (GABA), serotonin and acetylcholine (Aguilera, 1994). Once CRH and VP are released into the hypophyseal portal blood they travel to the anterior lobe of the pituitary gland where they stimulate the release of adrenocorticotrophic hormone (ACTH). ACTH is derived from a prohormone, proopiomelanocortin (POMC) (Joseph & Reichlin, 1987). ACTH travels through the systemic circulation to the adrenal cortex where it binds to membrane receptors on the adrenal cortex, located adjacent to the kidneys. This results in a net increase of cholesterol transport into the cell and the biosynthesis of cortisol (Mastorakos & Ilias, 2003). Cortisol is produced within minutes and is involved in negative feedback actions on its own secretion.

The steroid hormone cortisol is synthesized from cholesterol in the adrenal cortex. In short, mitochondrial CYP 11A1 converts cholesterol into pregnenolone, which is then converted into progesterone, a substrate for the formation of 17-hydroxyprogesterone (Rosol *et al.*, 2001). In the endoplasmic reticulum, hydroxylases convert 17-hydroxyprogesterone to 11-deoxycortisol, which is finally converted into cortisol. Cortisol is synthesized and released rapidly; it can be detected in the blood within minutes of ACTH stimulation (Joseph & Reichlin, 1987). The $T_{1/2}$ of cortisol in blood is

approximately 60 - 90 minutes and cortisol represents 80% of all glucocorticoid production, however, only around 10% circulates the body as free, unbound cortisol (Joseph & Reichlin, 1987; Rosol *et al.*, 2001).

1.3.2 Adverse Effects of Cortisol

Excessive and sustained cortisol secretion has been associated with depression, hypertension, osteoporosis and immunosuppression (Chrousos & Gold, 1998). Extreme production of cortisol can cause Cushing's Disease (Miller & O'Callaghan, 2002), a hormone disorder characterized by high levels of cortisol in the blood. Less severe excess of glucocorticoids can cause Cushingoid symptoms including central adiposity and other problems associated with metabolism (Miller & O'Callaghan, 2002). Recent literature has also suggested that increased cortisol is involved in the development of the metabolic syndrome which can lead to diseases such as T2D, cardiovascular disease, and stroke (Bjorntorp & Rosmond, 1999). Although the mechanisms of elevated HPA axis activity are unknown, two principle possibilities have been suggested: an elevated stimulation of cortisol synthesis and/or diminished feedback control of cortisol formation. Circumstances that cause physiological and psychological stress are statistically associated with increased HPA axis activity and result in stress-induced cortisol secretion (Bjorntorp & Rosmond, 1999).

1.3.3 Susceptible Populations

Chronic and acute stress are known to cause increased cortisol production and secretion (Aguilera, 1994; Blanchard *et al.*, 2001; Miller & O'Callaghan, 2002; Van Uum

et al., 2008). Stressors are defined as the actual or perceived threat to the organism (Schneiderman *et al.*, 2005). Causes of stress can come in many different packages and all depend on the individual and her or his coping mechanisms. Some known specific causes of stress are: socioeconomic status; race; lack of warmth and support from parents; living with a high level of conflict and violence; physical and sexual abuse; neglect; work overload; job uncertainty and unemployment; divorce/marital conflict; major medical illness; war; traumatic events; smoking; substance use; accidents; sleep problems; and eating disorders (Taylor *et al.*, 1997; Schneiderman *et al.*, 2005).

Children and adolescents who are exposed to stressors such as violence, abuse (including physical, sexual, emotional or neglect) and divorce or marital conflict have been well studied (Cicchetti & Toth, 2005). This abuse has been associated with negative performances in learning and school (Schneiderman *et al.*, 2005). Health problems in children can often stem from chronic stress caused by the family environment, including a lack of warmth and emotional support from parents and a high level of conflict and violence (Taylor *et al.*, 1997). For example, children with a history of physical abuse and neglect have an elevated mortality risk for all causes of death including homicide, transportation injury, other unintentional injuries and disease (Sorenson & Peterson, 1994). These events translate into adult years, often affecting the individual's ability to cope with subsequent stressors and can also cause multiple adverse health effects. One physiological pathway through which a chronically stressful family environment may cause health problems in children is via repeated interferences with homeostatic processes (Taylor *et al.*, 1997). For example in children, experimental manipulations of the presence of anger and conflict and the absence of emotional warmth and

responsiveness can disrupt patterns of cardiovascular and neuroendocrine regulation (Taylor *et al.*, 1997). Exposure to intense or chronic stressors during developmental years has long-lasting neurobiological effects and puts one at increased risk for anxiety and mood disorders, control problems, hypo-immune dysfunction, medical morbidity, structural changes in the central nervous system (CNS), and early death (Shaw, 2003).

Among adults, stressors can come in many different forms, including, but not limited to, the diagnosis of a major medical illness, the occurrence of a traumatic event, and the socioeconomic status of the individual. It is estimated that a large percentage of the general public (40 - 70%) are exposed to traumatic events throughout their lifetime (Norris, 1992). Traumatic events are defined as a group of events involving violent encounters with nature, technology, or humankind (Norris, 1992). The characteristics of an individual's community heavily influence the degree to which chronic stress is experienced (Taylor *et al.*, 1997). People, who have a lower socioeconomic status, tend to live in areas of higher crime rates, greater local fear of crime, fewer public services, and poorer transportation and recreational facilities (Taylor *et al.*, 1997). All these factors contribute to the chronic stress of an individual as they all make the basic task of living more difficult and time consuming. These areas are also associated with greater exposure to physical hazards such as air and water pollutants, hazardous wastes, pesticides, and industrial chemicals (Calnan & Johnson, 1985).

Some stressors, such as smoking, make it harder for the person to quit the stressful event, i.e. smoking. Nitric oxide is an inhibitory mediator of nicotine-induced HPA activity, and therefore provides a direct link between inflammatory processes and

the HPA activation stimulated by smoking (Steptoe & Ussher, 2006). The HPA axis has been implicated in addictive processes and therefore may lead smokers to fail in attempts to quit smoking, adding to the level of experienced stress.

1.3.4 Hair Biology and Hair Growth

Hair is an important protein filament that grows through the epidermis and protects the skin from the elements (Paus & Cotsarelis, 1999). Hair is characterized as thin, flexible tubes of dead fully keratinized epithelial cells which vary in colour, length, and diameter (Randall, 2007). Approximately 3 – 4 mm below the surface of the skin, hair follicles are embedded into the epidermal epithelium (Harkey, 1993). The hair follicle is richly innervated by a surrounding capillary system that provides the hair with necessary nutrients to grow (Pragst & Balikova, 2006). The hair follicle extends from the matrix cells located in the basement membrane to the mature hair shaft, seen outside the scalp. The hair shaft can be divided into 3 zones: the innermost zone, which is the site of biological synthesis of hair cells, surrounds the bulb; the keratogenous zone, the site of keratinisation where hair hardens and solidifies, is located directly above the bulb; and the permanent hair, where the hair shaft consists of dehydrated, cornified cells, is the final zone (Harkey, 1993). Matrix cells are composed of keratinocytes and melanocytes and form the germination centre at the bulb papilla, where proliferation and differentiation occur. The hair shaft is composed of three different layers, the cuticle, cortex and medulla, from outside to inside. Each hair has a sebaceous gland attached to it and its duct leads to the upper part of the root of the hair (Pragst & Balikova, 2006). Eccrine

sweat glands are located near the follicles and wet the hair without emptying into the follicles (Harkey, 1993).

Hair growth is cyclic but its rate is dependent on many different variables such as, race species, age, gender, body site, and seasons (Chamberlain & Dawber, 2003). Physiological states can also affect the hair growth cycle. Hair follicle growth generally goes through 3 cycles: anagen, catagen and telogen. Anagen is the growth phase of the cycle and it is proportional to the final length of the hair (Paus & Cotsarelis, 1999). This stage of hair growth typically lasts for approximately two years. The intermediate stage is the catagen stage, which generally only lasts for a couple of weeks and only begins once full hair length is reached. The final stage of hair growth is telogen. During the telogen stage, the hair shaft matures into club hair and will eventually be shed from the hair follicle (Paus & Cotsarelis, 1999). This stage generally lasts a couple of months. At a given time, the majority of hair follicles are in the anagen phase while the rest of hair is typically in the telogen phase; very few hairs are in the catagen phase at a given time (Chamberlain & Dawber, 2003). Scalp hair in humans grows at an estimated rate of 0.6 - 1.4 cm per month (Pragst & Balikova, 2006).

Incorporation of endogenous or exogenous compounds into hair occurs during the anagen phase. Endogenous and exogenous compounds can enter hair through various mechanisms. The main route of incorporation of cortisol into hair is by passive diffusion from blood capillaries into the growing cells (Pragst & Balikova, 2006). The hair bulb is richly vascularised, as the lower hair shaft from the base to just above the bulb is surrounded by a rich vascular plexus composed of long parallel vessels connected by

cross shunts (Harkey, 1993). However, cortisol can also be incorporated onto hair through diffusion of sweat or sebum secretions into the mature hair (Pragst & Balikova, 2006). Because the ducts of sebaceous glands discharge directly into the hair follicle of scalp hair, cortisol can pass directly into the hair follicle before it becomes mature hair (Harkey, 1993). The sebaceous glands are richly innervated; therefore cortisol can be easily incorporated into the sebum through the blood stream. Cortisol can also be incorporated into mature hair through contact with the external environment, such as the transfer of sweat from hands to hair.

Chapter 2: *Hypothesis and Objectives*

Walpole Island First Nation (WIFN) is located downstream from Sarnia and the industrial area known as Chemical Valley, along the St. Clair River at the mouth of Lake St. Clair. For decades, chemical valley has been the source of pollution in this important waterway system. Both bodies of water are important in all aspects of the WIFN community life, acting as a source of income, food, culture, and tradition. Due to historical spills into the St. Clair River and increasing concerns within the community, we conducted a community-based collaborative participatory baseline biomonitoring study to assess the level of exposure of the WIFN community to POPs and heavy metals, and to determine the adverse health effects these exposures are having on the community, such as increased stress from chemophobia. This study is the first step in understanding the current body burden of POPs and heavy metals within the WIFN community, in hopes that further epidemiological studies can be conducted to determine the contribution of historical and current exposure to environmental contaminants to the disease burden in this community.

The overarching hypothesis of this thesis is: some members of the WIFN community are exposed to sufficiently high concentrations of POPs and heavy metals from environmental sources including traditional foods that their health is adversely affected.

2.1 Questionnaire

Hypothesis: Several of the diseases or conditions reported by the WIFN in the questionnaire for themselves and their family members might be associated with and caused by exposure to environmental contaminants.

The specific objective of administering the questionnaire was to obtain information on health, disease burden, diet, and stress from WIFN volunteers about themselves and their family members, particularly their children.

2.2 Persistent Organic Pollutants

Hypothesis 1: Historical and current exposures of members of the WIFN to POPs are contributing to the increased incidence of T2D in members of this community.

Hypothesis 2: Concentrations of POPs in plasma lipids of the WIFN volunteers will be higher than concentrations reported in the literature representative of the general population, indicating higher exposure via consumption of traditional foods including fish caught in Lake St. Clair and the St. Clair River.

The specific objectives of this section of the thesis are:

1. To prepare a systematic review of the relationships between exposure to POPs and the incidence of diabetes, concentrating upon T2D.
2. To determine the baseline concentration of 91 POPs (20 OC pesticides and 71 PCBs) in plasma lipids of volunteers from the WIFN community to evaluate current exposures to these pollutants.

3. To compare concentrations of these 91 POPs in plasma lipids of WIFN volunteers to other populations reported in the literature, both historical and contemporary.

2.3 Heavy Metals

Hypothesis 1. Exposures to other metals will present lower risks for adverse health effects to members of the WIFN than exposure to methylmercury from eating fish.

Hypothesis 2. Heavy metal concentrations in blood will not correlate well with those in hair because of inefficient incorporation of non-organic metals into the growing hair strand.

Hypothesis 3. Concentrations of heavy metals in hair of WIFN volunteers will be higher than concentrations in groups used as reference populations - Japanese living in Toronto and women living in Ontario - indicating higher environmental exposures to heavy metals via the diet.

The specific objectives of this section of the thesis are:

1. To determine the baseline concentration of heavy metals in blood and hair of volunteers of the WIFN community to evaluate current exposures to these pollutants.
2. To determine the distribution ratios of several metals between blood and hair to evaluate the better matrix for analysis of heavy metals, and evaluation of exposure to these pollutants.

3. To compare the concentrations of heavy metals in hair of WIFN volunteers to those in hair of two groups used for reference purposes – Japanese living in Toronto and women living in Ontario.

2. 4 Cortisol

Hypothesis 1. Analysis of hair cortisol will serve as a biomarker for psychosocial stress in Walpole Island First Nation volunteers.

Hypothesis 2. Stress in the WIFN community, as indicated by concentrations of cortisol in hair of WIFN volunteers, will correspond with perceived stress within the community, as determined from the Perceived Stress Questionnaire.

Hypothesis 3. Elevated hair cortisol content is likely to be observed in those with chronic diseases, such as diabetes.

The specific objectives of this section of the thesis are:

1. To determine cortisol content in hair of WIFN volunteers as an index of psychosocial stress and to compare these data to hair cortisol content from a reference population living near London Ontario.
2. To compare the cortisol concentration of hair of WIFN volunteers with the results of the Perceived Stress Questionnaire.
3. To evaluate the effects of gender, smoking, and self-reported diabetes incidence on hair cortisol content.

Chapter 3: *Exposure to POPs and Incidence of Type 2 Diabetes*

Introduction

Diabetes Mellitus

Diabetes mellitus is a chronic metabolic disorder that has become a major health concern in the last century. There are two main types of diabetes mellitus. Type 1 diabetes is an autoimmune disease where cell-mediated destruction of pancreatic islet of Langerhans beta cells results in the loss of insulin production (Amos *et al.*, 1997). Type 1 diabetes most often develops in children, although it can occur at any age, and those with this disease require insulin injections to survive. T2D accounts for more than 90% of all diabetes cases in Canada. Contrary to type 1 diabetes, diagnosis of T2D generally occurs in adults over the age of 40 (Zimmet, 1999). T2D can often be initially managed with a change in diet and exercise, under which conditions its symptoms improve.

Type 2 Diabetes

T2D is characterized by insulin resistance and relative insulin deficiency (Amos *et al.*, 1997). Several criteria are used for the diagnosis of T2D. These include a glucose plasma concentration of 200 mg/dL (≥ 11.1 mmol/L; 11.1 mM) or more after a 2 h, 75 g oral glucose tolerance test; a value of 200 mg/dL or more on a random plasma glucose test with typical symptoms of diabetes; and a (venous) fasting plasma glucose concentration of 126 mg/dL (≥ 7.0 mmol/L) or more on more than one occasion (Mahler & Adler, 1999). Typical T2D symptoms include thirst, dry mouth, frequent urination,

increased hunger especially after eating, blurred eyesight, headaches, fatigue, unexplained weight lost and in rare occurrences, loss of consciousness. Impaired fasting glucose is defined as a fasting plasma glucose concentration between 110 and 125 mg/dL (Mahler & Adler, 1999).

Evidence suggests that the incidence of T2D increases with physical inactivity, obesity, age and genetic factors (Burrows *et al.*, 2000; Centers for Disease Control and Prevention (CDC), 2003; Kriska *et al.*, 2003; O'Rahilly *et al.*, 2005}. T2D is also associated with many other conditions, such as hyperinsulinemia, dyslipidemia, hypertension and visceral obesity. Precursors to the development of T2D are impaired glucose tolerance (IGT), insulin resistance, and the metabolic syndrome. The WHO has defined IGT as a 2 h plasma glucose concentration of 140 mg/dL or more, and of less than 200 mg/dL during an oral glucose test (The World Health Organization, 2003). One third of people diagnosed with IGT and impaired fasting glucose will ultimately suffer with T2D (Mahler & Adler, 1999). The metabolic syndrome is IGT, insulin resistance or T2D combined with hypertension, obesity, hypertriglyceridaemia (or low HDL) and microalbuminuria (Zimmet *et al.*, 2001). Complications can adversely affect the cardiovascular, neurological, cerebrovascular, ophthalmological and urinary systems.

T2D is one of the fastest growing epidemics worldwide (Zimmet *et al.*, 2001). Of great concern, it is affecting people at younger ages than ever before. Reports of adolescents and children being diagnosed with T2D are becoming common place (The World Health Organization, 2003). In 1985, an estimated 30 million people had diabetes throughout the world, which increased to approximately 171 million people by the year 2000 (The World Health Organization, 2003). By the year 2010, it is estimated there will

be 215 million cases of T2D on Earth (Zimmet, 1999). People diagnosed with diabetes require 2 - 3 times more health care resources than those without the disease, and diabetes is estimated to account for 15% of national healthcare budgets (The World Health Organization, 2003). In 1999 in Canada, an estimated total of \$619 649 000 Canadian were spent on health care for those suffering from T2D. These health care costs included hospital care, physician care, drugs and research (Katzmarzyk *et al.*, 2000).

Different factors have contributed to the rapid increase in prevalence of diabetes that is occurring today. These include: population growth, aging, urbanization, the increase in obesity, changes in diet, and physical inactivity (Wild *et al.*, 2004). Concomitant with urbanization and population growth comes increased pollution and the potential for exposure of more people to pollutants that may contribute to the diabetes disease burden.

T2D was first reported to be associated with POPs in individuals who were occupationally exposed. Wong *et al.*, (1984) reported that diabetes mellitus was the only cause of death with significant excess for the entire cohort among 3 579 white male chemical workers employed from 1935-1976 at 3 manufacturing plants that used PBBs, 1,2-dibromo-3-chloropropane and 2,3-dibromochloropropane, across the United States. Subsequently, this relationship was investigated within the general public. For example, a population in Eastern Slovakia previously exposed to PCBs and other POPs in high concentrations was reported to show an increased frequency of diabetes and other dysglycemias (Radikova *et al.*, 2004). Additional studies have been conducted on those occupationally exposed as well as in general populations exposed to environmental levels of POPs. These studies are discussed in more detail herein.

Polyhalogenated Aromatic Hydrocarbons

PHAHs are a group of chemicals that are known to occur as persistent pollutants. This group includes the PCBs, PBBs, PCDFs, PCDDs, chlorinated benzenes, PCNs, and chlorinated pesticides. Many of the planar PHAHs have similar toxicity and mechanisms of action to the dioxins, and act through the AHR. Chemicals classified as dioxin-like are PCDDs, PCDFs and planar PCB and PBB congeners. Other PHAHs have similar structures and show other functional similarities to dioxin, including the polyhalogenated naphthalenes and chlorinated benzenes (Van den Berg *et al.*, 1998). Figure 5 shows the chemical structures for several common PHAHs discussed in this review.

TCDD is the best studied PHAH and is the most toxic anthropogenic compound known (Poland & Glover, 1977). Dioxin-like compounds produce their toxicity through the AHR signaling network and as a consequence, cause similar health effects in animals and humans. Exposure to dioxins can result in reproductive alterations, immunotoxicity, teratogenicity, carcinogenicity and death (Schecter & Gasiewicz, 2003). In animal studies, toxicity of dioxins does not depend on the route of administration but rather is related to the dose and body burden of the chemical (DeVito & Birnbaum, 1995). All PHAHs are lipophilic and persistent in the environment because they resist most forms of degradation, including chemical, metabolic (biological) and physical (heat, sunlight). These physical-chemical properties allow dioxins and other stable PHAH compounds to undergo long range atmospheric transport, to be deposited in the environment and bioaccumulate and biomagnify in the food web. Therefore, the body burden of these chemicals is related to the weight and the fat content in the animal in addition to the amount of chemical exposure, which frequently occurs via fish consumption. In humans,

the $T_{1/2}$ of TCDD is estimated to be approximately 7 years, and although other dioxin-like compounds have shorter half-lives, they are still in the range of months to years (Gallo *et al.*, 1991).

TCDD occurred as a significant by-product in the herbicide Agent Orange that was sprayed by the United States (US) Air Force during the Vietnam War (Stone, 2007). PCDDs and PCDFs are also unwanted contaminants formed as by-products of industrial and combustion processes of chlorinated plastics (Jones, 1999). PCNs were first synthesized during World War 1 and PCBs were introduced to commerce shortly after in the late 1920's (Brinkman & Reymer, 1976; Agency for Toxic Substances and Disease Registry, 2000). Due to their chemical and thermal stability, PCBs were used in many different industrial applications as cooling fluids in hydraulic systems, lubricating oils, flame retardants and plasticizers in paints, adhesives, sealants and plastics (United Nations Environmental Programme, 1992). Because of their ability to accumulate in the environment, in wildlife, and in humans, the production of PCBs was banned in North America in 1977. However, as a result of their persistence, it is estimated that 70% of all PCBs ever produced are still in circulation in the environment (Erdal *et al.*, 2008).

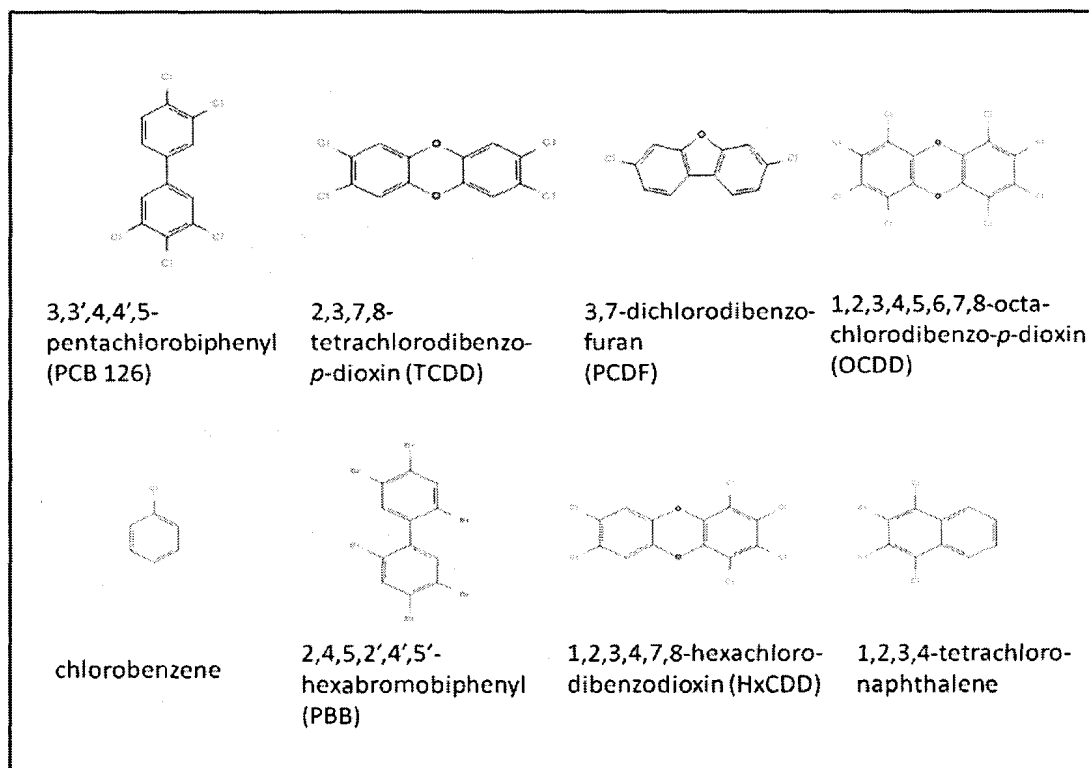


Figure 5: Examples of structures of PHAHs

Over the last decade, many scientific reports have attempted to evaluate the relationship between exposure to POPs, including PHAHs and OC pesticides, and the incidence of T2D because of the dramatic increase in T2D world-wide. This review will focus on the potential contribution of environmental exposures to polyhalogenated pollutants to the increasing incidence of T2D. Whereas some studies demonstrated a positive relationship between polyhalogenated pollutant exposures and increased risk for T2D, others did not find an association between these parameters. The objective, based on original literature reports, is to determine whether there is an association between environmental or occupational exposure to PHAH chemicals and the incidence of T2D.

Methods

Search Strategy, Study Selection and Data Extraction

A systematic review was performed to retrieve all published articles involving T2D and exposures to POPs. Original articles in any language were accepted into the study if their design included a control or comparison group and if the sample size was larger than 10 participants per arm. Case reports, or case series were excluded from consideration and only human studies were included.

Searches were independently conducted using the electronic databases PubMed, EMbase, Scopus, MEDLINE and Google Scholar. References from retrieved studies and reviews of the topic were further searched for additional papers not captured by our search strategy. Searches were conducted from the initiation of the databases up to December 31, 2008.

Two independent reviewers performed article inclusion and exclusion decisions and disagreements were resolved through a third impartial party. We further focused on the selected studies which specifically evaluated the following classes of PHAH chemicals: PCDDs, including TCDD; PCDFs; PCBs and PBBs; OC pesticides; polychlorinated diphenyl ethers; and PCNs.

Data extraction sheets were created and applied to all included articles. Information recorded included year of publication, type of study, comparison, study group and control group characteristics, diabetes diagnosis criteria, type of PHAH chemical involved, route of exposure, and nature of exposure. Because the endpoint was T2D, articles were excluded if the type of diabetes was thought to be type 1. Emails were sent to authors of articles where the type of diabetes was not specifically reported. These articles were then considered to be relevant to our analysis if the authors responded that most of the diabetes cases reported were type 2; if the subjects of the studies were all adults; if the authors used a phenotypical diagnosis by high insulin/c-peptide levels and obesity in individuals with diabetic glucose values; and if individuals had high fasting serum glucose levels after reports that subjects had developed diabetes subsequent to exposure to POPs. Articles that looked at mortality associated with T2D and time-to-onset of T2D were included in this systematic review, as were articles that evaluated the prevalence of T2D.

Meta-Analysis

Of all the studies included in this review, only the studies focusing on the chemical TCDD ($n = 6$) had sufficient information to be grouped together to perform a meta-analysis. For the rest of the studies, this was not the case and, therefore, we could

not perform a meta-analysis on them. The outcome of interest for the meta-analysis is the occurrence of diabetes after exposure to TCDD. Data was extracted and entered into 2 x 2 tables. Diabetes was the only outcome examined. Statistical analysis was done using Cochrane's Review Manager (version 4.3, Oxford: Cochrane Collaboration). Heterogeneity of effects was assessed using I^2 statistics and the data visualized on a forest plot. For all statistical analysis, a p value of 0.05 or less was considered to be significant.

The Naranjo Adverse Drug Probability Scale

The Naranjo Scale is a validated, systematic yet simple method to assess the causality of adverse drug reactions (ADR) (Naranjo *et al.*, 1981) and it was applied to all articles included in this review. As this analysis deals with environmental contaminants and not drugs, we modified the Naranjo Scale to fit to environmental contaminants such as POPs. Table 5 shows the modified ADR probability scale.

Several of the questions in the Naranjo Probability Scale are not relevant for environmental contaminants and were not used. These include: question 3: *Did the adverse drug reaction improve when the drug was discontinued or a specific antagonist administered?*; question 4: *Did the adverse drug reaction reappear when the drug was readministered?*; question 6: *Did the reaction reappear when a placebo was given?*; and question 9: *Did the patient have a similar reaction to the same or similar drug in any previous exposure?*).

Questions 2, 5, 7 and 8 were slightly modified and reworded to be relevant for exposure to environmental contaminants. The Naranjo ADR Scale is scored out of 13. However, after relevant modifications for environmental contaminants, the score is out of 8. The original Naranjo scoring system is as follows: definite $\geq 9/13$, probable 5/13 to

8/13, possible 1/13 to 4/13, doubtful $\leq 0/13$. For our purposes we used the same ratio to determine the scoring for the modified Naranjo Scale's scoring system: definite $\geq 5.5/8$, probable 3/8 to 5/8, possible 0.6/8 to 2.5/8, doubtful $\leq 0.6/8$.

Table 5: Modified ADR Scale for environmental contaminants.

| | <i>Yes</i> | <i>No</i> | <i>Do Not Know</i> | <i>Score</i> |
|---|------------|-----------|--------------------|--------------|
| 1. Are there previous conclusive reports on this reaction? | +1 | 0 | 0 | |
| 2. Did the adverse event appear after exposure to the environmental contaminant(s)? | +2 | -1 | 0 | |
| 5. Are there alternative causes (other than the environmental contaminant(s)) that could have on their own caused the reaction? | -1 | +2 | 0 | |
| 7. Was the environmental contaminant(s) detected in the blood (or other fluids) in concentrations known to be toxic? | +1 | 0 | 0 | |
| 8. Was the reaction more severe in people with higher concentrations of the environmental contaminant(s) in their blood (or other fluids), or less severe in people with lower concentrations of the environmental contaminant(s) in their blood (or other fluids)? | +1 | 0 | 0 | |
| 10. Was the adverse event confirmed by any objective evidence? | +1 | 0 | 0 | |
| Total Score | | | | / 8 |

Assessment of Study Quality

The included articles were also submitted to the methodological index for non-randomized studies (MINORS). MINORS is a validated methodological index to assess the quality of non-randomized, observational studies (Slim *et al.*, 2003) (Table 6). Two points are given for each question if the article reports and provides an adequate answer; 1 point is given if the article reports on the issue but the answer is inadequate; and no points are given if the article does not report the answer. The global ideal score is out of 24 for comparative studies (Slim *et al.*, 2003). The qualitative assessment of studies that found positive associations between exposures to PHAHs and the incidence of T2D was compared with the qualitative assessment of studies that did not find associations. The mean MINORS score of the articles that reported associations and those that did not were subjected to the Student's T Test to determine statistical differences.

Table 6: Methodological items for non-randomized studies**Scoring system:****Score**

0 = no, 1 = answered but not adequately, 2 = adequately answered question

1. Clearly stated aim: the question should be precise and relevant in the light of available literature.

2. Inclusion of consecutive patients: all patients potentially fit for inclusion (satisfying the criteria for inclusion) have been included in the study during the study period (no exclusion or details about the reasons for exclusion)

3. Prospective collection of data: data were collected according to a protocol established before the beginning of the study.

4. Endpoints appropriate to the aim of the study: unambiguous explanation of the criteria used to evaluate the main outcome, which should be in accordance with the question addressed by the study. Also, the endpoints should be assessed on an intention-to-treat basis.

5. Unbiased assessment of the study endpoint: blind evaluation of objective endpoints and double-blind evaluation of subjective endpoints. Otherwise reasons for not blinding should be stated.

6. Follow-up period appropriate to the aim of the study: the follow-up should be sufficiently long to allow the assessment of the main endpoint and possible adverse events.

7. Loss to follow up less than 5%: all patients should be included in the follow-up. Otherwise, the proportion lost to follow-up should not exceed the proportion experiencing the major endpoint.

8. Prospective calculation of the study size: information of the size detectable difference of interest with a calculation of 95% confidence interval, according to the expected incidence of the outcome event, and information about the level for statistical significance and estimates of power when comparing the outcomes.

Additional criteria in the case of comparative study

9. An adequate control group: having a gold standard diagnostic test or therapeutic intervention recognized as the optimal intervention according to the available published data.

10. Contemporary groups: control and studied group should be managed during the same time period (no historical comparison).

11. Baseline equivalence of groups: the groups should be similar regarding the criteria other than the studied endpoints. Absence of confounding factors that could bias the interpretation of the results.

12. Adequate statistical analyses: whether the statistics were in accordance with the type of study, with calculation of confidence intervals or relative risk.

Results

Twenty-four articles met the inclusion criteria established for this review (Table 7). Nine of the 24 articles explicitly stated that T2D was only considered as an end-point for diabetes (Table 8). The other 15 articles either did not differentiate between the 2 types of diabetes because the authors either could not differentiate between the types or they elected to study both forms of diabetes. Initially, these articles were analyzed separately, and the material obtained was subsequently synthesized as a whole. Because of different chemicals being evaluated and different endpoints of diabetes, it was judged impossible to combine all studies into a formal meta-analysis. Six studies focused specifically on TCDD and we conducted a meta-analysis on these studies alone (Table 9). In addition, we critically analyzed the remainder of the studies qualitatively and quantitatively.

Table 7: List of all studies included in the systematic review.

| REFERENCE # | AUTHORS | TYPE OF DIABETES Unknown / Type 2 | CHEMICALS |
|----------------|---------------------------------------|--|--|
| 1 | (Kouznetsova <i>et al.</i> , 2007) | Unknown | Dioxins/Furans PCBs Persistent pesticides |
| 2 | (Fierens <i>et al.</i> , 2003) | Type 2 | PCDD/PCDF PCBs |
| 3 | (Montgomery <i>et al.</i> , 2008) | Type 2 | OC Pesticides |
| 4 | (Rylander <i>et al.</i> , 2005) | Type 2 | CB-153 DDE |
| 5 | (Karouna-Renier <i>et al.</i> , 2007) | Unknown | PCDD/PCDF |
| 7 | (Lee <i>et al.</i> , 2006) | Unknown | PCB 153 HpCDD OCDD oxychlordane DDE <i>trans</i> -nonachlor |
| 9 | (Vasiliu <i>et al.</i> , 2006) | Type 2 | PBB |

| | | | |
|----|----------------------------------|---------|-------------------------|
| | | | PCB |
| 14 | (Steenland <i>et al.</i> , 1999) | Unknown | TCDD |
| 16 | (Zober <i>et al.</i> , 1994) | Unknown | TCDD |
| 17 | (Henriksen <i>et al.</i> , 1997) | Type 2 | TCDD |
| 18 | (Everett <i>et al.</i> , 2007) | Type 2 | HxCDD |
| | | | PCB 126 |
| | | | DDT |
| 21 | (Vena <i>et al.</i> , 1998) | Unknown | TCDD |
| 23 | (Calvert <i>et al.</i> , 1999) | Type 2 | TCDD |
| 26 | (Kim <i>et al.</i> , 2003) | Unknown | TCDD |
| 31 | (Morgan <i>et al.</i> , 1980) | Unknown | DDT |
| | | | OC Pesticides |
| | | | Toxaphene |
| | | | Chlordane |
| 32 | (Glynn <i>et al.</i> , 2003) | Unknown | PCB |
| | | | DDE |
| | | | HCB |
| | | | β HCH |
| | | | <i>trans</i> -nonachlor |

| | | | |
|----|---------------------------------------|---------|-------------------------|
| | | | oxychlordane |
| 34 | (Codru <i>et al.</i> , 2007) | Unknown | PCB |
| | | | DDE |
| | | | HCB |
| 35 | (Rignell-Hydbom <i>et al.</i> , 2007) | Type 2 | CB-153 |
| | | | DDE |
| 54 | (Jorgensen <i>et al.</i> , 2008) | Unknown | DPCB |
| | | | NPCB |
| | | | OC Pesticides |
| 59 | (Wang <i>et al.</i> , 2008) | Type 2 | Dioxins |
| | | | PCB |
| 60 | (Kang <i>et al.</i> , 2006) | Unknown | TCDD |
| 61 | (Consonni <i>et al.</i> , 2008) | Unknown | TCDD |
| 64 | (Uemura <i>et al.</i> , 2008) | Unknown | PCDD |
| | | | PCDF |
| | | | PCB |
| 68 | (Cox <i>et al.</i> , 2007) | Unknown | DDT |
| | | | DDE |
| | | | β HCB |
| | | | <i>trans</i> -nonachlor |

*Unknown type of diabetes = diabetes type not specified or unable to determine

Table 8: Studies conducted exclusively on individuals who have type 2 diabetes.

| RERERENCE | AUTHORS | CHEMICALS |
|-----------|---------------------------------------|----------------------------|
| # | | |
| 17 | (Henriksen <i>et al.</i> , 1997) | TCDD |
| 35 | (Rignell-Hydbom <i>et al.</i> , 2007) | CB-153 <i>p,p'</i> -DDE |
| 3 | (Montgomery <i>et al.</i> , 2008) | OC pesticides |
| 4 | (Rylander <i>et al.</i> , 2005) | <i>p,p'</i> -DDE CB-153 |
| 2 | (Fierens <i>et al.</i> , 2003) | PCDD PCDF PCBs |
| 9 | (Vasiliu <i>et al.</i> , 2006) | PBB, PCB |
| 18 | (Everett <i>et al.</i> , 2007) | HxCDD PCB 126 DDT |
| 23 | (Calvert <i>et al.</i> , 1999) | TCDD |
| 59 | (Wang <i>et al.</i> , 2008) | Dioxins PCB |

Table 9: Articles included in Meta-Analysis of TCDD and diabetes

| REFERENCE # | AUTHORS | TYPE OF DIABETES |
|-------------|----------------------------------|------------------|
| 16 | (Zober <i>et al.</i> , 1994) | Unknown |
| 17 | (Henriksen <i>et al.</i> , 1997) | T2D |
| 23 | (Calvert <i>et al.</i> , 1999) | T2D |
| 26 | (Kim <i>et al.</i> , 2003) | Unknown |
| 60 | (Kang <i>et al.</i> , 2006) | Unknown |
| 61 | (Consonni <i>et al.</i> , 2008) | Unknown |

Meta-Analysis of TCDD Studies

Although 7 studies focused specifically on TCDD, only 6 studies met the requirements for a meta-analysis. The study by Steenland *et al.*, (1999) did not report diabetes incidence in referent subjects and therefore statistical analysis could not be conducted for this study. The other 6 studies were then analyzed using Chi^2 (X^2) to determine the individual significance of each of the studies. Upon further analysis we found that these studies show contradictory results and therefore are not homogeneous and cannot be combined (Fig. 6; $I^2=96\%$).

On closer examination, it was noted that 2 of the studies are cases of acute occupational exposure, while the other 4 studies are cases of chronic exposure. Therefore we analyzed the 2 studies separately from the 4 other chronic exposure studies to determine if this showed any effect (Figs. 7 and 8).

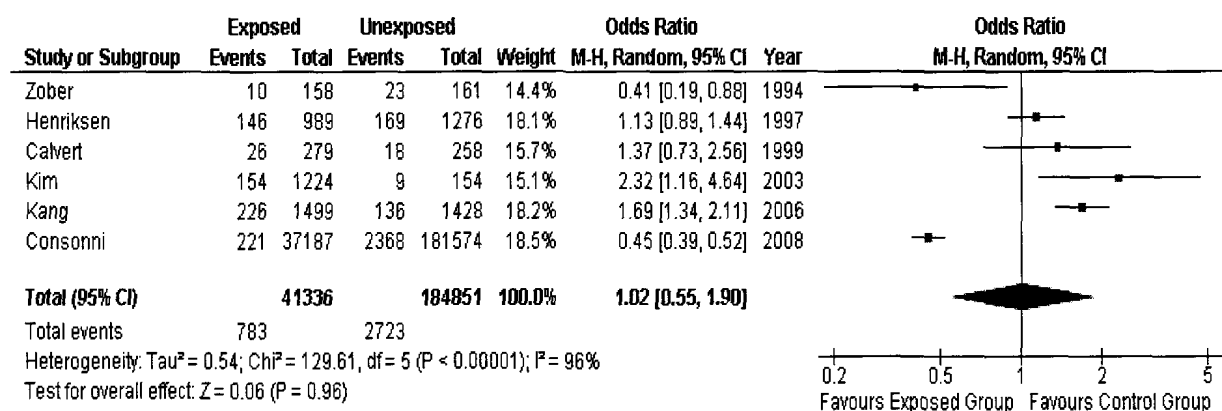


Figure 6: Meta-analysis of exposure to TCDD and occurrence of diabetes.

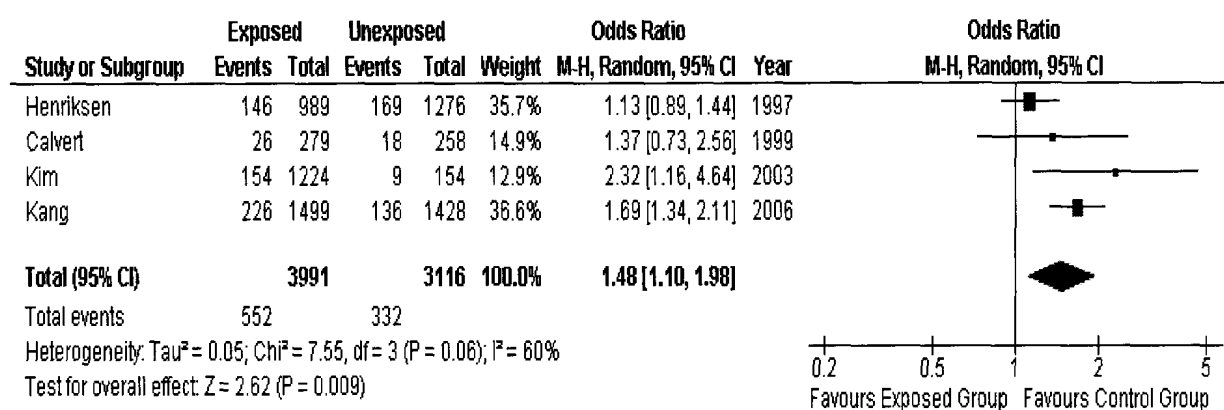


Figure 7: Chronic exposure to TCDD and the occurrence of diabetes.

