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DEFINING A HAIR MERCURY LOAEL FOR NEUROTOXIC RISK TO THE UNBORN CHILD THROUGH MATERNAL FISH CONSUMPTION

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DEFINING A HAIR MERCURY LOAEL FOR NEUROTOXIC RISK TO THE UNBORN CHILD THROUGH MATERNAL FISH CONSUMPTION

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Abstract

Methylmercury, whose primary source of exposure is via maternal fish consumption, can adversely affect human fetal neurodevelopment. The objectives of this thesis were threefold: 1) To systematically review the evidence of neurodevelopmental risks to the fetus from maternal fish consumption, to define a Lowest Observable Adverse Effect Level (LOAEL); 2) To measure mercury in hair of several groups including women of reproductive age, to define the proportion reaching this LOAEL; and 3) To investigate their perception of risk. We defined our LOAEL at 0.3μg/g maternal hair mercury. The Japanese population were the heaviest fish consumers, reflected by their high hair mercury content (1.7 μg/g) compared to Motherisk callers (0.41 μg/g), WIFN volunteers (0.23 μg/g) and Canadian women (0.15 μg/g). The negative perceptions of Motherisk callers were justified because 64% were above our LOAEL. Analysis of hair mercury content prior to pregnancy could be employed to protect the fetus.

Keywords: *methylmercury, fish consumption, pregnancy, prenatal exposure, neurotoxicity, chemophobia, risk perception, human, fetus, neurodevelopment, hair analysis*

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List of Abbreviations

Abbreviation	Meaning
ANOVA	Analysis of Variance
AOC	Area of Concern
ATP	Adenosine Triphosphate
ATDSR	Agency for Toxic Substances and Disease Registry
BD	Becton Dickinson
BSID	Bayley Scales of Infant Development
CAT	Cognitive Adaptive Test
CLAMS	Clinical Linguistic Auditory Milestone Scale
CNS	Central Nervous System
CV-AAS	Cold Vapour Atomic Absorption Spectrometry
CV-AFS	Cold Vapour Atomic Florescence Spectrometry
DDST-R	Denver Developmental Test-Revised
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GSH	Gluthathione
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IRTs	Interresponse Times
IQ	Intelligent Quotient

LCPUFAs	Long Chain Polyunsaturated Fatty Acids
LOAEL	Lowest Observable Adverse Effect Level
LHSC	London Health Science Centre
MRL	Minimal Risk Level
NBNA	Neonatal Behavioural Neurologic Assessment
NHANES	National Health and Nutrition Examination Survey
PCBs	Polychlorinated Biphenyls
POUCH	Pregnancy Outcomes and Community Health
Ppb	parts per billion
Ppm	parts per million
PPVT	Peabody Picture Vocabulary Test
PTWI	Provisional Tolerable Weekly Intake
RBCs	Red Blood Cells
RFNECP	Regional First Nations Environmental Contaminants Program
ROS	Reactive Oxygen Species
SCDS	Seychelles Child Development Study
TM	Therapeutic Monitoring
UWO	University of Western Ontario
VMI	Visual Motor Integration
VRM	Visual Recognition Memory
WIFN	Walpole Island First Nation

WRAVMA	Wide Range Assessment of Visual Motor Abilities
WHO	World Health Organization

Chapter 1: Introduction

Mercury is a widespread and persistent environmental pollutant. It occurs naturally and exists in three forms: elemental, inorganic and organic. These forms all have different toxicities and implications for human health. Methylmercury, a species of organic mercury, is the most toxic form to humans. The extensive use of mercury in many products and its emission from multiple industrial processes have resulted in well-documented occupational exposures, large scale poisonings and worldwide chronic exposures to low-level, environmental concentrations. This chapter is a literature review concerning the ecosystem health effects of methylmercury which serves as a foundation for the original work that follows.

1.1 The Biology of Methylmercury

1.1.1 Physical and Chemical Properties

Mercury, a heavy metal, is found in the environment in its elemental form and in various inorganic and organic complexes. Mercury has an atomic weight of 200.6, an atomic number of 80, a melting point of – 38.9°C, a boiling point of 356.6°C and a density of 13.6 (Chang 1997). At room temperature mercury exists as a shiny, silver-white, odourless liquid. When heated it becomes a colorless, odourless gas. It occurs in its natural state as mercuric sulfide. When mercury combines with carbon, organic mercury compounds are formed. Although there are other types of organic mercury, the emphasis in this report is on the most relevant in the environment, methylmercury.

Methylmercury is an organometallic cation with the chemical formula $[CH_3Hg]^+$, composed of a methyl group bonded to a mercury atom. It can be formed in both chemical processes and by micro-organisms in the environment. The mercuric ion in its

second oxidation state is responsible for nearly all organic chemical compounds of mercury and plays a large role in the toxicology of mercury (Clarkson & Magos 2006). The mercuric ion exhibits high affinity for thiol groups, particularly the sulfhydryl groups of the amino acid cysteine (Clarkson 1972). Methylmercury also complexes with the cysteinyl thiol of reduced glutathione (Ballatori & Clarkson 1985). The formation of complexes with these molecules plays a major role in the transport and disposition of mercury in the body, which will be further addressed.

1.1.2 Environmental Transport, Distribution and Transformation

Once mercury has been liberated and released into the biosphere as mercury vapour, it can be highly mobile, cycling between the earth's surface and the atmosphere. Mercury vapor is a very stable gas with approximate residence time of about 1 year in the atmosphere. Therefore, once released to the air, mercury can travel long distances and impact distant sites. There is a well recognized global cycle for mercury, whereby inter-conversion between the different forms of mercury occurs (figure 1). Mercury vapour that is emitted to the atmosphere from both natural and anthropogenic sources is converted to water-soluble forms, presumably inorganic mercury, and deposited by rain onto soil and water (Clarkson 2002). Some fraction of inorganic mercury may be reduced back to mercury vapour and re-emitted into the atmosphere. Fresh water lakes and oceans receive mercury from both atmospheric deposition and land run-off. The primary sinks for mercury in the biosphere are thought to be the earth's soils, water bodies and sediments.

Once either elemental or inorganic mercury is deposited into water, the mercury settles to the sediment and reacts with sulphate to form an insoluble mercuric sulfide precipitate (Gochfeld 2003). However, more importantly part of the inorganic mercury is converted to methylmercury compounds by microbial action (methanogenic bacteria) that live in aquatic ecosystems, accepted to be the first step in the aquatic bioaccumulation process (Clarkson 1997).

Once in its methylated form, mercury begins to ascend the aquatic food chain, disusing from its sites of synthesis to multicellular organisms in the form of zooplankton, which are then consumed by progressively bigger and bigger fish, attaining its highest concentrations in large, long-lived, predatory species such as snapper, pickerel, bass, pike, tuna, swordfish, tilefish, king mackerel and shark. Fish occupying higher trophic levels may contain mercury concentrations a million-fold greater than the surrounding water (Gochfeld 2003). When consumed by humans, these mercury-containing fish can result in an increased risk of adverse health effects especially in highly exposed or highly sensitive populations.

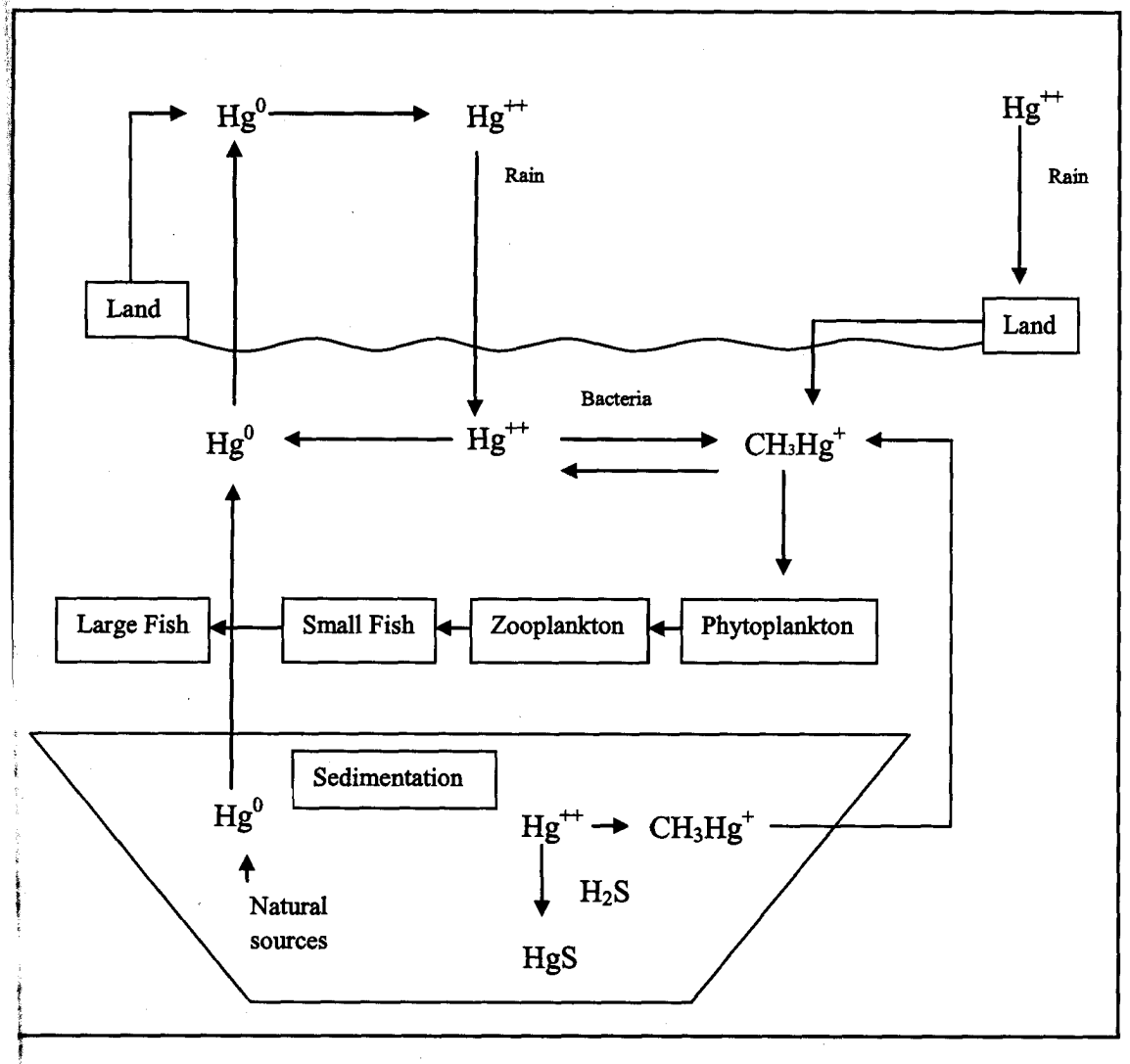

Fig. 1 Global cycle of mercury. Most of atmospheric mercury is in the form of monatomic vapour (Hg^0). Oxygenation takes place to form Hg^{++}. Mercury is brought to the earth surface primarily in rain water. Inorganic mercury is transformed to methylmercury in bodies of water resulting in bioaccumulation in the food chain.

1.1.3 Environmental and Dietary Sources

In the environment mercury emerges from both natural and anthropogenic sources. Deposition of atmospheric mercury, leaching from rocks, and anthropogenic sources all add to the mercury burden in bodies of water (WHO 1990). The most significant releases of mercury pollution are emissions to air, but mercury is also released from various sources directly to water and land. The major source of atmospheric mercury is the natural degassing of the earth's crust, however volcanic activity and weathering of rock containing mercury ore, are also important natural sources (Fitzgerald & Clarkson 1991).

Anthropogenic activities, both intentional and unintentional, contribute to the majority of the mercury (70%) found in the environment (Trasange *et al.* 2006). With the expansion of industry and technological advancement since the beginning of the industrial age, there has been an enormous increase in the emission of mercury into the environment. Human activity increases the amount of mercury released primarily through coal-combustion, mining of heavy metals, incineration and chlor-alkali plants. Mercury is used in a variety of industrial processes, manufacturing applications and in medical supplies such as thermometers, barometers, and sphygmomanometers (ATSDR 2007). It constitutes 50% of dental amalgams, and ethylmercury was once used as a vaccine preservative (Davidson *et al.* 2004). Potential sources of population exposure to mercury include inhalation of mercury vapours, ingestion of drinking water, exposure through dental fillings and medical treatments and most importantly consumption of contaminated foodstuffs.