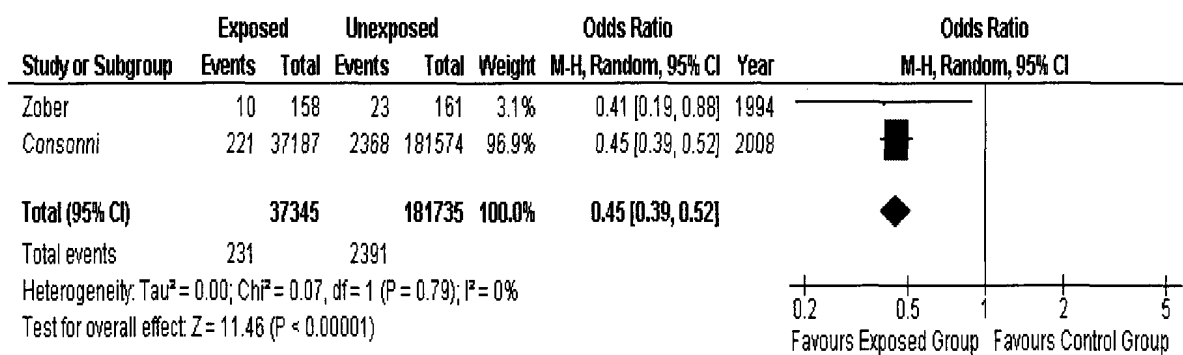


Figure 8: Acute exposure to TCDD and the occurrence of T2D.

Naranjo Score

The mean Naranjo Score for the 6 TCDD studies is 3.3 out of 8 (Table 10), which means a probable association between TCDD and diabetes. The Naranjo Scores for the 6 studies ranged from 1 to 5. As shown in Table 11, the mean Naranjo score for the 9 articles that specifically included only T2D as an endpoint was 3.1 out of 8, which also signals a probable association between exposure to PHAHs and T2D. Table 12 shows the mean Naranjo score for all articles included in this review. The mean Naranjo score was 2.5 out of 8, which indicates a possible association between exposure to PHAHs and T2D. The Naranjo scores ranged from 1, which constitutes a possible association to 5, which constitutes a probable association.

[illegible]

The following headings apply to the Naranjo Score.

1. Previous conclusive reports

As there are no conclusive reports in human epidemiological studies regarding exposure to PHAHs and the subsequent development of T2D, studies that showed positive associations between the development of precursors to diabetes, such as metabolic syndrome, hyperinsulinemia, and insulin resistance, and the incidence of T2D were used in our analysis.

Fujiyoshi *et al.*, (2006) conducted a molecular epidemiological study on the state of expression of selected biomarkers in adipose tissue samples from the Air Force Health Study. The individuals who participated in this study were veterans of Operation Ranch Hand, which involved spraying the herbicide Agent Orange for deforestation purposes during the Vietnam War. This herbicide unintentionally contained relatively high concentrations of TCDD and the Operation Ranch Hand veterans continue to have relatively high levels of dioxin residues years after exposure (Fujiyoshi *et al.*, 2006). GLUT-4 is a glucose transporter protein expressed mainly in insulin-responsive tissues, such as muscles and adipose tissue (Das, 1999). GLUT-4 plays an important role in glucose transport and metabolism and it has been hypothesized that a decrease in insulin-mediated glucose transport occurs in diabetics (Das, 1999). Tumor necrosis factor- α (TNF- α) is a major mediator of TCDD-induced cell inflammatory reactions, and it is produced by adipocytes in response to obesity (Kern *et al.*, 2002). TNF- α causes increased expression of transcription factor NF κ B and eventually leads to the down regulation of GLUT-4 (Fujiyoshi *et al.*, 2006). In the Ranch Hand veterans, the most recognizable gene expression changes were of the GLUT4:NF κ B transcript ratio in

response to the presence of dioxin in Ranch Hand veterans (Fujiyoshi *et al.*, 2006). In individuals exposed to low-to-medium concentrations of dioxin, a rise in NF κ B mRNA led to a decrease in GLUT4 mRNA. Therefore, the ratio is expected to be a more responsive biomarker than expression of either individual gene. Fujiyoshi *et al.*, (2006) provided definitive evidence for this diabetogenic shift in adipose tissue from Vietnam veterans who were exposed to TCDD while spraying Agent Orange.

These findings are in accordance with previous animal studies using mice models. Minokoshi *et al.*, (2003) found that a reduction of GLUT4 protein in muscle or adipose tissue causes insulin resistance in other insulin target tissues secondarily and increases the risk of developing T2D. Another *in vivo* study with mice conducted by Liu & Matsumura (1995) reported that adipose tissue is a primary target of TCDD toxicity; treatment with TCDD resulted in decreased levels of GLUT4 and lipoprotein lipase (LPL) mRNA gene expression. TCDD apparently decreased GLUT4 mRNA levels in adipose tissue through the AHR, which is the accepted mechanism of action for most TCDD toxicity (Liu & Matsumura, 1995).

This mechanistic research is supported by epidemiological studies that report a positive association between exposure to TCDD and the development of precursors of diabetes such as metabolic syndrome, insulin resistance, and hyperinsulinemia. Cranmer *et al.*, (2000) found that TCDD levels above 15 ng/g in blood lipids of healthy persons were highly correlated with excess risk of hyperinsulinemia. Another study based on the 1999-2002 NHANES data from a representative sample of the general US population, found positive associations between body burdens of specific groups of POPs (organochlorine pesticides including oxychlordan and *trans*-nonachlor) and incidence of

insulin resistance (Lee *et al.*, 2007a). As a result of this analysis, Lee *et al.*, (2007a) concluded that OC pesticides may be involved in the pathogenesis of insulin resistance and diabetes. In a related investigation, Lee *et al.*, (2007b) demonstrated that body burdens of dioxin-like (i.e. co-planar) PCBs (PCB 126, PCB 74 and PCB 118), non-dioxin-like (non-planar) PCBs (PCB 138, PCB 153, PCB 170, PCB 180, PCB 187), and OC pesticides (β -hexachlorocyclohexane) were positively associated with the occurrence of metabolic syndrome.

2. Type 2 diabetes appeared after exposure to PHAHs

Most of the articles included in this review did not mention whether diabetes developed before or after exposure, or the authors were unable to determine this due to study design (Vena *et al.*, 1998; Steenland *et al.*, 1999; Fierens *et al.*, 2003; Glynn *et al.*, 2003; Kim *et al.*, 2003; Rylander *et al.*, 2005; Codru *et al.*, 2007; Everett *et al.*, 2007; Karouna-Renier *et al.*, 2007; Rignell-Hydborn *et al.*, 2007; Consonni *et al.*, 2008; Jorgensen *et al.*, 2008; Uemura *et al.*, 2008).

In the Yucheng cohort study, Wang *et al.*, (2008) did not exclude those who had diabetes prior to exposure, although their study design allowed for this to be done. These authors studied the relationship between cumulative incidence of T2D and postnatal exposure to PCDFs and PCBs but decided there was enough evidence to conclude that the onset of T2D developed after exposure to these POPs. Kouznetsova *et al.*, (2007) were unable to determine when diabetes occurred relative to exposure because they studied the rate of hospitalization for diabetes in populations living near areas contaminated with PHAHs.

A few articles excluded individuals who reported having diabetes prior to the start of the investigation, or before the exposure period, from their analysis (Morgan *et al.*, 1980; Zober *et al.*, 1994; Henriksen *et al.*, 1997; Vena *et al.*, 1998; Calvert *et al.*, 1999; Vasiliu *et al.*, 2006; Montgomery *et al.*, 2008).

Questions 3 and 4 from the Naranjo Adverse Drug Probability Scale are not relevant to issues concerning adverse effects of environmental contaminants and are not considered here.

5. Possible alternative causes (other than exposure to PHAHs) that could have on their own caused T2D.

For each of the published studies included in this review, factors other than exposure to PHAHs could be causative for T2D. Known risk factors for diabetes include obesity (indicated by higher than normal BMI and waist circumference), race, gender, age, family history of diabetes (genetics), dietary and exercise habits and lifestyle. Although authors of these reports attempted to control for some or all of these confounding variables through adjustment of data and matching study cohorts to referent cohorts, it cannot be concluded for certain that exposure to PHAHs is the only contributor to the development of diabetes.

It is also impossible to control for environmental (or occupational) exposures to other types of environmental pollutants, including metals, that could occur throughout a lifetime and to effectively exclude these exposures from their contribution to an increased risk of developing diabetes. This is especially true for exposures to chemicals such as arsenic that cause oxidative stress. At elevated concentrations in drinking water, As has

been associated with the risk for T2D in Bangladesh (Rahman *et al.*, 1998), Mexico (Coronado-Gonzalez *et al.*, 2007) and Taiwan (Tseng *et al.*, 2000).

Question 6 from the Naranjo Adverse Drug Probability Scale is not relevant for environmental contaminants and is not considered further.

7. PHAHs were detected in the blood (or other tissues or fluids) in concentrations known to be toxic

The general North American population tends to have a baseline concentration of 1 – 2 ng/Kg TCDD in blood lipids (Schecter *et al.*, 2006). Most individuals also have a low body burden of other PHAHs, such as the PCBs, which complicates exposure issues because when an individual is additionally exposed to these chemicals from the environment, food, or the workplace, exposures to chemicals with similar mechanism of action can complicate assumptions about the associations between exposure to a specific chemical or chemical class and an endpoint such as increased risk for T2D.

Adverse effects from exposure to TCDD and other dioxin-like chemicals (PCDD/PCDF) have been reported at body burdens ranging from 10 – 500 ng/kg TCDD (Neuberger *et al.*, 1991; Smith *et al.*, 1992; Papke *et al.*, 1996; Landi *et al.*, 1998; Aylward & Hays, 2002). However, the body burden in the general population for polybrominated diphenylethers (PBDEs) is in the order of ng/g (ppb) lipid weight, which is approximately 50 - 200 times lower than that of PCBs (Covaci *et al.*, 2002). Because of the wide range of body burdens associated with TCDD toxicity and the diversity of toxic effects of this POP, we included articles that reported any body burden within the range described to be associated with T2D as having met criteria. However, biochemical and adaptive responses can occur at body burdens that are an order of magnitude lower

than those that cause “adverse” or toxicological effects as seen in T2D, where T2D and alterations in insulin and glucose metabolism have been associated with dioxin levels only 10-fold higher than those reported within the general population (Lee *et al.*, 2007a; Lee *et al.*, 2007b).

Most of the studies reported the body burden of various PHAHs as serum concentrations above 5 ng/kg toxic equivalent quotient (TEQ)/kg body weight or 1 - 2 ng/g bodyweight (Morgan *et al.*, 1980; Henriksen *et al.*, 1997; Fierens *et al.*, 2003; Glynn *et al.*, 2003; Rylander *et al.*, 2005; Kang *et al.*, 2006; Vasiliu *et al.*, 2006; Codru *et al.*, 2007; Cox *et al.*, 2007; Rignell-Hydbom *et al.*, 2007; Jorgensen *et al.*, 2008; Wang *et al.*, 2008). In most of these studies, the concentrations of PHAHs were normalized per g serum lipids. Only a few studies did not do this or did not report doing so in the article. Three studies did not find at least one subject with a PHAH concentration in plasma lipids over 1 – 2 ng/kg (Vena *et al.*, 1998; Kim *et al.*, 2003; Consonni *et al.*, 2008).

Montgomery *et al.*, (2008) did not report the body burden of PHAHs for their participants and it is unknown whether they even took blood samples from participants to determine body burden. Steenland *et al.* (1999) did not report the concentrations of PHAHs in participants, although they did take samples to establish a job-exposure matrix in which the concentrations of PHAH were required. As previously mentioned, Koutznetsova *et al.* (2007) only evaluated hospitalization rates and therefore did not collect any biological samples for assay of PHAHs within their study population.

Two studies reported concentrations of some PHAHs to be over the limit set, while other concentrations of PHAHs were not. In the study by Everett *et al.*, (2007), only DDT was found at concentrations sufficiently high to be given a score for the

Naranjo Scale, while hexachlorodibenzo-*p*-dioxin (HxCDD) and PCB 126 did not. In the study conducted by Calvert *et al.*, (1999), TCDD concentrations in the upper two quartiles were high enough to be given a score, whereas those in the lower two quartiles were not. Uemura *et al.*, (2008) reported concentrations above and below 5 ng/kg TEQ lipid for all the different POPs analyzed. For these three studies, a mark of 0.5 was assigned for the Naranjo Score.

8. T2D was more prevalent in people who had higher concentrations of POPs

In general, the higher the concentration of PHAHs in the body, the more prevalent the incidence of diabetes, or the higher the odds ratio, or relative risk for association of exposure with risk for developing T2D (Calvert *et al.*, 1999; Kim *et al.*, 2003; Rylander *et al.*, 2005; Kang *et al.*, 2006; Lee *et al.*, 2006; Cox *et al.*, 2007; Rignell-Hydbom *et al.*, 2007; Montgomery *et al.*, 2008; Uemura *et al.*, 2008; Wang *et al.*, 2008).

In 4 of the studies analyzed, an increase in body burden of PHAH was not positively associated with an increase in prevalence of diabetes (Zober *et al.*, 1994; Karouna-Renier *et al.*, 2007; Kouznetsova *et al.*, 2007; Jorgensen *et al.*, 2008). Kouznetsova *et al.*, (2007) did find a significant increase in the rate of hospitalization for diabetes among adults living in areas contaminated with toxic waste containing PHAHs, after controlling for confounders. However, because these authors looked exclusively at hospitalization rates and did not collect biological samples to determine body burden of PHAHs, they were unable to associate higher concentrations of PHAHs in subjects living near hazardous waste sites with an increased risk for developing diabetes.

A few studies reported a negative association between the incidence of T2D and exposure to PHAHs. In the Michigan PBB Cohort, it was found that the incidence of

T2D tended to decrease in men with increasing concentrations of PBB (Vasiliu *et al.*, 2006). Likewise, Morgan *et al.*, (1980) found that as the concentration of DDE and DDT increased in blood lipids of the exposed cohort, the incidence of diabetes decreased. Steenland *et al.*, (1999) also did not find excess mortality due to diabetes in their PHAH exposed cohort; rather they found a negative-exposure trend, suggesting that diabetes may be unrelated to TCDD exposure. Similarly, Codru *et al.*, (2007) reported a negative association between the serum concentration of Mirex and the incidence of diabetes in their study population.

To conclude this section, a few studies either did not report concentrations of PHAH in subjects or did not perform analysis on ranges of PHAH concentrations for association with the prevalence of diabetes (Vena *et al.*, 1998; Glynn *et al.*, 2003; Consonni *et al.*, 2008).

Question 9 from the Naranjo Adverse Drug Probability Scale is not relevant for environmental contaminants and is not considered further.

10. Type 2 diabetes was confirmed by objective evidence

Objective evidence was reported for the diagnosis of T2D for many of the studies. For the Yucheng cohort, medical records were obtained from the Taiwan Provincial Department of Health and follow-up interviews were conducted by phone to acquire medical information on individuals who had been diagnosed or treated by certified medical doctors (Wang *et al.*, 2008). Medical, hospital, and army records were also obtained by both Kang *et al.*, (2006) and Morgan *et al.*, (1980) to confirm self-reported diabetes diagnosis. Interviews and medical examinations were conducted in order to determine diabetes diagnosis and self-reported diabetes was confirmed by blood tests in

many studies (Henriksen *et al.*, 1997; Calvert *et al.*, 1999; Kim *et al.*, 2003; Lee *et al.*, 2006; Codru *et al.*, 2007; Everett *et al.*, 2007; Karouna-Renier *et al.*, 2007; Jorgensen *et al.*, 2008; Montgomery *et al.*, 2008; Uemura *et al.*, 2008). Other studies used international statistical classification of disease (ICD-9) codes to determine diabetes through death certificates (Zober *et al.*, 1994; Vena *et al.*, 1998; Steenland *et al.*, 1999; Kouznetsova *et al.*, 2007; Consonni *et al.*, 2008) but several did not confirm self-reported diabetes with any objective evidence (Fierens *et al.*, 2003; Glynn *et al.*, 2003; Rylander *et al.*, 2005; Vasiliu *et al.*, 2006; Cox *et al.*, 2007; Rignell-Hydbom *et al.*, 2007).

Quality Assessment

The mean Naranjo global score for all studies was 17.6 out of 24. Most articles failed to prospectively calculate the study sample size. Also, a few studies did not mention whether blinding was used during the assessment of diabetes or not, and reasons for not blinding were not discussed. Not many of the articles reviewed were follow-up studies and therefore, questions 6 and 7 applied to only a few studies. Overall the quality of these studies ranged between 13 and 22.

Discussion

Meta-Analysis

When the 6 studies were combined, it became apparent that they showed contradictory results and that they were heterogenic ($I^2 = 97\%$). Upon further examination it became apparent that 2 of the studies (Zober *et al.*, 1994; Consonni *et al.*, 2008) were both performed with populations that experience acute exposure to TCDD. Zober *et al.*, (1994) is a study on a group of employees working in a BASF

trichlorophenol unit of a chemical plant at the time of an uncontrolled decomposition reaction. The employees were either exposed during the chemical reaction or during clean-up (Zober *et al.*, 1994). Zober *et al.*, (1994) did not report any association between exposure to TCDD and diabetes; in fact, they reported that diabetes was found less in the exposed group when compared with the referent population. Consonni *et al.*, (2008) also studied a population with an acute exposure to TCDD. This population lived in and around Seveso Italy at the time when an industrial accident occurred in the building where 2,4,5-T, a herbicide, was produced (Consonni *et al.*, 2008). TCDD is a by-product in this process and it was emitted into the surrounding environment. However, when these 2 studies were analyzed separately, $I^2 = 0$. This indicates that all variability in diabetes is due to sampling error within these 2 studies and allows us to conclude that these studies are homogeneous.

The 4 articles that studied chronic exposure to TCDD were Henriksen *et al.*, (1997), Calvert *et al.*, (1999), Kim *et al.*, (2003) and Kang *et al.*, (2006). Henriksen, Kang and Kim studied Vietnam veterans exposed to Agent Orange, of which TCDD was a major contaminant. The Henriksen and Kang groups both studied US Vietnam veterans; Henriksen *et al.*, (1997) only evaluated those who acted as Ranch Hands, individuals involved in aerial spraying of the herbicide and cleaning the airplane application equipment, whereas Kang *et al.*, (2006) included those veterans of the Army Chemical Corps who were involved in the storage, preparation, and spraying of Agent Orange around the perimeter of base camps, and aerial spraying from helicopters (Kang *et al.*, 2001). Kim *et al.*, (2003) studied Korean Vietnam veterans who were exposed to TCDD while it was being sprayed. Calvert *et al.*, (1999) studied a group of employees who were

involved in the production of 2,4,5-trichlorophenol for more than 15 years. As mentioned above, TCDD was a contaminant in this product. When these 4 studies were analyzed separately, there still remained significant heterogeneity ($I^2=64\%$). Therefore the exposure to TCDD was not the only factor influencing the heterogeneity of the studies.

From these results, it appears that accidental exposure resulted in significantly decreased risk of developing diabetes, OR = 0.41 (0.19 – 0.88) for Zober *et al.*, (1994) and OR = 0.45 (0.39 - 0.52) for Consonni *et al.*, (2008). One possible explanation for this is with a single accident, as seen in both studies people are warned to avoid local food and water that could be contaminated with the chemical. These precautions help lower the risk of further exposure through food and water.

Three studies were on Vietnam veterans exposed to TCDD via Agent Orange. These studies were among those that reported positive relationships between exposure to TCDD and risk of developing diabetes. In fact, in our meta-analysis, Kim *et al.*, (2003) had an odds ratio of 2.32 (1.16 - 4.64), Kang *et al.*, (2006) had an odds ratio of 1.69 (1.34 - 2.11), and Henriksen *et al.*, (1997) had an odds ratio of 1.13 (0.89 - 1.44; 95% CI). There could be other factors playing a role in the relationship between diabetes and exposure to TCDD for these veterans. One possible explanation is the interaction between high cortisol concentrations in veterans who have posttraumatic stress disorder (PTSD) and diabetes. A study by Trief *et al.*, (2006) found that a large number and significant percentage of diabetes patients have co-morbid PTSD in a population of American veterans. Another study performed on adults living in the community found a

significant and potentially specific link between PTSD and self-reported diabetes (Goodwin & Davidson, 2005). Although the mechanism of these observations is not precisely known, Goodwin *et al.*, (2005) have provided some possible explanations.

First, diabetes could lead to PTSD, for example, after having a life-threatening episode; this is not applicable to what we have observed in our review. Second, early trauma can result in heightened activity of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in increased concentrations of corticotrophin-releasing hormone (CRH) and increased adrenocortotropic hormone (ACTH), which finally results in increased cortisol production (Goodwin & Davidson, 2005). The elevation of endogenous glucocorticoids is known to induce insulin resistance (Gold & Charney, 2002; Lundgren *et al.*, 2004). This is a possible explanation for the Vietnam veterans in our review; however, it is hard to know whether PTSD developed before or after diabetes. We know in these 3 studies that diabetes developed after the veterans were exposed to TCDD, but further examination is required to determine whether stress or PTSD occurred prior to or after the onset of insulin resistance and diabetes.

The Naranjo Score for the TCDD studies is 3.3 out of 8, indicating a possible association between diabetes and exposure to TCDD. For the 3 groups of studies evaluated (TCDD, T2D only and all studies combined), the TCDD studies had the highest association with diabetes found by the Naranjo Score.

Systematic Review Discussion

After application of the Naranjo Scale to all studies that met our inclusion criteria, a “possible association” was found between exposure to PHAHs and the risk of diabetes.

However, we found a probable association in the 9 studies that specifically looked at T2D with exposure to PHAHs. The mean Naranjo score calculated for the 9 T2D articles was 3.1 and the mean Naranjo Score calculated for all included articles ($n = 24$) was 2.5.

We compared the mean Naranjo Score of the studies that reported a positive association between exposures to POPs and diabetes ($n = 19$) to those studies that did not ($n = 5$) and we found there was no significant difference ($p = 0.9299$) between the two groups (Fig. 9). The mean Naranjo Score in the studies that reported an association was 2.5 ± 0.26 (SD) and the mean Naranjo Score for the studies that did not report an association was 2.5 ± 0.59 .

Of importance, of the 9 articles that only reported on T2D, 8 found significant positive associations between the exposure to some PHAHs and the incidence of T2D. Only 1 study did not find a positive association between any PHAH and the incidence of T2D in a population. This study of American factory workers employed in the production of 2,4,5-trichlorophenol or one of its derivatives, at 2 factories in the US, only included cases of diabetes that developed after starting work at the factory (Calvert *et al.*, 1999). Two other investigations did not find a positive association between the incidence of diabetes and exposure to specific PHAHs. One of these evaluated PBBs and PCBs in a Michigan cohort and found no overall positive association between exposure to PBB and the incidence of diabetes. However, only in women were higher PCB serum concentrations associated with a 2-fold increase in the incidence of T2D (Vasiliu *et al.*, 2006). The second study tested for possible associations between environmental exposures to *p,p'*-DDE, HxCDD or PCB 126 and the incidence of undiagnosed diabetes, diagnosed diabetes, and total diabetes (undiagnosed diabetes plus diagnosed diabetes), in

the NHANES cohort. Although all 3 compounds were positively associated with diagnosed diabetes, HxCDD was not positively associated with total diabetes (Everett *et al.*, 2007). For each of the 3 studies that did not find an association with the incidence of T2D, the PHAH was chemically different.

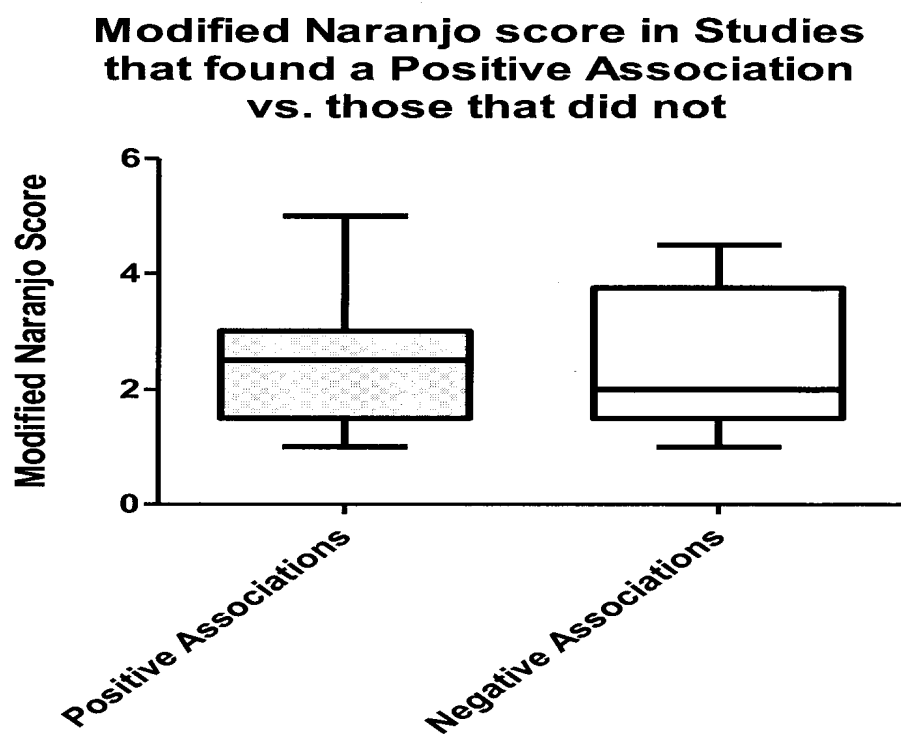


Figure 9: Comparison of Naranjo Scores between the studies that found an association and those that did not.

When one considers all the studies together, of the 24 publications, 5 did not find any association between T2D and exposures to PHAH, whereas 20 did report a positive association between these parameters. In addition to the study by Calvert *et al.*, (1999) described above, 4 others did not find an association between the risk for T2D incidence and PHAH exposures.

Members of a population that lived near the Escambia Treating Company (ETC) outside Pensacola, Florida were exposed to benzo(α)pyrene and PCDD/F for 40 years through ground water, soil, and air contamination (Karouna-Renier *et al.*, 2007). Although the rates of diabetes in the ETC cohort exceeded those of national averages at the time of the study, these authors were unable to find any significant association between the occurrence of diabetes and serum PCDD/F concentrations normalized to lipid content (Karouna-Renier *et al.*, 2007).

The IARC cohort is a population of workers from 12 plants that produced chemicals contaminated during synthesis with TCDD in the United States. Steenland *et al.*, (1999) evaluated exposure-responses in diabetes-induced deaths and failed to find any excess risk for diabetes. Their findings led them to conclude that exposure to TCDD may be unrelated to the incidence of diabetes. Zober *et al.*, (1994) studied a similar population of workers exposed to TCDD by fumes while cleaning up after a decomposition reaction at a trichlorophenol production facility (Zober *et al.*, 1994). Diabetes was found significantly less often in the exposed population (11% in the high TCDD group, 2.4% in the low TCDD group) than the reference population (14.3%) approximately 35 years after the accident.

Jorgensen *et al.*, (2008) studied a population of Greenland Inuit who were exposed to PHAHs through consumption of contaminated fish, and also found no association between the degree of exposure to PHAHs and the incidence of diabetes. The authors surmised that a possible reason for their results is the existence of a threshold for PHAH exposure. Inuit already have a very high prevalence of diabetes and due to their diet, characterized by high fish and sea mammal consumption which contain relatively high concentrations of POPs, have relatively high body burdens of PHAH, indicating significant exposures. Thus, it is possible that the exposure concentrations above such a threshold may not further increase risk for diabetes (Jorgensen *et al.*, 2008).

Two important trends arose from the studies included in this review: a gender difference within exposed cohorts and an increased prevalence of diabetes that resulted in significant associations between the incidence of diabetes and individuals highly exposed to PHAH.

Gender Difference

Five studies only included men (Zober *et al.*, 1994; Henriksen *et al.*, 1997; Steenland *et al.*, 1999; Kim *et al.*, 2003; Kang *et al.*, 2006) and two studies had primarily male participants (95% and 97%) because individuals in these cohorts were factory workers (Calvert *et al.*, 1999; Montgomery *et al.*, 2008). Of these 7 studies, significant associations were found in the 3 Vietnam veteran studies and one pesticide worker study. Kang *et al.*, (2006) found that US army veterans who were occupationally exposed to TCDD in Vietnam had significantly higher risks for diabetes. Likewise, Kim *et al.*, (2003) studied a Korean population of Vietnam veterans who were occupationally exposed to TCDD and found an excess frequency of diabetes, which remained significant

even after adjusting for age, smoking status, alcohol consumption, BMI, education and marital status. Henriksen *et al.*, (1997) found that the risk for being diagnosed with diabetes mellitus increased, the time-to-onset of diabetes decreased, and the severity of diabetes increased with increasing dioxin concentrations in veterans of Operation Ranch Hand. Montgomery *et al.*, (2008) studied pesticide applicators and reported positive associations between the incidence of diabetes and body burdens of the 7 chlorinated pesticides, aldrin, chlordane, heptachlor, dichlorvos, trichlorfon, alachlor and cyanazine. The other 3 studies did not find a significant association between diabetes and the degree of exposure to PHAH.

Two studies only included women and both concerned Swedish populations mainly exposed through consumption of contaminated fish. The first (Rignell-Hydbom *et al.*, 2007) found a significant association between the incidence of T2D and the degree of exposure to 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153), *p,p'*-DDE, and T2D in wives of fishermen. The second evaluated women from 12 different counties along the coast of Sweden and found that diabetics had significantly higher body burdens of HCB (Glynn *et al.*, 2003).

Of the studies that included both male and female participants and included gender analysis, significant associations were found between the degree of exposure to PHAH in women and the incidence of T2D (Rylander *et al.*, 2005; Vasiliu *et al.*, 2006; Consonni *et al.*, 2008; Wang *et al.*, 2008). Rylander *et al.*, (2005) also found that men had a positive association between the incidence of T2D and CB-153 exposures, but a more ambiguous pattern was observed with *p,p'*-DDE, where men had a stronger association than with CB-153. These authors did not offer a plausible biological

explanation for the differences. Vasiliu *et al.*, (2006) reported a 2-fold increase in the incidence of diabetes in exposed women with serum concentrations of PCBs above 5 ng/g, relative to a reference population. Women also were noted to have a linear association of increasing incidence for diabetes with PBB levels, which was not seen in males, but no explanation was offered for these gender differences. Wang *et al.*, (2008) reported a 2-fold increase in the prevalence of T2D among exposed Yucheng women when compared to the reference population. Similarly, Consonni *et al.*, (2008) found excess mortality from diabetes in females in all exposure categories, but more so in the middle exposure category when compared to males, in a 25-year follow-up to the Seveso incident. The exposure categories were determined by soil concentrations of TCDD after the accident. The highest exposure category had a mean TCDD soil concentration of $15.5 \mu\text{g}/\text{m}^2 - 580.4 \mu\text{g}/\text{m}^2$, the middle exposure category had a mean TCDD soil concentrations of $1.7 \mu\text{g}/\text{m}^2 - 4.3 \mu\text{g}/\text{m}^2$, and the lowest exposure category had a mean TCDD concentration of $0.9 \mu\text{g}/\text{m}^2 - 1.4 \mu\text{g}/\text{m}^2$ (Consonni *et al.*, 2008).

Lee *et al.*, (2006) did not compare genders in their analysis of NHANES data; however, they found that men tended to have lower concentrations of most POPs, especially OCDD, than the women studied. This finding is similar to that of Wang *et al.*, (2008): most excess body burdens of PCBs and PCDFs occurred in women with an average age of 25 years. This prompted these authors to rationalize that women have a higher fat composition than men, resulting in a longer half life of slowly metabolized lipophilic compounds, such as PHAHs, in adipose tissue. As obesity is also a risk factor for diabetes, the additional extra body burden of PHAHs could have additional significance in obese individuals (Wang *et al.*, 2008). However, this explanation does not

hold for the Michigan study, where Vasiliu *et al.*, (2006) found that more women than men were in the lowest exposure group, whereas more men than women were in the highest serum level group for both PBBs and PCBs. Wang *et al.*, (2008) also suggested that women may be more vulnerable to PCB and PCDF exposures as a result of higher estrogen levels than men, as some dioxin-like chemicals may exert their diabetogenic effects through an estrogen-dependent pathway.

Highly Exposed

In general, a higher prevalence of diabetes was found in individuals in the highest category of PHAH exposure. Thus, Calvert *et al.*, (1999) reported that 60% of those with the highest serum concentrations of TCDD, >1500 ng/kg serum lipid, had diabetes mellitus. From these data, this group of researchers concluded that workers with high body burdens of TCDD are at increased risk of T2D. Fierens *et al.*, (2003) also reported a significant increased risk of T2D in the most exposed individuals for dioxin, coplanar PCBs and 12 PCB congeners in their study. In fact, for those in the 90th percentile, the odds ratio for developing diabetes was 13.3 for exposure to coplanar PCBs, 7.58 for exposure to 12 PCB congeners, and 5.07 for exposures to dioxin.

For comparison, Wang *et al.*, (2008) found that the odds ratio for the incidence of T2D increased in those whose concentration of PCB 70 were in the upper quartile compared to the reference population. A similar trend was observed by Vasiliu *et al.*, (2006) in the Michigan cohort, where the incidence of diabetes significantly increased in women in the higher PCB concentrations by 2- to 2.3-fold, after adjustment for other risk factors. Uemura *et al.*, (2008) reported a significant association between the third highest

and highest quartiles of exposures to PCDD/Fs, dioxin-like PCBs and total dioxins with diabetes prevalence. A significant increase in the incidence of diabetes in individuals from the highest tertile of body burden compared to the lowest tertile for PCB-153 and PCB-74 exposures was reported by Codru *et al.*, (2007). In the same vein, Kang *et al.*, (2006) noted that American Vietnam veterans who had high body burdens of TCDD also had a higher prevalence of diabetes than those with a lower body burden of TCDD. Finally, Rignell-Hydbom *et al.*, (2007) reported significant positive trends between CB-153 and *p,p'*-DDE residues in wives of Swedish fishermen and the incidence of diabetes when exposure concentrations were characterized into quartiles.

Montgomery *et al.*, (2008) did not report body burden concentrations of the pesticides and herbicides studied. However, they did find an increase in the odds ratio for the increased incidence of diabetes in those who were in the highest quartile of cumulative days of use of pesticides compared to those in the lowest quartile. Kouznetsova *et al.*, (2007) studied hospitalization rates due to diabetes and determined that the rate was increased in individuals that lived near areas that contain one or more hazardous waste sites containing PHAHs and volatile organic chemicals, compared to individuals from areas with no hazardous waste sites.

In contrast, Steenland *et al.*, (1999) reported a statistically significant negative trend between risk for incidence of diabetes and cumulative exposure to TCDD. They noted that diabetes was more prevalent in those they evaluated who had lower amounts of TCDD exposure. The 25-year Seveso follow-up study of Consonni *et al.*, (2008) documented excess mortality from diabetes in women from all TCDD exposure zones, but this mortality was highest in zone B, characterized by intermediate exposures to

TCDD. This appears to contradict what is reported in other studies reviewed here. What one might have expected to observe in this population is a definite dose gradient between the 3 zones A (highest exposures) > B > R (the reference population with background concentrations of TCDD) (Consonni *et al.*, 2008). However, as noted by the authors, population size varied across the three zones, and this limited the power of the study and its ability to detect unusual relative risks, compromising the interpretation of the results.

Considering Bias against the Null Hypothesis

Overall, the overwhelming trend of the published studies is a positive association between exposure to POPs and the incidence of T2D, with a clear trend toward a dose response relationship, particularly at higher exposure levels. Yet, the existence of negative studies bears the question of the known publication bias against negative studies. Our analysis reveals that the quality score of the negative studies, 17.8 ± 0.73 (mean \pm SEM) did not differ ($p = 0.8619$; Fig. 10) from the score of the positive studies, 17.6 ± 0.61 . This allows us to conclude that publication bias did not play a role in our analysis.

**Qualitative Assessment Comparison of
Studies that Found an Association
and Those that Did Not**

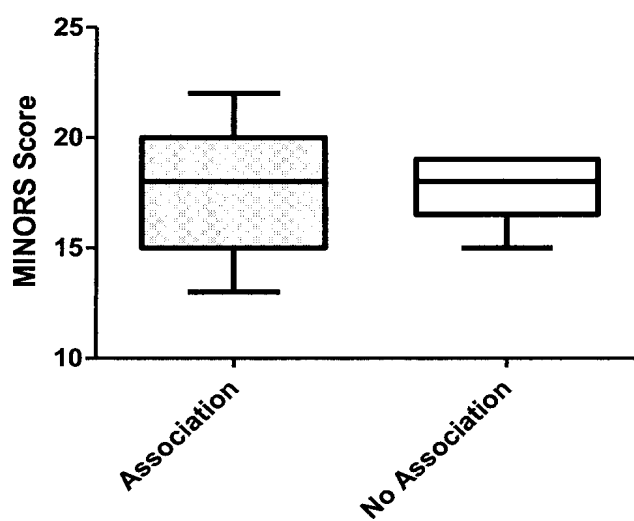


Figure 10: Qualitative assessment of articles

Biological plausibility

There are at least 3 plausible biological mechanisms: altered lipid metabolism; altered glucose transport; and/or alterations in the insulin signaling pathway that could contribute to the positive association between exposure to some PHAHs, including dioxins, and the increased incidence of T2D (Schechter & Gasiewicz, 2003).

In vivo and *in vitro* studies in experimental models have identified mechanisms by which TCDD and other PHAHs could potentially cause T2D and its precursors. For example, Marchand *et al.*, (2005) reported that TCDD treatment of human hepatoma (HepG2) cells increases the expression of the gene that codes for insulin like growth factor binding protein 1 (IGFBP-1). This in turn, leads to IGFBP-1 protein formation and secretion from these cells. Increased serum IGFBP-1 content has been shown to oppose insulin action, potentially through attenuation of the action of insulin growth factors (IGF) (Marchand *et al.*, 2005). Additional support for this explanation comes from transgenic mice expressing high levels of the rat IGFBP-1 gene. After their first week of life, these genetically modified mice become hyperinsulinemic and then develop fasting hyperglycemia (Crossey *et al.*, 2000). Moreover, transgenic mice expressing the human IGFBP-1 gene become hyperglycemic, then hyperinsulinemic and finally develop glucose intolerance in later life (Rajkumar *et al.*, 1996).

The wasting syndrome is a toxic manifestation of exposure to TCDD and other dioxin-like chemicals that can act through the AHR, and this syndrome is accompanied by decreased lipoprotein lipase activity, serum hyperlipidemia and decreased glucose uptake (Enan *et al.*, 1996). Urban *et al.*, (2007) recently reported that TCDD can cause

indirect (or secondary) damage that result in disorders of lipid metabolism, some of which are precursors to diabetes.

The AHR is a ligand-dependent transcription factor that regulates a large range of gene expression in a variety of species and tissues. TCDD is thought to act through the AHR to cause most of its toxic effects, so it is not surprising that it is also thought to be involved in the onset of T2D. Dioxin and dioxin-like chemicals bind first to the cytosolic receptor (Ah locus protein product), then translocate to the nucleus where they cause their changes in gene expression (Enan *et al.*, 1992). It has been proposed by Poland *et al.*, (1982) that TCDD most likely inhibits the transcriptional expression of GLUT genes after it binds to the cytosolic Ah-receptor. Enan *et al.*, (1992) showed that GLUT 4 was the most affected glucose transporter after treatment with TCDD. This is in agreement with the epidemiological studies that were done on the Veterans of Operation Ranch Hand (see above).