Dietary intake of fish and marine mammals is the primary source of mercury exposure by humans. For more than three decades, studies have shown a positive correlation between mercury concentrations in humans and fish consumption (Bjornberg *et al.* 2003) and methylmercury exposure is a major problem in heavy fish-eating populations (EPA 1997). In today's modern society with the general focus on good health, dietary habits have changed to advocate the benefits of eating fish, which suggests the possibility of methylmercury toxicity. Methylmercury is present in most if not all aquatic species including fish, shellfish and marine mammals such as pilot whales. Virtually all mercury in fish (75-95%) is in the form of methylmercury (Gochfeld 2003). The chemical form of methylmercury in fish tissues has recently been identified as attached to the thiol group of the cysteine residues in fish protein (Harris *et al.* 2003).

The concentrations of mercury vary widely across fish and shellfish species, with the mean values differing by as much as 100-fold (Keating *et al.* 1997). Mercury concentrations in most freshwater and marine fish are low (<0.5ppm: μg/g) and therefore do not pose as much of a risk to human health. What is of concern however, are the higher concentrations found in predatory fish species such as pike (250-300 μg/kg) and swordfish (1000 μg/kg) (Choi 1989). There are several factors that influence the concentration of methylmercury in these fish. The overall concentration depends not only on the pollution of surrounding waters, but also on the size, weight and age of each particular fish. As a result, critical elements in estimating methylmercury exposure and risk from fish consumption must include the species of fish consumed, methylmercury concentrations in the fish, the amount of fish consumed daily and the frequency of fish

consumption. These are all factors that play a role in influencing the health consequences that may arise from eating fish.

1.1.4 Human Kinetics, Metabolism and Excretion

In contrast to the other forms of mercury, methylmercury is readily absorbed by the gastrointestinal tract and is therefore the form of greatest concern to humans (Gochfeld 2003). Experimental studies on adult humans determined that approximately 95% of methylmercury ingested in fish is absorbed into the bloodstream (WHO 1990). 5% of the absorbed methylmercury is found in the blood compartment, preferentially accumulating in red blood cells (95%) (RBCs), where it has an elimination half-life of approximately 50 days (Kershaw *et al.* 1980). Once absorbed, methylmercury has a long whole body half-life of approximately 60-70 days in humans (Knobeloch *et al.* 2005).

The high mobility of methylmercury in the body is due to the formation of a complex with the amino acid cysteine (Clarkson & Magos 2006). The methylmercuric-cysteinyl complex is recognized by amino acid transport proteins as the essential amino acid methionine, and therefore gains its entry into cells on large neutral amino acids carriers. Other protein ligands for methylmercury include albumin, hemoglobin, keratin and tubulin (Cernichiari *et al.* 2007).

Due to its lipophillic nature and the fact that methylmercury forms water-soluble complexes with proteins containing thiol groups, peptides such as glutathione (GSH) and amino acids, it transports rapidly throughout the body within 4 days, including across the blood-brain barrier and placenta (Clarkson & Magos 2006). Experimental data on rats have shown that methylmercury gains entry into the endothelial cells of the blood-brain

barrier as a complex with cysteine (Kerper *et al.* 1992). The methylmercury-L-cysteine complex is transported by the L-system amino acid carrier (Kerper *et al.* 1992). Methylmercury concentrations are found in the brain within 5-6 days, where it is then slowly demethylated to inorganic mercury. Brain concentrations are about 5-fold and in scalp hair 250-fold the corresponding concentration in the blood (Clarkson & Magos 2006). Animal data indicate that concentrations in the fetal brain may be higher than in the maternal brain (Inouye *et al.* 1986), as well as higher in fetal RBCs compared to those from adults (Kuhnert *et al.* 1981).

Methylmercury is transported from liver cells into bile as a complex with reduced glutathione using glutathione carriers (Ballatori & Clarkson 1985; Ballatori *et al.* 1995). Because of its strong binding to cysteine-containing proteins, methylmercury is not readily eliminated, accounting for its long elimination half life.

Excretion of approximately 1% of the total body burden of mercury occurs daily. Fecal excretion accounts for most of the elimination (90%), with urinary excretion accounting for only a small contribution of total excretion (10%) (Clarkson & Magos 2006). The process of fecal excretion commences with the secretion of methylmercury from liver cells to bile, where GSH is hydrolyzed, resulting in the release of methylmercury as a complex with cysteine (Dutczak *et al.* 1991). Some methylmercury is redistributed to the liver, undergoing enterohepatic recycling, however some is secreted into the gastrointestinal tract where it comes in contact with microflora that are capable of breaking the carbon-mercury bond to release inorganic mercury (Rowland *et al.* 1978). Other investigators have reported that methylmercury is converted to inorganic mercury in phagocytic cells (Suda *et al.* 1993) and by liver microsomes (Suda & Hirayama 1992).

Inorganic mercury is poorly absorbed and thus remains the predominant form in the feces.

1.1.5 Neurotoxicity in Humans

Methylmercury is a well-established neurotoxin in humans that can have serious effects on the central nervous system (CNS), particularly during fetal development (Grandjean *et al.* 1994; Myers *et al.* 2000; Clarkson *et al.* 2003; Rice 2000). With sufficient exposure to high concentrations of any mercury-based toxin, damage to the CNS will occur, making it a major site of toxicity. Methylmercury holds a special position in that large populations are environmentally exposed to it and its toxicity is better characterized than for other organomercurials. On the basis of the body of evidence from human and animal studies, it can be concluded that neurodevelopmental deficits are the most sensitive and well-documented effects.

Neurotoxicity is defined as any adverse change in the development, structure, or function of the central or peripheral nervous system following exposure to a chemical agent (Slikker & Bowyer 2005). The exact mechanism by which methylmercury causes its neurotoxic effects is largely unknown, although a number of molecular targets have been proposed. Methylmercury and other organic mercurials are hydrophobic, allowing them to readily penetrate through cells which normally act as a barrier to toxins. Once in the brain, methylmercury is slowly metabolized into inorganic mercury. It is unclear whether methylmercury toxicity is caused by the parent compound itself, or due to inorganic mercury, its metabolite.

The main target tissue for methylmercury is the brain where the proliferation, division and migration of neuronal cells are inhibited and cytoarchitecture is disrupted (Castoldi *et al.* 2003; Johansson *et al.* 2007). Animal studies have shown damage to the cerebellar cortex and dorsal root ganglion, which are highly sensitive to the effects caused by mercury (Chang & Hartmann 1972; Jacobs *et al.* 1977). Other areas include the cerebral cortex, corpus striatum, thalamus, hypothalamus, organ of Corti, and peripheral nerves.

There is ample clinical evidence showing the increased vulnerability of the developing brain to the toxic effects of methylmercury (Chang 1980). The susceptibility of the fetus could be due to a number of factors including the high vulnerability of developmental processes to destruction by mercury (Choi 1989; Choi *et al.* 1978), and the presence of an incomplete blood brain barrier (Adinolfi 1985). In addition, the possible lack of a methylmercury excretory mechanism and the high levels of RBCs in the fetus could also potentiate prenatal toxicity (Rodier 1995; Sakamoto *et al.* 2002).

Analysis of blood taken from mother-infant pairs exposed to methylmercury, has shown elevated ratios in umbilical cord blood compared to maternal blood-mercury levels with an average ratio of 1.7 (Butler *et al.* 2006; Morrissette *et al.* 2004; Stern & Smith 2003). In the context of developmental neurotoxicity, once transplacental passage of methylmercury occurs, the toxic element has a greater affinity for the fetal CNS than in adults (Amin-Zaki *et al.* 1974), and therefore the fetus is much more sensitive to the neurotoxic effects of methylmercury than the adult (EPA 1997).

It is assumed that specific types of developmental effects might have different windows of vulnerability (Choi 1989). During the embryonic developmental stage, when there is no brain per se, there might be little sensitivity to methylmercury developmental neurotoxicity. During fetal stages of brain structure formation, methylmercury exposure is most likely to cause broad abnormalities in brain architecture. When the brain structure is basically established during late fetal development, methylmercury exposure is likely to cause more function-specific effects on brain architecture.

A spectrum of adverse health effects occurs following methylmercury exposure, with the severity depending largely on the dose. Toxic exposure to methylmercury results primarily in neurological damage in both adults and children, characterized by ataxia, sensory disturbances and changes in mental state (Takeuchi 1968). The best evidence for methylmercury toxicity in the fetus comes from well publicized cases of environmental mercury poisoning that occurred in Minamata Bay, Japan (Harada 1978;Yorifuji *et al.* 2008; Harada 1995; Ekino *et al.* 2007; Harada *et al.* 1999) and Iraq (Amin-Zaki *et al.* 1974; Greenwood 1985; Bakir *et al.* 1973; Rustam & Hamdi 1974). Data from these incidents affirm the fact that consumption of high concentrations of methylmercury in dietary sources by pregnant women can cause severe neurodevelopment sequelae in their offspring. The poisoning in Japan due to maternal consumption of contaminated fish caught from Minamata Bay first raised awareness of the toxic potential of methylmercury. Individuals presented with neurologic symptoms including paresthesias, ataxia, sensory disturbances, tremors, loss of physical coordination, hearing impairment, blindness, and death (Harada 1995). It is difficult to reconstruct the doses of

methylmercury that resulted in these effects, as measurements of hair and blood were not taken until methylmercury was found to be the source of the poisoning many years later.

In Iraq, following methylmercury exposure via consumption of homemade bread made from seed grain treated with methylmercury-containing fungicide, the most common effect in adults was paresthesia (Myers *et al.* 2000). Of the 6530 cases reported throughout the country, 459 deaths were attributed to methylmercury poisoning. Most severely affected individuals presented with ataxia, blurred vision, slurred speech, hearing difficulties, blindness, deafness and death (Marsh *et al.* 1987). Children presented with a range of effects from being slower to reach developmental milestones, to more severe disabilities including mental retardation, seizures disorders, in-coordination and cerebral palsy (ATSDR 1999). Such effects occured in children when their mothers remained healthy or only suffered minor symptoms (WHO 1990; Davis *et al.* 1994). Exposures that occurred in Japan and Iraq, in both adulthood and childhood, exemplified the latency period before the onset of mercury-effects; in Iraq from weeks to months and in Minamata, more than a year (Davis *et al.* 1994).

Toxic effects in the fetal brain differ when compared to the adult brain in terms of mechanism of action and outcome (ATSDR 1999). In the adult, methylmercury poisoning is characterized by damage to discrete anatomical areas of the brain, such as a loss of neurons from the visual cortex and disappearance of granule cells from the granule layer of the cerebellum (Choi *et al.* 1978). Autopsy cases from Iraq showed that neuronal cell division, migration and organization were disrupted (Choi *et al.* 1978). Furthermore, axonal degeneration has been associated with secondary myelin disruption of the peripheral nerve (Hunter & Russel 1954). Unlike focal damage in adults, the

developing brain shows diffuse and widespread damage (Castoldi *et al.* 2001). In the developing fetus, prenatal exposure can interfere with the growth and migration of neurons and has the potential to cause irreversible damage (EPA 1997).

At much lower doses typical of chronic maternal fish consumption, some effects might not be apparent in the first few years of age but may present later on, such as discrete decreases in Intelligent Quotient (IQ) or effects on the brain that may only be determined by the use of very sensitive neurological or neurobehavioural testing. These endpoints include attention, fine-motor function, language, visual-spatial ability and verbal memory (Grandjean *et al.* 1997). Perhaps if low methylmercury concentrations do cause adverse effects they may be apparent only on higher order cognitive functions that develop with maturity (Davidson *et al.* 2006).

Collectively, the above information emphasizes the high sensitivity of the CNS, especially during fetal development, to methylmercury and indicates that exposure to sufficient doses may cause adverse neurological effects to exposed individuals.

1.1.6 Biologic Plausibility: Proposed Mechanisms

Studies of neurotoxicity have traditionally involved either behavioural or pathological observations in animals treated for days or weeks with methylmercury (Atchison & Hare 1994). Mitochondrial changes, induction of lipid peroxidation, microtubule disruption, and disrupted protein synthesis have all been proposed as potential mechanisms ((National Research Council (U.S.) 2000). Exposure of rats to methylmercury *in vivo* causes an accumulation of the mercurial in mitochondria followed by biochemical and ultrastructural changes and disrupting several functions of

mitochondria, such as electron transport or respiration and oxidative phosphorylation, but this is not the primary mechanism for toxicity (Denny & Atchison 1994; Yoshino *et al.* 1966a).