Conclusion

In the meta-analysis of TCDD studies, we found increased odds of developing diabetes in populations with chronic exposure to TCDD compared with those populations who had acute (accidental) exposure to TCDD.

By the application of the modified Naranjo ADR Score to all studies that met the inclusion criteria for this systematic review, we found a possible association between exposure to PHAH and the prevalence of T2D. This positive association was stronger, becoming a probable association, in those studies that specifically evaluated the relationship between exposures to PHAH and T2D (Naranjo Score of 3.1) and for studies

that specifically evaluated the relationship between exposure to TCDD and diabetes (Naranjo Score of 3.3) when compared to all studies (Naranjo Score of 2.5).

A gender difference was also reported in a majority of the studies that evaluated this parameter by comparing PHAH body burdens between males and females with the incidence of diabetes. In the studies that included both males and females, females appeared to have a higher risk of developing T2D than did males. However, there were a few studies that showed the opposite effect for specific PHAHs. More studies are required to explain this difference and to draw firm conclusions.

From the qualitative analysis we conducted on the studies, it does not seem likely that publication bias plays a role in our conclusions. Following the precautionary principle in toxicology it seems prudent to us that exposure to high concentrations of PHAH should be avoided whenever possible due to their clear trend for association with the increased incidence of T2D.

Chapter 4: *Subjects and Methods*

This study was approved by the University of Western Ontario's Office of Research Ethics (see Appendix 1).

4.1 Health Questionnaire

A Walpole Island Healthy Survey Questionnaire was adapted from the 2006 Statistics Canada Aboriginal Children's Survey. The questionnaire was developed by collaboration and consultation among members of the multi-disciplinary Ecosystem Health Research Team from Walpole Island First Nation Health Centre, Walpole Island First Nation Heritage Centre and the Schulich School of Medicine & Dentistry, University of Western Ontario. The questionnaire is a detailed (24 page; Appendix 2) in-depth inquiry into the health status of participants and their children and grand children, and also asked questions regarding dietary habits, health problems, child care, child development, and community issues. The questionnaire also contains the Perceived Stress Scale (PSS) (Cohen *et al.*, 1983) questionnaire. This validated questionnaire has a scale measuring generalized perceptions of stress that was designed by Cohen and his colleagues (Cohen *et al.*, 1983). It is intended for use in a community population with at least a junior high school education and asks questions regarding current levels of experienced stress.

The health questionnaire was administered to participants on May 27, 2008 at the time of blood and hair sampling at the Walpole Island Health Centre. Participants filled out the questionnaire after donating hair and blood samples for the study.

4.2 Analytical Procedures

4.2.1 Measuring Persistent Organic Pollutants

Whole blood samples were collected from 20 volunteers and were analyzed for 91 different POPs. Blood was drawn by a physician or a trained research nurse who are all members of the WIFN Ecosystem Health Research Team. Blood for POPs analysis was collected in Becton-Dickinson Vacutainer® (BD) tubes (Becton-Dickinson Oakville, Canada). A total of 35 mL of blood was collected from each participant in the study. One tube (10 mL) of blood from 20 volunteers was separated into plasma, stored on ice and delivered immediately to the Great Lakes Institute for Environmental Research (GLIER), where analysis was conducted. One tube (5 mL) of whole blood was collected in an EDTA BD Vacutainer ® tube (Oakville, Canada) and used for analysis of heavy metals and metalloids (see below). The other blood samples (2 x 10 mL) were taken to the Robarts Research Institute, University of Western Ontario where they were frozen immediately in a -80° C freezer.

These samples are stored in specialized tubes for genomic and proteomic analysis, respectively (details of tubes used for their collection are required). Under the conditions of our UWO ethics approval (Principal Investigator, J.R. Bend; Review Number 13752E; valid Jan 8, 2008 – November 30, 2012 with annual updates) and with signed consent forms received, these samples may be stored up to 10 years for use in studies to evaluate the relationships between exposure to environmental contaminants and human health.

This is an optional part to our biomonitoring study and additional blood was only taken and stored if participants signed the informed consent forms prior to sampling.

The POPs selected for analysis included: 1,2,4,5-tetrachlorobenzene (1,2,4,5-TCB), 1,2,3,4-tetrachlorobenzene (1,2,3,4-tetrachlorobenzene), pentachlorobenzene (QCB), hexachlorobiphenyl (HCB), α , β , γ and δ hexachlorocyclohexane (α -HCH, β -HCH, γ -HCH and δ -HCH), octachlorostyrene (OCS), heptachlor-epoxide, oxychlordan, *trans*-chlordan, *cis*-chlordan, *trans*-nonachlor, *cis*-nonachlor, 1,1-bis-(4-chlorophenyl)-2,2-dichloroethylene (*p,p'*-DDE), 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene (*p,p'*-DDD), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (*p,p'*-DDT), dieldrin, Mirex and PCBs (IUPAC #: 17, 18, 28, 31, 33, 44, 49, 52, 70, 74, 82, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 153, 156, 158, 169, 170, 171, 177, 180, 183, 187, 191, 194, 195, 201, 205, 206, 208, 209). PCB congeners are chlorine-substituted biphenyl compounds. The individual congeners are identified by the number and position of chlorine atoms around the biphenyl rings. There are currently 209 possible PCB congeners ranging from mono-chlorinated biphenyls to deca-chlorinated biphenyls.

The extraction of POPs from the plasma sample was performed as previously described by Drouillard and Norstrom, 2000. Plasma (0.3 – 0.5g) was added to a 15 mL centrifuge tube and spiked with 200 pg of the six [^{13}C] PCB congeners used as internal standards. Methanol (100% plasma volume) and 6 M HCl (33% plasma volume) was added to the centrifuge tube to deproteinate the plasma sample (Drouillard & Norstrom, 2000). The sample was vortexed for 1 min, and diluted to 5 mL with distilled water.

Solid-phase extraction cartridges (Enviro 18; 1g; Supelco) were prewashed with hexane (6 mL), then acetone (6 mL), and were activated with 10 mL methanol. The POPs samples were then eluted from the column with 10 mL distilled water and was then loaded onto a C18 extraction column, which is used for lipophilic compounds. The column was washed under reduced pressure at 1 mL/min with water. A vacuum was used to remove excess water from the extraction column. The POPs were recovered from the extraction column by elution with 12 mL chloromethane: hexane (1:1). Enviro-Florisil SPE tubes (0.5 g; Supelco) were pre-cleaned with 10 mL hexane and used in the clean-up of concentrated extracts of the POPs to be analyzed (<2 mL). Clean-up was performed by loading the extracts onto the Florisil column and eluted with 14 mL hexane at a rate of 1 mL/min by gravity. The eluted extracts were then concentrated to 100 μ L and spiked with 200 pg [13 C] PCB 138 as a volume corrector. Blanks were performed using distilled water.

The recovered POPs were analyzed using a method similar to that described by Lazar and his colleagues (Lazar *et al.*, 1992) with a Hewlett Packard-5890 gas chromatograph with electron capture detection, equipped with a Hewlett Packard-3396 integrator and a Hewlett Packard -7673 autosampler. A 30 m x 0.25 mm i.d. with 0.25 mm DB-5 film thickness (J & W) column was used for POPs analysis at an injector temperature of 250°C. The carrier gas was helium at a rate of 30 cm/sec, measured at 100°C. The composition of the make-up gas was argon: methane (95:5) at 40 mL/min. The oven temperature program was: initial temperature, 100°C for 0.5 min; rate of temperature increase, 3°C/min; final temperature of 270°C, achieved at 8 min following

sample injection. The sample volume injected onto the column was 3 μL , using a splitless injection mode (Lazar *et al.*, 1992).

4.2.2 Measuring Heavy Metals

Hair (500 mg) and whole blood samples (5 ml) were collected from volunteers for heavy metal and metalloid analysis. All analyses were performed at the London Health Sciences Centre (LHSC) Trace Elements lab for metal analysis. The blood samples were analyzed for 6 heavy metals; one metalloid, arsenic (As); and 5 metals: antimony (Sb), cadmium (Cd), lead (Pb), nickel (Ni) and thallium (Tl) by inductively coupled mass spectrometry (ICP-MS). Blood was collected in an EDTA BD Vacutainer® tube (Oakville, Canada). Approximately 485 – 490 mg of hair was collected from the posterior vertex region of the scalp. Hair was cut with scissors as close to the scalp as possible. The hair was then taped, using 3M Micropore Surgical Tape (3M), to a clean sheet of paper, with the scalp end of the hair indicated on the paper by an arrow. The piece of paper was folded carefully, ensuring that the hair was not bent, and placed into an envelope. Hair samples were analyzed for 15 different heavy metals, one metalloid As; and 14 metals including Sb, aluminum (Al), barium (Ba), beryllium (Be), bismuth (Bi), Cd, Pb, Ni, palladium (Pd), platinum (Pt), Silver (Ag), Tl, titanium (Ti) and uranium (U) by ICP-MS.

Red blood cells (RBC) were separated and digested with equal amounts of HNO_3 and H_2O_2 and then diluted 20-fold with purified water (London Health Sciences Centre, 2008). Hair was washed 0.1% triton X, rinsed 3 times with purified water and dried in an oven at 70°C for 30 minutes. It was then digested with concentrated nitric acid (HNO_3)

for 1 hour (London Health Sciences Centre, 2008). Before analysis with HR-ICP-MS, the hair was diluted 10-fold by purified water. The procedure was done using the HR-ICP-MS instrument, ELEMENT (Finnigan MAT, Bremen, Germany) (London Health Sciences Centre, 2008). The ion source (ICP) operates at temperatures greater than 8000K and a double focussing magnetic sector mass spectrometer is used as a detector to separate the elements and their isotopes for subsequent detection and measurement (London Health Sciences Centre, 2008). Optimal instrumental conditions were applied, the resolution factor power was 1300 W and the carrier gas was argon with a flow of 1.05 L/min., outer gas flow of 14 L/min. and intermediate gas flow of 0.95 L/min. (Philp, *et al.*, 2003). The sensitivity for hair was less than 0.01 ng/g hair.

4.2.3 Measuring Cortisol

Hair was collected from the posterior vertex region on the back of the scalp from Walpole Island volunteers on May 28, 2008. The posterior vertex region was chosen because most hairs in this area have the same growth rate and because the proportion of hair in the telogen growth phase is low (Villain *et al.*, 2004; Pragst & Balikova, 2006). The posterior vertex is located just beneath the crown, at the back of the head. Approximately 10 - 15 mg of hair was collected, as the method used to analyze the hair samples for cortisol required a minimum of 10 mg. Before hair collection, scissors were cleaned with isopropyl alcohol to disinfect them and then dried thoroughly to prevent hair from sticking to them. Gloves were worn by persons collecting the hair samples to prevent contamination of the samples with sweat from hands.

Hair was cut with scissors as close to the scalp as possible. The hair was then taped using 3M Micropore Surgical Tape (3M) to a clean sheet of paper, with the scalp end of the hair indicated on the paper by an arrow. The piece of paper was folded carefully, ensuring that the hair was not bent, and placed into an envelope. The hair was then transported to Robarts Institute of Technology, where it was stored in the envelope at room temperature until analysis. Hair was stored for a maximum of one month before analysis. However, it is worth noting that organic substances, such as cortisol, can survive in hair for hundreds of years when it is protected from light and moisture (Villain *et al.*, 2004).

During sample preparation, hair was separated from the paper by slicing the tape and exposing hair. Hair was then transferred from the paper to the cutting device. Hair was aligned along the ruler of the cutting device, with the scalp end of the hair lined up against 0 cm. Hair was clamped down on the cutting device and one centimetre was cut using an Exacto knife. Once cut, the one cm portion of hair was transferred to a glass vial with a polyethylene cap lined with aluminum foil and weighed using an analytical balance. One mL of HPLC grade methanol (>98%) was added to each vial and hair was cut up finely using surgical scissors (Van Uum *et al.*, 2008). The vials were then sealed by a strip of parafilm wrapped tightly around the necks to prevent the escape of vapour. Once sealed, the vials were placed in a shaking incubator at 100 RPM and 50°C for 16 h (Van Uum *et al.*, 2008).

After incubation, the vials were cooled to room temperature, the methanol extract was quantitatively transferred to a clean borosilicate test tube, and the solvent removed

by heating the tube to 50°C under a gentle stream of nitrogen gas (Van Uum *et al.*, 2008). A white residue remained in the tube after methanol removal and this residue was dissolved in 250 µL of phosphate buffered solution (PBS) at pH 8.0, by vortexing the tube and PBS for 30 s (Van Uum *et al.*, 2008). The PBS solutions were then assayed using a commercially available salivary enzyme immunoassay (EIA) for cortisol (11-CORTIU-E01-SLV, Alpco Diagnostics, Salem, NH, USA) as per the manufacturer's directions with the exception that the assay was shaken at 100 RPM instead of the recommended 200 RPM (Van Uum *et al.*, 2008). The EIA assay was conducted on a flat bottomed, antibody coated 96-well plate (Alpco Diagnostics, Salem, NH, USA) and was read on a Vmax plate reader from Molecular Devices (Sunnyvale CA, USA). The Vmax plate reader evaluates the optical density of the colour, interpreting the results.

4.3 Statistical Analysis

The WIFN population was not normally distributed in the POPs data, heavy metals data, and cortisol data, which was determined because they did not pass the Shapiro-Wilk normality test. Because of this, the Mann-Whitney U test was used to determine whether there were variations in medians between 2 groups of data. The Kruskal-Wallis Test, followed by the Dunn's Multiple Comparison Test, were used to determine whether there were variations in medians between 3 or more cohorts in the heavy metal results. Correlations were done with the data by using Spearman Rank Correlations.

Due to the amount of Mann-Whitney U Tests done on all our data, we recognize that some significant results could be due to chance alone. In an attempt to correct for

this we applied the Bonferroni correction to all results found to be significant by the Mann-Whitney U Test.

Chapter 5: *WIFN Human Baseline Biomonitoring and Child Health Survey to Assess Risk to Exposure of Environmental Contaminants*

5.1 Introduction

5.1.1 Walpole Island

Walpole Island is located downstream of Sarnia on the St. Clair River at the mouth of Lake St. Clair. The St. Clair River is an important waterway in the Great Lakes Basin, joining Lake Huron in the north to Lake St. Clair in the south, which then feeds into the Detroit River and subsequently Lake Erie. A total of 6 islands make up the Walpole Island First Nation (WIFN) and Aboriginals have been living on these islands for more than 6000 years (Bowles, August 2005). The WIFN community consist of 3 different ancestral native peoples: the Ojibwa, Odawa (Ottawa) and Pottowatomi, all living in a political compact known as the *Three Fires Confederacy* (Stephens & Darnell, 2008). Walpole Island is also known as Bkejwanong, meaning “where the waters divide”. Walpole Island is home to one of the most diverse ecosystems in Canada and for centuries the WIFN has relied on the land and the river as a source of livelihood, entertainment, education, tradition, culture, income, and food. The ecosystems found on Walpole Island (tall grass prairies, oak savannahs, and wetlands) provide a habitat for many rare plants and animals and the WIFN community has successfully managed these diverse ecosystems for thousands of years (Bowles, August 2005).

Important sources of income for the community are also traditional lifestyle practices such as hunting and fishing (Stephens & Darnell, 2008). Their economy was devastated when the fishing industry was shut down in the 1970s for a decade because of the high concentrations of PCBs and heavy metals in the water. Sport fishing is also another major industry for the community and helps to bring in tourists from all over Ontario and Michigan, but it too is being adversely affected by the pollution. A survey done on anglers in Lake St. Clair showed that fishing in 2002 was 31% lower than a decade earlier (Wigle & Vincent, 2005). Currently, the WIFN is the only group holding a commercial fishing licence for Lake St. Clair and some families in the community exercise their right to fish for subsistence on the lake (Wigle & Vincent, 2005). However, because of the closure of the commercial fishing industry in the 1970's, and due to the continual pollution of the St. Clair River and Lake St. Clair, many community members are wary of the effects these pollutants are having on both the surrounding environment, wildlife, and the WIFN population.

5.1.2 Chemical Valley

The source of pollution and concern for the community is coming from an area known as Chemical Valley located within 60 km of Walpole Island. Chemical Valley is an industrial area in and around Sarnia, Ontario surrounding the St. Clair River on both the American and Canadian sides of the border. The St. Clair River has played an integral role in the development of Chemical Valley, as it has allowed for easy transportation of products to the United States and Windsor. The petrochemical industry, just one of the industries now found in Chemical Valley and the first to locate there,

began in this area in the 1860's in Petrolia and Oil Springs (Wigle & Vincent, 2005). Industrial expansion occurred in the 1940's, 50's, and 70's resulting in the development of more and larger refineries and chemical and manufacturing plants on both sides of the border. Today Chemical Valley includes approximately 60 industrial factories in both Canada and the US and accounts for more than 40% of Canada's total chemical industry (MacDonald & Rang, 2007).

By the 1960's the development, industrialization and habitation of Sarnia and the surrounding area resulted in increased environmental pollution (Wigle & Vincent, 2005). In 1984 - 1985 field studies were conducted to determine the concentration of heavy metals and POPs in the sediment of the St. Clair River. Elevated concentrations of Pb, Cd and several organic chemicals were discovered. These contaminants are continually stirred up by the dredging that occurs in the St. Clair River because it is part of the St. Lawrence Seaway (Wigle & Vincent, 2005). The St. Clair River was identified as an Area of Concern in the Great Lakes Basin by the International Joint Commission of the Great Lakes in 1985 (Wigle & Vincent, 2005). Since 1986, Environment Canada (EC) has been monitoring the St. Clair River for heavy metals and POPs (Wigle & Vincent, 2005). Samples of the river water and sediments have shown a large range of POPs, such as: PCBs, PAHs, dioxins, furans, OC pesticides, and PBDEs (Wigle & Vincent, 2005). In 1987 the *Great Lakes Water Quality Agreement (GLWQA)* resulted in the development of Remedial Action Plans (RAPs) for the St. Clair River (Wigle & Vincent, 2005). Over the last 2 decades, with an increase in governmental regulation and industrial awareness, pollution into the St. Clair River from point sources has decreased (Wigle & Vincent, 2005).

However, pollution is still a major concern in the area and justifiably so. There are currently 46 facilities on the Canadian side of the border within 25 km of Sarnia that are listed under the NPRI (MacDonald & Rang, 2007). In 2005, the facilities in Chemical Valley emitted 5.7 million kg of 'Toxic Air Pollutants', which include numerous chemicals that have been associated with reproductive and developmental disorders and cancer among humans (MacDonald & Rang, 2007).

With specific reference to POPs, in 2005, LANXESS Inc. released 1 362 500 kg of volatile organic compounds (VOCs) accounting for 23% of the total VOCs released in the area; Shell Canada released 2 kg of tetrachloroethylene, a known carcinogen and toxin; Royal Polymers released 5 501 tonnes of vinyl chloride, also a known carcinogen and toxin and LANXESS Inc. released 204 600 tonnes of chloromethane, a chemical known to disrupt respiration and reproduction (MacDonald & Rang, 2007). Lambton Generation Station, owned by Ontario Power Generation, released 0.183 g toxic TCDD equivalent, accounting for 85% of the total dioxins and furans release in the Sarnia area in 2005 (MacDonald & Rang, 2007). Between the years 2000 and 2004, 5 documented large chemical spills occurred in the St. Clair River. One occurred in December of the year 2000 when NOVA Corunna Chemical Plant spilt a large volume of aromatic hydrocarbons (ex. Benzene) in the environment, with some entering the St. Clair River (Wigle & Vincent, 2005). Downstream from the point source, the peak benzene concentration measured was 0.7 ppb, which is over the Ontario Drinking Water Standard of 0.5 ppb (Wigle & Vincent, 2005).

These POPs and heavy metal pollutants have not only been measured in the water and sediment, but also in the vegetation and wildlife that live in the St. Clair River and Lake St. Clair. Snapping turtle eggs obtained from the wetlands in the area are measured for PCDD, PCDF, OC pesticides, and PCBs as a way to monitor chemical contamination. Ashpole *et al.*, found that concentrations of PCDDs, PCDFs, and non-*ortho* PCBs in 1999 in 3 areas around Walpole Island were relatively low compared with other areas sampled in the Great Lakes basin and historical records from the St. Clair River. Another study done in 1986, examined the concentrations of QCB, HCB, and OCS in migratory and non-migratory ducks on Walpole Island. Hebert *et al.*, (1990) reported that concentrations in non-migratory ducks were higher than those found in migratory ducks and the concentrations of OCS in breast muscle of ducks were above the New York Department of Environmental Conservation (NYDEC) consumption guideline (OCS: 20 µg/kg) at the time of the study, putting the WIFN community at increased risk of exposure.

Edelstein (2004) has defined a 'contaminated community' as any residential area located within identified boundaries for a known exposure to some form of pollution. By this definition, Walpole Island qualifies as a contaminated community, and the consequences of being in this category can impact more than just the community's health. Many community members are worried about the risks of adhering to their traditional diet, culture, and lifestyle. What effect will exposure to these chemical contaminants have on their health and on the health of future generations?

5.1.3 Native American Health

There are many interconnected determinants affecting the health of Native North Americans such as: socioeconomic status, environmental conditions, and lack of access to health care services (MacMillan *et al.*, 1996).

There is an inverse relationship between low socioeconomic status and health that has been well documented in the literature (Syme & Berkman, 1976). Socioeconomic status plays a large role in where people live, what food they eat and the amount of time they spend taking care of themselves and other people. The unemployment rate for Native North Americans living on reserves from 1999 - 2003 was 27.7% compared to the Canadian rate of 7.3% (Health Canada, 2009). Employment factors into health by determining the kind of housing people live in, the quality of food they can afford to buy, their ability to travel to access better health care services if needed, and their ability to take time off work for themselves.

Another important socioeconomic factor involved in employment and health is education level. Between 1999 - 2003 in Canada, 36% of Native American youths completed a high school education, compared to 84.6% of the Canadian population; 49.7% of Native North Americans did not complete a high school education compared with 12% of the rest of Canadians (Health Canada, 2009). Education factors into health of an individual and a community in many different ways. Just being aware of health issues and healthy lifestyle practices is a large part of maintaining health. Anand *et al.*, (2001) showed that Native North Americans in Canada had a higher frequency of cardiovascular disease (CVD) and a greater burden of atherosclerosis compared with the

general Canadian population of European descent. However, these authors concluded that the Aboriginal population studied had significantly lower levels of education and employment and lived in poverty all of which are socioeconomic factors that are also associated with high rates of CVD and CVD risk factors (Anand *et al.*, 2001).

Access to medical services is a reoccurring health issue for Native North Americans. Macmillan *et al.*, (1996) have attributed this to geographic isolation and a shortage of trained personnel in a close proximity to Native American populations. Gao *et al.*, (2008) conducted a study of Aboriginal people with chronic kidney disease in an attempt to assess differences across the Canadian health care system. They found that Aboriginal people were almost twice as likely as non-Aboriginals to be admitted to the hospital for an ambulatory-care-sensitive condition and that aboriginals with severe chronic kidney disease were significantly less likely to have visited a nephrologist. These researchers concluded that a lack of access to specialized care results in less than optimal use of treatments that could reduce the risk of progression of kidney disease and therefore contributes to higher rates of end-stage renal disease. Diverty *et al.*, (1998) also conducted a study on a Native American population's accessibility to health care in the Yukon and Northwest Territories in Northern Ontario and reported that Aboriginals, when compared with non-Aboriginals, were less likely to assess their health as 'very good' or 'excellent' (Diverty & Perez, 1998). Other significant conclusions were that although very few Aboriginals reported a time in the past year when they needed health care advice and did not receive it, only 50% of Aboriginals studied consulted a doctor (GP) in the last year compared with 77% of provincial residents.

Native North Americans, especially those that follow a traditional lifestyle, are susceptible to exposure to environmental contaminants including POPs and heavy metals (MacMillan *et al.*, 1996). Researchers have concluded through chemical analysis of animals used for food, that traditional food is a major source of POPs (Deutch *et al.*, 2007). Correlations have been made between the intake of traditional food items and blood levels of OC pesticides by dietary surveys (Deutch *et al.*, 2007). Members of the Mohawk community, the Akwesasne people who are traditionally fish-eating people, live along the St. Lawrence River in Quebec, Ontario and New York State downstream from a chemical plant that is a Superfund hazardous waste site. Before advisories against fish consumption were put in place, Fitzgerald *et al.*, (2007) found that cumulative lifetime exposure to PCBs from local fish consumption was significantly associated with individual serum concentrations of total PCBs and several PCB congeners in this community. Another recent study published by Codru *et al.*, (2007) reported a significant association between serum PCB concentrations and the risk for diabetes in this Native American population.

Other adverse health effects stem from the deteriorating relationship between the community and its environment which can cause added stressors in the psychological, social, and cultural life of community members (Couch & Kroll-Smith, 1985). Couch (1985), defines this condition as an ecodisaster which can be either an immediate crisis or a long-term deterioration in the human system – ecosystem relationship that is perceived by the community, or parts of the community, as being detrimental to both the health and safety of the community members, the social relationships within the community, and the community's shared understandings, beliefs and ideas. The situation at Walpole Island

has many of the characteristics of an “ecodisaster”. Many community members report their fear of consumption of fish and water due to chemical contamination. Concurrently, as their fear increases, there is a loss of tradition, culture, and diet as well as a greater disconnect between the community and the environment as the community members steer away from hunting and fishing due to their fear. Anxiety and even delusion, as the tendency to hold onto the perception that the pollution is affecting them more greatly than is the actual case, can become a part of all or some of the community members (Couch & Kroll-Smith, 1985). The resulting stress can act as primary and secondary stressors on community members’ health (Section 1.3.2). One of the major reasons that this participatory, community-based biomonitoring project was requested by members of the Walpole Island Heritage Centre and the Walpole Island Health Centre was to better inform citizens about current exposure levels of POPs and heavy metals so that appropriate actions could be taken by the WIFN in response to risks involved and to decrease the perceived risks best described as chemophobia.

5.2 Results

5.2.1 Questionnaire

Of the total volunteers who participated in our study ($n = 57$), 52 filled out and returned the health questionnaire, a compliance rate of 91%. The relevant results are reported in Table 13.

There were 20 females and 32 males who filled out and returned the questionnaire. The mean age was 49.9 ± 17.2 (mean \pm SD). The maximum age of the

volunteers surveyed was 80 and the minimum age was 17. There was no difference in median ages of males (50.5 years) when compared to females (51.5 years) ($p = 0.3615$; Mann-Whitney U Test).

We wanted to ascertain the overall health of the volunteers, so we questioned them on their lifestyle, traditional diet, and general health. Fifty-six percent of the volunteers surveyed self-reported themselves as smokers. Of those who smoked, 59% were males and 41% were female. Also of importance, 33% of the surveyed volunteers reported that they had diabetes, compared with 56% who did not report having diabetes, 2% who said they may have diabetes, and 9% who did not answer. As for traditional diet, 79% of the volunteers reported that they eat fish, large game, and wild game birds on a regular basis, while only 24% reported that they regularly eat small game. Large game refers to larger animals such as deer or moose, while small game refers to animals such as rabbit or muskrat. Wild game birds refer to goose, duck, partridge or ptarmigan, all part of the Native American traditional diet. When asked about their current source of water, 79% of volunteers reported that they obtained their water from pipes. Of importance is the 33% of volunteers reported drinking bottled water on a regular basis and 12% of volunteers who reported getting their drinking water directly from the St. Clair River. We also asked participants about their past sources of water. Forty-eight percent reported obtaining their water in the past from both pipes and well sources of water. Of particular concern is the 44% of volunteers who reported getting their water directly from the St. Clair River in the past.

Volunteers were asked about their current health and the health of their children. The questionnaire included queries regarding attention deficit hyperactivity disorder (ADHD), anxiety/depression, asthma, autism, cerebral palsy, chronic bronchitis, diabetes, epilepsy, heart conditions/disease, kidney condition/disease, speech or language difficulties, and tuberculosis (TB). Out of the 52 volunteers who responded, most WIFN volunteers (43%) reported asthma in either themselves or their children (Table 14). There was no case of epilepsy reported by the WIFN volunteers in either themselves or their children. It should be noted that when asked about these health conditions, the question was stated for either themselves (the WIFN volunteer) or their child/children, so there is no way of knowing to whom the health condition applies.

Table 13: Characteristics of WIFN volunteers.

| WIFN Characteristics | Number / 52 (%) |
|-----------------------------------|------------------------|
| Total | 52 |
| Males | 32 (62%) |
| Females | 20 (38%) |
| Age (years) | |
| Mean (\pm SD) | 49.9 (17.2) |
| Minimum Age | 17 |
| Maximum Age | 80 |
| Years on Walpole Island | |
| Entire Life | 39 (75%) |
| 5 – 30 years | 5 (10%) |
| Less than 5 years | 1 (2%) |
| No Answer | 7 (13%) |
| Smoking Status | |
| Smokers | 29 (56%) |
| Males | 17 (33%) |
| Females | 12 (23%) |
| Non-Smokers | 21 (40%) |
| Males | 13 (25%) |
| Females | 8 (15%) |
| No Answer | 2 (4%) |
| Male | 2 (4%) |
| Diabetes | |
| Diabetes | 17 (33%) |
| No Diabetes | 29 (56%) |

| | |
|--------------------------------|----------|
| Maybe | 1 (2%) |
| No Answer | 5 (9%) |
| Breast Fed Children | |
| Yes | 26 (50%) |
| No | 13 (25%) |
| No Answer | 12 (23%) |
| Did not Know | 1 (2%) |
| Fish Consumption | |
| Eats Fish | 41 (79%) |
| Does Not Eat Fish | 6 (12%) |
| No Answer | 5 (9%) |
| Small Game Consumption | |
| Eats Small Game | 24 (46%) |
| Does Not Eat Small Game | 18 (35%) |
| No Answer | 10 (19%) |
| Large Game Consumption | |
| Eats Large Game | 41 (79%) |
| Does Not Eat Large Game | 7 (13%) |
| No Answer | 4 (8%) |
| Wild Bird Consumption | |
| Eats Wild Bird | 41 (79%) |
| Does Not Eat Wild Bird | 5 (9%) |
| No Answer | 6 (12%) |
| Current Water Source* | |
| Pipe | 41 (79%) |
| Well Water | 5 (9%) |

| | |
|-----------------------------------|----------|
| Water from St. Clair River | 6 (12%) |
| Bottled Water | 17 (33%) |
| Other | |
| Past Water Source* | |
| Pipe | 25 (48%) |
| Well Water | 25 (48%) |
| Water from St. Clair River | 23 (44%) |
| Bottled Water | 4 (8%) |
| Other | 4 (8%) |

*does not add up to 100 as people could answer more than one source

Table 14: Health problems reported by WIFN Volunteers for themselves and/or their children.

| WIFN Volunteers and their Children (N = 52) | | | | |
|--|----------------|---------------|------------------|----------------------|
| Condition | Yes (%) | No (%) | Maybe (%) | No Answer (%) |
| ADHD | 11 (21%) | 25 (48%) | 1 (2%) | 15 (29%) |
| Anxiety/Depression | 16 (31%) | 26 (50%) | 1 (2%) | 9 (17%) |
| Asthma | 22 (43%) | 21 (40%) | - | 9 (17%) |
| Autism | 1 (2%) | 39 (75%) | - | 12 (23%) |
| Cerebral Palsy | 2 (4%) | 42 (81%) | - | 8 (15%) |
| Chronic Bronchitis | 11 (21%) | 25 (48%) | - | 16 (31%) |
| Diabetes | 17 (33%) | 29 (56%) | 1 (2%) | 5 (9%) |
| Epilepsy | 0 | 43 (83%) | - | 9 (17%) |
| Heart Condition or Disease | 7 (13%) | 39 (75%) | - | 6 (12%) |
| Kidney Condition or Disease | 7 (13%) | 33 (64%) | - | 12 (23%) |
| Speech/Language Difficulty | 6 (12%) | 35 (67%) | 2 (4%) | 9 (17%) |
| TB | 3 (6%) | 38 (73%) | - | 11 (21%) |

5.2.2 POPs

Of the 91 different POPs analyzed only 34 were detected in blood serum samples. These include: β -BHC, γ -BHC, *cis*-chlordane, oxychlordane, *p,p'*-DDD, *p,p'*-DDE, HCB, Mirex, OCS, *cis*-nonachlor, *trans*-nonachlor, QCB and the PCB congeners: 49, 52, 74, 95, 99, 110, 118, 138, 153, 169, 170, 177, 180, 183, 187, 194, 195, 201, 206, 208, 209. PCB 105 could not be separated from PCB 132 so these PCB congeners are reported together, and PCB 156 could not be separated from PCB 171 so they are treated in the same manner. The sum concentration of all the PCBs was also determined. The following tables (Table 15 and Table 16) list the POPs concentrations in order of highest to lowest concentrations found and the number of WIFN samples each POP was found in. The concentrations of the OC pesticides are shown in Table 15 and the PCB congeners are represented in Table 16.

p,p'-DDE was found in the highest concentration in WIFN volunteers with a median concentration of 236.50 (59.35 - 3325) ng/g serum lipids. The other metabolite of DDT, *p,p'*-DDD, was only detected in 1 individual from the WIFN in a very low concentration, 5.68 ng/g lipid. The next highest concentration was of PCB 153, median (range): 47.12 (10.63 - 232.50) ng/g serum lipid, which was more than 4 times lower than the concentration of *p,p'*-DDE. The lowest concentration was found for PCB 105/132, which has a median concentration of 1.53 ng/g serum lipid. This was only reported in one individual and therefore does not have a range. *cis*-chlordane, *p,p'*-DDT, and PCB congeners 156/171, 169 and 49 were also reported in 1 individual. 1,2,3,4-TCB, 1,2,4,5-

TCB, α -BHC, *p,p'*-DDT, *trans*-chlordane and PCB congeners 18/17, 31/28, 33, 44, 70, 82, 87, 101, 128, 149, 158, 191, and 205 were not detected in any individuals.

Table 15: Median concentration and range of OC pesticides in ng/g lipid from highest to lowest concentration.

| Organochlorine Pesticides | | |
|---------------------------|---------------------------------|--|
| Name | Median (range) ng/g lipid | # detected in blood lipids (N = 20) |
| <i>p,p'</i> -DDE | 236.50 (59.35 - 3325) | 20 |
| <i>cis</i> -chlordane | 36.07 | 1 |
| γ -BHC | 36.01 (5.36 - 66.67) | 2 |
| HCB | 23.49 (10.26 - 53.66) | 20 |
| β -BHC | 23.09 (9.17 - 41.82) | 12 |
| <i>trans</i> -nonachlor | 21.58 (4.79 - 67.14) | 20 |
| Oxychlordane | 20.52 (8.14 - 50.83) | 18 |
| <i>cis</i> -nonachlor | 9.03 (5.07 - 14.38) | 7 |
| Mirex | 6.81 (4.44 - 18.49) | 17 |
| QCB | 5.90 (3.06 - 21.28) | 20 |
| <i>p,p'</i> -DDD | 5.68 | 1 |
| OCS | 4.46 (3.77 - 12.68) | 4 |
| 1,2,3,4-TCB | - | 0 |
| 1,2,3,4-TCB | - | 0 |
| α -BHC | - | 0 |
| <i>p,p'</i> -DDT | - | 0 |
| <i>trans</i> -chlordane | - | 0 |

Table 16: Median concentration and range of PCB congeners in ng/g lipid from highest to lowest concentration.

| PCB Congeners | | |
|--------------------|---------------------------------|--|
| Name | Median (range) ng/g lipid | # detected in blood lipids (N = 20) |
| SUM PCBs | 243.6 (81.46 - 871.1) | 20 |
| PCB 153 | 47.12 (10.63 - 232.5) | 20 |
| PCB 180 | 36.57 (6.88 - 171.8) | 20 |
| PCB 138 | 30.55 (9.38 - 155.4) | 20 |
| PCB 187 | 21.59 (12.46 - 95.0) | 15 |
| PCB 52 | 19.57 (8.82 - 39.11) | 20 |
| PCB 74 | 19.57 (8.14 - 20.0) | 3 |
| PCB 156/171 | 14.35 | 1 |
| PCB 169 | 14.11 | 1 |
| PCB 49 | 13.59 | 1 |
| PCB 118 | 13.22 (5.57 - 40.71) | 18 |
| PCB 99 | 12.05 (5.75 - 32.14) | 18 |
| PCB 95 | 10.70 (7.11 - 11.78) | 3 |
| PCB 110 | 10.22 (5.71 - 17.55) | 5 |
| PCB 170 | 10.16 (5.0 - 31.79) | 19 |
| PCB 194 | 10.0 (4.68 - 22.98) | 19 |
| PCB 201 | 9.48 (5.12 - 33.21) | 18 |
| PCB 177 | 9.30 (4.59 - 17.64) | 15 |
| PCB 206 | 8.19 (2.33 - 26.79) | 17 |
| PCB 208 | 7.56 (3.97-9.06) | 3 |
| PCB 183 | 6.69 (4.59-17.14) | 18 |
| PCB 195 | 6.14 (6.11 - 6.17) | 2 |
| PCB 209 | 5.81 (3.56 - 16.07) | 18 |
| PCB 105/132 | 1.53 | 1 |
| PCB 18/17 | - | 0 |
| PCB 31/28 | - | 0 |
| PCB 33 | - | 0 |
| PCB 44 | - | 0 |
| PCB 70 | - | 0 |
| PCB 82 | - | 0 |
| PCB 87 | - | 0 |
| PCB 101 | - | 0 |
| PCB 128 | - | 0 |
| PCB 149 | - | 0 |
| PCB 158 | - | 0 |
| PCB 191 | - | 0 |
| PCB 205 | - | 0 |

5.2.2.1 Effects of Age on Concentrations of POPs in Plasma Lipids

All of the POPs were analyzed for age dependent relationships using Spearman's Rank Correlation and the Bonferroni Correction. We found that *trans*-nonachlor and PCB 180 has a significant age-dependent relationship. Figure 11 shows the correlation for *trans*-nonachlor with age of the volunteers ($p < 0.001$). Figure 12 shows the significant correlation between PCB 180 concentration in serum and age of the volunteers ($p < 0.001$).

5.2.2.2 Diabetes

We examined the relationship between concentrations of POPs and the prevalence of diabetes in the community. The self-reported prevalence of diabetes within the volunteers from the WIFN community was 33%. Figure 13 shows the concentrations of OC pesticides in those who reported having diabetes compared to those who did not. Figure 14 shows similar data with the PCB congeners analyzed. As shown in Figure 13, there were trends for slightly higher concentrations of several OC pesticides in serum from diabetics, including QCB, *p,p'*-DDE and *cis*-nonachlor, whereas the reverse was true for Mirex. Similarly, there were trends for higher concentrations of several PCB congeners in serum from diabetics vs. non-diabetics and for total PCBs. However, this difference was only significant for PCB 177, a PCB found at relatively low concentrations in WIFN volunteers so that the biological significance of these observations is not clear.

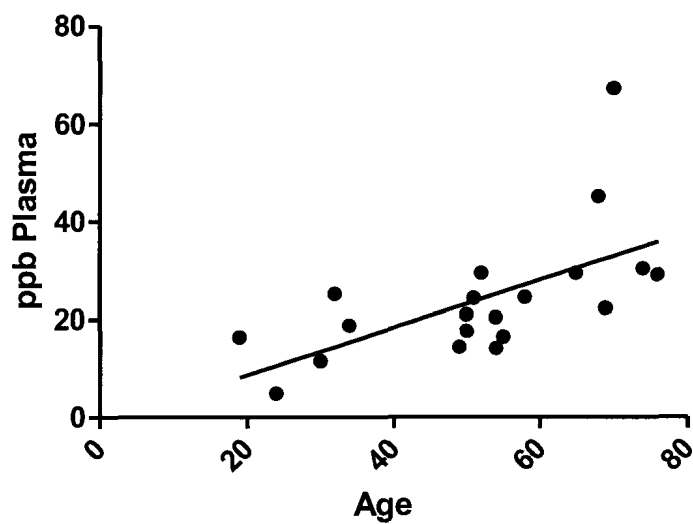


Figure 11: Relationship between *trans*-nonachlor and age (Spearman's Rank Correlation; $p < 0.001$).