Methylmercury causes membrane lipid peroxidation in nerve cells (Sarafian & Verity 1991). Since the brain is exceptionally sensitive to oxidative free radical injury, free radical-induced lipid peroxidation may well be involved in methylmercury-induced cell damage (Magos & Webb 1980). In cultured mouse neuroblastoma cells, methylmercury decreases GSH-S-transferase activity and GSH peroxidase activity, both required for the metabolism of reactive oxygen species (ROS) (Kromidas *et al.* 1990).

Methylmercury may also affect calcium homeostasis. The calcium ion plays a critical role in CNS cell death (do Nascimento *et al.* 2008) by activating catabolic enzymes and disturbing cytoskeletal organization. Methylmercury increases calcium levels in rat brain (Komulainen & Bondy 1987) and calcium channel blockers prevent the appearance of neurological disorders in rats (Sakamoto *et al.* 1996), implicating the importance of calcium homeostasis in methylmercury-induced neurotoxicity.

Methylmercury also directly affects the mechanism of neurotransmission, including the release and uptake of neurotransmitters, enzymatic neurotransmitter inactivation and post-synaptic events (Atchison 2005). Experimental data from several studies support the involvement of a glutamate-mediated excitotoxic mechanism in methylmercury neurotoxicity (Castoldi *et al.* 2001). Furthermore, methylmercury causes the release of other neurotransmitters such as dopamine, gamma-aminobutyric acid

(GABA), acetylcholine, and serotonin from rat brain synaptosomes (Komulainen & Tuomisto 1982; Minnema *et al.* 1989).

Another proposed mechanism for damage is disruption of microtubules in the neuronal cytoskeleton by methylmercury (Miura & Imura 1987). Microtubules are made from tubulin monomers and mercury binds to the thiols in the tubulin and blocks the depolymerization and repolymerization of microtubules and their assembly, potentially disrupting cellular processes (Sager *et al.* 1983; Vogel *et al.* 1989). In primary rat brain cells, methylmercury disrupts cell-cycle progression (Ponce *et al.* 1994). Proper microtubule function is critical for development of the CNS including cell proliferation, migration of post-mitotic neurons, formation of the cerebrum and cerebellum and axodendritic transport (Castoldi *et al.* 2001).

One of the proposed mechanisms of mercury toxicity is inhibition of protein synthesis (Yoshino *et al.* 1966b; Verity *et al.* 1977). However, no direct link between inhibition of protein synthesis and neuropathologic changes has been found. To date, there has been no definitive data that points to any one mechanism as the proximate cause for methylmercury-related neurotoxicity.

1.1.7 Biological Indicators of Exposure

Levels of total mercury in whole blood or scalp hair are the matrices of choice for the absorbed dose of methylmercury in adults (Cernichiari *et al.* 1995). Both are well-established and widely used matrices for clinical measurement of mercury exposure of an individual. The blood to hair ratio is 1:250 (WHO 1990). Blood monitoring is generally the method of choice for assessing the recent exposure to, or current body burden of

methylmercury. Hair monitoring can be used for retrospective cumulative exposure analysis (Clarkson & Magos 2006).

Cord blood, maternal blood and maternal hair have been used to predict prenatal exposure. There is an ongoing debate over which biomarker better reflects the concentration of methylmercury in the fetal brain. There are no direct data available on mercury concentrations in the fetal brain, therefore the biomarker concentrations must act as a surrogate for the unknown dose of methylmercury in the fetal brain.

Cord blood and maternal blood have been used with some frequency in accessing exposure to methylmercury in the developing fetus. Proponents of cord blood note that methylmercury in cord blood is in closer contact with the fetal brain and thus is the most relevant surrogate for the dose present in the fetal brain (Cernichiari *et al.* 2007). When using whole blood, one must assume that the mother is at steady state with respect to her body burden of methylmercury, to ensure that the ratio of methylmercury in the blood and brain is constant. The mean half-life of total mercury in human blood is approximately 50 days (Stern 1997), therefore blood mercury reflects relatively short-term exposure. Measures of methylmercury in whole blood are affected by the hematocrit (Cernichiari *et al.* 2007); 95% of methylmercury in human whole blood is found within red blood cells. Of the 5% found in plasma, most is bound to mercaptolalbumin and only 1% is bound to cysteine (Ancora *et al.* 2002). Therefore, only a small fraction of total mercury in whole blood accounts for the transportable species. The present detection limits for total mercury in blood is in the range of 0.1-0.3 ppb (National Research Council (U.S.) 2000).

Compared with blood, sampling of hair is noninvasive and analysis is easier to carry out due to the comparatively high mercury content (Airey 1983). Mercury in hair consists of 80% methylmercury and the remainder is inorganic mercury (Cernichiari *et al.* 1995). Once incorporated into the hair follicle, the concentration of methylmercury remains stable, which allows for a recapitulation of past mercury exposure (Cernichiari *et al.* 2007). Total mercury concentration in hair reflects methylmercury exposure and not inorganic exposure, as inorganic mercury is poorly accumulated in hair and the 20% found in hair is due to the conversion of methylmercury to inorganic mercury in the hair follicle (Lindberg *et al.* 2004). Studies on uptake into animal fur have suggested that methylmercury enters via the hair follicle and is accumulated only in the growing phase (Shi *et al.* 1990). Studies have shown that radioactive methylmercury is taken up by keratinocytes where it is deposited in the sulphur keratin proteins of hair (Zareba *et al.* 2008).

Hair grows at a growth rate of approximately 1.1 cm/month, however this varies within and among individuals (Boischio & Cernichiari 1998; Cernichiari *et al.* 1995; Grandjean *et al.* 1992), so hair has the advantage of providing a time record of mercury exposure throughout pregnancy. Hair analysis may show different exposure levels over time, potentially correlating with changes in fish intake, whereas cord blood gives the level only at delivery. A potential disadvantage is that hair may be subjected to external contamination and cosmetic treatments that may reduce the mercury concentrations (Dakeishi *et al.* 2005). However, results of a comprehensive study showed that this is not the case and no mercury is lost from hair due to cosmetic treatments (McDowell *et al.*

2004). Using the standard cold vapour techniques, the detection limit for total mercury is generally 0.01 to 0.04 μg/g hair (National Research Council (U.S.) 2000).

1.2 Mercury in Women of Reproductive Age

1.2.1 Global Reference Doses and Fish Consumption Guidelines

Current reference doses, which are maximum acceptable oral doses of a toxic substance, fluctuate across different regulatory bodies. The differences in guidelines among the various agencies are due to the use of different risk-assessment methods, data sets, and uncertainty factors (National Research Council (U.S.) 2000). As an example, the World Health Organization (WHO) guidelines allow for consumption of fish containing 0.47μg mercury/kg body weight/day (WHO 1990). In contrast, the U.S. Environmental Protection Agency (EPA) set a reference dose for methylmercury at 0.1 μg mercury/kg body weight/day, sufficient to protect against the most sensitive endpoint, adverse fetal neurobehavioral development (EPA 1997). This reference dose set by the EPA was only one fifth of the WHO dose, which, if followed, would drastically reduce seafood intake. However, the Food and Drug Administration (FDA) have recommended regulatory levels that are significantly less stringent than the EPA's reference dose, an acceptable daily intake for mercury of 0.4 μg mercury/kg body weight/day (FDA 2001). The Agency for Toxic Substances and Disease Registry (ATSDR) established a Minimum Risk Level (MRL) for methylmercury of 0.3 μg mercury/kg body weight/day which is 3 times the EPA's reference dose (ATSDR 1999). In 2004, the WHO in collaboration with the Food and Agriculture Organization (FAO) revised its recommendation for safe intake levels of mercury in food to 1.6 μg/kg body weight/ week, known as the Provisional Tolerable Weekly Intake (PTWI) (JECFA 2004). The PTWI was devised to protect the developing

embryo and thus is most applicable to pregnant women. In fact, the reference dose for mercury adopted by WHO is more than two times greater than EPA's reference dose.

In 2004 the U.S. EPA and FDA issued a warning for women of reproductive age, pregnant women, nursing mothers and young children to limit their fish intake to 12 ounces per week (2 servings) or 0.1 μg mercury/kg body weight/day due to potential mercury contamination in the fish (FDA 2004). Health Canada suggests that women chose fish such as salmon, char, herring, mackerel, sardines and trout as these fish have high omega-3 unsaturated fats and low mercury content (Health Canada 2008). To avoid high mercury exposure, Health Canada recommends limiting intake of tuna (fresh), shark, swordfish, marlin, orange roughy and escolar to 2 servings a month (Health Canada 2008). Regulatory bodies also advise women to limit their intake of canned (white) albacore tuna to 6 ounces per week (1 serving) as it is higher in mercury than various types of canned light tuna.

By following the guidelines put in place by these governmental bodies, fish consumers can be confident in reducing the harmful effects of mercury to themselves and their newborns, while at the same time maintaining a healthy diet including fish. However, varying reference doses and risk messages that are available to the public create confusion and uncertainty and have been construed by many as suggestions to completely avoid consumption of all fish.

1.2.2 Mercury Exposure in US Women: NHANES 1999-2000

Estimates of exposure to methylmercury using whole blood total mercury concentrations were assessed among 1,709 women who were participants in the U.S.

National Health and Nutrition Examination Survey (NHANES) in 1999 and 2000 (Mahaffey *et al.* 2004). These data are nationally representative and are based on analysis of cross-sectional data for the non-institutionalized, general U.S. household population. Results showed that blood mercury concentrations were 0.6 μg/L at the 50th percentile and ranged from concentrations that were non-detectable (5th percentile) to 6.7 μg/L (95th percentile). Blood mercury concentrations were 7-fold higher among women who reported eating 9 or more fish and/or shellfish meals within the past 30 days than among women who reported no fish and/or shellfish consumption during that time period. Blood methylmercury concentrations were ~1.5-fold higher among women 30–49 years of age than among women 16–29 years of age, which reflected their heavier fish consumption habits.

It was concluded that based on the distribution of blood mercury concentrations among the adult female participants in 1999–2000 NHANES and the number of U.S. births in 2000, > 300,000 newborns each year in the U.S. may have been exposed *in utero* to methylmercury concentrations higher than those considered to be without increased risk of adverse neurodevelopmental effects associated with methylmercury exposure.

Exposure to methylmercury was also assessed in U.S. women 16–49 years of age (n = 1,726) using hair mercury analysis during the 1999–2000 NHANES (McDowell *et al.* 2004). The geometric mean hair mercury content was 0.20 μg/g in these women. Among frequent fish consumers, geometric mean hair mercury levels were 3-fold higher for women (0.38 vs. 0.11 μg/g) compared with non-consumers. The total hair mercury

levels of NHANES children and women were generally lower than the levels reported in other studies of U.S. and international populations.

1.2.3 Decline in Fish Consumption by Women of Reproductive Age

Despite the benefits of fish consumption, many pregnant women avoid this important dietary source, possibly due to the history of adverse methylmercury events, or in response to public health warnings about toxic contamination of seafood. In response to many epidemiological studies showing negative health effects of maternal fish consumption, the U.S. Congress mandated that the National Academy of Sciences review data regarding the toxicological effects of mercury (National Research Council (U.S.) 2000). Subsequently, in January 2001, the U.S. FDA issued an advisory that counselled pregnant women to avoid consuming specified long-lived predatory fish, which may contain high levels of organic mercury, and to limit ingestion of all other fish (FDA 2001).

Following this advisory, a study was conducted to examine its repercussions (Oken *et al.* 2003). The investigators were able to show a significant decline in fish consumption in response to this well-publicised national mercury advisory. A cohort of 2235 pregnant women visiting obstetric offices in eastern Massachusetts, were surveyed before the advisory from April 1999 through December 2000 and after the advisory from April 2001 through February 2002. Subjects reported fish consumption on semi-quantitative food frequency questionnaires administered at each trimester of pregnancy. A reduction in total fish consumption of approximately 1.4 serving per month (95% confidence interval 0.7, 2.0) was observed in December 2000 to April 2001.

The results of this study suggest that pregnant women in this cohort acted in accordance with the federal guidelines to reduce fish consumption. In conclusion, these results suggest that a broadly disseminated health advisory may substantially change dietary behaviour among pregnant women, which may have significant unwanted health implications.

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Chapter 2: Study Purpose, Hypotheses and Objectives

2.1 Primary Research Question and Study Purpose

My primary research question to be examined was: Is there a risk to Canadian children from *in utero* methylmercury exposure through maternal fish consumption? Little is known about mercury exposure in the general population in Canada, especially in women of reproductive age.

2.2 Hypotheses

1. Hair mercury content of women of reproductive age who had called the Motherisk program for guidance would fall below our defined lowest observable adverse effect level (LOAEL) for detrimental effects of methylmercury on neurodevelopment because they were vigilant about fish consumption.
2. Members of the Japanese population residing in Toronto, due to their almost daily fish consumption, would have hair mercury content higher than our conservative LOAEL.
3. The St. Clair River has experienced historical mercury spills to such an extent that high exposures to mercury in the Walpole Island First Nation (WIFN) are possible in the highest fish consumers.
4. Women will have high perception of risk for damage to their babies from eating fish during pregnancy.

2.3 Objectives

Within the overall hypotheses listed above, this study had the following specific objectives:

1. To systematically review all relevant published research focusing on the effects of prenatal (fetal) mercury exposure through fish consumption on neurodevelopment; and to define a LOAEL for these adverse effects on child neurodevelopment resulting from fetal exposures.
2. To determine hair mercury concentrations and to evaluate them in relation to fish intake and our defined LOAEL.
3. To determine the baseline level of mercury in hair and whole blood in a limited number of volunteers in the WIFN.
4. To document maternal perceptions of health risks to the fetus due to eating mercury in fish.

2.4 Structure of Thesis Document

The remainder of this report is organized into 6 chapters and an appendix. In Chapter 3, a systematic review of the literature written on the neurodevelopmental effects caused by prenatal exposure through maternal fish consumption will be presented, including data from both longitudinal and cross-sectional studies and the derviation of our LOAEL. In chapter 4, results of the hair mercury analysis in 3 cohorts will be described in relation to their fish consumption habits and our defined LOAEL. Sources of uncertainty and limitations to this study are characterized. Perceptions on the potential risks of consuming fish during pregnancy are provided in chapter 5. In chapter 6, the mercury concentrations in whole blood and hair in members of the WIFN will be

reported, along with the significant correlation found between the two. Chapter 7 presents an overall discussion of the research, leading to the potential for hair mercury as a new indication for therapeutic monitoring. Conclusions to this present study as well as recommendations for potential future directions are explored in Chapter 8.

Chapter 3: A Systematic Review: Defining a LOAEL for Adverse Neurodevelopmental Effects of Prenatal Methylmercury Exposure through Maternal Fish Consumption

3.1 Background

Fish and other forms of seafood are important components of healthy diets (Mozaffarian & Rimm 2006). Fish consumption is particularly advantageous for pregnant women because it contains relatively high concentrations of omega-3 polyunsaturated fatty acids not commonly found in other foods, which are essential for the developing fetal brain (Hadders-Algra 2008; Makrides *et al.* 1995). The predominant drawback of fish consumption for expectant mothers is that some species of fish contain organic mercury at concentrations sufficient for high consumption to cause adverse developmental effects to the unborn child (Costa 2007; Dovydaitis 2008). Methylmercury, the most detrimental form of environmental mercury to humans, is produced from inorganic mercury by the action of anaerobic organisms that live in aquatic environments (Clarkson & Magos 2006). Since fish have limited ability to eliminate this contaminant, methylmercury bioaccumulates resulting in high concentrations in muscle of large and old predatory fish (Dorea 2008).

Methylmercury is a well-established neurotoxicant in humans that can have serious effects on the CNS, particularly during fetal development (Grandjean *et al.* 1994; Myers *et al.* 2000; Rice 2000). Due to its lipophillic nature, methylmercury readily crosses the placenta and occurs at higher concentrations in the fetal than maternal circulating blood, raising concerns about fish consumption by pregnant women (Morrissette *et al.* 2004). The main target tissue for methylmercury is the brain where the

division and migration of neuronal cells are inhibited and cytoarchitecture is disrupted (Castoldi *et al.* 2001; Johansson *et al.* 2007; Sanfeliu *et al.* 2003).

Using data collected after the Iraqi disaster (Amin-Zaki *et al.* 1974; Bakir *et al.* 1973), Clarkson and colleagues defined threshold toxicological levels associated with adverse effects to the fetus as low as 10 μg/g in maternal hair (Clarkson *et al.* 2003). Although a place to start in the assessment of dose-response of fetal damage by mercury, this threshold was based on clinical evidence of neurological damage and not on current neurodevelopmental testing techniques. The exposure scenerios are not comparable to the low-dose chronic exposure that the general North American population might experience.

For populations who are socioeconomically dependent upon fish consumption as a major dietary protein source, it is also prudent to consider the benefits derived from fish when evaluating the actual risk associated with methylmercury in fish consumed during pregnancy. Fish and other seafood contain beneficial nutrients such as omega-3 polyunsaturated fatty acids which are necessary for optimal neurologic development, however, presently there is no conclusive evidence that such supplements improve child neurocognitive achievements (Nettleton 1993, Helland *et al.* 2008). Despite the benefits of fish consumption, many pregnant women avoid this important dietary source due to the history of these adverse methylmercury events or in response to public health warnings about toxic contamination of seafood. One study showed a significant decline in fish consumption in response to a well-publicised national mercury advisory. A reduction in total fish consumption of approximately 1.4 servings per month (95% confidence interval 0.7, 2.0) by pregnant women (Oken *et al.* 2003).

Extrapolation of results from past mass poisoning environmental disasters such as, methylmercury-treated wheat in Iraq and chronic poisoning via seafood in Minamata, to determine thresholds of toxicological concern is scientifically tenuous and does not address the risk-benefit ratio of fish consumption by specific components of the general population, specifically pregnant women. Rice argues that derivation of a reference dose for methylmercury is inappropriate, given that there does not appear to be a threshold for adverse neuropsychological effects based on available data (Rice 2004). This issue should be addressed for maintenance of healthy diets of women of reproductive age so they might be very well informed as they make choices before and during pregnancy about safe consumption of fish, high in omega-3s and low in methylmercury. The objective of this study was to systematically review all relevant research focusing on the effects of prenatal mercury exposure through fish consumption on neurodevelopment following fetal exposure and to define a LOAEL for adverse effects on child neurodevelopment resulting from fetal exposure.

3.2 Methods

Search Strategy, Study Selection

This systematic review examined all published epidemiological studies of methylmercury exposure in infants and young children found in the Medline-Ovid, PubMed, Google Scholar, EMBASE and SCOPUS databases from their inception to September 30, 2008. The search was performed using *methylmercury, pregnancy, fish consumption, prenatal, in utero, neurotoxicity, neurodevelopment* and *human* as keywords. The references of all retrieved studies and available reviews of the topic were examined for additional studies not captured by our initial search strategy. The title and

abstract of the articles were screened for relevance by assessing the study population, exposure route and outcome. Articles not written in English were not included. We excluded studies if they did not define mercury exposure concentrations; if the participants were not prenatally exposed to mercury; if fish consumption was not the primary source of exposure; and if data were not available for outcomes associated with methylmercury exposure. We also excluded all studies that did not report original data. Two reviewers assessed independently which articles were to be included. In the case of discrepancies between the decisions of the two reviewers a third reviewer adjudicated the differences. Authors of original papers were contacted for additional information if there was an aspect of the study we wished to clarify. A list of all included and excluded articles can be found on the Ivey Chair website: (www.schulich.uwo.ca/moleculartoxicology).

Quality Assessment and Data Extraction

The quality of the studies was evaluated using the Methodological Index for Non-Randomized Studies (MINORS) (Slim *et al.* 2003). This scale consists of 12 questions for comparative studies and 8 questions for non-comparative studies that address different aspects of the quality of the methods used. Scores are expressed as total numbers (out of 24 for comparative studies and 16 for non-comparative studies) with a higher score representing a greater quality. There were 32 comparative (accessing groups with different exposure concentrations) and 16 non-comparative studies included in this review, each assessed by the same reviewer. The following information was extracted: study population size and age, number of subjects studied, patients' demographics,

presence of a comparison group, route and dose of exposure, different outcome measures, method of analysis and conclusions drawn.

Data Synthesis

For the purpose of defining a LOAEL for adverse neurodevelopmental effects of methylmercury, we have used the lowest maternal hair mercury level associated with any adverse neurodevelopmental effect reported in the papers accepted for analysis based on our preset criteria. Maternal content of mercury in hair was chosen as the biomarker for the extent (dose) of exposure to methylmercury because these values were reported by most studies, reflect longer exposure periods than do maternal whole blood values, and represent a less invasive method of sampling for concerned women who are planning to become pregnant. Microgram/gram (μg/g) was chosen as the standardized unit as most studies reported hair mercury content using this unit.

3.3 Results

We retrieved 67 articles in our initial broad search and overall 48 articles met the inclusion criteria and are included in this systematic review. Of the 48 articles, 30 were of longitudinal and 18 were of cross-sectional design. Of the 30 longitudinal articles, 11 involved work done in the original Seychelles longitudinal study and 2 articles were of the most recent Nutrition Study conducted in the Seychelles. Eight articles were of studies conducted in the main Faroe Island study and 2 from the second Faroe Islands longitudinal study. Another 2 articles described a longitudinal study conducted in the U.S. Finally, 2 of the included articles were of studies conducted in New Zealand and 2 in Poland.

Relevance of Studies

All accepted studies measured neuropsychological outcomes including: language, attention, IQ, memory, neuromotor development, visual-spatial ability, brainstem auditory evoked potentials, birthweight and gestational age. The use of different tests or testing parameters by the authors of the various studies precluded a formal meta-analysis (Tables 1 & 2).

Table 1. Characteristics of Longitudinal Studies

Location of Study	Year	Age Studied	Test (s) Used	Number of subjects	Exposure Measure	Median/ Mean Exposure Level	Effect Found-Y or N?	First Author
New Zealand	1986	4 years	DDST	31	MH	8.8 ppm	Y	Kjellstrom, T
	1989	6-7 years	CDS,BWRD, TLD, KMDAT, PVT, MSCD, WISC-R	73	MH	8.3 ppm	Y	Kjellstrom, T
Seychelles *-Main Cohort*	1995	6.5 months	FI test VRM, DDST-R	740	MH	5.9 ppm	N	Myers, G
	1997	19 months	DM, BSID	738	MH	5.8 ppm	N	Myers. G
	1995	29 months	BSID	736	MH	5.9 ppm	N	Davidson, P
	1998	66 months	MSCD, PLS Total Score, W-J A-T, Bender-Gestalt	711	MH	6.8 ppm	N	Davidson, P
	2003	108 months	WISC-III, W-J AT, CVLT, BNT, FT, Trailmaking, Grooved Pegboard, BBDT-VMI, CCPT	643	MH	6.9 ppm	N	Myers, G
	2008	10.7 years	BVMGT	613 -results for 346	MH	7.0 ppm	N	Davidson, P
Seychelles *-Pilot Cohort*	1995	5 to 109 weeks	DDST-R, NE	789	MH	6.6 ppm	Y	Myers, G
	1995	66 months	MSCD, PLS, W-J Tests	217	MH	7.1 ppm	N	Myers, G
	2000	108 months	WISC-III, CVLT, BNT, BBDT-VMI, WRAML, FT, Grooved Pegboard, Trailmaking	87	MH	7.8 ppm	N	Davidson, P
Seychelles Nutrition Study	2008	9 and 30 months	BSID-II	229	MH	5.7 ppm	Y	Strain, J Davidson, P
Faroe Islands	1993	Newborn	BW	997	CB	22.4 µg/L	Y	Grandjean, P
	1997	7 years	NE, VEP, BAEP, Postural Sway, NES FTT, NES HCT NES CPT, WISC-R, WISC-R Sim, WISC-R BD, BVMGT, CVLT, BNT	917	CB	22.9 µg/L	Y	Grandjean, P
	2001	7 years	FACT, VEPs, CPT, WISC, BVMGT, FT	917	CB	24.2 µg/L	N	Grandjean, P
	2003	7 years	NES2 FT, NES2 HECT NES2 CPT, WISC-R, BVMGT, BNT CVLT	917	CB	23.2 µg/L	Y	Grandjean, P
	2006	14 Years	NES CPT, CE, WISC-R, S-B CT, CCT, WMS-III SS	878	CB	22.5 µg/L	Y	Debes, F
Faroe Islands-2nd study	2001	Newborn	BW, GA	182	CB	20.4 µg/L	N	Grandjean, P
	1999	2 weeks	NE	182	MH,CB	4.08 µg/g 2.04 µg/L	Y	Stewerwald, U)
Poland	2006	12 months	BSID-II	233	MB, CB	0.55 µg/L 0.75 µg/L	Y	Jedrychowski, W
	2007	24 and 36 months	BSID-II	374	CB	NS	N	Jedrychowski, W
Other United States	2003	38 months and 54 months	MSCD	212	MH	0.5 ppm	Y at 38 months N at 54 months	Stewart, P
Massachusetts United States	2005	5.5-8.4 months	VRM	135	MH	0.55 ppm	Y	Oken,E
	2008	3 years	PPVT, WRAVMA	341	MB	3.8 ppm	Y	Oken, E
Brazil	2006	Birth and 6 months	NE, GDS	100	MH	5.4 ppm	N	Marques, R