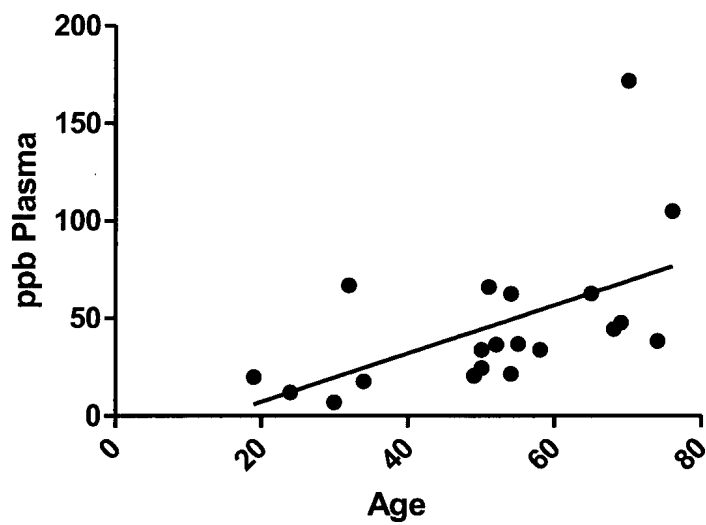


Figure 12: Relationship between PCB 180 and age (Spearman's Rank Correlation; $p < 0.001$).

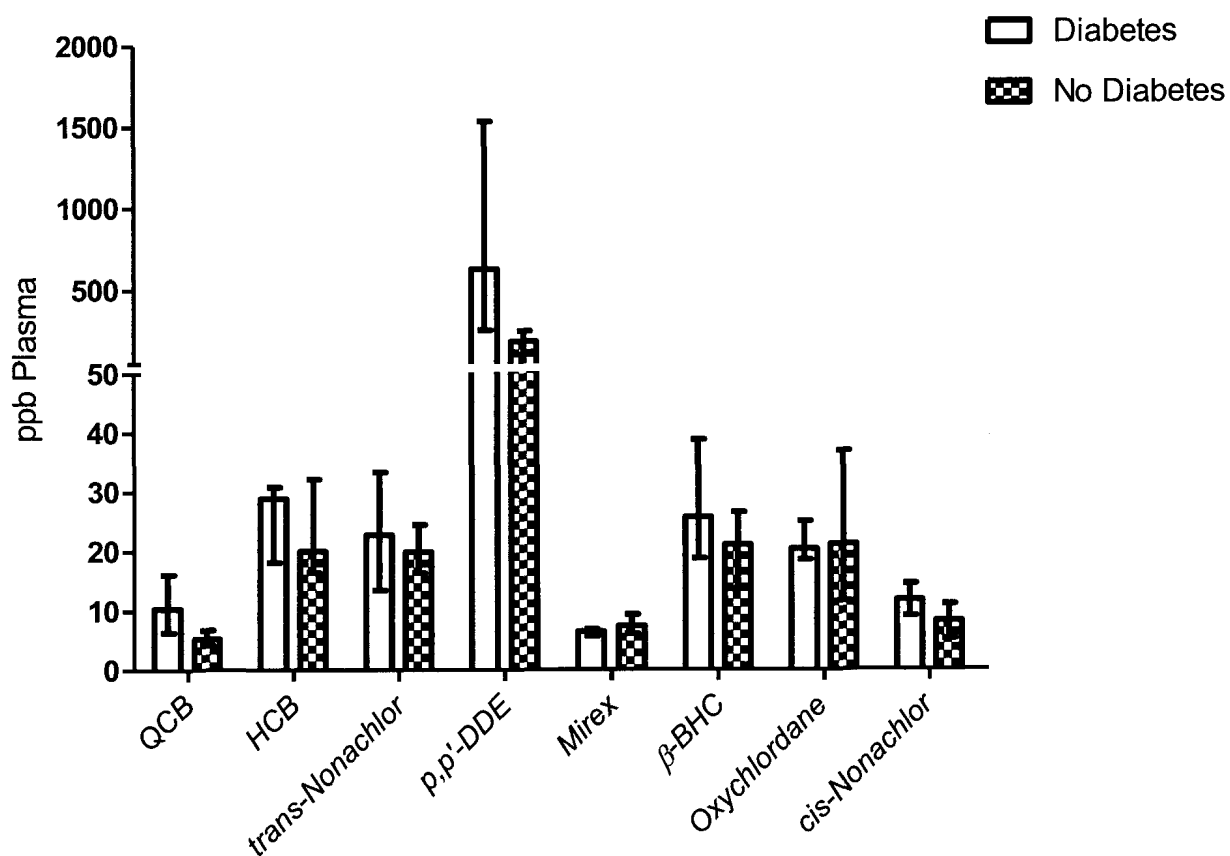


Figure 13: Median concentration and interquartile range of OC pesticide concentrations in serum lipids of WIFN volunteers who self-reported diabetes vs. those who did not (Mann-Whitney U Test; Bonferroni Correction).

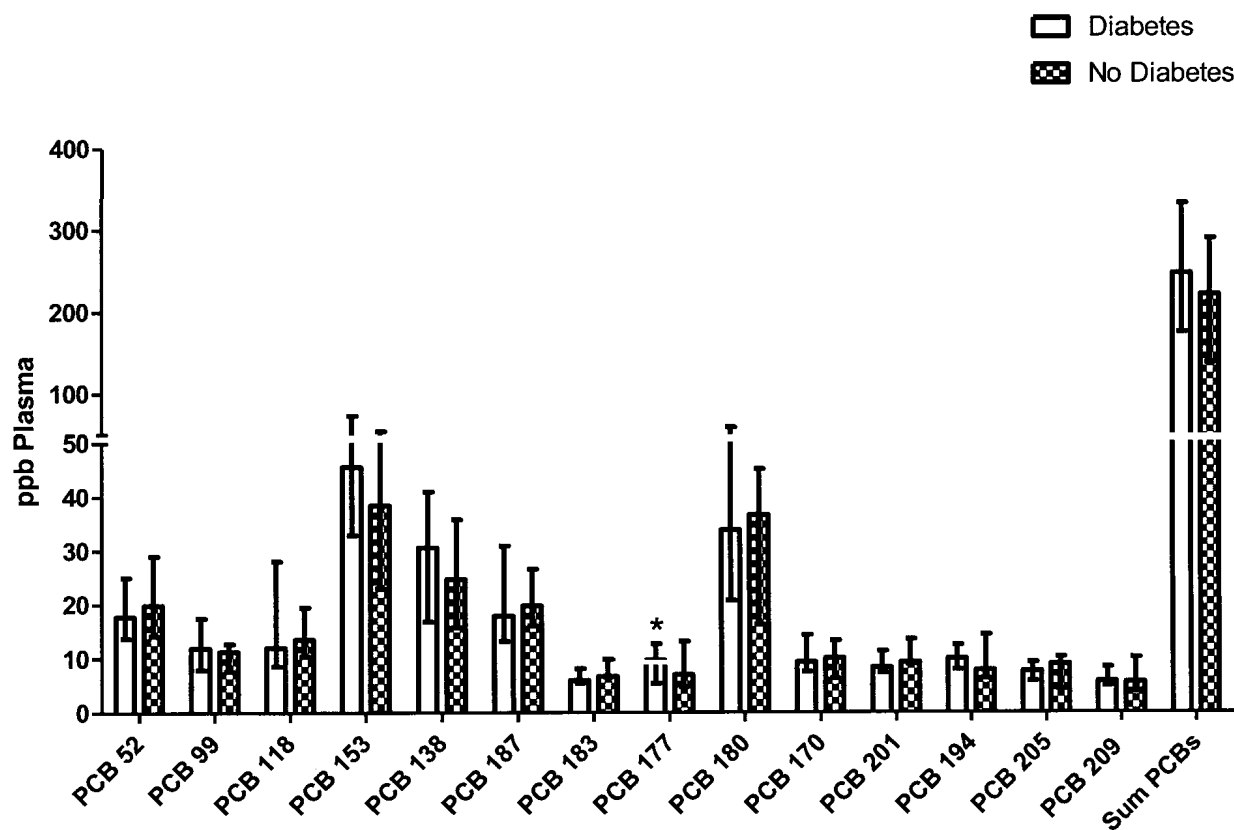


Figure 14: Median concentration and interquartile range in self-reported diabetics vs. non diabetics in WIFN volunteers (Mann-Whitney U Test; Bonferroni Correction; * $p < 0.01$).

5.2.2.3 Fish Consumption

Fish consumption analysis was performed on OC pesticide concentrations and PCB congener concentrations as POPs are known to accumulate in lipids of fish. We compared POP concentrations of WIFN volunteers who reported eating fish in the questionnaire to those who reported that they did not consume fish. A couple of OC pesticides were found in higher concentrations in the WIFN volunteers who reported eating fish when compared with those who did not. These OC pesticides are: HCB, OCS, *trans*-nonachlor, *p,p'*-DDE and *cis*-nonachlor (Fig. 15). OCS was only found in blood lipids of fish consumers. All individual PCB congeners were also found in higher blood lipid concentrations in fish consumers when compared with WIFN volunteers who do not eat fish (data not shown). However, none of these differences were significant.

5.2.2.4 Game Consumption

Likewise, POPs are also known to accumulate in wild game (small game, large game, and game bird), so we conducted analysis on game consumption as reported in the questionnaire by WIFN volunteers. We divided our analysis into large game (Fig. 16), small game (Fig. 17), and game birds (data not shown). A large proportion of WIFN volunteers (79%) consume large game and game birds, while less than half (46%) of the WIFN volunteers consume small game. Due to the small number of volunteers who reportedly did not consume game birds and had concentrations of OC pesticides and PCB congeners above the limit of detection, we were unable to perform game bird analysis.

There did not appear to be any difference between blood lipid concentrations of OC pesticides and PCB congeners in WIFN volunteers who reported eating large game vs. those who do not. Figure 16 shows the blood lipid concentrations of OC pesticides and total PCB congeners in WIFN volunteers who eat large game vs. those who do not. Individual PCB congener analysis is not shown.

Similarly there does not appear to be any difference between blood lipid concentrations of PCB congeners (specific individual congeners not shown) in WIFN volunteers who reported eating small game vs. those who do not. However, *trans*-nonachlor was found in higher concentrations in blood lipids of WIFN volunteers who reportedly do not consume small game compared to those who do (Fig. 17; $p < 0.05$). QCB, HCB and *trans*-nonachlor were also found in blood lipids at higher concentrations in WIFN volunteers who do not consume small game compared with those who do, although these differences were not significant.

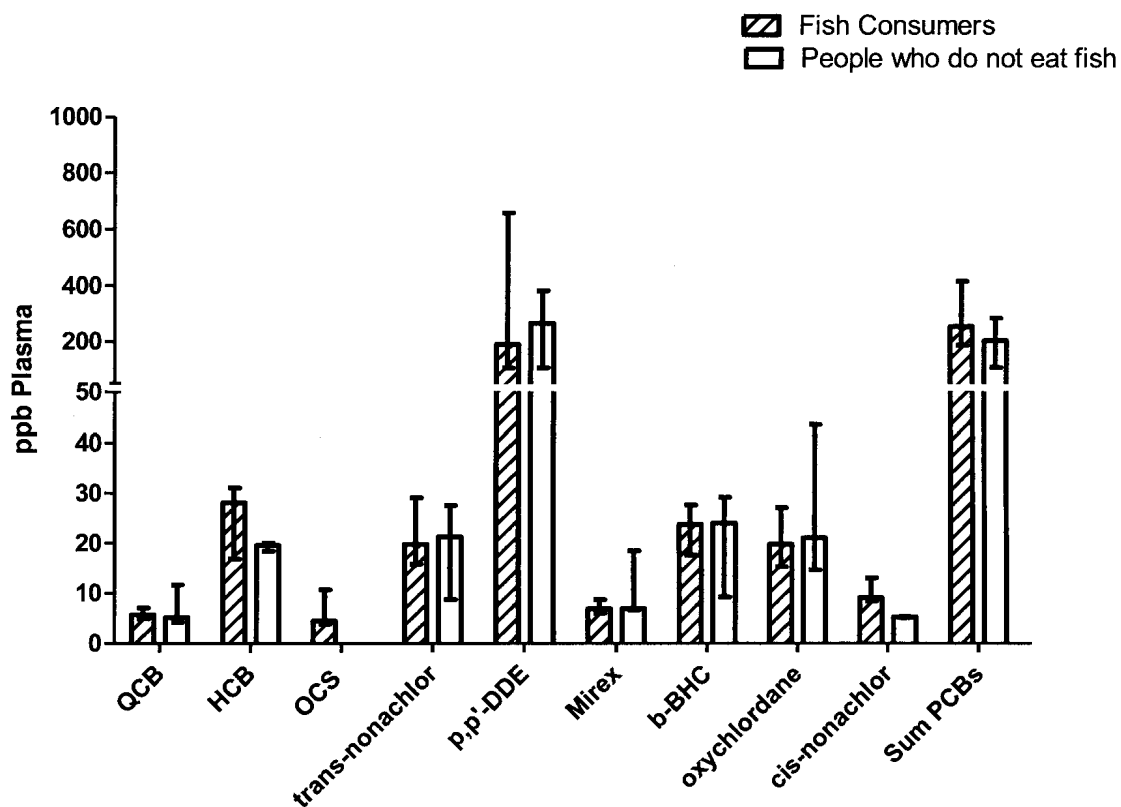


Figure 15: Median concentrations and interquartile range in blood lipids of WIFN volunteers who eat fish vs. those who do not (Mann-Whitney U Test; Bonferroni Correction).

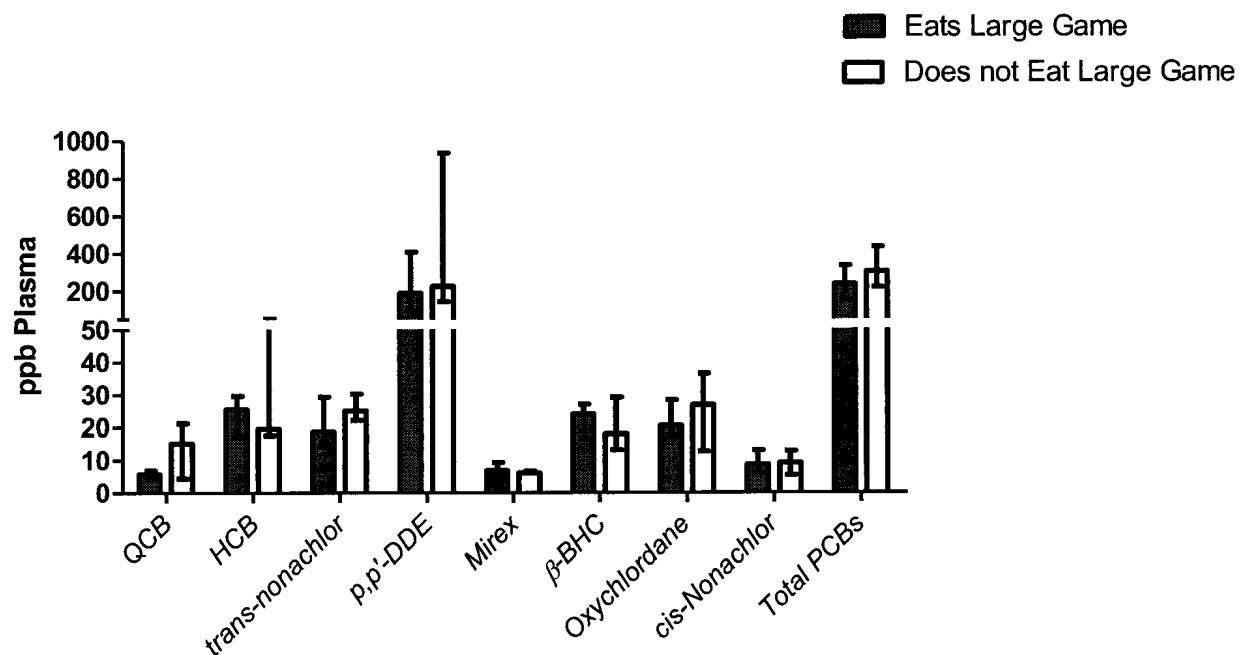


Figure 16: Median concentration and range of OC pesticides and total PCB concentrations in blood lipids of WIFN volunteers who eat large game vs. those who do not (Mann-Whitney U Test; Bonferroni Correction).

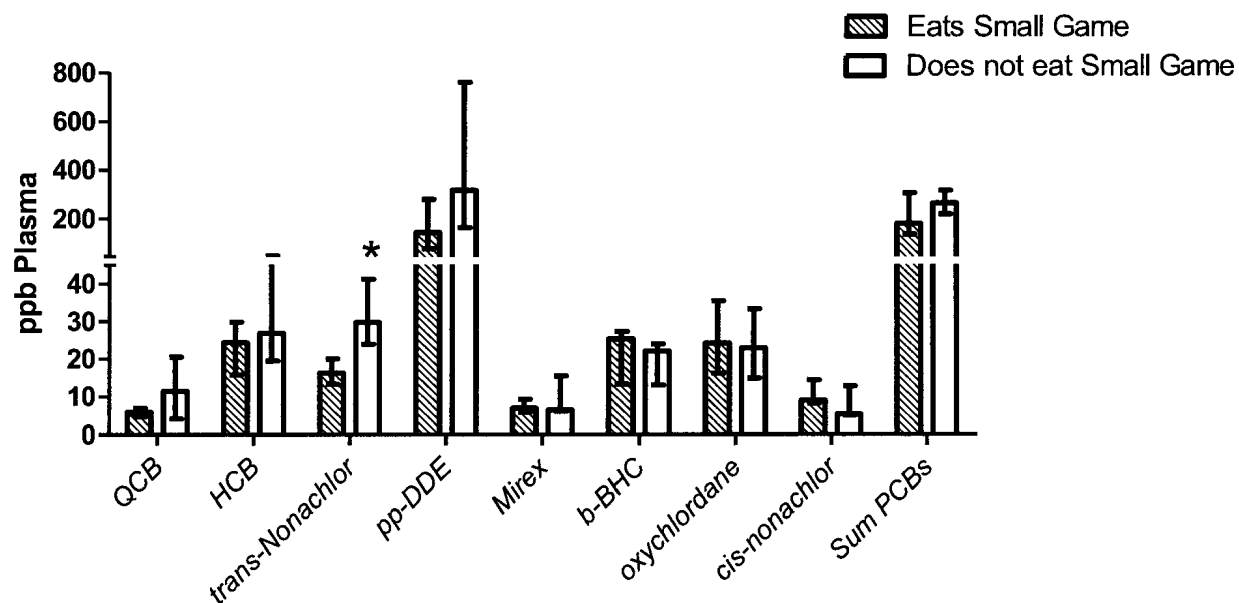


Figure 17: Median concentration and range of OC pesticides and total PCB concentrations in blood lipids of WIFN volunteers who reported eating small game vs. those who did not (Mann-Whitney U Test; Bonferroni Correction; * $p < 0.05$).

5.2.2.5 Gender

Women appeared to have slightly higher concentrations of OC pesticides: QCB, OCS, *trans*-nonachlor, β -BHC, *p,p'*-DDD (Fig. 18), and almost all individual PCB congeners. Males had slightly higher concentrations of Mirex, γ -BHC, oxychlordane, *cis*-chlordane and *cis*-nonachlor (Fig. 18). However, none of these differences were significant. Of note, *p,p'*-DDD and PCB congener 169 were only found in blood lipids of female WIFN volunteers. Similarly, *cis*-nonachlor, *cis*-chlordane, and PCB congeners 49, 95, 105/132, 156/171 and 208 were only found in blood lipids of male WIFN volunteers.

5.2.2.6 Smoking

The final analysis we performed was to determine whether smoking status had an effect on POPs concentrations. More than half the WIFN volunteers (56%) reported that they smoked. HCB, *trans*-nonachlor, *p,p'*-DDE, and Mirex were all found in higher concentrations in blood lipid of smokers when compared with non-smokers. However, Mirex was the only OC pesticide that showed a significant difference (Fig. 19; $p < 0.005$). Contrarily, β -BHC, oxychlordane, *cis*-nonachlor and total PCB congeners were found in higher concentrations in non-smokers when compared with smokers and this difference was significant for β -BHC (Fig. 9; $p < 0.0005$). There were no significant differences between concentrations of PCB congeners in blood lipids of smokers and non-smokers (data not shown).

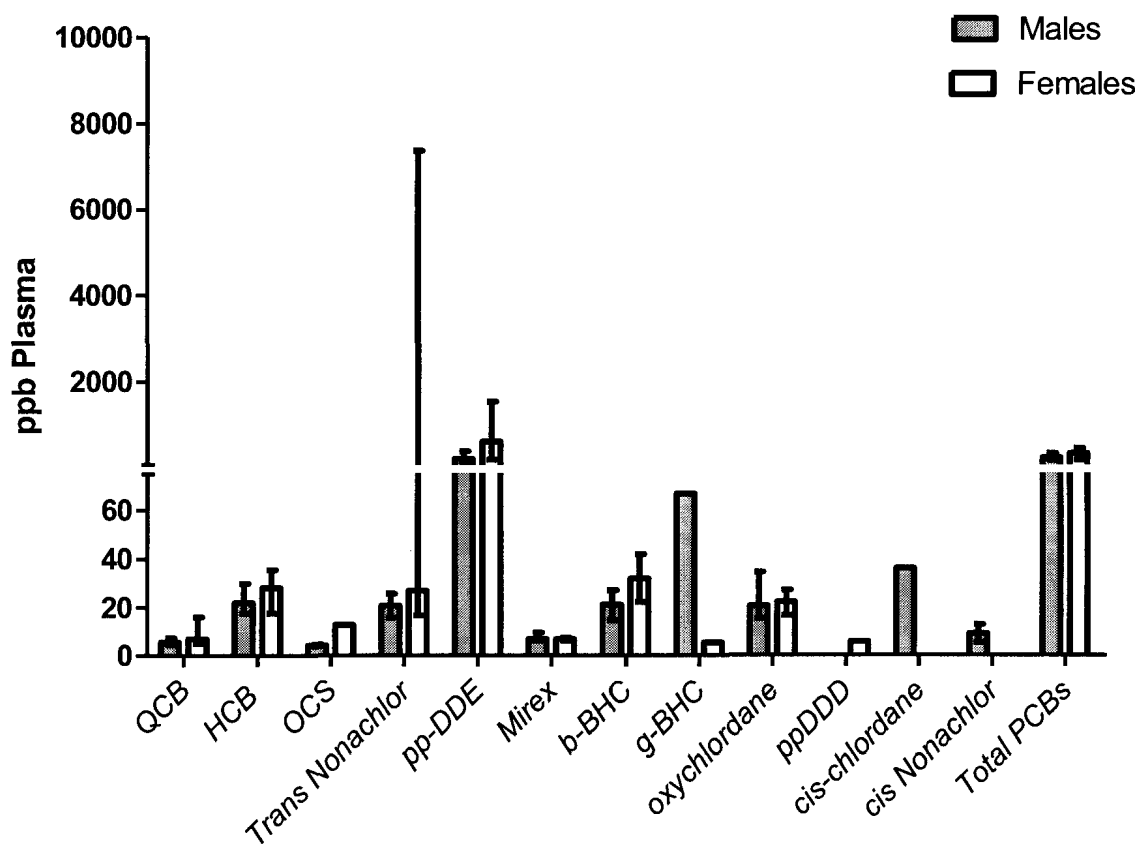


Figure 18: Median concentration and interquartile range in blood lipids of OC pesticides and total PCBs in male WIFN volunteers vs. female WIFN volunteers (Mann-Whitney U Test; Bonferroni Correction).

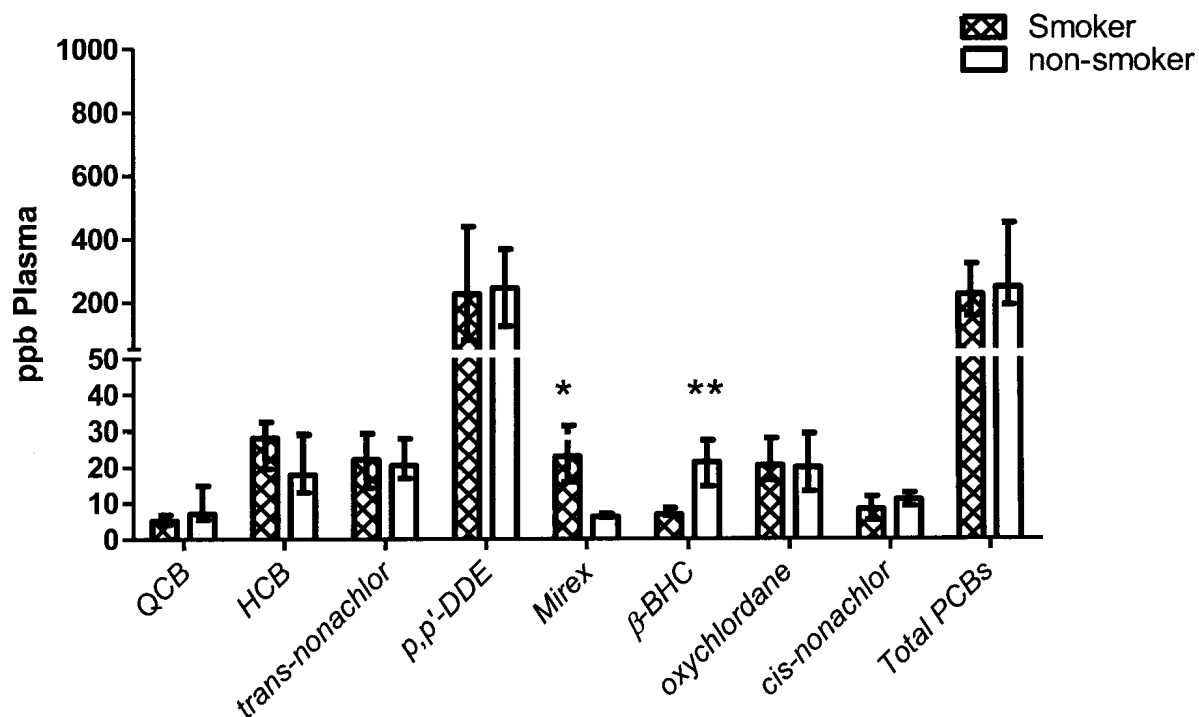


Figure 19: Median concentration and interquartile range in blood lipids of WIFN volunteers who self-reported smoking vs. those who did not (Mann-Whitney U Test; Bonferroni Correction; * $p < 0.005$; ** $p < 0.0005$).

5.2.3 Heavy Metals

Heavy metals were analyzed in both hair and whole blood samples of WIFN volunteers. Of the 57 total WIFN volunteers, hair samples were obtained from 55 volunteers and blood samples from 56 volunteers. There were 6 heavy metals analyzed in the whole blood samples: antimony (Sb), arsenic (As), cadmium (Cd), lead (Pb), nickel (Ni), and thallium (Tl) and there were 15 heavy metals analyzed in hair samples: aluminum (Al), Sb, As, barium (Ba), beryllium (Be), bismuth (Bi), Cd, Pb, Ni, palladium (Pd), platinum (Pt), silver (Ag), Tl, titanium (Ti), and uranium (U). Table 17 shows the concentrations of the 6 metals measured in both hair and blood (As, Cd, Pb, Ni, Sb, Tl) and the ratio of hair to blood for these metals. We found Cd to have the highest hair to blood ratio of 33.67, while Ni has the smallest hair to blood ratio of 0.20.

The following table (Table 18) lists the median concentrations of these heavy metals in relative order of toxic importance and then alphabetically. The heavy metal concentrations found in blood samples are shown on the right hand side of Table 18, those in hair are shown on the left hand side. Median concentrations are reported here because the WIFN population was not normally distributed.

Ni was found in the highest concentration in blood with a median of 798 320 (199 580 – 3.5×10^6) ng/L. The next highest heavy metal concentration in blood was Pb with a median (range) concentration of 10 360 (4 144 – 41 440) ng/L, which is considerably lower than the concentration of Ni in blood. The lowest concentration in blood was found for the heavy metal Tl, which has a median (range) concentration of 115.0 (60.0 – 230.0) ng/L.

Table 17: Heavy metal concentrations in hair and blood samples of WIFN volunteers.

| Heavy Metal | Median (range) Hair ppt | Median (range) Blood ppt | Ratio Hair : Blood |
|-------------|---------------------------------------|--|-----------------------|
| Sb | 11 000 (2 000 – 1.4×10^6) | 3 250 (40 – 5 118) | 3.38 |
| As | 14 000 (2 000 – 170 000) | 1 200 (203 – 29 940) | 11.67 |
| Cd | 20 000 (3 000 – 240 000) | 594 (202 – 5 085) | 33.67 |
| Pb | 300 000 (18 000 – 4.3×10^6) | 10 360 (4 144 – 41 440) | 28.96 |
| Ni | 160 000 (20 000 – 1.7×10^6) | 798 320 (199 580 – 3.5×10^6) | 0.20 |
| Tl | 300 (100 – 2 500) | 115 (60 – 230) | 20.0 |

Table 18: Median and range for heavy metal in hair and blood.

| Heavy Metal | Median (range) Hair (ng/kg) | # detected in hair (N = 55) | Median (range) Blood (ng/L) | # detected in blood (N = 56) |
|--------------------|---|--|---|---|
| Sb | 11 000 (2 000 - 1.4 x 10 ⁶) | 55 | 3 250 (40.3 - 5 118) | 56 |
| As | 14 000 (2 000 - 170 000) | 55 | 1 200 (202.5 - 29 940) | 56 |
| Cd | 20 000 (3 000 - 240 000) | 55 | 593.6 (201.6 - 5 085) | 56 |
| Pb | 300 000 (18 000 - 4.3 x 10 ⁶) | 55 | 10 360 (4144 - 41 440) | 56 |
| Ni | 160 000 (20 000 - 1.7 x 10 ⁶) | 55 | 798 320 (199 580 - 3.5 x 10 ⁶) | 56 |
| Tl | 300 (100 - 2 500) | 55 | 115.0 (60.0 - 230.0) | 56 |
| Al | 2.8 x 10 ⁶ (1.1 x 10 ⁶ - 1.1 x 10 ⁷) | 55 | | |
| Ba | 700 000 (100 000 - 3.3 x 10 ⁷) | 55 | | |
| Be | 800 (100 - 3 000) | 55 | | |
| Bi | 4 000 (1 000 - 2.1 x 10 ⁶) | 55 | | |
| Pd | 4 000 (1 000 - 130 000) | 55 | | |
| Pt | 400 (100 - 8 000) | 55 | | |
| Ag | 19 000 (2 000 - 500 000) | 55 | | |
| Ti | 100 000 (20 000 - 1.1 x 10 ⁶) | 55 | | |
| U | 10 000 (2 000 - 63 000) | 55 | | |

The highest heavy metal concentration measured in hair was for the heavy metal Al, which has a median (range) concentration of 2.8×10^6 ($1.1 \times 10^6 - 1.1 \times 10^7$) ng/Kg. The second highest heavy metal measured in hair was for Ba, which has a median concentration of 700 000 ($100\ 000 - 3.3 \times 10^7$) ng/Kg. The heavy metal found in the lowest concentration in hair was for Tl, which has a median concentration of 300 ($100 - 2\ 500$) ng/Kg. All heavy metals measured in hair (Ag, Al, As, Ba, Be, Bi, Cd, Ni, Pb, Pd, Pt, Sb, Ti, Tl and U) were detected in all hair samples (55).

We performed a Spearman's Rank Correlation between heavy metals in hair versus blood samples. Tl content in hair does not appear to be a good marker for Tl content in blood. We did not find a correlation for Tl in hair and blood samples (Fig. 20). However, we did note that there were appreciable amounts of blood Tl at low concentrations of hair Tl. This suggests the possibility of contamination of the blood samples with Tl either during or after blood collection. Similarly, we did not find a positive correlation between Cd concentrations in hair and blood samples (Fig. 21).

We did however find significant positive correlations in hair and blood for both As and Pb. Figure 22 shows a positive correlation between the concentrations of As in hair and whole blood samples (Spearman's R Correlation; $p < 0.05$). There were low concentrations of As in both hair and blood in the WIFN volunteers. Figure 23 also shows a significant correlation between hair and blood concentration of Pb (Spearman's Rank Correlation; $p < 0.0005$).

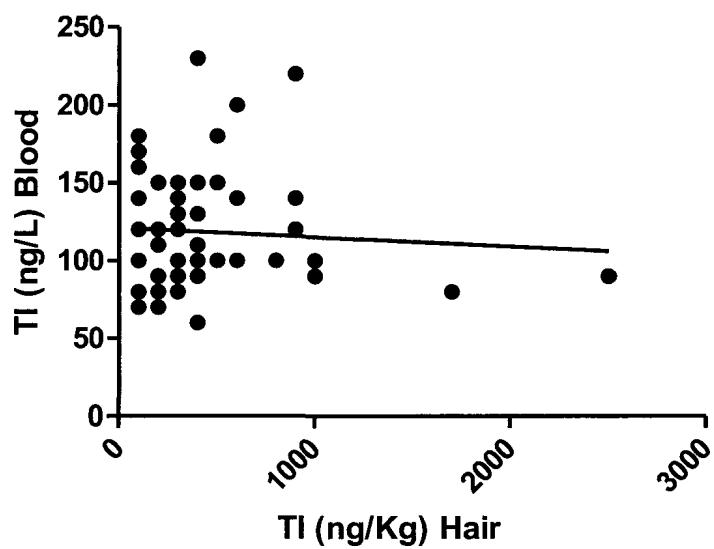


Figure 20: Relationship of thallium content in hair and blood (Spearman's Rank Correlation).

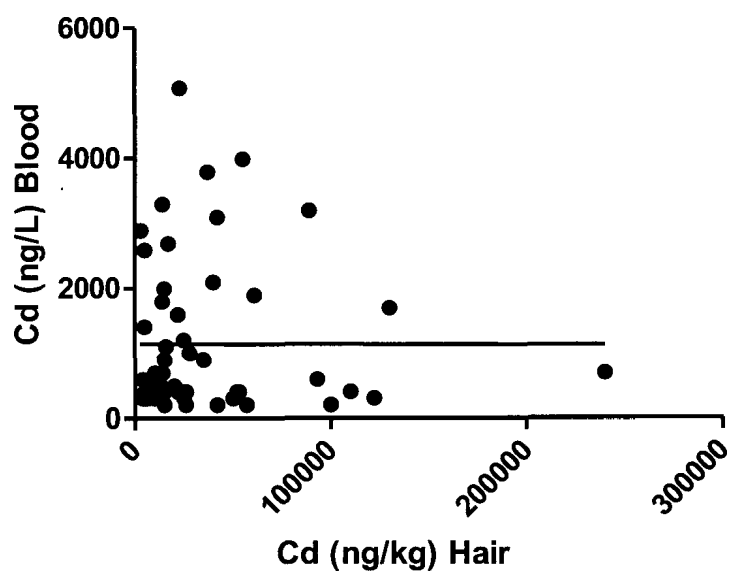


Figure 21: Relationship of cadmium content in hair and blood samples (Spearman's Rank Correlation).

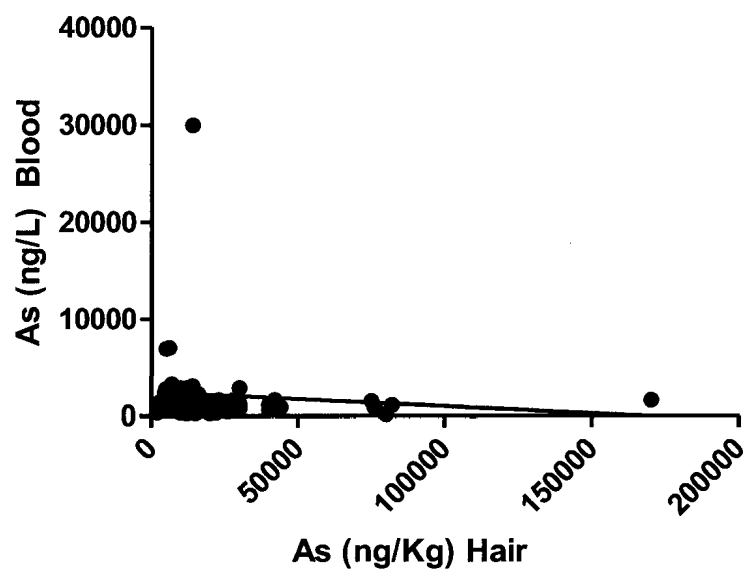


Figure 22: Relationship of arsenic content in hair and blood samples (Spearman's Rank Correlation; $p < 0.05$).

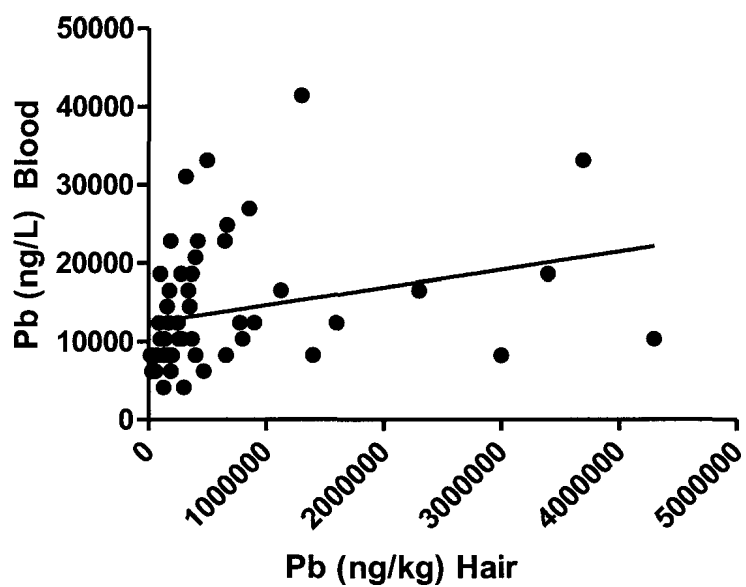


Figure 23: Relationship between lead content in hair and blood samples (Spearman's Rank Correlation; $p < 0.0005$).

5.2.3.1 Age

We attempted to correlate the heavy metal concentration in hair and blood separately with ages of the WIFN volunteers using Spearman's Rank correlation. There was no significant correlation between age and heavy metal concentration in hair or blood (data not shown).

5.2.3.2 Diabetes

We used a Mann-Whitney U Test followed by a Bonferroni Correction, to determine whether there were any significant differences between heavy metal concentrations in hair or blood of self-reported diabetics versus non-diabetics. As an example, Figure 24 compares the concentrations of Pb, As and Cd in hair of WIFN volunteers who either reported having or not having diabetes. There were no significant differences between concentrations of any of the heavy metals in hair (Fig. 24) or whole blood (data not shown) in diabetics vs. non-diabetics.

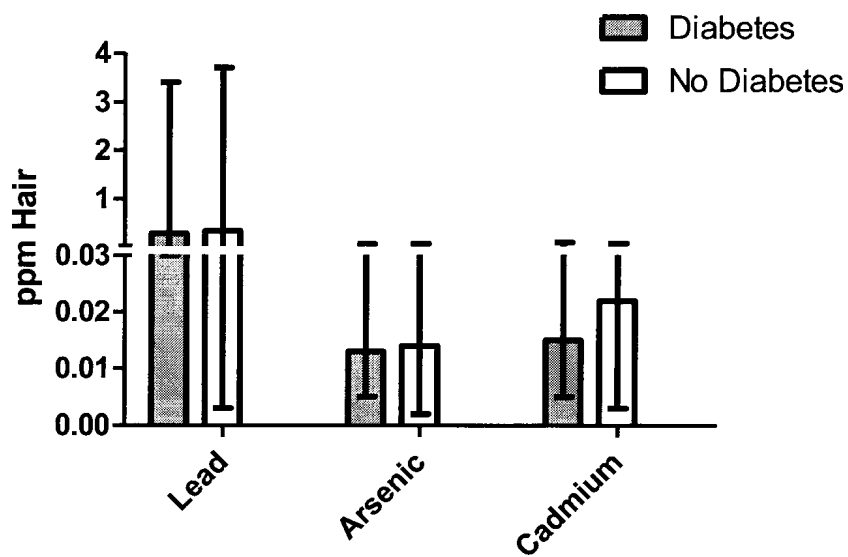


Figure 24: Median and range concentration of lead, arsenic and cadmium in hair of WIFN diabetics versus non-diabetics (Mann-Whitney U Test and Bonferroni Correction).