Table 2. Characteristics of Cross-sectional Studies

Title/Location of Study	*Year*	*Age Studied*	*Tests Used*	*Number of Subjects*	**Exposure Measure**	**Mean/Median Exposure Level**	**Effect-Y or N**	**Reference**
Canada	1983	12-30 months	NE, DDST	234	MH	6 ppm	Y	McKeown-Eyssen, G
Greenland	1990	Newborn	BW, GA	376	CB	21.0 µg/L	Y	Foldspang, A
Peru	1995	Newborn	NE	131	MH	8.3 ppm	N	Marsh, D
Greenland	1996	Newborn	BW	1106	CB	26.4 µg/L	N	Bjerregaard, P
Madeira	1999	6.4-7.4 years	NES2 FT, NES2 HECT NES2 CPT, WISC-R, S-B BMT, EP	149	MH	9.64 ppm	Y	Murata, K
French Guiana	2002	9 months-6 years	NE, MSCD, FT MLC, S-B CT, S-B BMT RPM	156	MH	12.7 ppm	Y	Cordier, S
Greenland	2002	7.5 years	NE, NES2 tests, WISC-R, S-B BMT, BAEP latency, VEP latency	43	MH	15.5 ppm	Y	Weihe, P
Tagum	2003	2 years	CAT, CLAMS	48	CH,CB	1.28 ppm 2.6 ppb	Y	Ramierez, G
Japan	2004	7 years	BAEP latency	327	MH	1.63 ppm	Y	Murata, K
Britain	2004	15 and 18 months	MCDI, DDST	1054	CT	0.01 ppm	N	Daniels, J
Italy	2004	18 months	DDST-II	120 To date:52	MH	NS	Y	Barbone, F
Canada	2004	Newborns	BW, GA	454	CB	70.5 nmol/L	N	Lucas, M
Canada	2005	9 years	NE, GMF, CE	110	CB	15.9 ppm	N	Despres, C
Canada	2006	5 to 6 years	VEP	110	CB	82.4 nmol/L	Y	Saint-Amour, D
United States	2006	9.5 years	DRLS	167	MH	0.56 ppm	Y	Stewart, P
China	2007	3 days	NBNA test	384	MH,CB	1.25 ppm 5.58µg/L	Y	Gao,Y
United States	2007	Newborns	GA	1,024	MH	0.29 ppm	Y	Xue,F
United States	2008	12,24,26,48 months	GA, BW, BSID	329	CB	7.82 µg/L	Y	Lederman, S

Abbreviations Used in Tables 1 and 2

BAEP- Brainstem Auditory Evoked Potentials
BM-Blood mercury
BNT-Boston Naming Test
BSID-Bayley Scales of Infant Development
BW-Body Weight
BVMGT-Bender Visual Motor Gestalt Test
BWRD- Burt Word Recognition Test
CAT- Cognitive Aptitude Test
CB-Cord Blood
CCPT-Connor's Continuous Performance Test
CCT-Children's Category Test
CDS- Clay Diagnostic Survey
CE- Catsys Equipment
CLAMS- Clinical Linguistic Auditory Milestone Scale
CT- Cord Tissue
CVLT- California Verbal Learning Test
DDST-Denver Developmental Screening Test
DM-Developmental Milestones
DRLRS-Differential Reinforcement of Low Rates Schedules
EBRS- Everts Behaviour Rating Scale
EP-Evoked Potentials
FI test VRM- Fagan Infant test of Visual Recognition and Memory
FT-Finger Tapping
GA-Gestational Age
GDS-Gessell Developmental Schedules
GMF-Gross Motor Function
HM-Hair mercury
KMDAT-Key Math Diagnostic Arithmetic Test
MB-Maternal Blood
MCDI-MacArthur Communicative Development Inventory
MH-Maternal Hair
MLC-McCarthy Leg Coordination
MSCD- McCarthy Scales of Children's Abilities
NBNA- Neonatal Behavioural Neurologic Assessments
NE-Neurological Exam
NES CPT- Continuous Performance Test
NES FTT-Neurobehavioural Evaluation System Finger Tapping Test
NES HCT- Hand-eye Coordination Test
NS-Not specified
PDI- Psychomotor Developmental Index
PLS Total Score- Preschool Language Scale
PPVT- Peabody Picture Vocabulary Test
RPM-Raven Progressive Matrices
S-B BMT-Stanford-Binet Bead Memory Test
S-B CT-Stanford-Binet Copying Test
TLD- Test of Language and Development
VEP-Visual Evoked Potentials
VRM-Visual Recognition Memory
WISC-R BD-Block Designs
WISC-R-DS-Digit Spans
WISC-R Sim- Wechsler Intelligence Scale for Children-Similarities
WISC-R- Wechsler Intelligence Scale for Children, Revised
WISC-III-Wechsler Intelligence Scale for Children-III edition
W-J A-T- Woodcock-Johnson Tests of Achievement
WMS-III SS- Wechsler Memory Scale-III Spatial Span
WRAML-Wide-Range Assessment of Memory and Learning
WRAVMA Wide Range Assessment of Visual Motor Abilities

Quality of Studies

The mean global score for comparative studies on scale from 0 to 24 was 19. For non-comparative studies the mean global score ranging from 0 to 16 was 13. Most articles failed to describe the sample size and did not calculate the confidence intervals or relative risk.

Outcome of Studies

All 48 of the included studies evaluated the risk of prenatal methylmercury exposure on neurodevelopment. Of the 18 cross-sectional studies, 12 articles reported an adverse dose-dependent effect. One article found an effect associated only with postnatal mercury exposure. Of the 9 longitudinal studies analyzed, 5 studies found a detectable effect of methylmercury on brain function of the child. These latter (positive effect studies) included 2 separate longitudinal studies conducted in the Faroe Islands, one study each conducted in the U.S. and in New Zealand and the most recent study conducted in the Seychelles. The original longitudinal study conducted in the Seychelles Islands found no adverse associations between perinatal exposure to mercury and deficits in child neurodevelopment, however the more recent longitudinal study of the exposed children at higher ages did. A study conducted in Poland found an effect which was lost upon re-analysis.

Defining our LOAEL

From review of the data it is apparent that neurodevelopmental abnormalities occur in children following a range of gestational exposures, from maternal consumption of highly contaminated fish (maternal hair: 0.3-12.7 μg/g) (Cordier *et al.* 2002; Davidson *et al.* 2000; Despres *et al.* 2005; Gao *et al.* 2007; Grandjean *et al.* 1998; Kjellstrom *et al.*

1986; Kjellstrom *et al.* 1989; McKeown-Eyssen *et al.* 1983; Murata *et al.* 1999; Myers *et al.* 1995; Myers *et al.* 2003; Oken *et al.* 2005; Ramirez *et al.* 2003; Steuerwald *et al.* 2000; Stewart *et al.* 2006; Strain *et al.* 2008; Weihe *et al.* 2002; Xue *et al.* 2007), cord blood: 0.75 -25.7 μg/L (Barbone *et al.* 2004; Debes *et al.* 2006; Foldspang & Hansen 1990; Grandjean & Weihe 1993; Grandjean *et al.* 1997; Grandjean *et al.* 2001; Grandjean *et al.* 2003), and maternal blood: 3.8 μg/L (Oken *et al.* 2008).

Data from the NHANES conducted in the U.S. in 1999-2000 reports that consuming fish 1-2 times a month corresponds to a geometric mean total mercury concentration of 0.20 μg/g in hair and 1.05 μg/L in blood in women of reproductive age. Fish consumption ≥3 times a month corresponds to a geometric mean mercury content of 0.38 μg/g in hair and 1.94 μg/L in blood. No fish consumption corresponded to a mean total mercury content of 0.11 μg/g in hair and 0.51 μg/L in blood (McDowell *et al.* 2004; Schober *et al.* 2003).

The LOAEL of 0.3 μg/g mercury in maternal hair (that corresponds to 0.75 μg/L mercury in cord blood) was selected for purposes of this risk characterization from inspection of data that appear in Tables 3 and 4. We defined our LOAEL from all of the studies analyzed, at 0.3 μg/g mercury in maternal hair, meaning that in included studies, different adverse neuropsychological end points were detected when maternal hair mercury levels exceeded 0.3 μg/g.

Detailed Findings:

Longitudinal Studies

The effects of prenatal mercury exposure from maternal fish consumption on child neurodevelopment were measured prospectively in the Seychelles Child

Development Study (SCDS). In the original pilot study (Myers *et al.* 1995), evaluation consisted of a neurological examination and the administration of the Denver Developmental Screening Test-Revised (DDST-R), a test that determines development in 5 domains: personal-social, fine motor, adaptive, language, and gross motor, in children up to 6 years of age (Davidson *et al.* 1995a). This test identifies children from birth to 6 years of age whose developmental progress may be abnormal or questionable. It was suggested, after combining abnormal and questionable results from the DDST-R, that there was a concentration-dependent effect of mercury on the scores of the developmental test (Myers *et al.* 1995). Interpretation of these data was compromised, however, because the neuropsychological scores were not standardized.

The results of this study led to a more detailed and thorough examination (Myers *et al.* 1995). A subsequent pilot cohort was evaluated at 66 and 108 months of age. At 66 months a negative association between prenatal mercury exposure and 4 neurodevelopmental endpoints was found. However, once outliers and highly influential scores were removed an association was no longer observed (Myers *et al.* 1995). At 108 months of age the pilot cohort was re-tested and an association was found, this time in the beneficial direction (Davidson *et al.* 2000). Enhanced performance was seen for 3 endpoints: the Boston Naming Test (Kaplan *et al.* 1983) and 2 tests of visual motor coordination; the grooved pegboard task (Heaton *et al.* 2004) and the Beery-Buktenica Developmental Test of Visual Motor Integration (VMI) (Beery & Buktenica 1967). Authors explained that other constituents in the fish may be causing positive developmental outcomes rather than expected deficits.

Following this pilot study, the SCDS recruited children for the main cohort in 1989. The investigators examined the main cohort of 779 mother-infant pairs 6 times after birth at 6.5 (Myers *et al.* 1995), 19 (Davidson *et al.* 1995b), 29 (Davidson *et al.* 1995b), 66 (Davidson *et al.* 1998; Young *et al.* 2004), 108 (Myers *et al.* 2003) months of age and again at 10.7 years of age (Davidson *et al.* 2008) using a battery of tests. The mean (SD) maternal hair mercury for the entire cohort was 6.8 (4.5) μg/g and the mean child hair level at 66 months was 6.5 (3.3) μg/g (Myers *et al.* 2000). Results of this longitudinal study to date reveal no statistical association between neurodevelopmental deficits and prenatal methylmercury exposure. No delays in developmental milestones (age at walking and talking) were noted (Myers *et al.* 1997), in fact Seychellois children both walked and talked slightly earlier than U.S. children. Out of 21 endpoints measured, only one showed a possible adverse association with prenatal methylmercury exposure and this was found only in boys. Specifically, boys exposed to prenatal methylmercury took longer to complete a grooved pegboard task using their non-dominant hand than did non-exposed children (Myers *et al.* 2003). Neuropathological specimens from stillbirths and deaths in the Seychelles were also examined (Lapham *et al.* 1995) and no association was found between maternal hair mercury content and degree or type of histological change within this range of exposures. In contrast to negative findings, beneficial associations were reported in the longitudinal studies. Two endpoints, language function and attention showed enhancements with increasing methylmercury levels (Davidson *et al.* 1998). These associations remained after adjustment for confounding variables.

Recently investigators have expanded the original SCDS study to examine the association between long-chain polyunsaturated fatty acids (LCPUFAs), methylmercury

and infant development in 229 children at 9 and 30 months of age (Strain *et al.* 2008). They found prenatal methylmercury exposure to be associated with lower Bayley Scales of Infant Development (BSID-II) test scores at 30 months of age only when the LCPUFA measures were included in the regression analysis. Only the Psychomotor Developmental Index of the BSID-II was affected. The authors went on to state that not adjusting for nutritional factors may have masked the detrimental effects of methylmercury on development. In a companion paper, the combined impact of LCPUFAs and methylmercury suggested contradictory influences on child development scores (Davidson *et al.* 2008). Recent studies in the Seychelles have focused on nutrients in fish that might influence a child's development, including LCPUFAs, iodine, iron, and choline. Preliminary findings from these studies suggest that the beneficial influence of nutrients from fish may act as confounders and may help to counter any adverse effects of methylmercury on the developing nervous system (Myers *et al.* 2007).

The most recent study conducted by Bonham and colleagues characterized the diets of pregnant women in the Seychelles to determine the contribution of fish to intakes of nutrients important for fetal and neonatal development (Bonham *et al.* 2008). They found that fish consumption was lower than in previous surveys, perhaps suggesting a move towards a more Westernised diet. Compared with women who ate more fish, lower intakes of a number of nutrients important during pregnancy for fetal development (Fe, Zn, Se and iodine) were observed in women who did not consume fish. The investigators went on to state caution to regulatory bodies that promote the limitation of fish consumption during pregnancy.

One of the studies most comparable to those in the Seychelles is the prospective study completed in the Faroe Islands. In 1993, Grandjean and Weihe investigated whether intrauterine exposure to methylmercury would affect the birthweight of these Faroese children (Grandjean & Weihe 1993). In this series of studies, cord blood was chosen as the tissue matrix for analysis of mercury. High mercury concentrations in the cord blood were associated with increased birthweight, however the authors noted that it was unlikely to be related to the mercury exposure but may be related to other constituents in fish that might prolong gestation.

A total of 917 children aged 7 completed the first follow-up examination. Contradictory to the study done in the Seychelles Islands, the investigators of the Faroese Birth Cohort did find a concentration-dependent effect of mercury on development. The geometric mean cord blood mercury concentration was 22.9 μg/L (Interquartile Range: 13.4-41.3 μg/L) (Grandjean *et al.* 1997). Neurophyschological dysfunctions were found, most pronounced in language, attention and memory.

In a second report of this series, a case-control study, 113 children aged 7 whose mothers had hair mercury concentrations between 10 and 20 μg/g, were compared to 272 children whose mothers had mercury content below 3 μg/g. Findings revealed that the higher exposed case group showed mild decrements in motor function, language and memory compared to the lower exposed group, and to a lesser extent in visuospatial functions (Grandjean *et al.* 1998). The most important conclusion from this study was that a negative effect on neurodevelopment was found at maternal mercury hair concentrations which up to that time were considered to be safe (i.e 10-20 μg/g).

The final Faroe Island study investigated whether 'peak' exposures may have affected these mercury-dependent associations found previously, in the cohort of 7-year-old children (Grandjean *et al.* 2003). Results revealed that children showed deficits in 8 of 16 neuropsychological tests, which were related to cord blood mercury content. These findings remained after exclusion of 61 children with the greatest degree of variable intrauterine exposure levels, resulting in only a slight increase in the association. Thus, the association was not the result of variable exposures.

The investigators went on to examine whether visual contrast sensitivity was associated with prenatal mercury exposure, to determine whether these observed neuropsychological dysfunctions were secondary to adverse effects on the visual pathways (Grandjean *et al.* 2001). The investigators found that contrast sensitivity was not associated with mercury exposure and therefore, the mercury-effects reported previously were unlikely due to mercury-induced visual dysfunction.

As fish is the primary source of exposure it is difficult to determine whether mercury is the only cause of fish-related effects. To explore whether or not Polychlorinated Biphenyl (PCB) exposure may act as a confounding variable to these neurobehavioural effects that were associated with methylmercury exposure, another study was conducted (Grandjean *et al.* 2001). The authors found a possible confounding effect. Children in the highest tertile of mercury exposure were found to have PCB-associated deficits. The authors concluded that PCB exposure might augment the neurobehavioural deficits at increased levels of mercury exposure.

Children were re-assessed at age 14, and methylmercury exposure was again significantly associated with deficits in motor, attention and verbal tests (Debes *et al.*

2006). Higher prenatal mercury exposure was associated with lower finger tapping scores, increased reaction time, and lower cued naming scores. These results were in accordance to what was found previously in this cohort at age 7. The associations in all studies remained after adjustment for covariates.

Murata and colleagues studied brainstem auditory evoked potential latencies in this cohort and found that in accordance to data seen previously at age 7 (Murata *et al.* 1999) latencies of peaks III and V increased with increasing cord blood mercury concentration at age 14 (Murata *et al.* 2004). The persistence of increased brainstem auditory evoked potential latencies at both ages 7 and 14, indicates that neurotoxic effects caused by intrauterine methylmercury exposure may be irreversible.

A second longitudinal study was conducted using a different cohort of 182 pregnant women from the Faroe Islands. It was found that concentrations of mercury and PCBs did not appear to affect gestational length or birthweight (Grandjean *et al.* 2001). Evidence reported by Steuerwald and colleagues, showed that a 10-fold increase of cord-blood mercury was associated with a decrease in neurologic optimality score of 2.0 when these newborn infants were re-tested at 2 weeks of age (Steuerwald *et al.* 2000). The neurologic optimality score is the number of main items in the neurologic examination rated optimal (out of 60) (Prechtl 1977).

Similar to the Seychelles and Faroe Island studies, results from a study conducted in New Zealand show inconsistent results. Initial concern for methylmercury exposure came from preliminary studies done in New Zealand which found that mercury levels in some commercial fish were up to 10 times higher than the legal limit (0.5 mg Hg/kg) (Mitchell *et al.* 1982). In order to investigate whether consuming this mercury containing

commercial fish would cause potential adverse effects, a cohort of 11000 pregnant women was established and hair samples were initially collected. Ultimately, however, the high-exposure group of the study was selected from among the frequenct fish consumers; approximately 1000 of these mothers had consumed fish more than 3 times per week and 73 had an average hair mercury level above 6 μg/g. The first stage follow-up was completed when the children were 4 years old (Kjellstrom *et al.* 1986). Assessment consisted of 4 different function sectors: gross motor, fine motor, language and personal-social of the DDST (Frankenburg *et al.* 1971), as well as a vision test (Sheridan & Gardiner 1970) and a sensory test (Ayres & Tickle 1980), to compare the high exposure case group with the control group. It was found that a high proportion of the case group had abnormal (n=2) and questionable (n=14) results compared to the control group (n=1 and 4, respectively). Sensory tests that were used also indicated more deficiencies among higher exposed children.

The investigators noted a new finding; effects can be measured at maternal hair mercury levels lower than what was considered to be safe at the time (6 μg/g). It was concluded that a significant dose-response relationship existed between mean hair mercury content during pregnancy and results of the DDST. However, mismatching of age and ethnicity occurred and was described by Marsh and colleagues as a possible reason for the differences in DDST scores (Marsh 1994).

A second follow-up study was completed at age 6, using 61 of the original 73 children (Kjellstrom *et al.* 1989). In this investigation, each child of the high exposure group was matched with 3 control children representing the low exposure group; 1 child with maternal hair mercury level of 3-6 μg/g and 2 children with maternal hair mercury

in the range of 0-3 μg/g. This time a battery of psychological tests was used testing 5 dimensions of the child's physical and mental development: academic attainment, language development, fine and gross motor coordination, intelligence and social adjustment. The main finding of this study was that an average hair mercury content during pregnancy of 13-15 μg/g, was associated with decreased performance on a variety of psychological tests. This study included matching for the number of fish dinners during pregnancy

A more recent longitudinal study was conducted in Poland (Jedrychowski *et al.* 2006). The higher exposure group of infants (Mean=0.75 μg/L cord blood) had lower performance scores then the lower exposed group (Mean=0.56 μg/L) on the BSID-II (Bayley 1993). Subsequent testing at age 24 and 36 months of age did not reveal the same adverse results and the performance deficit observed at 12 months of age was found to be of borderline significance (Jedrychowski *et al.* 2007).

In a U.S. cohort, gestational maternal fish intake was positively associated with higher Visual Recognition Memory (VRM) scores (Oken *et al.* 2005). However, when mercury concentrations found in hair were incorporated into the analysis a negative association was found (Mean=0.55 μg/g maternal hair) at 6 months of age. When re-tested at age 3 years using different tests, an adverse association of mercury with test scores on 2 developmental tests; the Peabody Picture Vocabulary Test (PPVT) (Dunn & Dunn 1997) and Wide Range Assessment of Visual Motor Abilities (WRAVMA) (Adams & Sheslow 1995) was found (Oken *et al.* 2008). Contrary to these findings, a study conducted in Brazil found no association of mercury exposure (median= 5.4 μg/g

maternal hair) with developmental delays (Marques *et al.* 2007). The cohort of 100 infants was examined at age 6 months using the Gesell Developmental Schedules.

Cross-sectional Studies

Of the studies reviewed, 18 were cross-sectional, in that children were only tested on one occasion. In all studies the potential association between prenatal methylmercury exposure through fish consumption and neurological deficit was examined. Five studies examined the possible effect of methylmercury on intrauterine growth, as indicated by low birthweight and/or gestational length. Birthweight and gestational age outcomes were included in this review as they are important predictors of the survival and health status of the newborn (Wilcox & Skjaerven 1992). Research from Greenland found a dose-dependent effect of methylmercury on birthweight but not on gestational length (Foldspang & Hansen 1990) and concluded that methylmercury may be active in a mechanism of growth retardation of fetal tissues (when there are high levels of methylmercury exposure). In a recent study with 1,024 women from the Pregnancy Outcomes and Community Health (POUCH) study in Michigan, investigators found that compared to women delivering full term, women who delivered before 35 weeks were more likely to have hair mercury concentrations at or above the 90th percentile (>0.55 μg/g) (Xue *et al. 2007). The other 4 studies evaluated found that the contaminants did not* appear to affect either of these two outcome parameters (Bjerregaard & Hansen 1996; Grandjean *et al.* 2001; Lederman *et al.* 2008; Lucas *et al.* 2004).

A re-examination of 48 children from the original Tagum study (Ramirez *et al.* 2000), which correlated mercury in maternal and cord blood, breast milk, meconium, and

infants' hair with neurodevelopment was completed when the children were 2 years old. In this follow-up study, the mercury level in cord blood was negatively correlated with the scores from the cognitive adaptive test and clinical linguistic auditory milestone scale (CAT/CLAMS) (Wachtel *et al.* 1994) used (Ramirez *et al.* 2003). The scores of the 88 controls used were significantly higher than the scores of the subjects. The authors concluded that prenatal mercury exposure is correlated with lower scores in neurodevelopmental screening, especially for the linguistic pathway.

Consistent with these reported deficits, Barbone and colleagues found that the fine motor adaptive score on the DDST was inversely related to maternal hair mercury content (Barbone *et al.* 2004), suggesting a relationship between neurodevelopment and prenatal methylmercury exposure from maternal fish consumption. However, the study was limited by the small sample size. This result may be related to a previous study conducted in 1983, of the effects of mercury in children in relation to maternal fish consumption (McKeown-Eyssen *et al.* 1983). The investigators carried out a neurologic examination on 234 Cree Children aged 12 to 30 months and found an association between mercury exposure and abnormality of tendon reflexes only in boys. A dose-response relationship was not found. No other neurologic disorders were associated with exposure to methylmercury. More recently a study of 384 3 day-old neonates was conducted in China using a neonatal behavioural neurologic assessment (NBNA) test (Gao *et al.* 2007). The investigators found decreased behavioural ability linked to increased prenatal methylmercury both in umbilical cord blood and maternal hair. This effect was once again only seen in boys.

In 2002, Cordier and colleagues (Cordier *et al.* 2002) studied children in the French-Guyanese Amazon by forming 3 groups of different exposure levels; children from the upper Maroni served as the high exposure group, Camopi as the medium exposure group and Awala as the low exposure group. Both a standard neurologic exam and other neurophyschological tests were used in the investigation. There were no major neurologic abnormalities observed in the children, however a dose-dependent relationship between mercury concentration and increased deep tendon reflexes, poorer coordination of legs and decreased performance on the Stanford-Binet Copying score were found. The authors also noted that there was a gender by dose interaction, such that boys were more likely to show these deficits than girls.