5.2.3.3 Fish Consumption

We analysed heavy metal concentrations in hair and blood in fish consumers vs. people who do not eat fish. Differences were not found in hair (Fig. 25) or blood (data not shown).

We also examined the relationship between male fish consumers compared with female fish consumers (Fig. 26). We found a significant difference in the concentration of Pb ($p < 0.0001$), As ($p < 0.0001$) and Cd ($p < 0.005$) in male fish consumers versus female fish consumers. Male fish consumers had significantly higher concentrations of these 3 heavy metals in hair compared to female fish consumers. We also found significantly higher concentrations of Tl ($p < 0.0005$) and Sb ($p < 0.0001$) in male fish consumers when compared with female fish consumers (Mann-Whitney U Test and Bonferroni Test) (results not shown). We did not find any significant difference between heavy metal concentration in the blood of male and female fish consumers (figure not shown).

Of the 41 WIFN volunteers who answered the question “do you eat fish”, only 6 males responded saying they did not eat any fish. We therefore looked to see if there was any difference in heavy metal concentration in hair of WIFN male volunteers who reported that they ate fish with those who reported they did not eat fish. We did not find any significant difference in heavy metal concentration in males of WIFN volunteers who eat fish and those who do not (figure not shown). We did not find any significant difference in heavy metal concentration in blood in male fish consumers compared with males who do not consume fish (figure not shown).

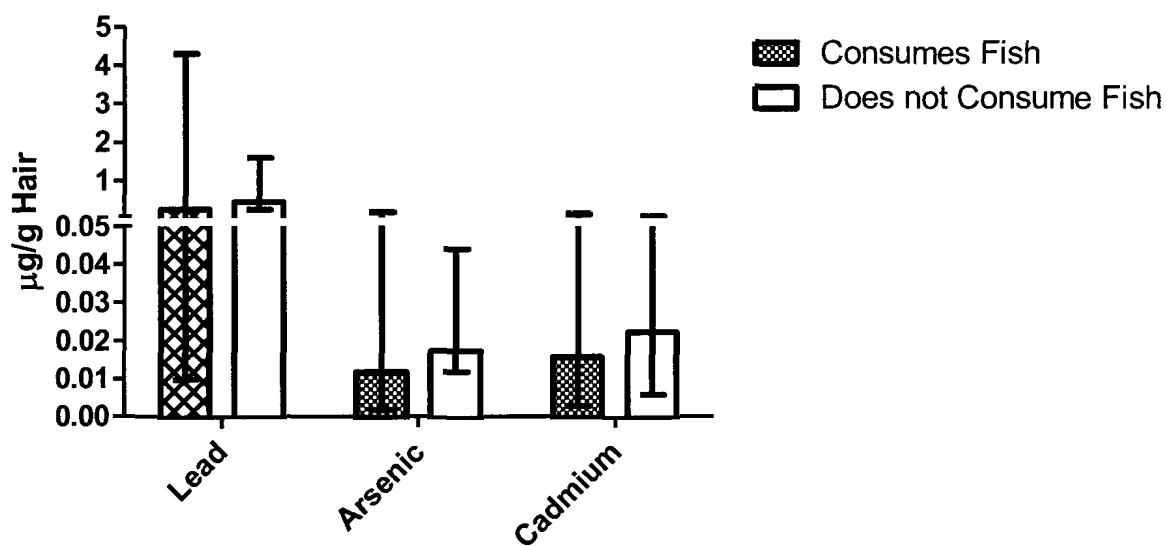


Figure 25: Median concentrations and range of lead, arsenic and cadmium in male WIFN fish consumers versus non-fish-consumers (Mann-Whitney U Test and Bonferroni Correction).

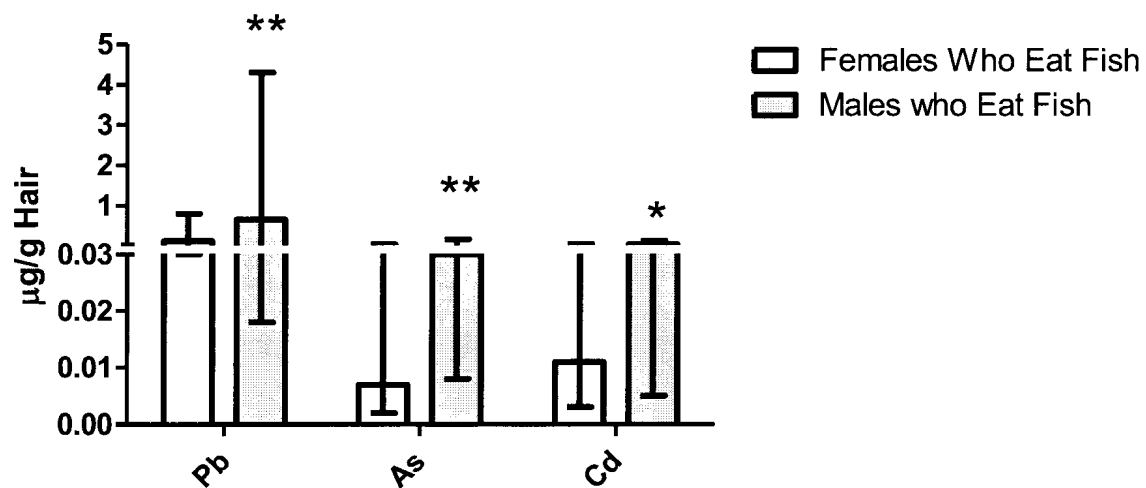


Figure 26: Median concentration and range of WIFN male fish consumers versus WIFN female fish consumers (Mann-Whitney U Test; * $p < 0.005$; ** $p < 0.0001$).

5.2.3.4 Game Analysis

We also examined the relationship between game consumption and heavy metal concentration. Figure 27 shows that we did not find any relationship between game bird consumption and As, Cd and Pb in hair. We also did not find any game bird consumption relationship with any other heavy metal in hair or blood samples.

Figure 28 shows As, Cd and Pb concentrations in hair of small game consumers and WIFN members who do not consume small game. Concentrations of As was found significantly higher in hair of those who ate small game compared to those who did not ($p < 0.05$). Sb was also found in significantly higher concentrations in the hair of those who ate small game compared with those who did not (not shown; $p < 0.005$). Pd was found in significantly higher concentrations in the hair of those who did not eat small game compared with those who did (not shown; $p < 0.01$). We did not find any significant relationships between small game consumers and concentrations of heavy metals in blood.

Figure 29 shows As, Cd and Pb concentrations in hair of large game consumers compared with WIFN members who do not consume large game. We did not find any significant relationship between heavy metal concentration in hair or blood in large game consumers versus WIFN members who do not consume large game.

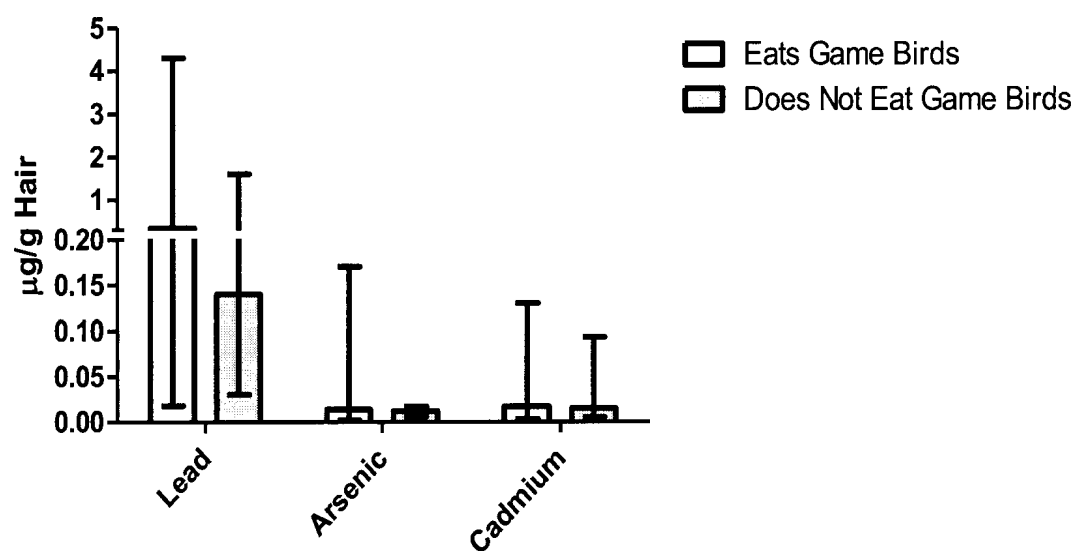


Figure 27: Median concentration and range in WIFN game bird consumers versus non-game bird consumers (Mann-Whitney U Test and Bonferroni Correction).

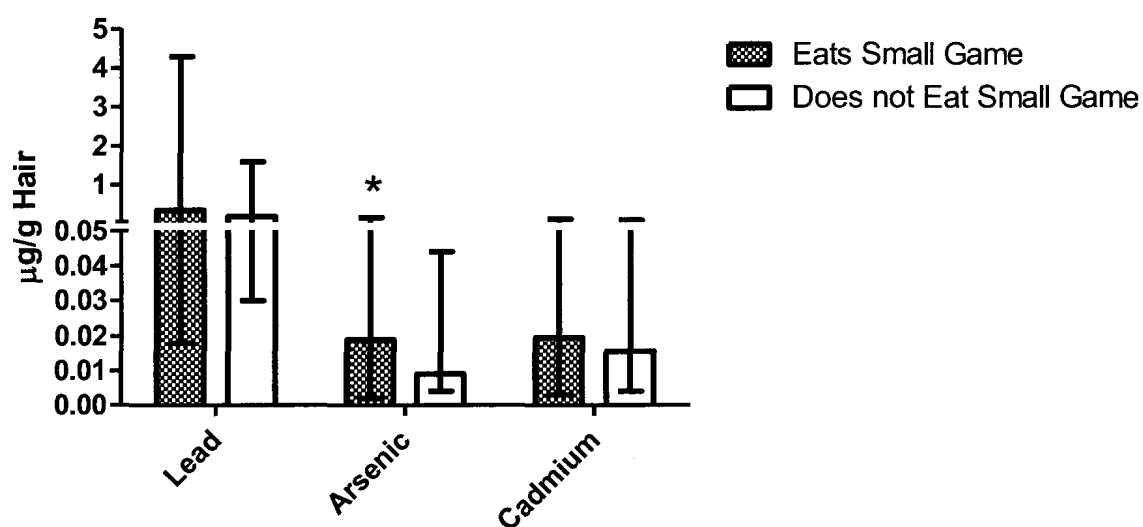


Figure 28: Median concentration and range of lead, arsenic and cadmium in WIFN hair of small game consumers and small game non-consumers (Mann-Whitney U Test and Bonferroni Correction; $p < 0.05$).

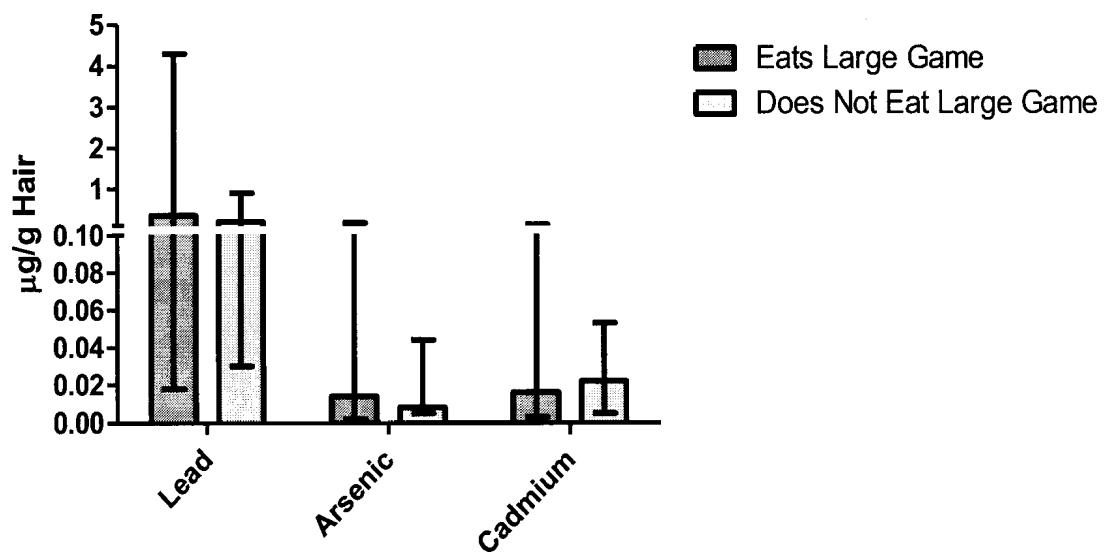


Figure 29: Median concentration and range in hair of WIFN large game consumers versus those who do not eat large game (Mann-Whitney U Test and Bonferroni Correction).

5.2.3.5 Gender

We evaluated gender effects on heavy metal concentrations in WIFN hair and blood samples. Of potential toxicological relevance, we found that Pb (Mann-Whitney U Test; $p < 0.0001$), As (Mann-Whitney U Test; $p < 0.0005$) and Cd concentrations (Mann-Whitney U Test; $p < 0.01$) in hair were all significantly higher in males compared to females (Fig. 30). We also noted that males had significantly higher concentrations of Tl (Mann-Whitney U Test, Bonferroni Correction; $p < 0.0005$) and Sb (Mann-Whitney U Test, Bonferroni Correction; $p < 0.0001$) in hair than females (data not shown). On the contrary, female WIFN volunteers had higher concentrations of Ba and Pd (Mann-Whitney U Test, Bonferroni Correction; $p < 0.05$) in hair than males (data not shown). In agreement with the hair data, males also had significantly higher concentrations of Pb in their blood than did females (Mann-Whitney U Test, Bonferroni Correction; $p < 0.01$) (data not shown). However, this was the only heavy metal in blood to show a significant gender difference.

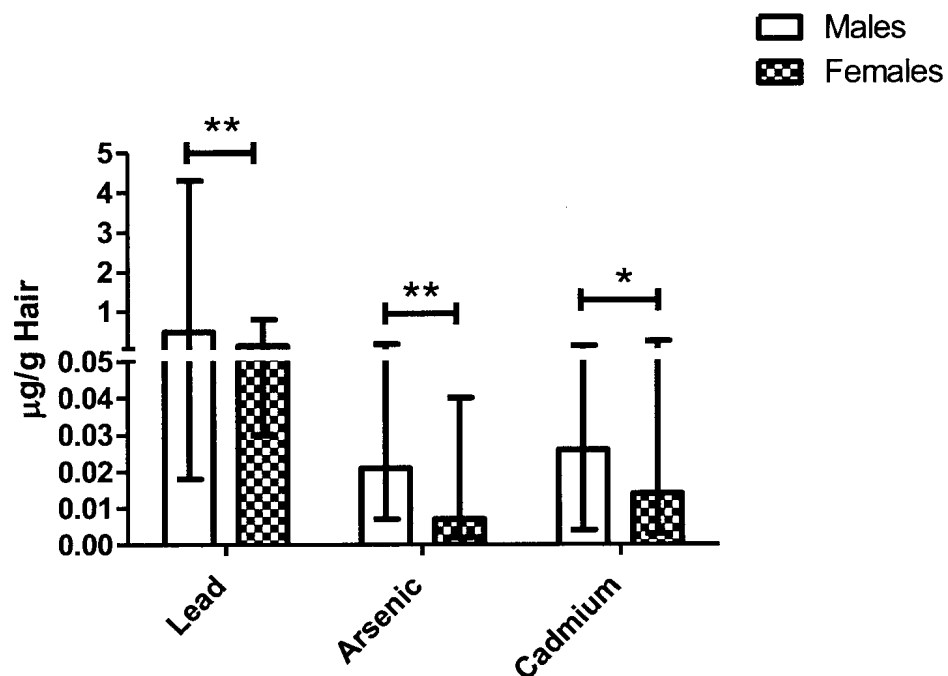


Figure 30: Median concentration and range in hair of WIFN males versus females in lead, arsenic, and cadmium (Mann-Whitney U Test and Bonferroni Correction; * $p < 0.01$; ** $p < 0.0001$).

5.2.3.6 Smoking

In an attempt to determine if there was a difference in concentrations of heavy metals in hair or whole blood of WIFN volunteers in smokers vs. non-smokers, we conducted Mann-Whitney U Test data analysis. Of some surprise, we did not find any difference in blood or hair Cd content in smokers vs. non-smokers and this was also true for As (Fig. 31). The only difference noted in this evaluation was a higher concentration of Pb in blood, but not hair, of smokers versus in the blood of non-smokers (Mann-Whitney U Test, Bonferroni Correction; $p < 0.01$) (data not shown). There was not a significant difference in content for any other heavy metals in hair or blood samples of smokers vs. non-smokers (data not shown).

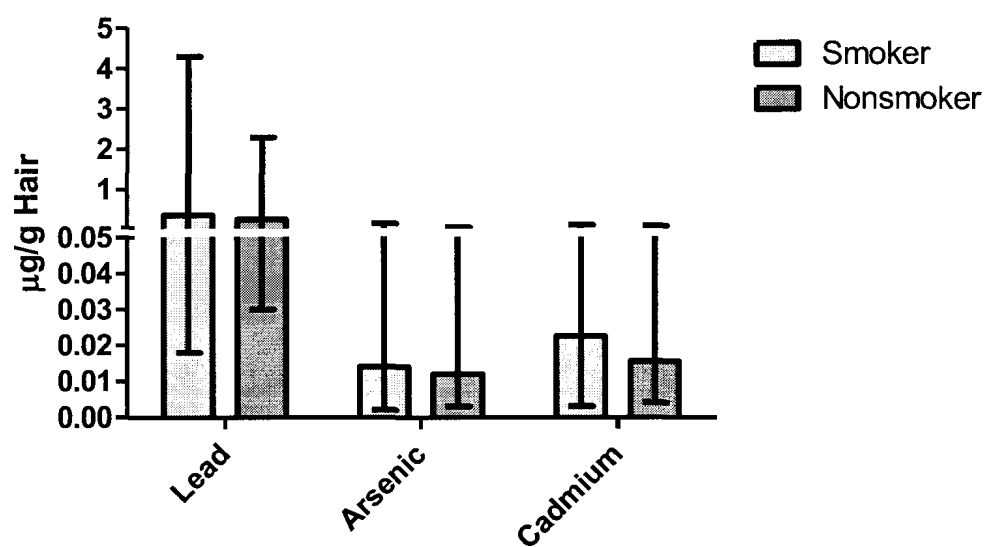


Figure 31: Median concentration and range in lead, arsenic, and cadmium of hair from WIFN smokers vs. non-smokers (Mann-Whitney U Test and Bonferroni Correction).

5.2.4 Cortisol

Cortisol concentrations found in volunteers from the WIFN population was compared to a reference population recruited by the London Health Sciences Centre. The reference population consisted of 32 healthy Caucasians (21 females and 11 males), living in and around London Ontario. The WIFN volunteers ($n = 40$) had a median (range) cortisol concentration of 176.5 (93.0 – 273.0) ng/g hair, which was significantly higher when compared to the median (range) cortisol concentration of 116.0 (26.0 – 204.0) ng/g hair of the reference population ($n = 32$) (Fig. 32; Mann Whitney U Test; $p < 0.0001$). The WIFN cohort was not normally distributed and therefore, medians are reported (D'Agostino & Pearson Omnibus normality test).

However, when comparing the PSS scores of the WIFN volunteers with the reference population, there was no significant difference (Fig. 33; Mann-Whitney U Test; $p = 0.2475$). The WIFN cohort had a median PSS score of 15 and the reference cohort had a median PSS score of 16.0. We did not find a correlation between WIFN hair cortisol concentrations and their PSS scores (Fig. 34; Spearman's Rank Correlation; Spearman $R = 0.02143$).

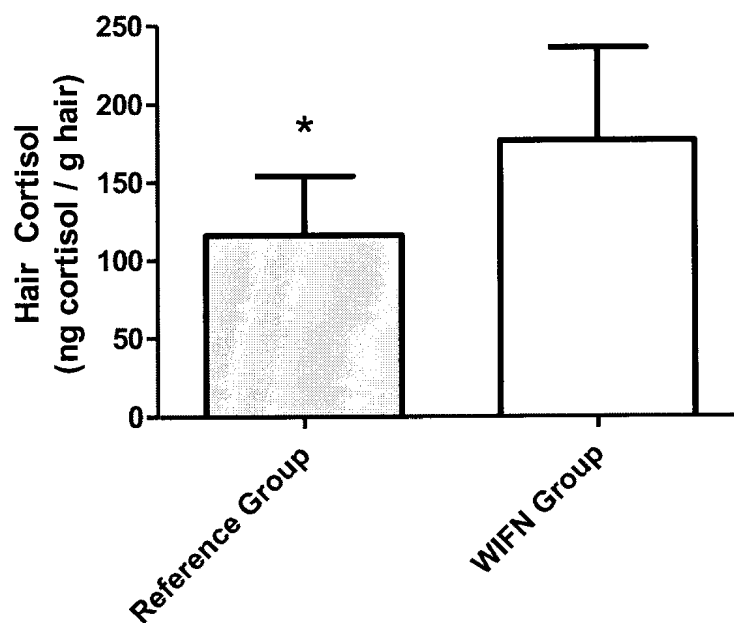


Figure 32: Median WIFN and reference group hair cortisol plus range.

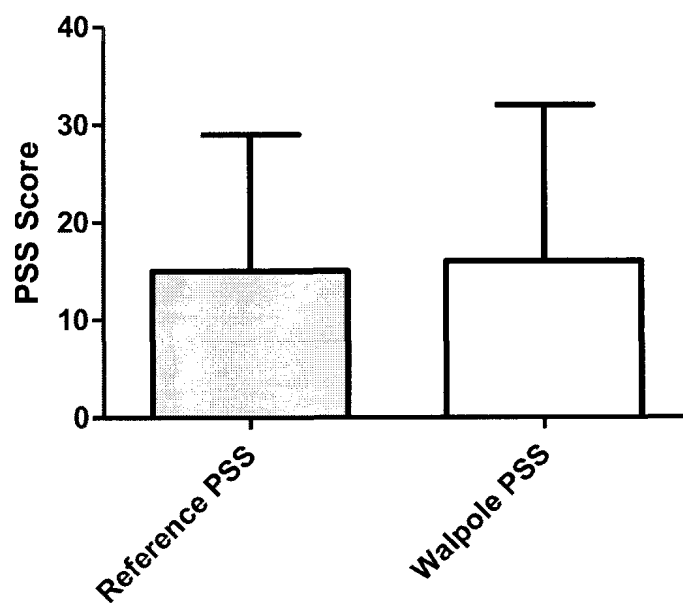


Figure 33: Median WIFN and reference group PSS score plus range.

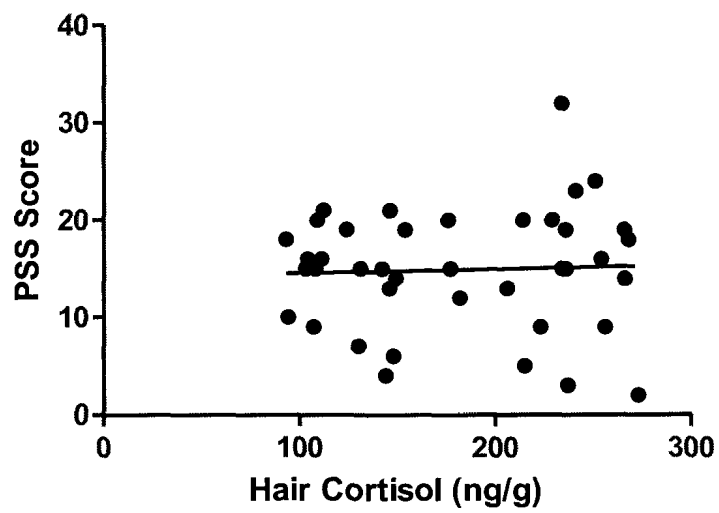


Figure 34: Relationship between WIFN hair cortisol and PSS score (Spearman's Rank Correlation).

Men ($n = 25$) in the WIFN population had a median cortisol concentration of 215.0 ng/g hair (Fig. 35). The WIFN women ($n = 15$) had a median cortisol concentration of 131.0 ng/g hair, which remained significantly less when compared to the men after Bonferroni correction ($p < 0.05$; Mann-Whitney U Test and Bonferroni correction).

As smoking is a confounder for cortisol, we wanted to determine whether there was a difference in cortisol concentrations of smokers ($n = 24$) compared to non-smokers ($n = 14$) in the WIFN population (Fig. 36). We did not find a significant difference when we compared the median cortisol concentration of smokers, 218.5 ng/g hair, to the median cortisol concentration of non-smokers, 147.5 ng/g hair ($p = 0.0930$; Mann-Whitney U Test).

We wanted to determine whether smoking played a role in the gender difference seen in the WIFN cohort. To do this we compared male smokers ($n = 15$) to male non-smokers ($n = 9$) and female smokers ($n = 9$) to female non-smokers ($n = 5$) (Fig. 37). There was no significant difference ($p = 0.0525$; Mann Whitney U Test) between the median cortisol concentrations of male smokers (234.0 ng/g hair) and male non-smokers (177.0 ng/g hair). We did not find a significant difference ($p = 0.7972$; Mann-Whitney U Test) between median cortisol concentration of female smokers (142.0 ng/g hair) and female non-smokers (130.0 ng/g hair). We then compared cortisol concentrations in WIFN male smokers to female smokers (Fig. 38). The cortisol concentration in the male smokers was significantly higher than the cortisol concentration in the female smokers even after the Bonferroni correction ($p < 0.05$; Mann-Whitney U Test).

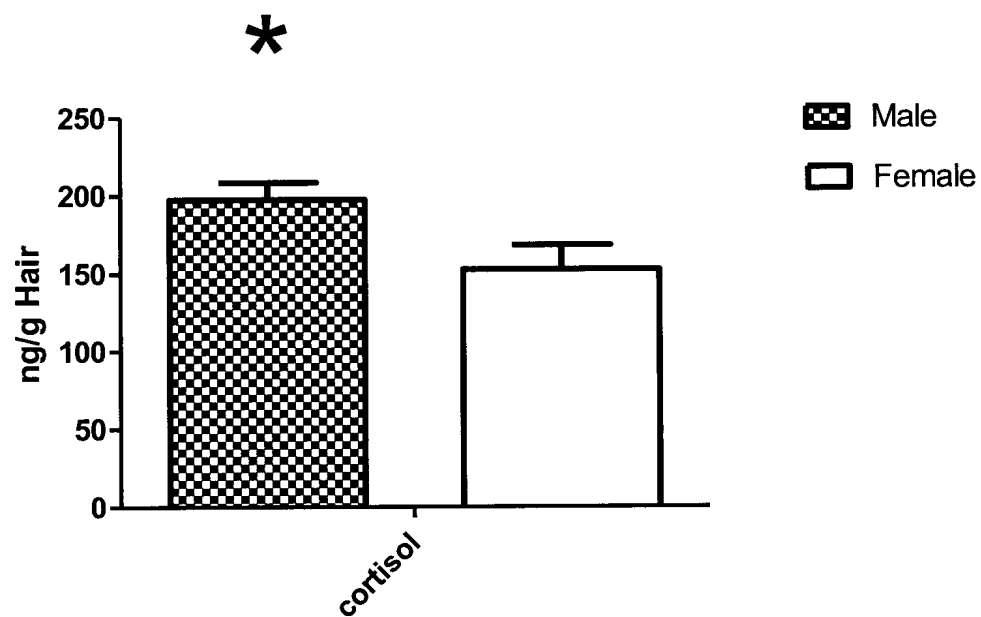


Figure 35: Median cortisol concentration in WIFN Males vs. Females (Mann-Whitney U Test; Bonferroni Correction; * $p < 0.05$).

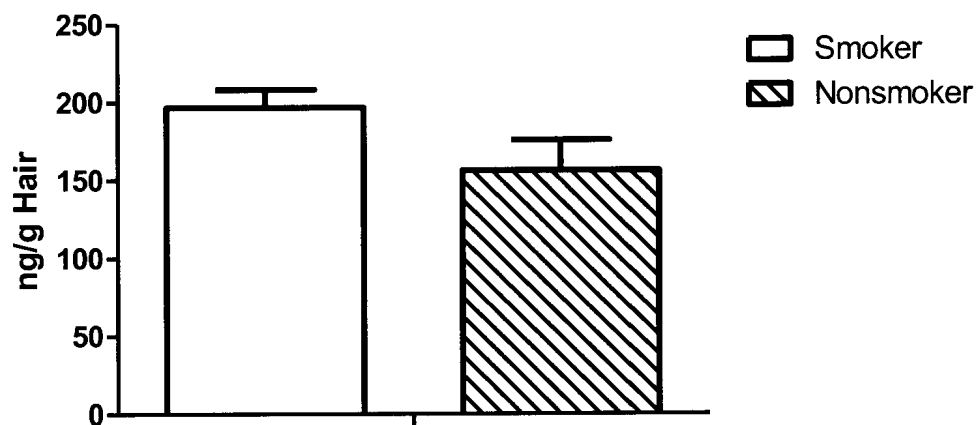


Figure 36: Median and range hair cortisol concentration in WIFN smokers vs. non-smokers (Mann-Whitney U Test; Bonferroni Correction).

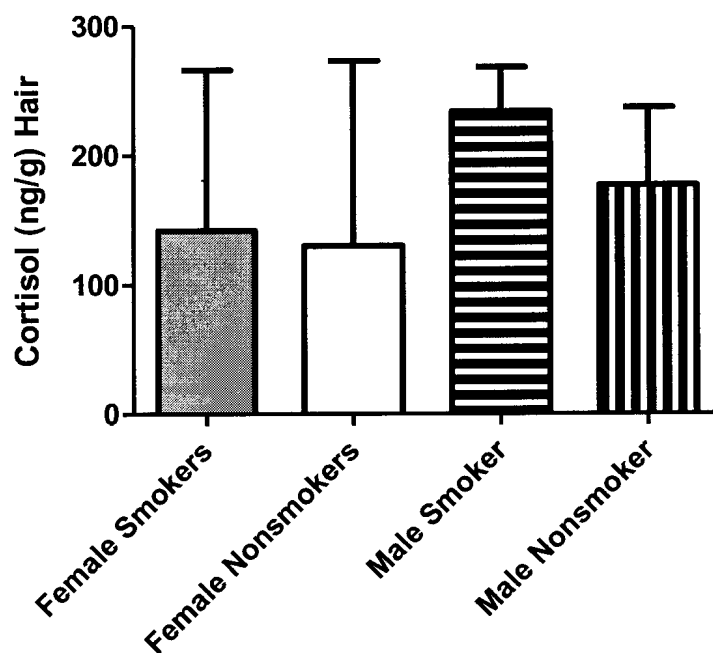


Figure 37: Median and range hair cortisol concentrations in WIFN Male and female smokers and WIFN male and female non-smokers (Mann-Whitney U Test; Bonferroni Correction).

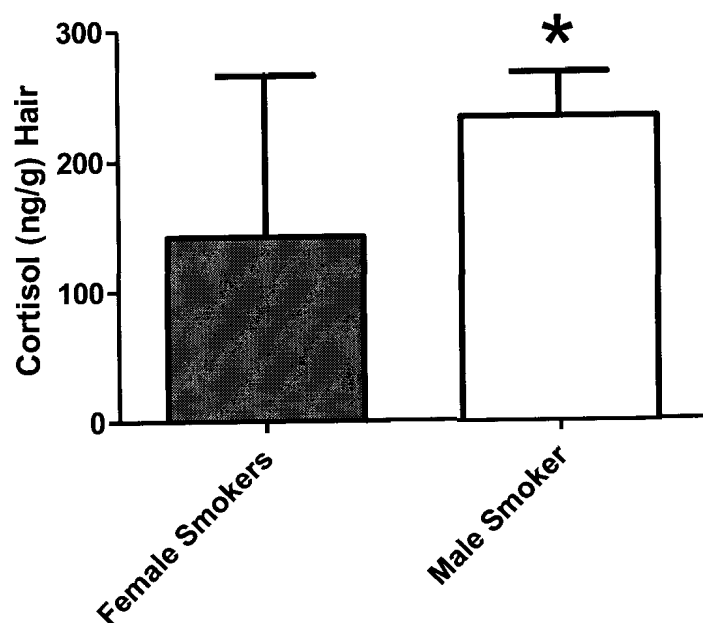


Figure 38: Median and range cortisol concentration in WIFN male smokers compared to WIFN female smokers (Mann-Whitney U Test; Bonferroni Correction; * $p < 0.05$).

There was no significant difference in cortisol concentration of female smokers and female non-smokers (graph not shown). We also examined the relationship of PSS scores of smokers and their cortisol concentrations and the PSS scores of non-smokers and their cortisol concentrations. We did not find a correlation (graph not shown).

Finally, we examined the cortisol concentration in WIFN volunteers who self-reported having diabetes ($n = 10$) on the health questionnaire and those who did not ($n = 24$) (Fig. 39). We found a significant difference ($p < 0.05$; Mann-Whitney U Test) in median cortisol concentration in diabetics (245.0 ng/g hair) when compared with non-diabetics (165.0 ng/g hair).

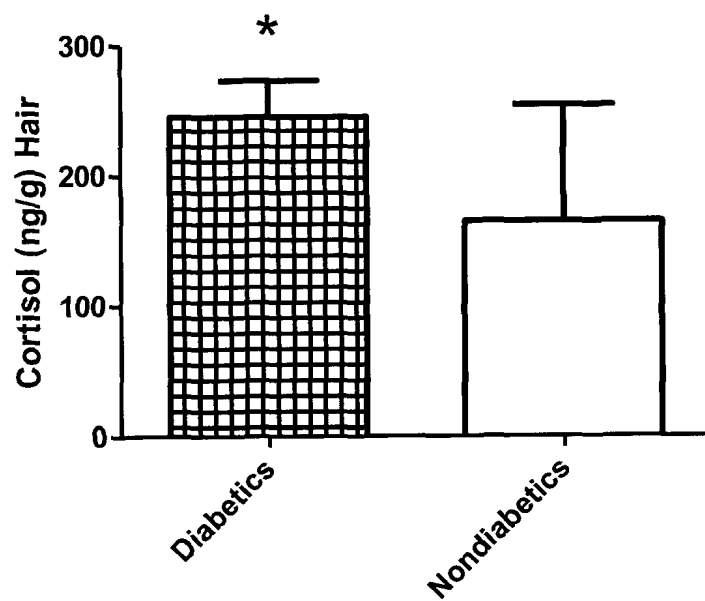


Figure 39: Median cortisol concentration and range in diabetics vs. non-diabetics (Mann-Whitney U Test; Bonferroni Correction; * $p < 0.05$).

5.3 Discussion

5.3.1 Questionnaire

A total of 52 volunteers who donated blood and hair answered and returned the health questionnaire. Most WIFN volunteers (75%) reported that they have been living on Walpole Island for their entire life. Only 1 WIFN volunteer reported living on Walpole Island for less than 5 years and approximately 10% of WIFN volunteers have lived on Walpole Island for 5 – 30 years, not encompassing life spans.

Of the 52 volunteers who responded to the questionnaire, 32 (62%) were male and 20 (38%) were female. This percentage did not match the trend seen in Registered Indians in the *1999-2003 Determinants of Health Statistical Profile on the Health of First Nations in Canada*. Here it was reported that 50.8% of Registered Indian were female and 49.2% of Registered Indians were male (Health Canada, 2009). According to the *1999-2003 Determinants of Health Statistical Profile on the Health of First Nations in Canada*, there are approximately 717 276 Registered Indians living in Canada and approximately 60.3% of them live on the reserve (Health Canada, 2009). The mean age of volunteers was 50, and the ages ranged from the youngest being 17 years old to the oldest being 80 years old. The *1999-2003 Determinants of Health Statistical Profile on the Health of First Nations in Canada* reported that nearly half (49.1%) the population of Registered Indians is under the age of 25 years and that males outnumbered females in all age groups 29 years of age or younger (Health Canada, 2009). Of the 7 WIFN volunteers

who were under the age of 29, 6 were male volunteers, which seem to follow the same trend as the Registered Indians in Canada.

Of these 52 volunteers who responded to the questionnaire, 29 (56%) self-identified themselves as smokers and 21 (40%) did not. This is slightly lower than the rate of smokers among First Nations living on reserve which was 58.8% in the *1999-2003 Determinants of Health Statistical Profile on the Health of First Nations in Canada* (Health Canada, 2009). Both the WIFN rate of smoking and the Health Canada reported rate of smoking among Aboriginals living on reserves are higher than the smoking rate among the Canadian population, which is 24.2% (Health Canada, 2009).

Of the WIFN volunteers who smoked (56%), 33% were male, and 23% were female. Health Canada also reported a gender difference between smoking rates in First Nations people living on the reserve. Health Canada found that 59.3% of on-reserve First Nations people were males, while 58.3% were female (Assembly of the First Nations/First Nations Information Governance Committee, 2007). However, the gender gap that we found for smokers in the WIFN volunteers was more consistent with the gender gap found in the general Canadian population, which is reported as being 26.5% of smokers are male compared to 22.0% of smokers are female (Assembly of the First Nations/First Nations Information Governance Committee, 2007). Aside from the well known adverse health effects caused by smoking (increased risk for cancer, cardiovascular disease and atherosclerosis), smoking has also been linked more recently with mental health and depression (Daniel *et al.*, 2004; Hutchinson *et al.*, 2008) and, of much relevance to our project, with T2D (Daniel & Cargo, 2004).

Of the health problems we asked about - ADHD, anxiety/depression, asthma, autism, cerebral palsy, chronic bronchitis, diabetes, epilepsy, heart conditions/disease, kidney conditions/disease, speech/language difficulties and TB - the most common health issue in WIFN volunteers and their children was asthma. Approximately 22 (43%) of WIFN volunteers reported asthma in themselves or their children. The next most prevalent health condition was diabetes at 33% which was just slightly more reported than anxiety/depression (31%). The least common health condition in the WIFN volunteers and/or their children was epilepsy, of which no cases were reported. Autism (2%), cerebral palsy (4%) and TB (6%) were also reported in low numbers.

According to an Assembly of First Nations report: *First Nations Regional Longitudinal Health Survey (RHS) 2002/03, Results for Adults, Youth and Children Living in First Nations Communities*, the most common health condition seen in adult First Nations people is arthritis, one condition we did not inquire about. However, heart disease is the second most common health condition occurring in approximately 19.5% of First Nations adults over the age of 60 (Assembly of the First Nations/First Nations Information Governance Committee, 2007). We found 13% of the WIFN volunteers reported either having a heart disease/condition or having a child/children with heart disease/condition. The Assembly of First Nations (2007) also reported asthma prevalence as 13.3% and 13.4% in First Nations people between the age of 50 - 59 years and over the age of 60 years respectively, while 9.4% of First Nations people between the ages of 17 - 29 years and 9% of First Nations people between the ages of 40 - 49 years reported asthma. Approximately 43% of the WIFN volunteers reported either having

asthma or having a child/children who have asthma, which seems consistent with statistics reported by the Assembly of First Nations (2007).

A total of 17 of the 52 (33%) WIFN members reported having diabetes while 29 out of 52 (56%) reported not having diabetes. Compared with the general Canadian population who were surveyed in 2005, the WIFN volunteer's diabetes prevalence was 6 times higher (Health Statistics Division, Statistics Canada, 2006). Approximately 5 % of the general Canadian population had diabetes in the year 2005 (Health Statistics Division, Statistics Canada, 2006). This is consistent for what is reported among other First Nations populations in Canada. In 2000, Health Canada found that First Nations people have a 3 – 5 times higher prevalence of diabetes when compared with the general Canadian population (Health Canada, 2000b).

Regardless of the prevalence of this disease in the community, diabetes is a significant concern in First Nations communities. This subgroup of the Canadian population tend to have earlier onset of diabetes, greater severity of diabetes at diagnosis, high rates of diabetic complications, and lack of accessible services (Health Canada, 2000a). It is also estimated that the actual number of First Nations people with diabetes is 2 – 3 times greater than the reported prevalence (Health Canada, 2000a) so that this disease is a major cause of morbidity and mortality in this group of Canadians.

However, there may be other factors influencing the occurrence of diabetes in the WIFN population. One of these factors could be the exposure to POPs that has the potential to increase risk of developing T2D. Another First Nations population at Akwesasne, which is located along the St. Lawrence River in New York, Ontario and

Quebec, has seen an increased risk of diabetes within their community due to exposure to PCBs through consumption of contaminated fish. This First Nations population is downstream from the Aluminum Company of America (ALCOA) which uses Aroclor 1248, a PCB mixture, in its heat-transfer equipment (Fitzgerald *et al.*, 2007). A study done on this population reported a significantly increased risk of diabetes in First Nations members exposed to PCB 153 and PCB 74 (Codru *et al.*, 2007). PCB 74 was also found to be most closely related to rates of fish consumption in this population (Fitzgerald *et al.*, 2007). There may be similar factors affecting the health of the WIFN population that should be looked into more closely, like the concentrations of POPs in local fish that are regularly consumed by members in the community.

We asked WIFN volunteers whether their children were breast fed as infants. This is relevant to our study since POPs have the potential to be passed to the infant through breast milk. Twenty-six (50%) of WIFN volunteers reported that they breast fed their child/children, whereas 13 (25%) said they did not. The rest either did not know, or did not answer the question.

Bend *et al.* (2006) reported that 31.7% of WIFN community members less than 20 years of age and 29.6% of WIFN adults ate fish during a 4 month fish consumption survey period. In our study of WIFN adults over 18 years of age, we found that 41 out of 52 volunteers (79%) reported eating fish. This difference could be attributed to our small sample size, which cannot be generalized to the entire community; also, we just asked members if they consumed fish, whereas the study done in 2006 had participants keep a fish consumption diary, so reporting bias may play an important role in our results.

We also inquired about current and past sources of drinking water. Most WIFN volunteers (79%) obtain their drinking water from pipes, while 33% drink bottled water, 9% said they have well water, and 12% said they obtain their water from the St. Clair River. However, for past sources of water, 48% of WIFN volunteers said they got their water from pipes and wells, while 44% said they got their drinking water directly from the St. Clair River. This figure is disturbing. However, follow-up questions regarding the time period for using water from the St. Clair River were not asked to better ascertain risk of exposure to pollutants.

5.3.2 POPs

The POP found in the highest concentration in the WIFN volunteers is *p,p'*-DDE, which is a metabolite of DDT. DDT has been used as an insecticide worldwide to combat mosquitoes and the spread of malaria, although the WHO have very recently recommended that it not be used any more for this purpose because of adverse effects to human health and the environment. DDT was banned from use as an insecticide in the mid 1970's in Canada and in 1973 in the US (Turusov *et al.*, 2002). Technical grade DDT contains 65 - 80% of the *p,p'*-DDT isomer, 15 - 21% *o,p'*-DDT isomer and up to 4% *p,p'*-DDD isomer and DDT breaks down to *p,p'*-DDE in the environment as well as in living organisms (Rogan & Chen, 2005). DDE is the form predominantly found in humans, especially if exposure to DDT has not occurred for quite some time. The $T_{1/2}$ of DDE in humans is 7 – 11 years. Due to the use pattern of DDT in North America, concentrations of DDT and DDE in humans increase with age (Rogan & Chen, 2005). It has been estimated that it would take 20 years for an exposed person to DDT to

completely eliminate DDT from his/her body, however DDE is thought to persist throughout the person's lifetime (Turusov *et al.*, 2002). Because we found DDE in high concentrations in the WIFN volunteers and did not find any detectable DDT in any of the volunteers, we know that the WIFN volunteers were exposed to DDT a long time ago when this compound was being widely used as an insecticide in Canada.

To determine the relative levels of exposure in the WIFN community, we initially compared these results to the National Health and Nutrition Examination Survey (NHANES), which is a national representation of the non-institutionalized United States population (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). The data from NHANES came from the 1999-2000 and 2001-2002 surveys.

Table 19 and Table 20 compare the WIFN data to the NHANES data. Those chemicals missing from these tables were either not measured in the NHANES survey or were not found in concentrations over the limit of detection.

Table 19: Concentration of OC Pesticide Residues in WIFN Volunteers compared to those in the US NHANES Cohort. Adapted from (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005).

| Chemical Name | WIFN Data | NHANES Data | Where WIFN Lies in NHANES Data | NHANES Data | Where WIFN Lies in NHANES Data |
|-------------------------|------------------------------|---|-------------------------------------|---|-------------------------------------|
| | Geometric Mean ng/g lipid | 1999-2000 Geometric Mean (95% CI) ng/g lipid | | 2001-2002 Geometric Mean (95% CI) ng/g lipid | |
| <i>p,p'</i> -DDE | 265.30 | 260 (226-298) | 50 th | 295 (267-327) | 50 th |
| <i>trans</i> -nonachlor | 21.11 | 18.3 (16.7-20.0) | 50 th – 75 th | 17.0 (15.2-18.9) | 50 th – 75 th |
| β -BHC | 20.91 | 9.68 (<LOD-10.9) | 75 th | *Not calculated | 90 th |
| Oxychlordan | 20.77 | *Not calculated | 75 th | 11.4 (<LOD-12.5) | 75 th |
| Mirex | 7.44 | *Not calculated | - | *Not calculated | 90 th |

*Not calculated: the proportion of results below the limit of detection was too high to provide a valid result.

Table 20: Concentration of PCB congener residues in WIFN volunteers compared to those in the US NHANES cohort. Adapted from (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005).

| PCB Congener | WIFN Geometric Mean ng/g lipid | NHANES 1999-2000 Geometric Mean (95%CI) ng/g lipid | Where WIFN Lies in NHANES Data | NHANES 2001-2002 Geometric Mean (95% CI) ng/g lipid | Where WIFN Lies in NHANES Data |
|-----------------|---|---|--|--|--|
| PCB 153 | 48.34 | Not calculated | < 90 th | 27.2 (24.7-30.1) | 50 th – 75 th |
| PCB 180 | 35.77 | Not calculated | 75 th | 19.2 (17.4-21.1) | 50 th – 75 th |
| PCB 138 | 30.68 | [†] Not calculated | < 90 th | [†] 19.9 (18.0-22.0) | 50 th – 75 th |
| PCB 187 | 24.05 | Not calculated | 95 th | Not calculated | 90 - 95 th |
| PCB 52 | 18.77 | Not calculated | - | Not calculated | > 95 th |
| PCB 74 | 14.72 | Not calculated | <90 th | Not calculated | 75 th |
| PCB 118 | 14.71 | Not calculated | 75 th | Not calculated | 75 th |
| PCB 156/171 | 14.35 | *Not calculated | 90 th – 95 th | *Not calculated | 90 th |
| PCB 169 | 14.11 | Not calculated | < 90 th | 17.9 (16.0-19.9) | < 50 th |
| PCB 99 | 12.20 | Not calculated | 90 th | Not calculated | < 90 th |
| PCB 170 | 10.89 | Not calculated | < 50 th | Not calculated | <75 th |
| PCB 194 | 10.22 | - | - | Not calculated | 75 th |

*only for PCB 156 [†]NHANES data reported 138/158 together

The geometric mean concentration of *p,p'*-DDE found in the WIFN volunteers was within the 50th percentile of the NHANES data for both the 1999 - 2000 and 2001 - 2002 surveys. However, the geometric mean concentration of *p,p'*-DDE was way above geometric mean concentrations of *p,p'*-DDT and *p,p'*-DDE (taken together) of 2.66 ppb and 2.38 ppb reported by Tsuji *et al.* (2005) in a female and male from a First Nations community in the James Bay region of Northern Ontario, Kashechewan, respectively.

Compared with the NHANES cohort from 2001 - 2002 data, the geometric mean concentration of PCB 153 was between the 50th and 75th percentile. PCB 180 and 138 were detected in the next highest concentrations. The geometric mean of PCB 180 fell within the 75th percentile of the 1999-2000 cohort and between the 50th and 75th percentile of the 2001 - 2002 cohort. There were many geometric mean concentrations of POPs that were higher in the WIFN population when compared with the NHANES cohort (*trans*-nonachlor, β -BHC, oxychlordane, PCB 153, PCB 180, and PCB 138). However, they all fell within the percentile concentrations of the populations except for one PCB congener, PCB 52. The WIFN population had a geometric mean concentration of 18.77 ng/g lipid (15.83 - 22.25; 95% CI). The 1999 - 2000 NHANES cohort did not detect concentrations of PCB 52 over the limit of detection in most of the US citizens analysed so a geometric mean was not calculated. However, the 95th percentile for PCB 52 had a geometric mean of 16.2 ng/g lipid (14.3 - 17.2; 95% CI).

When we compared the concentration of OC pesticides in the WIFN volunteers to concentrations of OC pesticides measured in the NHANES data from 1999 - 2000 and 2001 - 2002, we found, based on the geometric mean concentration in each group, that β -BHC in the WIFN group fell within the 90th percentile of the 2001 - 2002 NHANES

cohort. Excluding Mirex, the geometric mean concentrations of the rest of the OC pesticides we analyzed in the WIFN volunteers fell within the 50 - 75th percentiles of the NHANES data.

β -BHC was used as a fungicide and in the synthesis of other chemicals. It also forms as a by-product in the production of the insecticidal γ -BHC isomer (commonly known as Lindane) (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). Although β -BHC is not used anymore, approximately 10 million tons of technical HCH were released throughout the world between 1948 - 1997; β -BHC accounts for 5 - 14% of the chemical HCH (Persistent Organic Pollutants Review Committee (POPRC), 2007). β -BHC is also very persistent in the environment, occurs in the food web, especially in the Arctic, and accumulates in humans.

We found a geometric mean concentration of β -BHC of 20.9 (16.1-27.1) ppb (95% CI) in WIFN volunteers. This concentration was higher than that recently reported in a Canadian population of First Nations people in the James Bay area. The highest geometric mean concentration reported in the study by Tsuji *et al.* (2005) was 0.21 ppb in a Hamilton male and 0.13 ppb in a Hamilton female. Hamilton residents were used as a reference for the First Nation data. We found a median concentration of 23.06 ppb (9.17-41.82; range) in the WIFN volunteers for β -BHC, presumably reflective of the higher use of this chemical as a fungicide in southern Ontario than in northern Ontario.

This median concentration of β -BHC in the WIFN volunteers was also higher than reported previously for another Canadian group of sport fishermen from Montreal. Kosatsky *et al.* (1999) reported a median concentration of 0.02 (0.01 - 0.03) ppb (range)

in high fish consumers and a median concentration of 0.01 (0.01 - 0.03) ppb (range) in low fish consumers. However, when we compared the WIFN data to a recent study done outside of Canada, (Zamir *et al.* 2009), on different groups living throughout Bangladesh, we found that the WIFN median concentrations fell within concentrations reported and were a lot lower than most β -BHC concentrations. The highest median concentration reported by Zamir *et al.* (2009) was 1 400 ppb in a group of transformer workers and the lowest median concentration was 4 ppb in a group of male Bangladesh fishermen from Dhaka University (Zamir *et al.*, 2009). Although the WIFN volunteers appear to have slightly higher median concentrations of β -BHC when compared with the general American population (NHANES data) and different sub populations from the Canadian population, they have considerably lower concentrations when compared with different sub populations from Bangladesh, allowing us to conclude that, although the WIFN were exposed, their exposure was comparable to that of the general North American population. In the NHANES data the β -BHC content of blood lipids is decreasing with time indicating that exposures are decreasing and we expect, but do not know for certain, that a similar effect is occurring in individuals living in southern Ontario.

The geometric mean concentration of Mirex in serum lipids of the WIFN volunteers was found within the 90th percentile of the NHANES data from 2001 - 2002. Mirex is a pesticide that was used in the southern US to control fire ants, termites, and for other pests worldwide, as well as being used as a flame-retardant additive in plastics, rubber, paint, and electrical goods (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005; United Nations Environmental Programme, 2006). Fortunately, Mirex was never registered as an agricultural pesticide in Canada

because fire ants and termites are not major problems here. The main sources of Mirex in Canada came from chemical manufacturing and fire production plants in New York State via releases into the Niagara River and the Oswego River (United Nations Environmental Programme, 2006). This POP chemical has been banned from production and use since 1977 in the United States (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). Mirex is biologically active, accumulates in the food web, and is very persistent in the environment. The general Canadian public has a low exposure rate to Mirex from food. However, there is an exception for subpopulations that are partly or entirely dependent on a diet of traditional foods consisting of fish and/or fish-feeding birds from Lake Ontario or the St. Lawrence River (United Nations Environmental Programme, 2006). Mirex accumulates in fatty tissues and is not easily metabolized, so if volunteers from the WIFN were exposed to Mirex, even years ago when it was still in production, it would remain as a body residue and would have been detected in our study (Centers for Disease Control and Prevention & Department of Health and Human Services, July 2005). We reported a geometric mean concentration of 7.4 (6.3 - 8.8) ppb (95% CI) for Mirex.

We also compared this concentration to that of another population of First Nation people living in Kashechewan in the James Bay area in Canada investigated by Tsuji *et al.* (2005). This team of researchers reported a highest geometric mean concentration for Mirex of 0.40 ppb in a male and a highest geometric mean concentration of 0.28 (0.19 - 0.38) in a female from this community. The median (range) concentration of Mirex in the WIFN volunteers was 6.81 (4.44 - 18.49) ppb which was also higher than the median concentration of 0.01 ppb (0.00 - 0.15; range) for heavy fish consumers and 0.01 (0.00 -

0.04; range) for low fish consumers within a population of sport fish consumers in the Montreal area (Kosatsky *et al.*, 1999).

The geometric mean concentrations for several of the PCB congeners in the blood lipids of the WIFN volunteers fell within the 90th and 95th percentiles of the NHANES data for these same chemical pollutants. More specifically, the WIFN geometric mean concentration of 24.05 (17.31 – 33.42) for PCB 187 was within the 95th percentile of the 1999-2000 NHANES data and within the 90th-95th percentile in 2001 - 2002 NHANES data; 18.77 (15.83 - 22.25) for PCB 52 which was greater than 95th percentile in 2001 - 2002 NHANES data; 14.35 for PCB 156/171 which was between the 90-95th percentile in 1999 - 2000 and within the 90th percentile in 2001 - 2002 NHANES data; and 12.20 (9.84 – 15.13) for PCB 99 which was within the 90th percentile in 1999 - 2000 and less than the 90th percentile in 2001-2002 NHANES data.

PCB congeners were manufactured as mixtures and the most common mixture manufactured and sold was the Aroclor series. PCBs are no longer produced in the US or in Canada. However, they were produced for around 50 years and used for a wide range of commercial and industrial applications.

The WIFN volunteers had higher median concentrations of PCB 187, 52, 156/171 and 99 compared with an Ojibwa First Nations population from a reserve near the Great Lakes and a population of Great Lakes Fishermen reported by Chiu *et al.* (2004). However, the median concentration of PCB 187, 52, 156/171 and 99 were comparable to median concentrations reported in a population of Bangladeshi decent in a Vietnamese population, and in a population of high-local-fish consumers living in the Montreal area

reported by Kosatsky *et al.* (1999). Table 21 shows the WIFN concentrations of these 4 PCB congeners compared with the Objibwa First Nations population and Great Lakes Fishermen population in ppb on the left hand side. The right hand side of Table 21 shows the WIFN concentrations of these 4 PCB congeners compared with a population of Bangladeshi decent, a Vietnamese population, and a population of high-local-fish consumers living in the Montreal area in ppm.

Table 21: Concentrations of PCB 52, 99, 156/171 and 187 ppb in 6 populations.

| PCB Congener | | | | | | |
|--------------------------------|---|--|-----------------------------|--|---|--|
| ppb Median (range) | | | ppm Median (range) | | | |
| WIFN | Objibwa First Nation (Chiu <i>et al.</i> , 2004) | Great Lakes Fishermen (Chiu <i>et al.</i> , 2004) | WIFN | Bangladeshi (Kosatsky <i>et al.</i> , 1999) | Vietnamese (Kosatsky <i>et al.</i> , 1999) | High-level local (Montreal- area) fish consumers (Kosatsky <i>et al.</i> , 1999) |
| PCB 187 | | | PCB 187 | | | |
| 21.59 (12.46- 95.00) | 0.3 (0.02- 0.8) | 0.4 (0.2-0.8) | 0.02 (0.0- 0.10) | 0.04 (0.01-0.17) | 0.02 (0.01-0.06) | 0.02 (0.01-0.32) |
| PCB 52 | | | PCB 52 | | | |
| 19.57 (8.82- 39.11) | 0.3 (0.02- 0.8) | 0.4 (0.2-0.8) | 0.02 (0.01- 0.04) | 0.04 (0.01-0.17) | 0.02 (0.01-0.06) | 0.02 (0.01-0.32) |
| PCB 156/171 | | | PCB 156/171 | | | |
| 14.35 - | - | - | 0.01 | 0.01 (0.00-0.04) | 0.00 (0.00-0.01) | 0.00 (0.00-0.02) |
| PCB 99 | | | PCB 99 | | | |
| 12.05 (5.75- 17.55) | 0.4 (0.03- 1.2) | 0.7 (0.03-1.2) | 0.01 (0.0- 0.03) | 0.03 (0.01-0.06) | 0.01 (0.00-0.02) | 0.02 (0.01-0.33) |

The relatively high concentrations of specific POPs such as DDE, Mirex, and PCBs in the WIFN volunteers is not surprising considering the concentrations of POPs and PCBs in sediment measured at different locations around Walpole Island. Sediments were measured from four channels [Fig. 40: Chenal Ecarte (1), Johnson (2), Chematogen (3), and Bassette (4)] in Walpole Island as well as Goose Lake (5). PCB concentrations measured in sediment from Walpole Delta were significantly higher than concentrations from sediments from the entire St. Clair Delta (Great Lakes Institute for Environmental Research and Department of Biological Science, University of Windsor 2006). The concentration of *p,p'*-DDT found in sediments in the Chenal Ecarte and downstream from Bassette Channel exceeded the Ontario Ministry of Environment (OMOE) lowest effect level (LEL) of 8 ng/g dry weight (Great Lakes Institute for Environmental Research & Department of Biological Science, University of Windsor, 2006). The concentrations of POPs in sediments were also compared to those in sediments measured at the same sites in 2004. There was an increase of total PCBs, *p,p'*-DDT, and HCB concentrations from a sampling site at the top of the Chancel Escarte and one midway down the channel from 2004 - 2006 (Great Lakes Institute for Environmental Research & Department of Biological Science, University of Windsor, 2006).

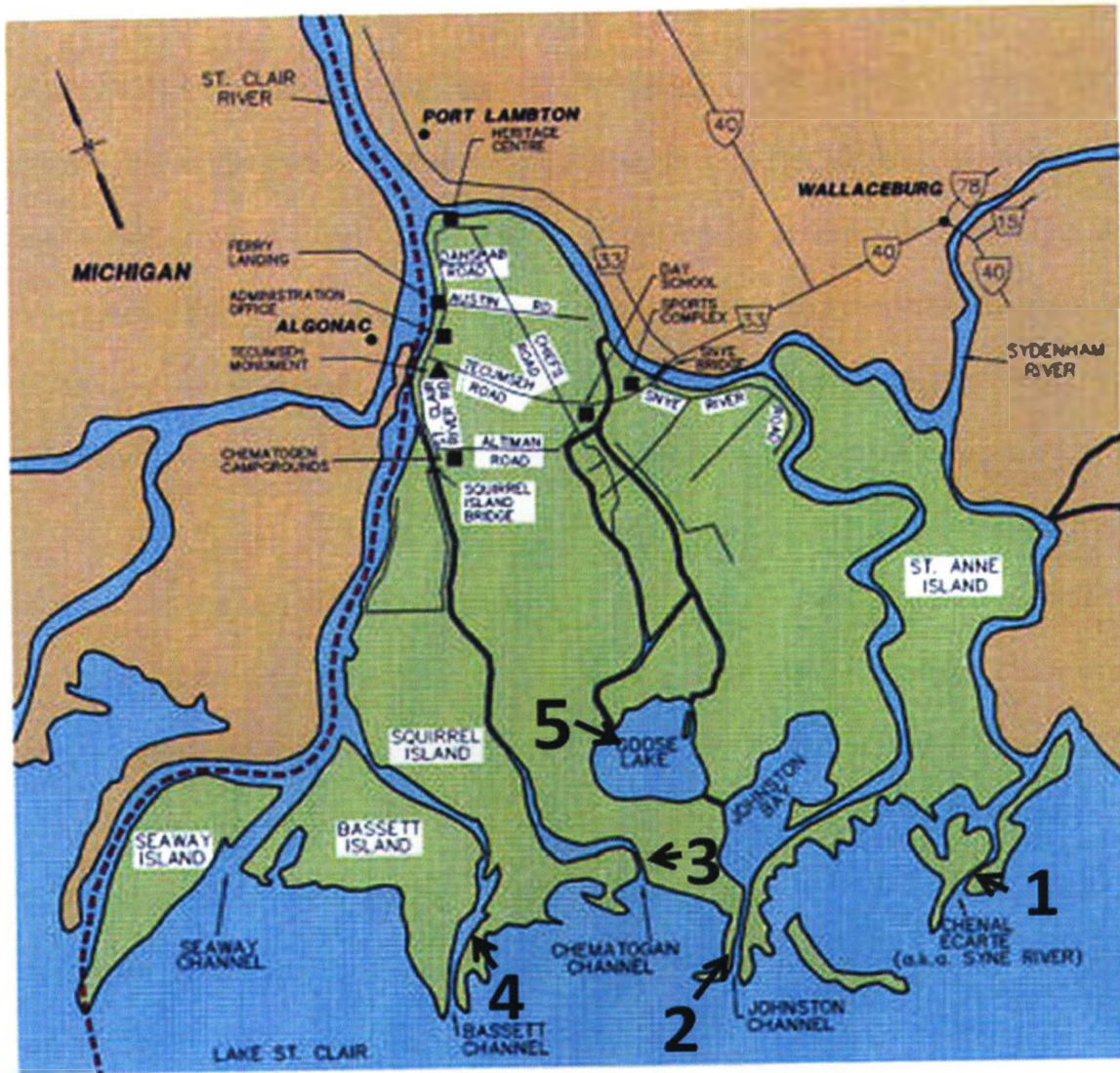


Figure 40: Map of Walpole Island. Adapted from (Bkejwanong.com 2005).

With regard to residues in blood lipids, we found a significant relationship between age of WIFN volunteers and concentration of *trans*-nonachlor. The older the WIFN volunteer, the higher concentration of *trans*-nonachlor found. This is consistent with what we know about POPs in general - they persist in the body, accumulating and biomagnifying over time. *Trans*-nonachlor is a component of the pesticide chlordane that was used as an insecticide in agriculture and to control termites. Chlordane was completely phased out in North America in 1995, but many restrictions were placed on its use beginning in the 1980's (Commissions for Environmental Cooperation in North America). As the majority of volunteers who had concentrations of *trans*-nonachlor in their blood lipids were over the age of 40, the exposures were most likely from using chlordane during farming or gardening, or consuming contaminated vegetables, grains, or game, and fish while chlordane was still in use. PCB 180 was another POP that showed a positive relationship with age in WIFN volunteers. PCB 180 is a large volume octachlorobiphenyl (OCB), accounting for 7% of the PCB mixture, Clophen A60 (Koss *et al.*, 1993).

Recent literature has reported relationships between POPs and the risk for diabetes (Kang *et al.*, 2006; Lee *et al.*, 2007; Rignell-Hydbom, Rylander, Hagmar 2007; Lee *et al.*, 2008; J. E. Michalek & Pavuk, 2008). However, we did not find any significant relationship between OC pesticide residues measured in blood lipids and the self-reported incidence of diabetes in WIFN volunteers. We did find a significant relationship between PCB 177 and diabetes in WIFN volunteers ($p < 0.01$; Bonferroni correction). However, it is quite possible that this association was found only by chance because PCB 177 was found at relatively low concentration in blood lipids of the WIFN volunteers. We did not

find any other study in the literature that reported a relationship between the incidence of diabetes and PCB 177. However, there are many studies that report a relationship between PCB concentrations in general and diabetes (Glynn *et al.*, 2003; Fierens *et al.*, 2003; Vasiliu *et al.*, 2006; Codru *et al.*, 2007; Jorgensen *et al.*, 2008; Wang *et al.*, 2008)

We found a significant relationship between *trans*-nonachlor and the self-reported consumption of small game. *Trans*-nonachlor has been recently measured in many fish, small game, and large game in the literature. Tsuji *et al.* (2007) reported concentrations (mean concentration 0.13-0.23 µg/Kg wet mass in Snow geese and 0.14-0.25 µg/Kg wet mass) in Godwits of *trans*-nonachlor in the breast tissue of game birds in south western Hudson Bay and western James Bay in Canada. *Trans*-nonachlor was reported in pintail ducks, mallard ducks, snow geese, and Canadian geese and elevated concentrations were found in spring harvested mallard males when compared with the fall-harvested mallard ducks (Tsuji *et al.*, 2007). *Trans*-nonachlor was found in long-tailed ducks and their eggs, from the Beaufort Sea., but not at elevated concentrations (Franson *et al.*, 2004).

We also found a significant relationship between volunteers who smoked and their plasma lipid concentration of Mirex ($p < 0.05$) and β -BHC ($p < 0.01$), after using Bonferroni Correction. Although many pesticides were used in tobacco in the 1970's, Mirex was never one of them (Dorough & Atallah, 1975). A study by Deutch *et al.* (2003) reported that the mean concentrations of POPs were higher (but not statistically so) in smokers and previous smokers when compared with non-smokers. Deutch has also co-authored other studies where he has reported a positive relationship between cigarette smoke and high POPs concentrations (Deutch & Hansen, 1999; Deutch & Hansen, 2000).

He offers some explanation for the relationship we saw with Mirex: that smoking may act on human POP accumulation by causing a lower BMI through enzymatic systems (Deutch *et al.*, 2003). In most cases, the concentration of POPs in smokers and non-smokers was similar. In a few cases, we found POPs in lower median concentrations in smokers when compared with non-smokers, significantly so for β -BHC, so that a lower BMI could explain these data.

5.3.3 Heavy Metal

The heavy metals Al, Ba and Pb were found at the highest concentration in hair of WIFN volunteers, and Ni, Pb and Sb were found at the highest concentrations in whole blood. Average concentrations of these metals in samples of the Earth's crust are as follows: 80.0 $\mu\text{g/g}$ Ni, 14-16.0 $\mu\text{g/g}$ Pb, 0.11 $\mu\text{g/g}$ Cd, 1.5 $\mu\text{g/g}$ As (Pekey 2006; Alomary & Belhadj, 2007). As Ni is commonly found in the Earth's crust and is a major natural trace element, it is not surprising that it is one of the heavy metals with high concentrations in WIFN blood. The incidence of other heavy metals in the Earth's crust is rarer when compared with Ni and exposure to these metals was most likely through anthropogenic sources, such as pollution. Ni is mainly used in industry in the production of stainless steel and nickel alloys. Ni-containing food is also the main source of Ni exposure in most environments, whereas drinking water is normally an additional minor source.

In a recent sampling of sediment from 6 locations around Walpole Island, Cd and Ni were the only metals found in concentrations exceeding the lowest effects levels set by the Ontario Ministry of Environment (Great Lakes Institute for Environmental Research

& Department of Biological Science, University of Windsor, 2006). The highest sediment concentration of Cd and Ni found near the Walpole Island exceeded the OME's lowest effects levels. Ni was the only metal found in high concentrations in the sediment as well as in higher concentrations than most of the other metals in the hair and blood samples. Three metals: As, Bi, and Sb were not found in detectable concentrations in the sediment at all 6 sediment sampling sites (Great Lakes Institute for Environmental Research & Department of Biological Science, University of Windsor, 2006). One major known source of Pb in the sediment is environmental release from Ethyl Canada. In 1985, the highest concentration of Pb found in sediment from the St. Clair River was 330 mg/kg dry mass, a concentration well above the probable effect level for fresh water of 91.3 mg/kg established in 2002 by the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life by the Canadian Council of Ministries of the Environment (Griffiths, 1991). The Dow Chemical Plant is also a probable historical source of Ni pollution in sediments of the St. Clair River (Griffiths, 1991).

There are additional sources that could be contributing to environmental exposure of these heavy metals other than sediment and wildlife contamination. Ba is commonly used as barium sulphate, an essentially insoluble X-ray contrast agent that is ingested by patients. However, this form of barium is not absorbed by the intestinal lumen (Jourdan *et al.*, 2001) so it is unlikely to contribute to Ba in either hair or blood samples from the WIFN volunteers. On the other hand, Ba is also found in drinking water that is taken from lakes and streams in the United States and presumably, also Canada (Wones *et al.*, 1990).

Environmental Cd that is bioavailable for humans is largely found in foods such as fish, meat, and fruit, and most human exposures come from the diet (Zenzes *et al.*, 1995). Other than diet, cigarette smoke (primary or secondary) is a major source of Cd exposure and Cd content of hair or blood from heavy smokers is higher than in non-smokers (Unkiewicz-Winiarczyk *et al.*, 2009).

Human exposure to the toxic metalloid, As, is generally from deep-welled drinking water or contaminated groundwater. However the diet is also a source of As exposure (Yost *et al.*, 1998; Schoof *et al.*, 1999; Del Razo *et al.*, 2002).

We compared concentrations of 6 metals in the hair of the WIFN volunteers with concentrations in samples of their whole blood to determine whether hair concentrations of these metals were sensitive biomarkers for these elements (As, Cd, Pb, Ni, Tl, Sb) in blood. We found a positive relationship between the concentration of Pb in hair and blood samples after performing a Spearman Rank Correlation analysis (Spearman r^2 value = 0.07024). This confirmed the earlier report of Foo *et al.* (1993) who also found a significant positive correlation between the concentration of Pb in hair and blood of a sample population, allowing them to conclude that hair is a good index of exposure for Pb. Hair is a relatively novel approach to determining concentrations of most heavy metals, except for mercury. Hair is advantageous in that for most populations it is a relatively non-invasive procedure, it is easily collected, transported, and stored, and it is relatively inexpensive to sample large populations. However, there are also some limitations to hair testing for environmental contaminants. Exogenous contamination with heavy metals is thought to be one major limitation that can be minimized by

thoroughly washing the external surface of the hair (Morton *et al.*, 2002). Endogenous deposition of heavy metals can come from sebum, sweat, air pollution residues, and residues from pharmaceutical and cosmetic products. Other limitations of sampling exposure to environmental contaminants by incorporation into hair are the difference between genders, the difference between racial groups, the effect dying hair has on the incorporation of the analyte into hair, and the difference of incorporation in hair between different regions on the scalp (Rodrigues *et al.*, 2008).

We also found a correlation between As in hair and blood (Spearman r^2 value = 0.009747). Relationships have been found between As content in hair and blood in hedgehogs, but no correlation has been seen between As content in human hair and blood (Vermeulen *et al.*, 2009). Hair grows at a rate of approximately 1 cm. per month. Therefore, consecutive 1 cm. segments of hair presumably allows us to determine the previous month's worth of exposure to heavy metals and other contaminant that have been incorporated into the hair. However, one important point to keep in mind is that there is approximately a 20 d lag between the concentration of trace elements in the first cm. closest to the scalp and the corresponding average monthly blood levels, as hair takes approximately 20 d to protrude externally out of the scalp (Clarkson & Magos, 2006). Therefore, fluctuation in dietary intake of trace elements or exposure to heavy metals over time could explain the lack of correlation between hair and blood levels, since blood and hair were collected on the same sampling day (Rodrigues *et al.*, 2008).

One way we assessed exposure was by comparing concentrations of heavy metals in blood to concentrations reported in the literature as normal and toxic. For this we used

the *Guide to the Interpretation of Analytical Toxicology Results and Mass/Amount Concentration Conversion Factors for Some Metals and Trace Elements* (Flanagan *et al.*, 2001). The heavy metals shown in Table 22 are the 5 heavy metals measured in whole blood, with whole blood values for comparison. Ni is not shown in Table 22 because the data presented by Flanagan *et al.*, (2001) did not have concentrations for whole blood, only plasma.

In all heavy metals except for As and Cd, the WIFN volunteers are below the concentrations thought to be normal and the concentration where serious toxicity occurs. However, 1 WIFN volunteer is over the concentration thought to be normal for As, but still under the concentration where serious toxicity will occur. This individual has an As concentration of 29.94 $\mu\text{g/L}$ in blood, which is higher than the normal concentration of 10 $\mu\text{g/L}$ and lower than the serious toxic concentration of 50 $\mu\text{g/L}$. One other volunteer is slightly higher than the concentration considered normal for Cd. His/her concentration in blood is 5.08 $\mu\text{g/L}$ compared to the normal concentration of 5.00 $\mu\text{g/L}$, but it is still way below the concentration above which serious toxicity occurs, 20 $\mu\text{g/L}$.

Although the median concentrations of As, Ni, and Pb were found to be the highest of the metals measured in blood from the WIFN volunteers, these were still present at relatively low concentrations. We compared the metal concentrations in blood with “therapeutic or normal plasma concentrations” and “plasma concentrations associated with serious toxicity” and found that As was the only metal (of the 3 found in highest concentration) that fell in the ‘normal’ or ‘therapeutic’ range, while the rest of the heavy metals were present at concentrations lower than the ranges given (Flanagan *et al.*,

2001). Cd was also found in the 'normal' or 'therapeutic' range. Both these heavy metals could be in these slightly elevated ranges due to recent exposure to cigarette smoke (Unkiewicz-Winiarczyk *et al.*, 2009).

Table 22: Heavy metal concentration in blood of WIFN volunteers relative to toxic concentrations.

| Heavy Metal | "Normal" Plasma Concentration (less than) (Flanagan <i>et al.</i> , 2001) | Plasma Concentration Associated with Serious Toxicity (Flanagan <i>et al.</i> , 2001) | Median WIFN Concentration Whole blood (min-max) | # of volunteers over the normal plasma concentration (concentration $\mu\text{g/L}$) |
|----------------------------|---|--|---|--|
| Sb (whole blood) | 10 $\mu\text{g/L}$ | 200 $\mu\text{g/L}$ | 3.25 (0.04 – 5.11) | 0 |
| As (whole blood) | 10 $\mu\text{g/L}$ | 50 $\mu\text{g/L}$ | 1.20 (0.20 – 29.94) | 1 (29.94) |
| Cd (whole blood) | 5 $\mu\text{g/L}$ | 20 $\mu\text{g/L}$ | 0.59 (0.20 – 5.08) | 1 (5.08) |
| Pb (whole blood) | 100 $\mu\text{g/L}$ | 600 $\mu\text{g/L}$ | 10.36 (4.14 – 41.44) | 0 |
| Tl (whole blood) | 2 $\mu\text{g/L}$ | 50 $\mu\text{g/L}$ | 0.02 (0.01-0.05) | 0 |

Another way to assess exposure of the WIFN volunteers to heavy metals was to compare the heavy metal concentrations in hair with individuals from 2 other populations in Ontario. The first population is a group of Japanese Canadians (N = 23) living in Toronto and the second population is a group of Canadian women (N = 20) living in central Ontario. All three groups were below the reference range, determined by the LHSC Trace Elements Lab and indicated by the dotted line on each of the graphs, for all 15 heavy metals.

The median concentration of Ti in the WIFN population hair samples was significantly less (One Way ANOVA, $p < 0.05$) than those in the Japanese group and the group of Ontario women (Fig. 41). The median concentration of the WIFN volunteers was 0.1 (0.07 - 0.17) $\mu\text{g/g}$ hair (interquartile range) compared to the median concentration of the Japanese which was 0.26 (0.20 - 0.38) $\mu\text{g/g}$ hair and the median concentration of the Ontario women which was 0.27 (0.20 - 0.38) $\mu\text{g/g}$ hair. The Ti content of hair from all 3 groups was within the reference range (0.00 – 0.40 $\mu\text{g/g}$). The numbers of subjects with no detection of Ti in their hair were 55 of 55 for the WIFN volunteers; 23 of 23 for the Japanese group; and 20 of 20 for the Ontario women group.

The WIFN volunteers also had significantly lower concentrations of U in their hair compared to the Japanese group living in Toronto (Fig. 42; One Way ANOVA, $p < 0.05$). All 3 groups had lower U concentrations in hair than the upper value of the reference range 0.0 - 0.06 $\mu\text{g/g}$ hair. The median concentration of U in the hair collected from the WIFN volunteers is 0.01 (0.01 - 0.03) $\mu\text{g/g}$ compared with 0.04 (0.03 - 0.06) $\mu\text{g/g}$ hair for the Japanese group and 0.02 (0.01 - 0.04) $\mu\text{g/g}$ in the Ontario women group.

The numbers of subjects with no detection of U in their hair were 55 of 55 for the WIFN volunteers; 23 of 23 for the Japanese group; and 20 of 20 for the Ontario women group.

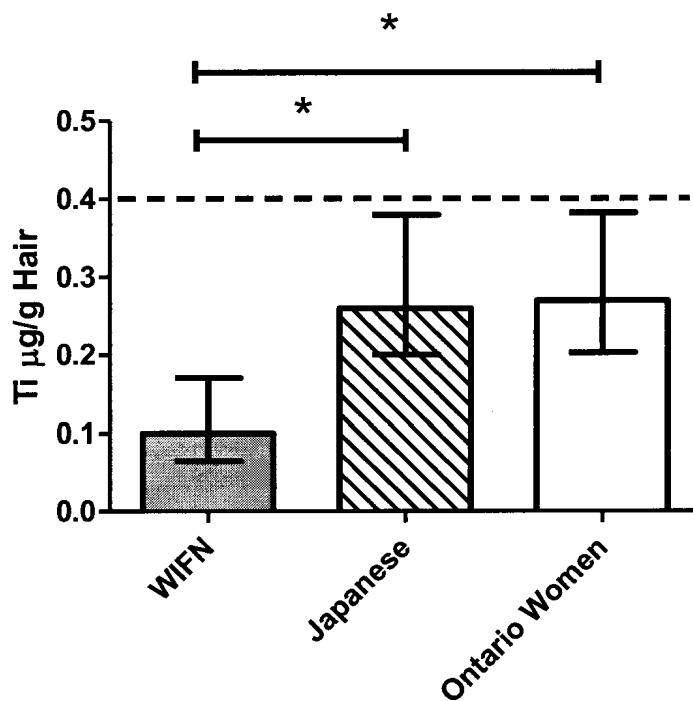


Figure 41: Median concentration and interquartile range of titanium in WIFN hair versus 2 other groups. One Way ANOVA * $p < 0.05$. Reference Range 0.0 – 0.4 µg/g hair (LHSC Trace Elements Lab).

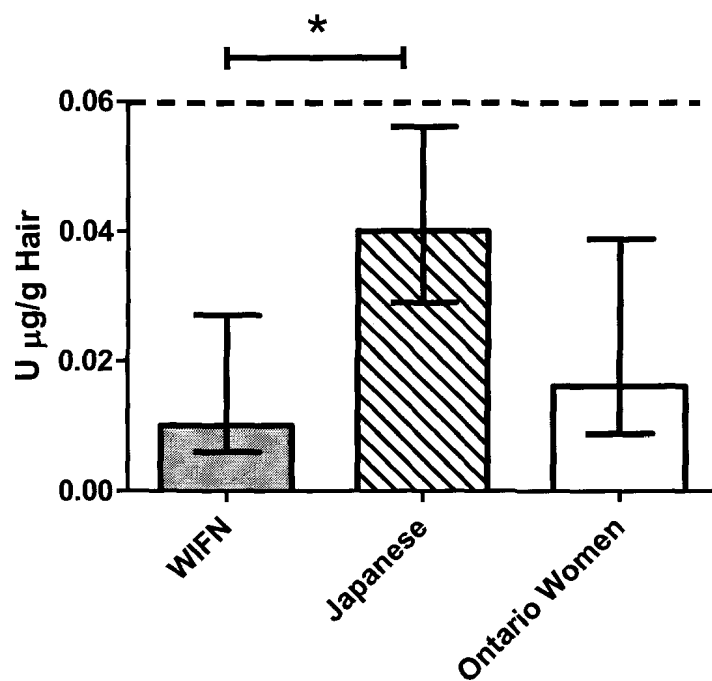


Figure 42: Median and interquartile range concentration of Uranium in WIFN versus 2 other groups. One Way ANOVA * $p < 0.05$. Reference Range 0.0 – 0.06 µg/g hair (LHSC Trace Elements Lab).