In the same year, a study was undertaken using 43 children of 7.5 years of age from Greenland (Weihe *et al.* 2002). The test procedure was similar to the previous study by Cordier and fellow investigators. The clinical examination showed no obvious deficits, however the neurophysiological tests showed possible exposure-linked deficits. Namely, brainstem auditory evoked potentials (BAEP) (Chiappa 1982) were prolonged in the higher exposed group. Prolonged BAEP latencies were also found in two other studies (Murata *et al.* 1999; Murata *et al.* 2004). The first study examined 149 children between the ages of 6-7 from the island of Madeira. The mean peak latency III at 40 Hz was increased when maternal hair mercury exceeded 10 μg/g. The second study examined 327 Japanese children aged 7 years and compared them to 113 children from the original Madeira study. The BAEP latencies were shorter in the Japanese children (median maternal hair mercury: 1.65 μg/g) compared to the 7 year old Madeiran children (median maternal hair mercury: 10.9 μg/g).

When 102 preschool children aged 5 and 6 years from Nunavik, Canada were examined, alterations of visual evoked potential latencies (Halliday 1992) were associated with methylmercury exposure (Saint-Amour *et al.* 2006). In a different study, when responses to intermittent reinforcement schedules were examined in 9.5 year old children, investigators found that prenatal methylmercury exposure impaired performance, with lower interresponse times (IRTs) and fewer reinforcements (Stewart *et al.* 2006). In addition, exposure to PCBs or lead predicted impairments of similar magnitude. In a recent study conducted in lower Manhattan after September 11 2001, cord mercury was found to be negatively associated with the BSID-II psychomotor score at 36 months of age and verbal and full IQ scores at 48 months of age, only after adjusting for the positive effects of fish consumption during pregnancy (Lederman *et al.* 2008) .

Many studies were unable to confirm these neurodevelopmental effects caused by prenatal mercury exposure. In a study of 110 preschool children, investigators found no link between gross motor development and prenatal exposures to mercury, lead or PCBs (Despres *et al.* 2005). However, tremor amplitude was associated with blood mercury concentrations found at the time of testing (postnatal exposure) which confirmed an earlier effect reported previously among adults (Beuter *et al.* 1999). A British prospective study also did not find an association between total mercury concentrations in cord tissue and neurodevelopment at 15 and 18 months of age (Daniels *et al.* 2004) using the MacArthur Communicative Development Inventory for assessment (Feldman *et al.* 2000). The lack of mercury effects is consistent with a study done by Marsh and colleagues, where the investigators found no association between methylmercury levels

and neurodevelopmental abnormalities (Marsh *et al.* 1995). However, the study design only included a neurological exam.

Data Synthesis

Analysis of all accepted cross-sectional and longitudinal studies reveals a very close agreement of mean LOAEL for neurocognitive effects at 0.3 and 0.5 μg/g hair mercury, respectively (Tables 3 and 4).

Table 3. Longitudinal Studies with Positive Associations: Exposures and Outcomes

Mean Prenatal Exposure Level	Associations with Increasing MeHg Exposure	Study Reference
MATERNAL HAIR (μg/g; ppm)		
MH: 0.55	Lower Visual Recognition Memory score; lower infant cognition	**Oken, 2005**
MH: 4.08	Decreased neurologic optimality score	**Stewerwald, 1999**
MH: 5.9	Decreased Psychomotor Developmental Index (PDI) score of the BSID-II	**Strain, 2008**
MH: 6.6	Lower developmental score	**Myers, 1995**
MH: 6.9	Decreased performance on grooved pegboard, Improved scores on Connor's teacher rating scale, assessing behaviour	**Myers, 2003**
MH: 7.8	Enhanced performance on Boston Naming Test and two tests of visual motor coordination, Decreased performance on grooved pegboard	**Davidson, 2000**
MH: 8.3	Low performance on psychological tests: Test Of Language Development and the Wechsler Intelligence Scale for Children-Full Intelligence Quotient	**Kjellstrom, 1989**
MH: 8.8	Abnormal and questionable results on Denver Developmental Test in the fine motor-adaptive and language sectors	**Kjellstrom, 1986**
MH: 12.5	Deficits in motor function, language, memory	**Grandjean, 1998**
MATERNAL BLOOD (μg/L)		
MB: 3.8	Decreased test scores on Peabody Picture Vocabulary Test assessing receptive vocabulary and Wide Range Assessment of Visual Motor Abilities	**Oken, 2008**
Cord Blood (μg/L)		
CB: 0.75	Lower mental developmental index (MDI) and psychomotor developmental index (PDI) on the Bayley Scales of Infant Development	**Jedrychowski, 2006**
CB: 22.5	Deficits on finger tapping, reaction time on a continued performance task and cued naming	**Debes, 2006**
CB: 22.6	Increased brainstem auditory evoked potential latencies	**Murata, 2004**
CB: 22.9	Deficits in language, attention and memory	**Grandjean, 1997**
CB:23.2	Deficits in language, attention, memory, visuospatial function and motor coordination	**Grandjean, 2003**
CB: 24.2	Increased birthweight	**Grandjean, 1993**
CB: 25.7	Boston Naming Test, California Verbal Test, Continuous Performance Test reaction time	**Grandjean, 2001**

Table 4. Cross-Sectional Studies with Positive Associations: Exposures and Outcomes

Mean/Median prenatal exposure MeHg levels	**Associations with Increasing MeHg Exposure**	**Study References**
Maternal Hair (µg/g; ppm)		
MH: 0.29	Decreased gestational age	**Xue, 2007**
MH: 0.56	Lower Interresponse Times and fewer reinforces assessing behavioural performance	**Stewart, 2006**
MH: 1.25	Decreased behavioural ability for males	**Gao, 2007**
MH: 1.28	Decrements on Cognitive Adaptive Test and Clinical Linguistic Auditory Milestone score	**Ramirez, 2003**
MH: 1.5	Prolonged Brainstem Auditory, Evoked Potential latencies	**Weihe, 2002**
HM: 1.7	Tremor amplitude with postnatal level	**Despres, 2005**
MH: 6.0	Abnormality of tendon reflexes in males	**McKeown-Eyssen, 1983**
MH: 9.64	Increased Brainstem Auditory Evoked Potential latencies	**Murata, 1999**
MH: 12.7	Increased tendon reflexes, Poorer leg coordination, Decreased Stanford-Binet Copying score assessing processing speed	**Cordier, 2002**
Cord Blood (µg/L)(1 µg/L = 4.638 nmol/L)		
CB: 7.82	Decreased Psychomotor scale at 38 months and Verbal, Performance and Full IQ score at 48 months	**Lederman, 2008**
CB: 23.9	Alterations of Visual Evoked Potentials responses	**Saint-Amour, 2007**
CB: 21.0	Low birthweight	**Foldspang, 1990**
NS	Lower fine motor adaptive score on the Denver Developmental test	**Barbone, 2004**

3.4 Discussion

The past 30 years of research have demonstrated mixed results pertaining to the effects of prenatal methylmercury exposure on child development. While some studies have shown adverse effects, others showed positive developmental outcomes. However, important differences with respect to study design and sample characteristics may have contributed to these discrepant findings, as could the presence of other toxic contaminants such as PCBs in the consumed fish. Regulatory bodies have performed benchmark dose analysis on a number of endpoints from 3 longitudinal prospective studies conducted in the: Seychelles, Faroe Islands and New Zealand. These are revisited below to allow readers insight into our synthesis of the LOAEL.

The Seychelles Child Development Study (SCDS) began in the early 1980s. There are numerous reasons that led investigators to choose the Seychelles as their study site. Importantly, Seychellois people consume ocean fish daily (over 80% of Seychellois women) and there is minimal local industry with no known local point source for pollution with mercury (Shamlaye *et al.* 1995). To date, this investigation has found no clinically significant adverse effects of mercury on neurodevelopment through 10.7 years of age, from prenatal exposure using a battery of age-appropriate developmental tests (Davidson *et al.* 2008). Out of 21 endpoints measured only one showed a possible adverse association with prenatal methylmercury exposure (Myers *et al.* 2003). When performing 20 tests, one expects by chance alone that one of them will be significant at $P< 0.05$.

Overall, the longitudinal data obtained from the SCDS suggests a pattern of beneficial, rather than harmful effects of prenatal methylmercury exposure. This may be a

result of two factors: either the nutritive value of daily fish intake or not being able to make appropriate adjustments for nutritional confounders (Davidson *et al.* 2008). The SCDS is an ongoing study that is providing health officials with accurate data pertaining to the developmental risks of mercury exposure through fish consumption that must be considered when discussing regulatory aspects of fish consumption by pregnant women.

In the Faroe Islands, a cohort of 1022 single births was established at 3 hospitals between 1986 and 1987. The Faroe Islands is a North Atlantic fishing community of approximately 42,000 inhabitants that include pilot whale meat as a main part of their diets (Julshamn *et al.* 1987), 79% of the mothers reported that they ate at least one whale dinner a month (Grandjean *et al.* 1992). Overall, the results from these studies suggest that maternal mercury exposure through the consumption of fish during pregnancy does cause widespread effects on brain function and thus is associated with detectable neuropsychological deficits (Grandjean *et al.* 1997). However, consumption of fish or whale blubber containing other persistent organic pollutants, including PCBs, may also be contributing to these developmental deficits (Grandjean *et al.* 1997).

In 1978, a research project aimed at studying the developmental effects of prenatal mercury exposure via fish consumption was carried out in a group of New Zealand children. There was a tendency of children with mean maternal hair mercury levels above 6 μg/g to perform less well on psychological tests, than the control population (Kjellstrom *et al.* 1986). Children with abnormal or questionable results on the Denver test at age 4 tended to perform the poorest on the Wechsler IQ test at age 6 (Kjellstrom *et al.* 1989). This confirmation of results suggested that the effects of mercury exposure on children's development and mental functions may be long-lasting.

These associations remained after controlling for a large number of confounding variables.

Differences between Studies

Numerous reasons have been proposed for the conflicting results between the 3 different studies. A major difference among the studies is the source of methylmercury. In the Seychelles people mainly eat oceanic fish, whereas habitants in the Faroe Islands generally eat whale meat and blubber. The exposure in New Zealand is via consumption of shark meat used in fish and chips. The whale and shark meat consumed in the Faroes and New Zealand contain much higher levels of methylmercury; 1.6 μg/g and 2.2 μg/g on average, respectively (Julshamn *et al.* 1987) compared to 0.3 μg/g in ocean fish consumed in the Seychelles (Cernichiari *et al.* 1995).

A critical issue is the possibility of concomitant exposure to other neurotoxicants. The blubber of whale meat is contaminated with PCBs as well as methylmercury (Bourre & Aguilar 1993). These persistent PCB chemicals could also be responsible for some or all of the neurodevelopmental effects found in the Faroe Islands study. The third difference among the studies lies in the tissue selected for analysis of prenatal mercury exposure. In the Seychelles and New Zealand studies maternal hair was used, whereas in the Faroe Islands studies investigators used umbilical cord blood as the tissue for analysis. In combination with these differences the execution and test selection, choice of endpoint and age at testing used in the 3 longitudinal studies varied.

It should be noted that in observational studies, the presence of confounding factors can distort the true relationship between exposure to mercury and actual toxic-effect (Choi *et al.* 2008). The relationship between the benefits and risks of fish

consumption is an example of negative confounding; underestimating the true association between two factors. The potential harmful effects caused by methylmercury may be attenuated by protective effects of nutrients and omega-3 fatty acids also present in fish. Unfortunately, the majority of cohort studies in this field either focused on contaminant risks or on nutrients benefits (Budtz-Jorgensen *et al.* 2007). In a recent study, investigators applied structural modeling to the original Faroe Islands data and found that after adjusting for the benefits conferred by maternal fish consumption, previously reported regression coefficients changed toward a larger mercury effect (Budtz-Jorgensen *et al.* 2007). In the New Zeland study, confounding was partially controlled for, as mothers were matched with the same high fish consumption and high mercury exposure and therefore the data may be affected to a lesser degree of confounding from nutrient intake (Kjellstrom *et al.* 1989). The problem may be more serious with the Seychelles study, where the average fish intake is high (Clarkson & Strain 2003). As stated previously, in a recent Seychelles study investigators found prenatal methylmercury exposure to be associated with an adverse effect only when the LCPUFA measures were included in the regression analysis (Strain *et al.* 2008). Not adjusting for nutritional factors may have masked the detrimental effects of methylmercury on development in the Seychelles study. Controlling for intake of omega-3 fatty acids and other confounding variables is crucial in methylmercury studies, in order to obtain a more precise estimate of the exposure effect. Perhaps the original results of these 3 studies would be more similar if the confounding effects of both nutrients and methylmercury, present in fish could be separated (Budtz-Jorgensen et al. 2007).