The median Cd concentration in hair of the WIFN volunteers was higher than in the Japanese or Ontario women groups. This difference was not significant due to large variations in Cd content of hair (Fig. 43; One Way ANOVA; $p = 0.044$). The median concentration of Cd in the WIFN volunteers was 0.02 (0.01 - 0.04) $\mu\text{g/g}$ hair compared with the median concentration of the Japanese cohort 0.01 (0.01 - 0.03) $\mu\text{g/g}$ hair and the Ontario Women cohort 0.01 (0.01 - 0.02) $\mu\text{g/g}$ hair. All three group concentrations were below the upper value of the reference range 0.15 $\mu\text{g/g}$ hair. The numbers of subjects with no detection of Cd in their hair were 55 of 55 for the WIFN volunteers; 23 of 23 for the Japanese group; and 20 of 20 for the Ontario women group.

The median of Pb hair concentration in the WIFN volunteers was also higher when compared to the Japanese group and the Ontario women group. This difference was also not significant due to large variations in this parameter (Fig. 44; One Way ANOVA; $p = 0.306$). The median concentration of Pb in hair of the WIFN volunteers was 0.3 (0.13 - 0.67) $\mu\text{g/g}$ hair compared to the Japanese group of 0.44 (0.27 - 0.80) $\mu\text{g/g}$ hair and the Ontario women group of 0.28 (0.13 - 0.49) $\mu\text{g/g}$ hair. The median of all 3 groups were below the upper limit of the reference range of 1.5 $\mu\text{g/g}$. The numbers of subjects with no detection of U in their hair were 55 of 55 for the WIFN volunteers; 23 of 23 for the Japanese group; and 20 of 20 for the Ontario women group.

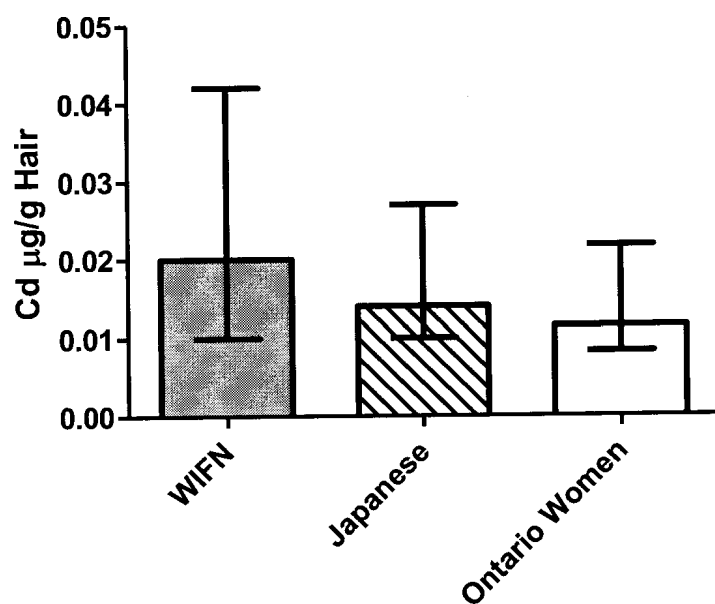


Figure 43: Median and interquartile range concentration of cadmium in WIFN hair versus hair from 2 other groups. One Way ANOVA. Reference range 0.0 - 0.15 µg/g hair (LHSC Trace Elements Lab).

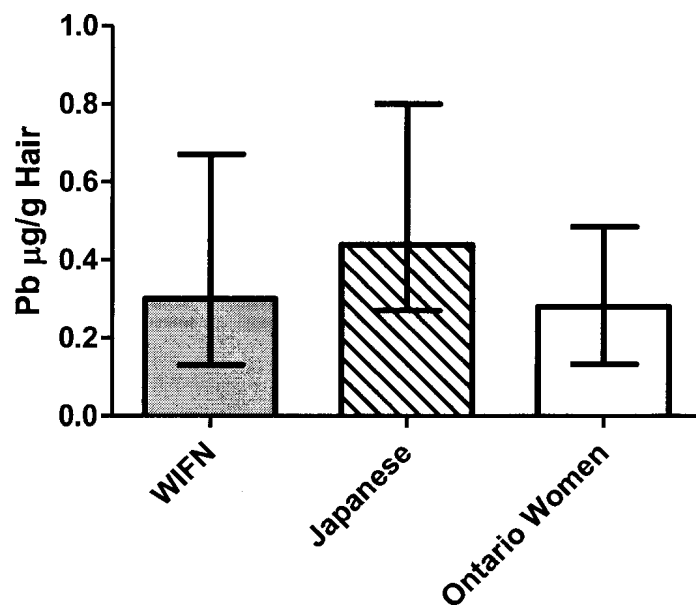


Figure 44: Median and interquartile range of lead in hair of WIFN populations versus 2 other groups. One Way ANOVA. Reference range 0.0 – 1.5 µg/g hair (LHSC Trace Elements Lab).

The median concentration of As in hair of the WIFN volunteers was 0.01 (0.01 - 0.03) $\mu\text{g/g}$ hair. This was significantly lower than the median concentration of the Japanese group: 0.04 (0.02 - 0.06) $\mu\text{g/g}$ hair, but was quite similar to the hair content of As in the hair from the Ontario women group: 0.02 (0.01 - 0.02) $\mu\text{g/g}$ hair (Fig. 45; One Way ANOVA; $p < 0.05$). The median As concentrations for all 3 groups were below the upper limit of the reference range of 0.15 $\mu\text{g/g}$ hair. The numbers of subjects with no detection of As in their hair were 55 of 55 for the WIFN volunteers; 23 of 23 for the Japanese group; and 20 of 20 for the Ontario women group.

The concentrations of most of the heavy metals (Al, As, Bi, Pd, Pt, Ag, Ti, and U) analyzed in hair from WIFN volunteers were significantly lower than those concentrations found in hair from members of a heavy fish-eating Japanese group residing in the Toronto area. Concentrations also tended to be lower for Sb, Ba, Cd and Pb in hair of the WIFN group, but these differences were not significant. On the other hand, WIFN subjects had significantly higher concentrations of Al, Bi, and Tl in their hair than did a group of women living in Ontario. There was also a trend towards higher hair content of As, Ag, Ba, Cd, Pb, and Sb in WIFN volunteers compared with the Ontario women group.

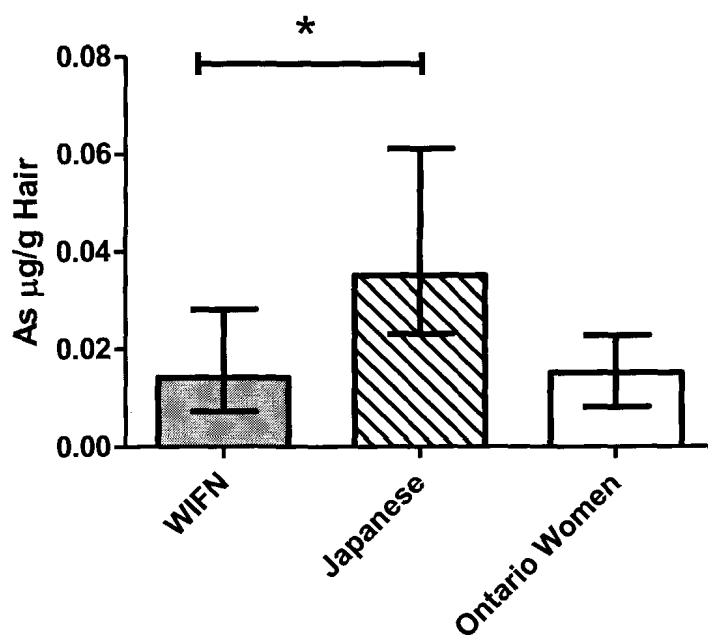


Figure 45: Median and interquartile range concentration of arsenic in WIFN hair versus 2 other groups. One Way ANOVA (* $p < 0.05$). Reference range 0.0 - 0.15 µg/g hair (LHSC Trace Elements Lab).

A logical reason for the differences seen in heavy metal content of hair from these various groups is the relative amount of fish consumption. The Japanese group represent a group of heavy fish consumers, whereas the Ontario women represent a population of light to no fish consumers, with the average WIFN volunteer falling between these two extremes. Also, another potential reason for the difference could be geographical. The Japanese living in and around Toronto represent an urban population while the WIFN and the Ontario women are more rural. Air pollution could play an important role in the Japanese group's exposure to heavy metals that is lower for the other groups. Air pollution from Chemical Valley does not affect the WIFN population and is not a significant source of exposure for them, as the air currents move South to North effectively taking polluted air away from Chemical Valley and Walpole Island.

We expect that Cd content is higher in hair of the WIFN subjects than in the Japanese and the Ontario women because of exposure to cigarette smoke. In this regard, we expect that exposure of WIFN volunteers to second hand smoke is the reason we did not find a significant difference in Cd content between smoking and non-smoking WIFN volunteers.

We did not find any effect of age on heavy metal content of heavy metals in the hair or blood of WIFN volunteers. Of interest, other recent studies have reported a positive relationship between Cd and age. Horiguchi *et al.* (2004) reported that young women (ages 20 - 39) absorbed Cd at a much higher rate than older women who did not seem to absorb it as well, but excreted it more effectively. Our analysis did not show any sort of relationship with age and this could be because the modest sample size skewed the

results as we did not have a good distribution of age. In addition, 56% of our volunteers were smokers. It has been hypothesized that populations who have been exposed to high concentrations of Cd in their diet (like the Japanese), absorb Cd more efficiently than non-exposed populations (Engstrom & Nordberg, 1979). This may also be true for populations exposed to high concentrations of Cd through first-hand and second-hand smoke. If the lungs in populations exposed to higher concentrations of Cd became more efficient at absorbing Cd, then people smoking or living with those who smoked would have higher concentrations of Cd throughout their life, regardless of age. However, further research needs to be performed to confirm this hypothesis.

We did not find a significant relationship between the concentration of metals measured in hair or blood and the prevalence of self-reported diabetes. Several articles reporting positive associations between the risk of developing T2D and exposure to As have appeared recently (Tseng *et al.*, 2000; Navas-Acien *et al.*, 2006; Coronado-Gonzalez *et al.*, 2007; Navas-Acien *et al.*, 2008). For example, most of these positive studies have been performed in countries where people are drinking water contaminated with As in concentrations greater than 100 ppb. However, Navas-Acien *et al.* (2008) evaluated the relationship between organic and inorganic As and T2D in US adults and found that increasing concentrations of total urine As were positively associated with T2D prevalence. Of interest, there was an insignificant association for urine dimethylarsinate (a metabolite of inorganic As) and no association between urine arsenobetaine (organic As found in sea-food) and the prevalence of T2D (Navas-Acien *et al.*, 2008). Their results, that inorganic As is associated with T2D, whereas at least one form of organic As is not, are supported by other epidemiological studies in the literature

on populations exposed to high concentrations of inorganic As in their drinking water in Bangladesh, Mexico, and Taiwan which also have enhanced prevalence of T2D (Rahman *et al.*, 1998; Tseng *et al.*, 2000; Coronado-Gonzalez *et al.*, 2007).

We did not find a significant relationship between the content of As in hair or blood of the WIFN volunteers and the incidence of self-reported diabetes. There are two important contributing factors to our results. First, a very small group of individuals were studied, especially since the WIFN population is not exposed to high As concentrations. Second, the molecular species of As was not evaluated in the WIFN volunteers so that the proportion of inorganic As and organic As in the blood and hair samples are unknown. It is likely that the WIFN are exposed more to organic As, as this is the species found most often in seafood, fish, and shell fish (Francesconi & Edmonds, 1997). If the WIFN population has a significantly higher proportion of organic than inorganic As in their blood and hair, a positive relationship between As content and the incidence of diabetes is not expected (Navas-Acien *et al.*, 2008)

Cd is another heavy metal that has recently been hypothesized to play a role in diabetes mellitus. Cd appears to aggravate diabetic glomerulonephropathy (Buchet *et al.*, 1990; Bernard *et al.*, 1991). However, Horiguchi *et al.* (2004) did not find a relationship between diabetics and cadmium absorption, and even concluded that diabetes mellitus does not affect Cd absorption. A link between Cd and T2D is not unexpected because Cd, like inorganic As, is known to cause oxidative stress, which is a likely contributor to diabetes (Stohs & Bagchi, 1995; Stohs *et al.*, 2001).

We found that As, Sb, Cd, and Tl concentrations of hair from male WIFN volunteers were significantly higher than those in females, whereas the opposite was true for Ba and Pd. In whole blood, only Pb content was significantly higher in males compared to females. We examined the effect smoking status had on concentrations of As, Sb, Cd, Tl, Pb, and Ba in hair and blood in male and female WIFN volunteers. Figure 46 shows the concentration of Pb, As, and Cd in hair of WIFN male smokers, male non-smokers, female smokers, and female non-smokers. We did not find a significant difference between concentrations of any heavy metal in hair of male smokers when compared to male non-smokers, or in hair of female smokers when compared to female non-smokers (Mann-Whitney U Test; Bonferroni Correction).

Likewise, we did not find a significant difference between female smokers compared with female non-smokers for concentrations of heavy metals in blood. We did however, find a significant difference between male smokers' and male non-smokers' blood concentration of Cd (figure not shown; Mann-Whitney U Test; Bonferroni Correction; $p < 0.0005$). One possible explanation for this is that male volunteers could have recently smoked before having their blood drawn.

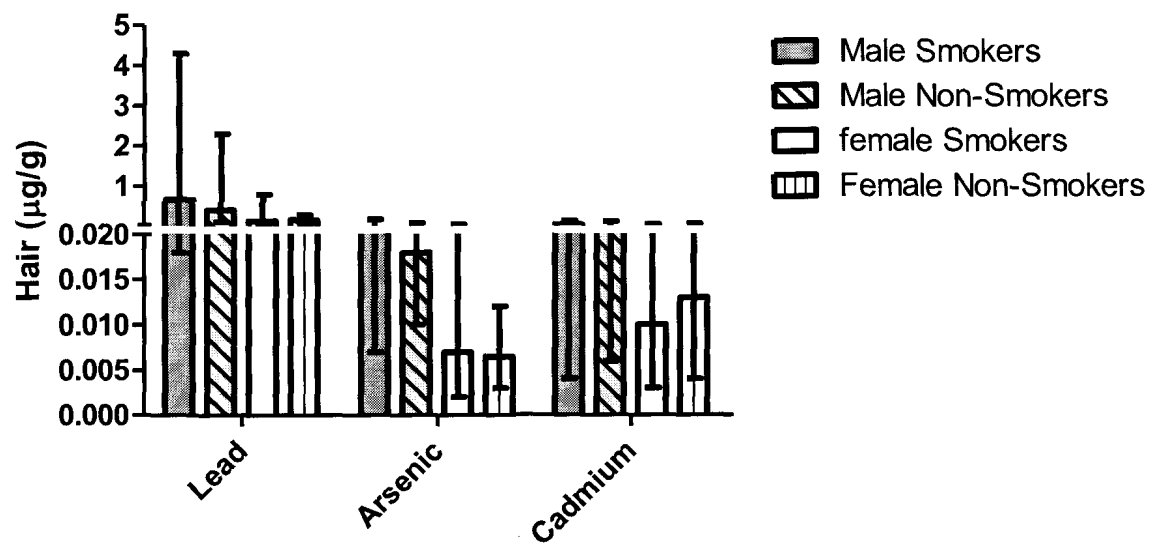


Figure 46: Median concentration and range of lead, arsenic, and cadmium in WIFN male smokers, male non-smokers, female smokers, and female non-smokers (Mann-Whitney U Test; Bonferroni Correction).

As, Cd, Cr, Pb, Hg, Ni, and Se are all heavy metals found in Canadian and imported tobacco (Hammond & O'Connor, 2008). Average concentration of heavy metals per Canadian cigarette are 151.4 ng for As, 205.4 ng for Ni, 257.7 ng for Pb, and 929.7 ng for Cd. Average concentrations of these metals per imported cigarette are 169.6 ng for As, 823.3 ng for Ni, 257.0 for Pb, and 765.8 for Cd (Hammond & O'Connor, 2008).

For Cd, cigarette smoke is a major source of exposure. We did not find any significant difference of blood or hair content of Cd or As in WIFN smokers compared to non-smokers. We did find a higher content of Pb in blood of WIFN smokers than in non-smokers. In the study by Hammond *et al.* (2008), Pb was found in the second highest concentration (16.7 ng/cigarette) of the 3 metals found in cigarette smoke, below Cd concentrations (57.6 ng/cigarette), but higher than Hg (3.2 ng/cigarette) concentrations. Pappas *et al.* (2007) found 2.0 – 6.5 times higher mean concentrations of Cd in smoke of counterfeit cigarettes when compared with authentic cigarette smoke. They also found mean Tl concentrations 1.4 - 4.9 times higher in smoke of counterfeit cigarette smoke when compared with authentic cigarette smoke and mean Pb concentrations 3.0 - 13.8 times higher in counterfeit cigarette smoke when compared with authentic cigarette smoke (Pappas *et al.*, 2007). Although Tl has not historically been associated with cigarette smoke, it has been identified as a contaminant of cigarettes in recent studies as it is becoming a more prevalent environmental contaminant, getting into the water system as a result of mining and industrial processes (Pappas *et al.*, 2007).

We did not ask the WIFN volunteer what brand of cigarettes they smoked, how often they smoked, or the time between their last cigarette and the time sampling. The Pb concentrations could have been associated in the blood of WIFN smokers compared to non-smokers, if the volunteers smoked frequently before the time of sampling, as blood is a measure of acute exposure. Also, the brand of cigarettes the WIFN volunteers smoked, may have given us more information about the concentrations of specific heavy metals in blood and their relationship to smokers versus non-smokers.

Dietary Cd and As are major sources of exposure for humans. As exposure through drinking well water is a major concern for humans in many different areas of the world. However, for Cd, the efficiency of absorption through inhalation is much higher (25 - 50%) than it is for that of ingestion (1 - 10%) (Horiguchi *et al.*, 2004). Although Cd exposure through smoking is a major concern, Cd exposure through diet is a concern for general world population.

We did not find any significant difference between heavy metal concentrations in blood or hair samples of fish consumers when compared with people who do not eat fish. More males reported that they consumed fish than females so we examined the relationship between male fish consumption compared with female fish consumption. We found that male fish consumers had significantly higher concentrations of Pb ($p < 0.0001$), As ($p < 0.0001$) and Cd ($p < 0.005$) in their hair when compared with female fish consumers. We did not find a significant difference between the concentrations of these metals in hair of male fish consumers compared with male non-fish consumers and, therefore, the difference could be due to other factors, such as smoking.

We also did not find a difference in heavy metal concentration in blood of male fish consumers when compared with female fish consumers. There were not enough females who reported they did not eat fish to conduct analysis on female fish consumers compared to female non-fish consumers.

5.3.4 Cortisol

We found a significant difference in the median concentration of cortisol in hair of WIFN volunteers when compared to that of a reference population from London Ontario ($p < 0.0001$). Ours is the first study we are aware of to determine the cortisol concentration of hair from a First Nation community as a biomarker for stress so we are unable at this time to say whether the difference in cortisol concentrations observed is due to a genetic variation or to psycho-social stress. The WIFN do have higher concentrations of cortisol in their hair, indicating higher levels of stress. Some reasons for stress could be due to the incidence of diseases on the island such as diabetes (33% of volunteer's self-reported diabetes), socio-economic status or chemophobia, as discussed in Section 1.3.

A study by Laudenslager *et al.* (2008) analyzed salivary cortisol concentrations of American Indians living in and around a Northern Plains reservation who had a lifetime diagnosis of post traumatic stress disorder (PTSD). These authors determined that the American Indian women had elevated salivary cortisol concentrations, but with a diurnal pattern similar to matched female controls (Laudenslager *et al.*, 2008). From this study, it is difficult to determine an explanation for the increased cortisol concentrations in the Native Indian women, whether it was due to living with PTSD or ethnicity. The male

American Indians did not show any significant differences in salivary cortisol concentrations compared to their matched controls (Laudenslager *et al.*, 2008).

Other reasons for higher cortisol concentrations in the WIFN population may be due to high smoking rates and/or the metabolic syndrome. The metabolic syndrome and increased obesity is thought to affect cortisol concentrations by alliterating the hypothalamic-pituitary-adrenal axis in response to feeding by causing glucocorticoid receptor dysfunctions, or by causing an increment in cortisol turnover (Parra *et al.*, 2006).

Acute smoking is known to increase ACTH and cortisol levels in the body (Seyler *et al.*, 1984; Steptoe & Ussher, 2006). Smoking affects the secretion of cortisol because there are nicotinic binding sites within the paraventricular nucleus of the hypothalamus, part of the pathway for cortisol secretion (Kellar *et al.*, 1999). Nicotine stimulates the release of ACTH, which then continues along the pathway enhancing production and release of cortisol. However, we did not find any significant differences between the median cortisol concentrations of hair from WIFN smokers compared with non-smokers. Yeh *et al.* (1989) also studied whether there were differences in cortisol concentrations of smokers versus non-smokers in urine samples over a 24-h period and did not find any significant differences between smokers and non-smokers in urinary free cortisol or 11-deoxycortisol (Yeh & Barbieri, 1989). These investigators offered two reasons why chronic smoking may not affect the amount of cortisol secreted. First, after acute cortisol peaks with cigarette inhalation, the HPA axis could compensate by allowing a steroid 'nadir' that is lower than the usual steroid baseline; or second, the diurnal adrenal pattern

is changed in smokers, with a change in the nocturnal rise in the cortisol level to compensate for the acute elevations during the day (Yeh & Barbieri, 1989).

We did not find any significant differences in cortisol concentration in smokers versus non-smokers, which could be explained by the theories put forth by Yeh *et al.* (1989). By monitoring hair cortisol in our study, we evaluated cortisol secretion over the last month from sampling, unlike urine cortisol which only shows cortisol secretion over the last 24 h. Even more importantly, hair cortisol secretion is not subjected to diurnal variations that are characteristic of saliva, serum, and urine analysis.

Endogenous and exogenous glucocorticoids are reported to contribute to diabetes and/or impaired glucose tolerance (Conn & Fajans, 1956; West, 1959; Perley & Kipnis, 1966; Rizza *et al.*, 1982). Friedman *et al.* (1996) studied carbohydrate and lipid metabolism in patients with Cushing's syndrome characterized by endogenous hypercortisolism. They found that individuals with Cushing syndrome had an elevated glucose response compared with obese non-Cushingoid individuals (Friedman *et al.*, 1996). There appears to be an adaptive process by which glucocorticoids are initially toxic to the β -cell and cause a relative decrease in glucose-induced insulin secretion (Friedman *et al.*, 1996). Although the WIFN volunteers did not have cortisol concentrations in ranges considered to be pathological, their elevated cortisol concentrations could contribute to an increased risk for diabetes. A novel observation of our study was that WIFN volunteers with self-reported diabetes had significantly higher cortisol concentrations in their hair compared to WIFN volunteers who did not have diabetes. Perhaps this can be explained by the theory of Friedman *et al.* (1996).

We also found a significant gender difference in the cortisol content of hair of WIFN volunteers with men having higher concentrations than women (Mann-Whitney U Test; $p < 0.05$). There are other reports of gender differences in cortisol concentrations throughout the literature. Most of these reports, however, indicate higher glucocorticoid levels in females rather than males after HPA axis stimulation (Kudielka & Kirschbaum, 2005). Laudenslager *et al.* (2009) found that female American Indians had significantly elevated concentrations in salivary cortisol compared to controls, whereas male American Indians did not. However, most psychological stress studies have revealed either that there are no significant gender differences, or that there are higher cortisol responses in young men after exposure to acute real-life psychological stress or controlled laboratory tests (Kudielka & Kirschbaum, 2005). In our situation, however, the mean age of the WIFN volunteers was 49.9 years and acute stress would not have affected the cortisol concentrations that we determined from the hair samples. A study by Kirschbaum *et al.* (1999) showed that men had larger ACTH increases than women, which supported the idea of enhanced hypothalamic drive in men that could account for higher hair cortisol content (Roelfsema *et al.*, 1993).

Because 59% of the WIFN smokers were males, we also compared hair cortisol content in male smokers versus female smokers and found that levels were significantly higher in males than females (Mann-Whitney U Test; $p < 0.05$). Therefore, the gender difference we observed in cortisol concentration in male hair vs. female hair might be confounded by the significant difference in concentration of hair from male smokers vs. female smokers. However, we did not find any significant differences when we compared hair cortisol content in male smoker's versus non-smokers, or in female

smokers versus non-smokers, demonstrating that the major differences observed were in fact due to gender differences in smokers and non-smokers.

When we asked the WIFN volunteers to rate their “perceived stress” in the PSS questionnaire, we did not find a significant difference between what the WIFN volunteers reported and what members of our reference group reported. As the PSS is self-reporting, there is the possibility that self-reporting bias affected the results. The volunteers may not feel that they are experiencing more stress than what they consider to be normal and, therefore, did not indicate that they are feeling stressed. Also, if the high cortisol concentrations are in part due to other factors, such as gender, genetics, diabetes or smoking, then the WIFN volunteers would not perceive high levels of stress in their daily activities, as indicated by their PSS Scores. Given the well documented enhanced psychosocial stress in First Nation communities, we favour the former explanation for the observed differences in hair cortisol content between the WIFN volunteers and the reference group.

Chapter 6: *Conclusions and Summary of Suggestions for Future Research*

6.1 Conclusions

The overall hypothesis is that some members of the WIFN community are exposed to high enough concentrations of POPs and heavy metals from environmental sources, including traditional foods, that their health is adversely affected. To this end, we measured concentrations of POPs in plasma samples from 20 volunteers from the WIFN community; we measured heavy metal concentrations in 56 whole blood samples and 55 hair samples from WIFN volunteers and cortisol concentrations in 40 hair samples from WIFN volunteers. Concentrations were then compared with literature populations or referent populations to assess exposure.

6.1.1 Questionnaire

We had a compliance rate of 91% with the Health Questionnaire. However, in the questionnaires returned, many questions were skipped therefore making it hard to draw any conclusive results. Comments regarding the Health Questionnaire from WIFN volunteers inform us that the questionnaire itself was too long and at times confusing to answer.

Due to the similarity in PSS scores between the WIFN volunteers and the reference group, we question whether or not the PSS Questionnaire is appropriate for use in Ecosystem Health research with special populations, such as a First Nations

population. Also, the lack of correlation between the results obtained by the PSS Questionnaire and hair cortisol concentrations in the WIFN group reinforces this conclusion.

6.1.2 POPs

Our specific objectives were to: prepare a systematic review of the relationship between exposure to POPs and the incidence of diabetes, concentrating on T2D; to determine the baseline concentrations of 91 POPs in plasma lipids of volunteers of the WIFN community to evaluate current exposure of these pollutants and to compare concentrations of these 91 POPs in plasma lipids of the WIFN volunteers to those in other populations reported in the literature, both historical and contemporary. We were able to systematically review the relationship between exposure to POPs and the incidence of diabetes and to conclude that there is a possible association. We also found a gender difference, in that females appeared to have a higher risk of developing T2Ds than males in the studies that included both males and females. We were also able to establish a baseline of 91 POPs in plasma samples of 20 WIFN volunteers which can be used in further ecosystem evaluative studies on the Walpole Island and Chemical Valley area. We found that most WIFN volunteers' POPs concentrations were within concentrations reported in the NHANES study, a representation of the general American public. However, there were a couple of WIFN individuals with high enough concentrations that adverse health effects could be possible. These individuals will be counselled by a physician.

6.1.3 Heavy Metals

Our specific objectives were to: determine the baseline concentration of heavy metals in blood and hair of WIFN volunteers to evaluate current exposure to these pollutants; to determine the distribution ratios of several metals between blood and hair to evaluate the better matrix for analysis of heavy metals, and to compare concentrations of heavy metals in hair of WIFN volunteers with two groups used for reference purposes – Japanese living in Toronto and women living in Ontario. We were able to determine the baseline concentrations of heavy metals in both hair and blood samples of WIFN volunteers. We determined that hair was a good marker for blood for the heavy metal Pb, but not for any other heavy metal measured in both hair and blood (As, Cd, Ni, Sb, Tl).

6.1.4 Cortisol

Our specific objectives were to: determine cortisol content in the hair of WIFN volunteers as an index of psychosocial stress and to compare these data with cortisol content from a referent population living near London Ontario; to compare cortisol concentration of hair of WIFN volunteers with the results of the perceived stress questionnaire (PSS) and to evaluate the effects of gender, smoking and self-reported diabetes incidence on cortisol content. The cortisol concentration of the WIFN volunteers was significantly higher than the cortisol concentration in the referent population. Although we are unsure of the exact reasons for elevated cortisol concentrations in the WIFN volunteers, elevated stress due to disease burden, socio-economic status or chemophobia are all possible contributing factors. We also found a

significant relationship between cortisol concentration and diabetics, gender and smoking.

We have concluded that smoking contributes, but is not solely responsible for the elevated hair cortisol concentrations in male compared with female WIFN volunteers. We did find hair cortisol concentrations in male smokers significantly higher than in hair cortisol concentrations of female smokers. However, further work is required in this area to prove or disprove our hypothesis.

6.2 Summary of Suggestions for Future Research

6.2.1 Questionnaire

A shorter more precise questionnaire designed in tandem with members from the WIFN community would be beneficial to elucidate more concrete results. Also more questions into the health of the individuals who volunteer for the study and separate questions regarding the health of their children will help elucidate more information regarding the overall health of the community.

We also feel that the PSS Questionnaire was not an adequate method for determining perceived stress in the WIFN community. If further cortisol analysis is done with this population, an adapted form of the PSS Questionnaire may be beneficial to better determine stress levels within the community.

6.2.2 POPs

WIFN volunteers had the option of agreeing to a future study, where a portion of the blood taken (20 mL) at the time of sampling was frozen and stored at the Robarts Research Institute, University of Western Ontario. The samples were to be frozen for a period of up to 10 years, at which time, if they had not been used, the samples would be destroyed. The purpose of storing the sample is to perform genomic and proteomic analysis on validated biomarkers for POPs exposure to further evaluate the relationship between exposure to environmental contaminants and health.

A follow-up study should be conducted with the community to continually monitor the community's exposure to POPs. Now that a baseline of POPs has been established in a sub-sample of the population of the community, it will be easy to determine whether exposure to POPs is increasing or decreasing. This is also another way to monitor pollution from Chemical Valley. Finally, by conducting a large epidemiological study, conclusions can be made about exposure to POPs and adverse health effects experienced by members of the community. Unfortunately, our sample size was too small to draw conclusions with any degree of certainty.

6.2.3 Heavy Metals

The Japanese group living in and around Toronto, had much higher concentrations of most heavy metals measured when compared to the women living in Ontario and the WIFN volunteers. Not much information was obtained from the Japanese group regarding smoking status, where they lived, where they got their water from and overall health, so many questions are left unanswered for why their

concentrations are higher. One future study may be with the Japanese group to determine reasons for high concentrations of heavy metals, and if it is affecting their overall health. Once reasons for high concentrations are elucidated and reported back, a future follow-up study can be conducted on hair samples from the population to determine whether concentrations of heavy metals are decreasing.

A similar follow-up study can be conducted with the WIFN community to determine whether concentrations of heavy metals are decreasing. This is one way to monitor exposure of the community as well as monitor pollution from Chemical Valley.

6.2.4 Cortisol

As far as we know from the literature, this is the first study ever to measure cortisol concentrations in a First Nations population. More studies need to be done on First Nations populations to determine whether the difference we found between the WIFN volunteer's cortisol concentration and the referent group's cortisol concentration is strictly due to stress or whether there are other factors playing a role on cortisol production and secretion, such as racial differences, socio-economic differences and/or geographical differences.

We also found a significant relationship between diabetes and cortisol concentration. Further research into this area is required to determine the direction of this relationship. For example, is diabetes affecting cortisol secretion or is cortisol secretion somehow effecting individuals in such a way that they are more prone to getting diabetes, or is there a secondary factor working on the both cortisol secretion and the pathway for

developing diabetes, such as genetic factors, that puts Native American's at higher risk for higher cortisol concentrations and for developing diabetes. A larger epidemiological study could be done in attempt to clear up some of these questions. This is specifically prevalent today, as many studies have found relationships between T2D and exposure to POPs. One of these studies is with Vietnam veterans who were apart of Operation Ranch Hand. If cortisol concentration is somehow affecting the development of T2D, then this could also be playing a part in the Ranch Hand study. The veterans could have higher concentrations of cortisol from PTSD from fighting in the Vietnam War. However, more research needs to be done before any firm conclusions can be made.

After results have been reported back to the community, a follow-up study should be completed to determine changes in lifestyle of the WIFN volunteers. If their fear of chemicals has been reduced due to the results of the study and whether or not they continue to feel stressed. A follow-up PSS questionnaire and hair sampling to analyze for cortisol concentration will help to determine whether or not the community's stress level has decreased.

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Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. J. Bend

Review Number: 13752E

Review Level: Expedited

Review Date: November 7, 2007

Protocol Title: Baseline Biomonitoring Studies and a Survey of Child-Youth Health as Prerequisites to Epidemiological Studies to Assess the Health Risk of the Walpole Island First Nation Community from Exposure to Environmental Contaminants

Department and Institution: Pathology, Seibens Drake Research Institute

Sponsor:

Ethics Approval Date: January 8, 2008

Expiry Date: November 30, 2012

Documents Reviewed and Approved: UWO Protocol, Letters (2) of Information and Consent (Biomonitoring, Health Status)

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

- a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- b) all adverse and unexpected experiences or events that are both serious and unexpected;
- c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. John W. McDonald

Ethics Officer to Contact for Further Information

☐ Janice Sutherland ☐ Jennifer McEwen ☐ Grace Kelly ☒ Denise Grafton

This is an official document. Please retain the original in your files.

cc: ORE File
LHR

UWO HSREB Ethics Approval - Initial
V. 2007-10-12 (ptApprovalNoticeHSREB_initial)

13752E

Page 1 of 1

Appendix 2 – Health Questionnaire

CODE: _____

May 27, 2008

HEALTH SURVEY

INTRODUCTION

We are members of a multi-disciplinary research team that is currently conducting community-based collaborative research with the Walpole Island Health Centre and the Walpole Island Heritage Centre, regarding the possible health hazards of exposure to environmental contaminants in the Walpole Island First Nation Community (WIFN).

No identifying information will be written on the survey. Research members will assign a unique identifier to each survey to enable identification without collecting identifying information.

This survey was adapted from the Aboriginal Children's Survey 2006 by Stats Canada.

PART 1: IDENTIFICATION

In the spaces provided below please answer the following questions about yourself and your children.

A1. What is your date of birth? *day/month/year*

1 ____ Don't know

2 ____ Declined

A2. What is your child/children's date(s) of birth?

1 ____ Don't know

2 ___ Declined

A3. What is the sex of your child/children?

1 ___ Declined

A4. How many times have you and your child/children

moved, that is changed your usual place of
residence?

___ ___ *Times*

1 ___ Never

2 ___ Don't know

3 ___ Declined

PART 2: ADULT AND CHILD QUESTIONNAIRE

B. HEALTH AND WELL-BEING

The following are questions about you and your child/children's health.

B1. In general, would you say your health is 1 ___ Excellent

2 ___ Very good

3 ___ Good

4 ___ Fair

5 ___ Poor

7 ___ Don't know

8 ___ Declined

**B2. In general, would you say your child/
children's health is/are**

- 1 ___ Excellent
2 ___ Very good
3 ___ Good
4 ___ Fair
5 ___ Poor
7 ___ Don't know
8 ___ Declined

B3. How much did your child/children weigh at birth?

Accept respondent's best estimate.

Pounds Ounces

OR

___ *Grams*

- 3 ___ Don't know
4 ___ Declined

**B4. In the past 12 months, have you or your child/children
visited any of the following about physical, mental, emotional
or spiritual health:**

Exclude at time of birth for babies.

a. A family doctor, general practitioner
or pediatrician?

1 ___ Yes

→

2 ___ No

3 ___ Don't know

b. How many times in
the past 12 months?

4 ____ Declined ____ Times

b. A nurse, including community health nurse, public health nurse or nurse practitioner separate from doctor's visits?

1 ____ Yes → b. How many times in the past 12 months?

2 ____ No

3 ____ Don't know

4 ____ Declined ____ Times

c. A medical specialist such as a surgeon, allergist or orthopaedist?

1 ____ Yes → b. How many times in the past 12 months?

2 ____ No

3 ____ Don't know

4 ____ Declined ____ Times

d. A traditional Aboriginal healer?

By "Aboriginal", we are referring to First Nations, Métis or Inuit.

1 ____ Yes → b. How many times in the past 12 months?

2 ____ No

3 ____ Don't know

4 ____ Declined ____ Times

B5. During the past 12 months, did you or your child/

children have to visit the Urgent Care clinic or

Emergency Department?

1 ____ Yes → b. How many times in the past 12 months?

2 ____ No

3 ____ Don't know

4 ____ Declined ____ Times

B6. During the past 12 months, was there

a time when you or your child/children

wanted health care or medication and could 1 ___ Yes → b. How many times in
 not get it? 2 ___ No the past 12 months?
 3 ___ Don't know
 4 ___ Declined ___ ___ Times

B7. In general, are you or your child/children's

physical activity limited by a health condition? 1 ___ Yes → b. How many times in
 2 ___ No the past 12 months?
A health condition may 3 ___ Don't know
include a disability or a long term condition. 4 ___ Declined ___ ___ Times

B8. Do you or your child/children have any of the following long-term conditions that have lasted or are expected to last 6 months or more?

Complete all parts of question when applicable.

a. Lactose intolerance or
trouble digesting milk?

1 ___ Yes →
 2 ___ No
 3 ___ Maybe
 4 ___ Don't know
 5 ___ Declined

b. Did you get a diagnosis
from a doctor, nurse or health
professional?

1 ___ Yes →
 2 ___ No
 3 ___ Don't know
 4 ___ Declined

c. Have you/your child
received
treatment?

1 ___ Yes
 2 ___ No
 3 ___ Don't know
 4 ___ Declined

B9. Do you or your child/children have any food, digestive, respiratory or other allergies? If no skip to B10.

a. Food or digestive allergies?

b. Did you get a diagnosis
from a doctor, nurse or

c. Have you/your child
received

| | health professional? | treatment? |
|------------------|----------------------|------------------|
| 1 ___ Yes → | 1 ___ Yes → | 1 ___ Yes |
| 2 ___ No | 2 ___ No | 2 ___ No |
| 3 ___ Maybe | 3 ___ Don't know | 3 ___ Don't know |
| 4 ___ Don't know | 4 ___ Declined | 4 ___ Declined |
| 5 ___ Declined | | |

a. Respiratory allergies (such as hay fever)?

1 ___ Yes →

2 ___ No

3 ___ Maybe

4 ___ Don't know

5 ___ Declined

b. Did you get a diagnosis from a doctor, nurse or

health professional?

1 ___ Yes →

2 ___ No

3 ___ Don't know

4 ___ Declined

c. Have you/your child received

treatment?

1 ___ Yes

2 ___ No

3 ___ Don't know

4 ___ Declined

a. Any other allergies?

1 ___ Yes →

2 ___ No

3 ___ Maybe

4 ___ Don't know

5 ___ Declined

b. Did you get a diagnosis from a doctor, nurse or

health professional?

1 ___ Yes →

2 ___ No

3 ___ Don't know

4 ___ Declined

c. Have you/ your child received

treatment?

1 ___ Yes

2 ___ No

3 ___ Don't know

4 ___ Declined

B10. Do you or your child/children have Asthma or Chronic Bronchitis or Tuberculosis?

If no skip to B11.

a. Asthma?

- 1 ☐ Yes →
- 2 ☐ No
- 3 ☐ Maybe
- 4 ☐ Don't know
- 5 ☐ Declined

b. Did you get a diagnosis
from a doctor, nurse or
health professional?

- 1 ☐ Yes →
- 2 ☐ No
- 3 ☐ Don't know
- 4 ☐ Declined

c. Have you/your child
received
treatment?

- 1 ☐ Yes
- 2 ☐ No
- 3 ☐ Don't know
- 4 ☐ Declined

a. Chronic Bronchitis?

- 1 ☐ Yes →
- 2 ☐ No
- 3 ☐ Maybe
- 4 ☐ Don't know
- 5 ☐ Declined

b. Did you get a diagnosis
from a doctor, nurse or
health professional?

- 1 ☐ Yes →
- 2 ☐ No
- 3 ☐ Don't know
- 4 ☐ Declined

c. Have you/your child
received
treatment?

- 1 ☐ Yes
- 2 ☐ No
- 3 ☐ Don't know
- 4 ☐ Declined

a. Tuberculosis?

- 1 ☐ Yes →
- 2 ☐ No
- 3 ☐ Maybe
- 4 ☐ Don't know
- 5 ☐ Declined

b. Did you get a diagnosis
from a doctor, nurse or
health professional?

- 1 ☐ Yes →
- 2 ☐ No
- 3 ☐ Don't know
- 4 ☐ Declined

c. Have you/your child
received
treatment?

- 1 ☐ Yes
- 2 ☐ No
- 3 ☐ Don't know
- 4 ☐ Declined

B11. Do you or your child/children have Diabetes, hypoglycemia or low blood sugar?**If no skip to B12.**

a. Diabetes?

1 ___ Yes →

2 ___ No

3 ___ Maybe

4 ___ Don't know

5 ___ Declined

b. Did you get a diagnosis

from a doctor, nurse or

health professional?

1 ___ Yes →

2 ___ No

3 ___ Don't know

4 ___ Declined

c. Have you/your child

received

treatment?

1 ___ Yes

2 ___ No

3 ___ Don't know

4 ___ Declined

a. Hypoglycemia or low blood

pressure?

1 ___ Yes →

2 ___ No

3 ___ Maybe

4 ___ Don't know

5 ___ Declined

b. Did you get a diagnosis

from a doctor, nurse or

health professional?

1 ___ Yes →

2 ___ No

3 ___ Don't know

4 ___ Declined

c. Have you/your child

received

treatment?

1 ___ Yes

2 ___ No

3 ___ Don't know

4 ___ Declined

B12. Do you or your child/children have a heart or kidney condition? If no skip to B13.

a. Heart condition or disease?

b. Did you get a diagnosis

from a doctor, nurse or

health professional?

c. Have you/your child

received

treatment?

1 ___ Yes →

2 ___ No

3 ___ Maybe

4 ___ Don't know

5 ___ Declined

1 ___ Yes →

2 ___ No

3 ___ Don't know

4 ___ Declined

1 ___ Yes

2 ___ No

3 ___ Don't know

4 ___ Declined

a. Kidney condition or disease?

b. Did you get a diagnosis
from a doctor, nurse or
health professional?c. Have you/your child
received
treatment?

1 ___ Yes →

2 ___ No

3 ___ Maybe

4 ___ Don't know

5 ___ Declined

1 ___ Yes →

2 ___ No

3 ___ Don't know

4 ___ Declined

1 ___ Yes

2 ___ No

3 ___ Don't know

4 ___ Declined

B13. Do you or your child/children have epilepsy? If no skip to B14.

a. Epilepsy?

b. Did you get a diagnosis
from a doctor, nurse orc. Have you/your child
received
treatment?

1 ___ Yes →

2 ___ No

3 ___ Maybe

4 ___ Don't know

5 ___ Declined

1 ___ Yes →

2 ___ No

3 ___ Don't know

4 ___ Declined

1 ___ Yes

2 ___ No

3 ___ Don't know

4 ___ Declined

B14. Do you or your child/children have Cerebral Palsy, Down Syndrome or Spina Bifida? If no skip to B15.

a. Cerebral Palsy?

b. Did you get a diagnosis
from a doctor, nurse orc. Have you/your child
received
treatment?

1 ___ Yes →

2 ___ No

3 ___ Maybe

4 ___ Declined

5 ___ Refused

1 ___ Yes →

2 ___ No

3 ___ Don't know

4 ___ Declined

1 ___ Yes

2 ___ No

3 ___ Don't know

4 ___ Declined

B15. Do you or your child/children have Anxiety, Depression or Attention Deficit Hyperactivity Disorder? If no skip to B16.

a. Anxiety or depression?

b. Did you get a diagnosis
from a doctor, nurse,c. Have you/your child
received

health professional or school?

treatment?

1 ___ Yes →

2 ___ No

3 ___ Maybe

4 ___ Don't know

5 ___ Declined

1 ___ Yes →

2 ___ No

3 ___ Don't know

4 ___ Declined

1 ___ Yes

2 ___ No

3 ___ Don't know

4 ___ Declined

a. Attention Deficit Hyperactivity
Disorderb. Did you get a diagnosis
from a doctor, nursec. Have you/your child
received

health professional or school?

treatment?

1 ___ Yes →

2 ___ No

3 ___ Maybe

4 ___ Don't know

5 ___ Declined

1 ___ Yes →

2 ___ No

3 ___ Don't know

4 ___ Declined

1 ___ Yes

2 ___ No

3 ___ Don't know

4 ___ Declined

B16. Do you or your child/children have Autism? If no skip to B17.

a. Autism?

b. Did you get a diagnosis

c. Have you/your child

| | | |
|------------------|---|------------------------|
| | from a doctor, nurse, health professional or school? | received treatment? |
| 1 ___ Yes → | 1 ___ Yes → | 1 ___ Yes |
| 2 ___ No | 2 ___ No | 2 ___ No |
| 3 ___ Maybe | 3 ___ Don't know | 3 ___ Don't know |
| 4 ___ Don't know | 4 ___ Declined | 4 ___ Declined |
| 5 ___ Declined | | |

B17. Do you or your child/children have speech or language difficulties? If no skip to B18.

| | | |
|--|---|--|
| a. Speech or language difficulties? | b. Did you get a diagnosis from a doctor, nurse, health professional or school? | c. Have you/your child received treatment? |
| 1 ___ Yes → | 1 ___ Yes → | 1 ___ Yes |
| 2 ___ No | 2 ___ No | 2 ___ No |
| 3 ___ Maybe | 3 ___ Don't know | 3 ___ Don't know |
| 4 ___ Don't know | 4 ___ Declined | 4 ___ Declined |
| 5 ___ Declined | | |

B18. Do you or your child/children have any other long term condition? If no skip to B19.

| | | |
|---|---|--|
| a. Any other long term condition or disease? | b. Did you get a diagnosis from a doctor, nurse or health professional? | c. Have you/your child received treatment? |
| 1 ___ Yes → | 1 ___ Yes → | 1 ___ Yes |
| 2 ___ No | 2 ___ No | 2 ___ No |
| 3 ___ Maybe | 3 ___ Don't know | 3 ___ Don't know |
| 4 ___ Don't know | 4 ___ Declined | 4 ___ Declined |
| 5 ___ Declined | | |

B19. Do you or your child/children take any of the following medications?

a. Ventolin, inhalers or puffers for asthma?

b. How often?

1 ___ Yes →

01 ___ More than once a day

2 ___ No

02 ___ Once a day

3 ___ Don't know

03 ___ More than once a week

4 ___ Declined

04 ___ Once a week

05 ___ At least once per month

06 ___ At least once per year

07 ___ Less than once a year

08 ___ Don't know

09 ___ Declined

b. Anti-convulsants, anti-epileptic or anti-seizure pills?

b. How often?

1 ___ Yes →

01 ___ More than once a day

2 ___ No

02 ___ Once a day

3 ___ Don't know

03 ___ More than once a week

4 ___ Declined

04 ___ Once a week

05 ___ At least once per month

06 ___ At least once per year

07 ___ Less than once a year

08 ___ Don't know

09 ___ Declined

c. Insulin or other drugs for diabetes?

b. How often?

1 ___ Yes →

01 ___ More than once a day

2 ___ No

3 ___ Don't know

4 ___ Declined

02 ___ Once a day

03 ___ More than once a week

04 ___ Once a week

05 ___ At least once per month

06 ___ At least once per year

07 ___ Less than once a year

08 ___ Don't know

09 ___ Declined

d. Traditional First Nations, Métis or Inuit
medicines?

1 ___ Yes



2 ___ No

3 ___ Don't know

4 ___ Declined

b. How often?

01 ___ More than once a day

02 ___ Once a day

03 ___ More than once a week

04 ___ Once a week

05 ___ At least once per month

06 ___ At least once per year

07 ___ Less than once a year

08 ___ As needed

09 ___ Don't know

e. Allergy medications?

1 ___ Yes



2 ___ No

3 ___ Don't know

4 ___ Declined

b. How often?

01 ___ More than once a day

02 ___ Once a day

03 ___ More than once a week

04 ___ Once a week

05 ___ At least once per month

06 ___ At least once per year

07 ___ Less than once a year

08 ___ Don't know

09 ___ Declined

f. Medications for attention deficit disorder?

b. How often?

1 ___ Yes →

01 ___ More than once a day

2 ___ No

02 ___ Once a day

3 ___ Don't know

03 ___ More than once a week

4 ___ Declined

04 ___ Once a week

05 ___ At least once per month

06 ___ At least once per year

07 ___ Less than once a year

08 ___ Don't know

09 ___ Declined

g. Other medications?

b. How often?

1 ___ Yes →

01 ___ More than once a day

2 ___ No

02 ___ Once a day

3 ___ Don't know

03 ___ More than once a week

4 ___ Declined

04 ___ Once a week

05 ___ At least once per month

06 ___ At least once per year

07 ___ Less than once a year

08 ___ Don't know

09 ___ Declined

B20. Do you or your children smoke or are you or your children exposed to smoke in the home?

1. ☐ Yes
2. ☐ No
3. ☐ Decline

C – FOOD & NUTRITION

Please answer the following questions about the food you and your child/children eat.

C1. a. Was your child/children ever breast-fed?

- | | | |
|---------------------------------------|--|---|
| 1 <input type="checkbox"/> Yes | → | b. For how long? |
| 2 <input type="checkbox"/> No | | <input type="text"/> months or <input type="text"/> years |
| 3 <input type="checkbox"/> Don't know | 1 <input type="checkbox"/> Less than one month | |
| 4 <input type="checkbox"/> Declined | 2 <input type="checkbox"/> Don't know | |
| | 3 <input type="checkbox"/> Declined | |

C2. a. Was your child/children ever fed bottle- formula?

- | | | |
|---------------------------------------|---|---|
| 1 <input type="checkbox"/> Yes | → | b. For how long? |
| 2 <input type="checkbox"/> No | | <input type="text"/> months or <input type="text"/> years |
| 3 <input type="checkbox"/> Don't know | 1 <input type="checkbox"/> Less than one month. | |
| 4 <input type="checkbox"/> Declined | 2 <input type="checkbox"/> Don't know | |
| | 3 <input type="checkbox"/> Declined | |

C3. On average, how often (in terms of a weekly basis) do you and your child/children consume the following foods and beverages?

Number of times Reporting Period

- a. Fish, eggs and meat, such as beef,

pork or poultry?

___ ___ Times → 1 ___ per day
 1 ___ Never 2 ___ per week
 2 ___ Don't know 3 ___ per month
 3 ___ Declined 4 ___ per year

b. Fruits and vegetables?

Number of times Reporting Period

___ ___ Times → 1 ___ per day
 1 ___ Never 2 ___ per week
 2 ___ Don't know 3 ___ per month
 3 ___ Declined 4 ___ per year

c. Tap Water?

Number of times Reporting Period

___ ___ Times → 1 ___ per day
 1 ___ Never 2 ___ per week
 2 ___ Don't know 3 ___ per month
 3 ___ Declined 4 ___ per year

d. Bottled water?

Number of times Reporting Period

___ ___ Times → 1 ___ per day
 1 ___ Never 2 ___ per week
 2 ___ Don't know 3 ___ per month
 3 ___ Declined 4 ___ per year

- e. Traditional or country foods such as berries, game animals, bannock or fry bread?

Number of times Reporting Period

____ Times → 1 ____ per day
 1 ____ Never 2 ____ per week
 2 ____ Don't know 3 ____ per month
 3 ____ Declined 4 ____ per year

- f. Homemade soup, such as corn soup, stew, fish soup or boiled moose or deer soup?

Number of times Reporting Period

____ Times → 1 ____ per day
 1 ____ Never 2 ____ per week
 2 ____ Don't know 3 ____ per month
 3 ____ Declined 4 ____ per year

- g. Large game animals such as deer or moose?

Number of times Reporting Period

____ Times → 1 ____ per day
 1 ____ Never 2 ____ per week
 2 ____ Don't know 3 ____ per month
 3 ____ Declined 4 ____ per year

- h. Small game animals such as rabbit or muskrat?

Number of times Reporting Period

____ Times → 1 ____ per day
 1 ____ Never 2 ____ per week

2 ___ Don't know 3 ___ per month
 3 ___ Declined 4 ___ per year

i. Game birds such as goose,
 duck, partridge or ptarmigan?

Number of times Reporting Period

___ ___ Times \longrightarrow 1 ___ per day
 1 ___ Never 2 ___ per week
 2 ___ Don't know 3 ___ per month
 3 ___ Declined 4 ___ per year

j. Salt or fresh water fish?

Number of times Reporting Period

___ ___ Times \longrightarrow 1 ___ per day
 1 ___ Never 2 ___ per week
 2 ___ Don't know 3 ___ per month
 3 ___ Declined 4 ___ per year

D- Developmental Milestones

For preschool children only

D1. Has your child/children ever looked for someone

or something that was lost

1 ___ Yes

\rightarrow

b. At what age in months?

or out of sight? 2 ___ No

3 ___ Don't know

4 ___ Declined

___ ___ Months

D2. Has your child/children sat up by

Himself/herself?

1 ___ Yes

\rightarrow

b. At what age in months?

2 ___ No

3 ___ Don't know

4 ___ Declined ___ ___ Months

D3. Has your child/children started walking**On his/her own?**

1 ___ Yes → b. At what age in months?
 2 ___ No

3 ___ Don't know

4 ___ Declined ___ ___ Months

D4. Has your child/children ever run?

1 ___ Yes → b. At what age in months?
 2 ___ No

3 ___ Don't know

4 ___ Declined ___ ___ Months

D5. Has your child/children made a line with a**crayon, stick or other object?**

1 ___ Yes → b. At what age in months?
 2 ___ No

3 ___ Don't know

4 ___ Declined ___ ___ Months

D6. Has your child/children ever expressed his/her**needs using a single word?**

b. How often?

c. At what age in

1 ___ Yes → 01 ___ All the time months?

2 ___ No 02 ___ Most of the time

- 3 ___ Don't know 03 ___ Sometimes ___ ___ Months
 4 ___ Declined 04 ___ Rarely
 05 ___ Don't know
 06 ___ Declined

D7. Has your child/children ever shown by his/her actions that he/she understands the names of common objects?

- 1 ___ Yes
 2 ___ No
 3 ___ Don't know
 4 ___ Declined

D8. Has your child/children ever expressed his/her needs using 2 to 3 words?

b. How often?

- 1 ___ Yes → 01 ___ All the time
 2 ___ No 02 ___ Most of the time
 3 ___ Don't know 03 ___ Sometimes
 4 ___ Declined 04 ___ Rarely
 05 ___ Don't know
 06 ___ Declined

D9. Has your child/children ever counted 3 objects correctly?

- 1 ___ Yes
 2 ___ No
 3 ___ Don't know
 4 ___ Declined

D10. Has your child/children ever expressed**his/her needs using full sentences?**

1 ___ Yes →

2 ___ No

3 ___ Don't know

4 ___ Declined

b. How often?

01 ___ All the time

02 ___ Most of the time

03 ___ Sometimes

04 ___ Rarely

05 ___ Don't know

06 ___ Declined

E – SCHOOL – For 4 to 5 year olds only**E1. Is your child/children currently attending school?** 1 ___ Yes*Pre- Kindergarten and kindergarten is to be included.*

2 ___ No

3 ___ Don't know

4 ___ Declined

} If no or
 } don't know
 } go to F

E2. What school grade is your child/are your children in?*Kindergarten is to be included.*

1 ___ Junior Kindergarten/Preschool/K-4

(generally 2 years before grade 1)

2 ___ (Senior) Kindergarten/Primary/K-5

(generally 1 year before grade 1)

3 ___ Grade 1

4 ___ Grade 2

5 ___ Grade 3

- 6 ____ Grade 4
 7 ____ Grade 5
 8 ____ Grade 6
 9 ____ Grade 7
 10 ____ Grade 8
 11 ____ High school (Grades 9 –12)
 12 ____ Don't know
 13 ____ Declined

F – CHILD CARE

F 1. Does your child/children's main child care

1 ____ Yes

arrangement promote First Nations,

2 ____ No

traditional and cultural values and customs?

3 ____ Don't know

4 ____ Declined

G – LEARNING AND ACTIVITIES

The following are some questions about activities your child/children may do.

G1. How often does your child/children . . .

a. Play outside during the warm weather, for example spring and summer months?

1 ____ more than once a day.

2 ____ once a day.

3 ____ more than once a week

4 ____ once a week

5 ____ at least once per month.

6 ____ at least once per year.

7 ___ less than once per year.

8 ___ Don't know

9 ___ Declined

b. Play outside during the cold weather, for example, fall and winter months?

1 ___ more than once a day.

2 ___ once a day.

3 ___ more than once a week

4 ___ once a week

5 ___ at least once per month.

6 ___ at least once per year.

7 ___ less than once per year.

8 ___ Don't know

9 ___ Declined

c. Hear stories?

1 ___ more than once a day.

2 ___ once a day.

3 ___ more than once a week

4 ___ once a week

5 ___ at least once per month.

6 ___ at least once per year.

7 ___ less than once per year.

8 ___ Don't know

9 ___ Declined

d. Sing songs?

1 ___ more than once a day.

2 ___ once a day.

3 ___ more than once a week

4 ___ once a week

5 ___ at least once per month.

6 ___ at least once per year.

7 ___ less than once per year.

8 ___ Don't know

9 ___ Declined

e. Read or look at books?

1 ___ more than once a day.

2 ___ once a day.

3 ___ more than once a week

4 ___ once a week

5 ___ at least once per month.

6 ___ at least once per year.

7 ___ less than once per year.

8 ___ Don't know

9 ___ Declined

f. Participate in or attend traditional First Nations, Métis or Inuit activities such as

singing, drum dancing, fiddling, gatherings, ceremonies or church services?

1 ___ more than once a day.

2 ___ once a day.

3 ___ more than once a week

4 ___ once a week

5 ___ at least once per month.

6 ___ at least once per year.

7 ___ less than once per year.

8 ☐ Don't know

9 ☐ Declined

g. Participate in seasonal activities such as gathering wild plants for example, berries, sweet grass, roots or participate in seasonal gatherings (e.g. maple sugar, cedar ceremonies, gathering medicinal plants?)

1 ☐ more than once a day.

2 ☐ once a day.

3 ☐ more than once a week

4 ☐ once a week

5 ☐ at least once per month.

6 ☐ at least once per year.

7 ☐ less than once per year.

8 ☐ Don't know

9 ☐ Declined

IF CHILD/CHILDREN IS/ARE UNDER 1 YEAR OLD GO TO G2

h. Take part in hunting, fishing, trapping or camping?

1 ☐ more than once a day.

2 ☐ once a day.

3 ☐ more than once a week

4 ☐ once a week

5 ☐ at least once per month.

6 ☐ at least once per year.

7 ☐ less than once per year.

8 ☐ Don't know

9 ☐ Declined

i. Engage in active play such as running, jumping or climbing?

- 1 ___ more than once a day.
- 2 ___ once a day.
- 3 ___ more than once a week
- 4 ___ once a week
- 5 ___ at least once per month.
- 6 ___ at least once per year.
- 7 ___ less than once per year.
- 8 ___ Don't know
- 9 ___ Declined

j. Do arts and crafts?

- 1 ___ more than once a day.
- 2 ___ once a day.
- 3 ___ more than once a week
- 4 ___ once a week
- 5 ___ at least once per month.
- 6 ___ at least once per year.
- 7 ___ less than once per year.
- 8 ___ Don't know
- 9 ___ Declined

k. Role play for example play house or superhero?

- 1 ___ more than once a day.
- 2 ___ once a day.
- 3 ___ more than once a week

- 4 ___ once a week
- 5 ___ at least once per month.
- 6 ___ at least once per year.
- 7 ___ less than once per year.
- 8 ___ Don't know
- 9 ___ Declined

l. Count?

- 1 ___ more than once a day.
- 2 ___ once a day.
- 3 ___ more than once a week
- 4 ___ once a week
- 5 ___ at least once per month.
- 6 ___ at least once per year.
- 7 ___ less than once per year.
- 8 ___ Don't know
- 9 ___ Declined

m. Tell stories?

- 1 ___ more than once a day.
- 2 ___ once a day.
- 3 ___ more than once a week
- 4 ___ once a week
- 5 ___ at least once per month.
- 6 ___ at least once per year.
- 7 ___ less than once per year.
- 8 ___ Don't know
- 9 ___ Declined

n. Does your child/children swim in Lake St. Clair/St. Clair River and/or play on the beach?

- 1 ___ more than once a day.
- 2 ___ once a day.
- 3 ___ more than once a week
- 4 ___ once a week
- 5 ___ at least once per month.
- 6 ___ at least once per year.
- 7 ___ less than once per year.
- 8 ___ Don't know
- 9 ___ Declined

G2. How often does your child/children and the following people talk or play together, focusing attention on each other for five minutes or more?

His/her mother

- a. Birth Mother
 - 1 ___ more than once a day.
 - 2 ___ once a day.
 - 3 ___ more than once a week
 - 4 ___ once a week
 - 5 ___ less than once a week
 - 6 ___ never
 - 7 ___ not applicable.
 - 8 ___ Don't know
 - 9 ___ Declined

- b. Step Mother
 - 1 ___ more than once a day
- (including common-law
 - 2 ___ once a day.
- step parent)
 - 3 ___ more than once a week
 - 4 ___ once a week

5 ___ less than once a week

6 ___ never.

7 ___ not applicable

8 ___ Don't know

9 ___ Declined

c. Adoptive Mother

1 ___ more than once a day.

2 ___ once a day.

3 ___ more than once a week

4 ___ once a week

5 ___ less than once a week

6 ___ never

7 ___ not applicable.

8 ___ Don't know

9 ___ Declined

d. Guardian or Foster Mother

1 ___ more than once a day.

2 ___ once a day.

3 ___ more than once a week

4 ___ once a week

5 ___ less than once a week

6 ___ never

7 ___ not applicable

8 ___ Don't know

9 ___ Declined

His/Her Father

e. Birth Father

1 ___ more than once a day.

- 2 ___ once a day.
- 3 ___ more than once a week
- 4 ___ once a week
- 5 ___ less than once a week
- 6 ___ never
- 7 ___ not applicable
- 8 ___ Don't know
- 9 ___ Declined

f. Step father
(including common-law
step parent)

- 1 ___ more than once a day.
- 2 ___ once a day.
- 3 ___ more than once a week
- 4 ___ once a week
- 5 ___ less than once a week
- 6 ___ never
- 7 ___ not applicable.
- 8 ___ Don't know
- 9 ___ Declined

g. Adoptive Father

- 1 ___ more than once a day.
- 2 ___ once a day.
- 3 ___ more than once a week
- 4 ___ once a week
- 5 ___ less than once a week
- 6 ___ never
- 7 ___ not applicable
- 8 ___ Don't know
- 9 ___ Declined

h. Guardian or Foster Father

- 1 ___ more than once a day.
- 2 ___ once a day.
- 3 ___ more than once a week
- 4 ___ once a week
- 5 ___ less than once a week
- 6 ___ never.
- 7 ___ not applicable
- 8 ___ Don't know
- 9 ___ Declined

i. his/her brothers and sisters

- 1 ___ more than once a day.
- 2 ___ once a day.
- 3 ___ more than once a week
- 4 ___ once a week
- 5 ___ less than once a week
- 6 ___ never.
- 7 ___ not applicable
- 8 ___ Don't know
- 9 ___ Declined

j. his/her grandparents

- 1 ___ more than once a day.
- 2 ___ once a day.
- 3 ___ more than once a week
- 4 ___ once a week
- 5 ___ less than once a week .
- 6 ___ never
- 7 ___ not applicable.

8 ___ Don't know

9 ___ Declined

k. his/her aunts and uncles 1 ___ more than once a day.

2 ___ once a day.

3 ___ more than once a week

4 ___ once a week

5 ___ less than once a week

6 ___ never

7 ___ not applicable

8 ___ Don't know

9 ___ Declined

l. his/her cousins

1 ___ more than once a day.

2 ___ once a day.

3 ___ more than once a week

4 ___ once a week

5 ___ at least once per month.

6 ___ never

7 ___ not applicable

8 ___ Don't know

9 ___ Declined

m. elders

1 ___ more than once a day.

2 ___ once a day.

3 ___ more than once a week

4 ___ once a week

5 ___ less than once a week.

6 ___ never

7 ___ not applicable

8 ___ Don't know

9 ___ Declined

n. his/her friends

1 ___ more than once a day.

2 ___ once a day.

3 ___ more than once a week

4 ___ once a week

5 ___ less than once a week

6 ___ never.

7 ___ not applicable

8 ___ Don't know

9 ___ Declined

o. other - specify

H- COMMUNITY

H1. How long have you and your child/children been living in the community?

H2. How many people currently live in your home?

H3. What is your current water source?

1 ___ piped water

2 ___ well water

3 ___ water taken directly from river

4 ___ bottled water

5 ___ other

H4. What water source did you and your family use when you were growing up?

1 ___ piped water

2 ___ well water

3 ___ water taken directly from river

4 ___ bottled water

5 ___ other

PART 3: Stress Questionnaire

I. Perceived Stress Scale

The questions in this scale ask you about your thoughts and feelings **during the last month.**

I1. In the last month, how often have you been upset because of something that happened unexpectedly?

- 0 ___ Never
 - 1 ___ Almost never
 - 2 ___ Sometimes
 - 3 ___ Fairly Often
 - 4 ___ Very Often
-

12. In the last month, how often have you felt that you were unable to control the important things in your life?

- 0 ___ Never
 - 1 ___ Almost never
 - 2 ___ Sometimes
 - 3 ___ Fairly Often
 - 4 ___ Very Often
-

13. In the last month, how often have you felt nervous or, "stressed?"

- 0 ___ Never
 - 1 ___ Almost never
 - 2 ___ Sometimes
 - 3 ___ Fairly Often
 - 4 ___ Very Often
-

14. In the last month, how often have you felt confident about your ability to handle your personal problems?

- 0 ___ Never
 - 1 ___ Almost never
 - 2 ___ Sometimes
 - 3 ___ Fairly Often
 - 4 ___ Very Often
-

15. In the last month, how often have you felt that things were going your way?

- 0 ___ Never
 - 1 ___ Almost never
 - 2 ___ Sometimes
 - 3 ___ Fairly Often
 - 4 ___ Very Often
-

16. In the last month, how often have you found that you could not cope with all the things that you had to do?

- 0 ___ Never
 - 1 ___ Almost never
 - 2 ___ Sometimes
 - 3 ___ Fairly Often
 - 4 ___ Very Often
-

17. In the last month, how often have you been able to control irritations in your life?

- 0 ☐ Never
 - 1 ☐ Almost never
 - 2 ☐ Sometimes
 - 3 ☐ Fairly Often
 - 4 ☐ Very Often
-

I18. In the last month, how often have you felt that you were on top of things?

- 0 ☐ Never
 - 1 ☐ Almost never
 - 2 ☐ Sometimes
 - 3 ☐ Fairly Often
 - 4 ☐ Very Often
-

I19. In the last month, how often have you been angered because of things that were outside of your control?

- 0 ☐ Never
 - 1 ☐ Almost never
 - 2 ☐ Sometimes
 - 3 ☐ Fairly Often
 - 4 ☐ Very Often
-

I10. In the last month, how often have you felt that difficulties were piling up so high that you could not over come them?

- 0 ☐ Never
 - 1 ☐ Almost never
 - 2 ☐ Sometimes
 - 3 ☐ Fairly Often
 - 4 ☐ Very Often
-

End of questionnaire.

Thank you for your participation.

Appendix 3: Letter of Information – Questionnaire to

Assess Health Status of Children and Fish Consumption

Research Project Title: Baseline Monitoring to Prepare for Epidemiological Studies to Assess the Health Risk of the Walpole Island First Nation Community from Exposure to Environmental Contaminants

Start Date: December 15, 2007

End Date: July 31, 2008

Principal Investigator: Jack Bend, Ph.D.

LETTER OF INFORMATION – QUESTIONNAIRE TO ASSESS HEALTH STATUS OF CHILDREN AND FISH CONSUMPTION

Introduction

We are members of a multi-disciplinary research team that is currently conducting community-based collaborative research with the Walpole Island Health Centre and the Walpole Island Heritage Centre, regarding the possible health hazards of exposure to environmental contaminants in the Walpole Island First Nation Community (WIFN). We are inviting you to participate in this study.

Purpose of the letter

This letter is to inform you about our community-based research project, funded through the Assembly of First Nations and Health Canada's Environmental Contaminants Program. We will be analyzing mercury and persistent organic pollutant (POPs) levels in blood or hair to help determine current exposures to these contaminants. Other parts of the study are designed to determine the health status of children and youths between 6 months and 18 years of age in the Walpole Island community from information obtained by questionnaire and in health records at the Walpole Island Health Centre. We will also

be determining what WIFN community members who are 18 years of age and older think about environmental contaminants and their health by personal interviews.

This specific part of the study involves a questionnaire you will be asked to complete that includes questions about the status of the health of your children and also about the amount of fish and game your family eats on a regular basis.

Purpose of the Questionnaire

The aims of this questionnaire are to understand the health status of your children between 6 months and 18 years of age, and to learn more about the amount of fish and game that you and members of your family eat. We are asking these questions to learn more about the amount of disease in the children of the WIFN community. The questions about how much fish and game you eat are being asked because the most common source of environmental contaminants such as mercury and persistent organic pollutants in the diet is fish, and to a lesser extent, game.

Voluntary Participation:

Participation in this health status questionnaire is voluntary and will have no effect on the care or services you receive at Walpole Island Health Centre. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time.

You will be asked to complete a questionnaire some time before the end of our current study, on July 31, 2008. There will normally be someone available to help you if you have questions, either in person or by telephone. (You can call either the Walpole Island Health Centre at 519-627-0765 or the Heritage Centre at 519-627-1475 for assistance). This questionnaire will take approximately 30 minutes of your time to complete.

Confidentiality

The information collected will be used for research purposes only, and neither your name nor information, which could identify you, will be used in any publication or presentation of the study results. All information collected for the study will be kept confidential. The year of birth and gender will be collected for each child as part of the health status questionnaire for follow up, however, this information will only be accessible to members of the research team. Names of volunteers will not be used in any publication or

presentation of the research and results will be grouped collectively and anonymously before it is presented to members of the WIFN community or elsewhere.

Risks and Benefits

There are no direct benefits to participation in the study. A more personal risk may be involved in completing the questionnaire.

The risk of the questionnaire is that it is an intrusion into the WIFN community. Although we are asking for volunteers, parents may feel pressured to participate in the study, for the health of their children, their land and community. Also, there may be no definitive conclusions to present to the community after this study is completed, by July 31, 2008, so that WIFN citizens are no better informed about health risks arising from exposure to environmental contaminants. This, in turn, might prompt many not to consent to any further studies.

In case of concerns by members of the community regarding the content of the questionnaire, counselling will be provided to those individuals concerned, through the Health Centre by Rosemary Williams, or if there are specific questions about health risks of environmental contaminants, by one of the research team's clinical toxicologists, Dr. Michael Rieder or Dr. Gideon Koren.

Questions

If you have any questions about the conduct of this study or your rights as a research participant you may contact the Manager, Office of Research Ethics, The University of Western Ontario at 519-661-3036 or ethics@uwo.ca. This letter is yours to keep for future reference.

Thank you, Meegwetch,

Jack Bend, The University of Western Ontario
and Members, Ecosystem Health Research Team

Members, Ecosystem Health Research Team

1. Walpole Island First Nation

Dr. Dean Jacobs, Director, WIFN Heritage Centre; *Naomi C. Williams*, Environmental Technologist, Heritage Centre; *Rosemary Williams*, Nurse Manager, WIFN Health Centre

2. University of Western Ontario

Jack Bend, Professor, Pathology; *Jorge Burneo*, Assistant Professor, Neurology; *Bradley Corbett*, Adjunct Research Professor, Sociology; *Regna Darnell*, Distinguished UWO Professor, Anthropology; *Carol Herbert*, Professor, Family Medicine; *Julie Hill*, BHSc (Hons), MSc Student; *Gideon Koren*, Ivey Chair in Molecular Toxicology; *Michael Rieder*, Professor, Paediatrics; *Katie Schoeman* BSc (Hons), MSc Student; *Kathy Speechley*, Associate Professor, Epidemiology & Biostatistics; *Christianne Stephens*, MA, PhD student, Medical Anthropology, McMaster; *Charles Trick*, Ivey Chair in Ecosystem Health.

Appendix 4: Letter of Information – Biomonitoring

Study

Baseline Monitoring to Prepare for Epidemiological Studies to Assess the Health Risk of the Walpole Island First Nation Community from Exposure to Environmental Contaminants

LETTER OF INFORMATION - BIOMONITORING

Introduction

We are members of a multi-disciplinary research team that is currently conducting community-based collaborative research with the Walpole Island Health Centre and the Walpole Island Heritage Centre, regarding the possible health hazards of exposure to environmental contaminants in the Walpole Island First Nation Community (WIFN). We are inviting you to participate in this study.

Purpose of the letter

This letter is to inform you about our community-based research project, funded through the Assembly of First Nations and Health Canada's Environmental Contaminants Program. We will be analyzing mercury and persistent organic pollutant (POPs) levels in blood or hair to help determine current exposures to these contaminants. Other components of the study are designed to determine the health status of children and youths in the Walpole Island community between 6 months and 18 years of age from information obtained by questionnaire, by interviews and in health records at the Walpole Island

Health Centre. The primary health indicators of interest include: health status in infancy and children, low birthweight and premature births, congenital anomalies, cerebral palsy in males and females and ratio of male to female children born per year. These endpoints will help to inform whether or not exposures to environmental contaminants are having any adverse health effects on the Walpole Island First Nation Community. You will be asked to provide informed consent before you provide a sample of blood or hair for analysis, before you are given a questionnaire to complete, or before members of the research team are given access to any of your health records at the Health Centre.

Purpose of the study

The aims of this study are:

1. To determine concentrations of mercury and persistent organic pollutants (POPs) in blood and hair samples of volunteers from the WIFN Community who provide their informed consent.
2. To continue our study of the social and cultural problems related to mercury and POPs exposure in the WIFN community. This will involve examining the perceptions of health risks caused by mercury and POPs by personal interview. We will be asking if you wish to participate in this component of the study when you donate blood and hair, and if so you will be contacted for an interview by a member of the research team before June 2008, when the study will be completed and the analysis of data will begin.

Voluntary Participation:

Participation in this study is voluntary and will have no effect on the care or services you receive at Walpole Island Health Centre. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time.

You will be asked to donate 2 samples of blood (1 x 5 ml) and a hair sample (500 mg) for analysis of mercury, and one blood sample (1 x 10 ml) for the analysis of 91 persistent organic chemicals, including pesticides such as DDT, and the PCBs.

Ms. Cindy Langford, a member of our research group and a Registered Nurse will be collecting the blood and hair samples at the Walpole Island Health Centre. We will schedule appointments for the collection of blood at the Health Centre to minimize the inconvenience to volunteer blood and hair donors.

We estimate that it will take approximately 15 minutes to donate blood and hair.

We also request that you answer a questionnaire while you are at the Health Centre to donate blood and hair. This includes questions about your fish consumption and the status of your children's health and will require approximately 30 minutes to complete. Since eating fish is the major source of mercury and POPs consumed in the diet, individuals donating blood and hair will be asked how much fish they routinely consume per week, as well as the most fish in terms of meals per week that they eat at any time of the year. Another part of our project is also concerned with children's health, and that is why we are asking for the information about the health of your children.

OPTIONAL PARTICIPATION:

An optional part of this study, if you consent, is to store 2 additional samples of blood (2 x 10 ml) for future evaluation of protein biomarkers (1 x 10 ml) and genetic markers in white blood cells (1 x 10 ml) related to the impacts of environmental pollutants on human health when they become available and less expensive, in the future. These blood samples will be stored up to 10 years. You have the option of consenting to this part of the study by signing

your consent on the second page of the Biomonitoring consent form in the box titled Optional Study.

Confidentiality

The information collected will be used for research purposes only, and neither your name nor information, which could identify you, will be used in any publication or presentation of the study results. Data obtained from charts and questionnaires will be assigned unique identifiers by Head Nurse Rosemary Williams of the Walpole Island Health Centre, and will be stored in electronic format at the Walpole Island Health Centre. Only members of the research team will have access to the coded data. All information collected for the study will be kept confidential. Blood and hair samples will be coded immediately upon collection by Head Nurse Williams and only coded samples will be sent for analysis and for storage. However, a master list will be kept by Ms. Williams to allow identification and removal of your coded charts or samples should you wish to withdraw from the study.

For individuals not willing to have their blood samples stored for subsequent research related specifically to relationships between exposure to environmental contaminants and health status, any remaining blood samples will be incinerated after analysis is complete. For individuals willing to have samples of blood stored for additional future studies, these samples will be stored in a locked ultralow freezer (-80°C) in the Department of Pathology, Schulich School of Medicine & Dentistry, University of Western Ontario for up to 10 years. The data arising from the study will be retained indefinitely.

Coded data from both the charts and the biological samples will be stored in a password-protected database at the Walpole Island Health Centre and in the Department of Pathology in the Schulich School of Medicine and Dentistry. The data will be stored at UWO only until it has been analysed and the research has been completed. The data at the Walpole Island Health Centre will be maintained indefinitely.

Risks and Benefits

There are no direct benefits to participation in the study. The only physical risks are those associated with having blood taken. Giving blood can be painful and may result in the risk of bruising and a small risk of infection.

The risks of the questionnaires and interviews are that these are intrusions into the WIFN community. Although we are asking for volunteers, families and individuals may feel pressured to participate in the study, for the health of their children, their land and community. Also, there may be no definitive conclusions to present to the community after this biomonitoring and survey study is completed, by July of 2008, so that WIFN citizens are no better informed about health risks arising from exposure to environmental contaminants. This, in turn, might prompt many not to consent to any further studies.

In case of concerns by members of the WIFN community regarding the content of the interviews or test results, counselling will be provided to those individuals concerned, through the Health Centre by Rosemary Williams or by the team's clinical toxicologists, Drs. Michael Rieder and Gideon Koren.

Questions

If you have any questions about the conduct of this study or your rights as a research participant you may contact the Manager, Office of Research Ethics, The University of Western Ontario at 519-661-3036 or ethics@uwo.ca. This letter is yours to keep for future reference.

[Signature]

Members, Ecosystem Health Research Team

1. Walpole Island First Nation

Dr. Dean Jacobs, Director, WIFN Heritage Centre; *Naomi C. Williams*, Environmental Technologist, Heritage Centre; and *Rosemary Williams*, Nurse Manager, WIFN Health Centre

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Jack Bend, Professor, Pathology; *Jorge Burneo*, Assistant Professor, Neurology;

Bradley Corbett, Adjunct Research Professor, Sociology; *Regna Darnell*, Distinguished UWO Professor, Anthropology; *Carol Herbert*, Professor, Family Medicine; *Julie Hill*, BHSc (Hons), MSc Student, Ecosystem Health Program; *Gideon Koren*, Ivey Chair in Molecular Toxicology; *Michael Rieder*, Professor, Paediatrics; *Katie Schoeman* BSc (Hons), MSc Student, Ecosystem Health; *Kathy Speechley*, Associate Professor, Epidemiology & Biostatistics; *Christianne Stephens*, MA, PhD student, Medical Anthropology, McMaster; *Charles Trick*, Ivey Chair in Ecosystem Health.