Defining the LOAEL

The neurotoxicity caused by high concentrations of methylmercury exposure has been well documented from a disastrous epidemic of methylmercury poisoning that occurred in rural Iraq in 1972, due to the ingestion of home-made bread prepared from wheat treated with methylmercury as a fungicide (Bakir *et al.* 1973). A dose-response curve was generated between prenatal exposure and developmental milestones which suggested that prenatal exposure as low as 10 μg/g mercury in maternal hair may be associated with adverse health effects (Cox *et al.* 1989). However, this threshold was for severe neurological damage and is not applicable for fish consumption at levels which are a thousand times lower. The aim of this systematic review of the literature was to define a threshold level for adverse effects on brain development informed by recent research findings. The ultimate goal was to define a LOAEL for mercury exposure of pregnant women via fish consumption for well accepted and validated endpoints of altered neurodevelopment. In this context the LOAEL denotes the lowest concentration of exposure found by observation which causes an adverse alteration in neurodevelopment of a target human being distinguishable from normal (control) populations of the same species and under defined conditions of exposure.

All included studies examined whether subclinical effects were detectable at much lower mercury exposure concentrations then those seen in Minamata and Iraq. The lack of consistency of results reported among both longitudinal and cross-sectional studies makes defining a LOAEL a difficult task. Overall, however, cross-sectional studies reported the lowest level of hair mercury associated with an adverse neurodevelopmental effect as 0.3 μg/g (ppm). It is important to note this is the lowest hair

content of mercury associated with alterations in neurodevelopment in any of the studies reviewed, and that negative effects were not always observed at this hair content. It is acknowledged that comparisons across studies are limited by heterogeneity of study designs across these longitudinal and cross-sectional studies, as well as inherent variability in the different populations. Variations in methods of accessing exposure, neurologic tests administered, age at testing, source of exposure and statistical analyses all may add to the variations in results. Some analyses were also limited by the incomplete adjustment for confounders such as poor prenatal and maternal nutrition, maternal substance abuse, exposure to other environmental toxins, and unstable home environments. Various studies found an effect on the development of children whereas some did not, further adding to the confusion. Some studies even reported beneficial outcomes. It is not surprising that research using different test measures and study designs will provide inconsistent results and conclusions.

However, in ensuring the health and development of fetuses and babies, it is most reasonable to use the precautionary principle and to set a LOAEL based on the lowest level of maternal hair mercury reproducibly associated with a measurable adverse outcome. An example of the precautionary principle is that regulatory agencies and governments demand that levels of human exposure to medicinal drugs are 100^{th} (California; 1000^{th}), the minimal toxic levels found in animals. Importantly, this suggested LOAEL is in the range shown in studies of different populations of women of reproductive age around the globe, thus highlighting the potential importance of monitoring mercury exposure in these populations (Bjornberg *et al.* 2003; Johnsson *et al.* 2004; Karouna-Renier *et al.* 2008; Knobeloch *et al.* 2005; McDowell *et al.* 2004).

Other methods may be utilized to evaluate the potential risk of methylmercury, such as the risk: benefit ratio, thus also incorporating the potential benefits of omega-3 fatty acids. However, it is important to stress that, at present time the benefits of these fatty acids on child neurodevelopment are not proven, and, in fact, a recent long term randomized clinical trial has failed to show such beneficial effects (Helland *et al.* 2008). Hence, any such risk: benefit ratio must await conclusive human data showing benefit. In contrast, mercury neurotoxicity is proven, and must be addressed applying the precautionary principle.

3.5 Conclusion

The potential effect of low-level exposure of methylmercury in the environment to fetuses and children is a complex societal and research issue. Although methylmercury is a well-known neurotoxin found in fish, exposure standards are still under active debate. Our review of the literature exemplifies the inconsistency of results regarding whether or not consumption of methylmercury in fish causes neurodevelopmental defects. The Faroe Islands study has demonstrated a correlation between prenatal exposure to methylmercury from seafood consumption and neuropsychological deficits (Grandjean *et al.* 1997). This conclusion was supported by results from the New Zealand study (Kjellstrom *et al.* 1989). However, another longitudinal study of equivalent merit conducted in the Seychelles, found no apparent developmental risk from consuming ocean fish (Myers *et al.* 2003) and the conclusions of this study are supported by other cross-sectional studies found in the literature. Importantly, some of the studies have shown that nervous system domains involving fine motor function, attention, learning and memory may be affected by methylmercury (Grandjean *et al.* 1997). In populations

with heavy fish consumption, where the risk of neurodevelopmental deficits may be higher, it is unclear whether removing fish from the diet entirely would be more harmful then continuing to consume contaminated fish. The potential health benefits of fish consumption attributed mainly to omega-3 fatty acids found within fish are also being investigated (Bulliyya 2002; Dewailly *et al.* 2003; Guldner *et al.* 2007; Hu *et al.* 2002). Our LOAEL at 0.3 μg/g of mercury in maternal hair allows for a balanced approach so that the risks and benefits are considered prior to making recommendations for altering dietary habits for pregnant women or those planning pregnancy.

The precautionary principle, underlying the toxicological determination of any LOAEL always assumes and makes allowances for residual unknown effects and confounders. Yet, after the completion of almost 50 controlled studies, it seems unlikely that future research will tilt this proposed LOAEL in a major manner. It is suggested that, rather than basing future guidelines on the amounts of fish consumed by women, that regional or even personal measurements of maternal hair mercury be adopted as the most clinically valid guide because of potential individual variations in the toxicokinetics of methylmercury in consumed fish.

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Chapter 4: Comparing Hair Mercury Content of Women of Reproductive Age with our Derived Lowest-Observable-Adverse-Effect-Level for Neurodevelopmental Effects of Prenatal Mercury Exposure through Maternal Fish Consumption

4.1 Background

Compared with other types of meat, fish are a lean source of high quality protein and n-3 polyunsaturated fatty acids which are considered essential for optimal perinatal growth of the developing fetal brain (Makrides *et al.* 1995). The predominant drawback of fish consumption for expectant mothers is that all fish contain traces of methylmercury, a known developmental neurotoxin. Methylmercury concentration varies widely among fish species, and top predatory fresh-water fish from mercury-contaminated lakes typically contain the highest concentrations (Akagi *et al.* 1998).

Serious poisoning events in which large numbers of people were affected by high doses of methylmercury in Japan (Harada 1995) and Iraq (Bakir *et al.* 1973), demonstrate that consumption of high concentrations of methylmercury in foodstuffs by pregnant women can cause severe neurodevelopment problems in their offspring (Grandjean *et al.* 1994). However, the implications of much lower level exposures, such as those occurring in fish-eating populations today, on neurodevelopment remain an active area of concern. The threshold that will adversely affect the developing fetus is still under debate.

In a recent systematic review of 48 longitudinal and cross-sectional studies, it was apparent that neurodevelopmental abnormalities occur in children following a range of

gestational exposures however, a LOAEL of 0.3 μg/g mercury in maternal hair was evident in data from included studies (Schoeman *et al.* 2009). The objective of the present study was to examine fish consumption habits and mercury exposure (via assay of hair mercury content) in a cohort of women of reproductive age and compare it to our defined LOAEL.

4.2 Subjects and Methods

The study cohort consisted of 3 groups; 1) Canadian women of reproductive age who had phoned the Motherisk program in 2006-2007 for information on the safe consumption of fish during pregnancy (n=22), 2); Canadian women of reproductive age who did not consult the Motherisk program (n=20); and 3) Japanese citizens residing in Toronto who typically consume large amounts of seafood as part of their normal diet (n=23).

The Motherisk program in Toronto, Canada, provides evidence-based information on potential risks associated with prenatal exposure to pharmaceutical drugs, recreational drugs and to chemicals that occur as environmental contaminants. Upon approval by the Research Ethics Committee at the Hospital for Sick Children in Toronto, women were identified using a prospectively collected database, who had called Motherisk for information regarding seafood, fish and/or mercury consumption during pregnancy. Women were excluded from the study if they refused participation, could not be reached by telephone, had insufficient English language skills that made it difficult to communicate over the phone, confirmed that they did not call about "mercury" in fish, or had other mercury exposures (e.g. occupational). A sample of middle class women, representing mainstream Canadians, was established by contacting them over the phone

or email from July through November 2008 (Control-Canadian). All of these women resided in Southwestern Ontario, had highschool educations, with majority having a university education and were between the ages of 20-37. Between the months of September 2008 and January 2009, the third cohort (Control-Japanese) was established through announcement of the study to a group of Japanese researchers and their families in Toronto. We also approached both a Japanese restaurant and a Japanese fish market in Toronto, from which 5 workers agreed to participate in the study. Men were included in the Japanese cohort as the sample size was limited.

Participants were asked questions pertaining to their seafood consumption habits. To estimate methylmercury exposure participants were first asked how often they consumed seafood (number of servings) and the approximate size of each serving. Fish consumption was converted to meals-per-month. They were then asked about the types of seafood that they typically consumed. All subjects were asked to provide a hair sample. Hair was chosen as the matrix for analysis of mercury, as it reflects exposure concentrations over time, with each 1 cm of growth representing on average 1 month of growth. All consenting participants received a package containing a written consent form for hair analysis, a brochure outlining how to cut hair and a self-addressed stamped envelope.

Individual mercury intake estimates were calculated using average mercury concentrations for species of retail fish and shellfish sold in Canada provided by Health Canada (Health Canada 2008). A weight of 150 g was used as an average portion size (Bureau of Chemical Safety Health Canada 2007). To estimate methylmercury exposure the gram-per month amount was multiplied by the characterisitic methylmercury

concentration of each species (μg/g) and then was summed across species to give the average intake of methylmercury by each individual (μg/month).

Hair analysis

Analysis of the 65 collected hair samples was conducted at the London Health Sciences Centre (LHSC) Trace Elements Laboratory (London, ON). The instrument used was a Finnigan MAT Element High Resolution Inductively Coupled Plasma Mass Spectrometer (ICP-MS) that performs multi-element analysis in solution. ICP-MS analysis is an exellent technique for the clinical analysis of trace elements, achieving a detection limit of 0.01 μg/g hair.

Data Analysis

The relationship between the number of servings of fish eaten and hair mercury content, and the estimated dose of mercury and hair mercury content, were evaluated using correlation analysis. Because the data was not normally distributed, a Kruskal-Wallis test was used to compare group medians for hair mercury concentrations, number of servings and estimated dose of mercury among the 3 cohorts, then a Mann-Whitney U-test.

4.3 Results

Women in the Motherisk group consumed significantly less seafood while pregnant compared to when not pregnant (fig. 2 and fig. 3). Overall the majority of subjects consumed salmon, as the fish of choice and canned tuna at least 1-2 times a month when not pregnant. When comparing fish consumption habits among the 3 groups the median number of servings of fish per month of each group differed significantly

between the Japanese group and Motherisk callers ($p<0.001$); the highest among the Japanese group (10 servings), followed by the Motherisk callers (4). The Japanese also differed significantly from the Canadian women (3) ($p<0.0001$) (fig. 4). Using Motherisk callers' reports, their median consumption during pregnancy (rather than at the time of hair analysis) was 2 servings. The estimated median mercury intakes from fish consumption of the 3 groups followed the same trend: 130.0, 54.4, and 24.5 μg/month, respectively (fig. 5).

The median hair mercury concentrations of the groups differed significantly ($p<0.0001$) between the Japanese population (1.7 μg/g) and Motherisk callers (0.41 μg/g), and between the general population of Canadian women (0.15 μg/g) and the Motherisk callers ($p<0.001$) (fig. 6). Analysis shows no difference in these relationships between males and females in the Japanese cohort. Using the Motherisk caller's reports, their mercury hair levels during pregnancy would have a median of 0.28 μg/g (table 5).

Combining data across all 3 groups, the number of servings of fish significantly correlated with hair mercury content (Spearman $r=0.73$, $p<0.0001$; fig. 7). The estimated dose of mercury correlated stronger with hair mercury content (Spearman $r=0.81$, $p<0.0001$; fig. 8).

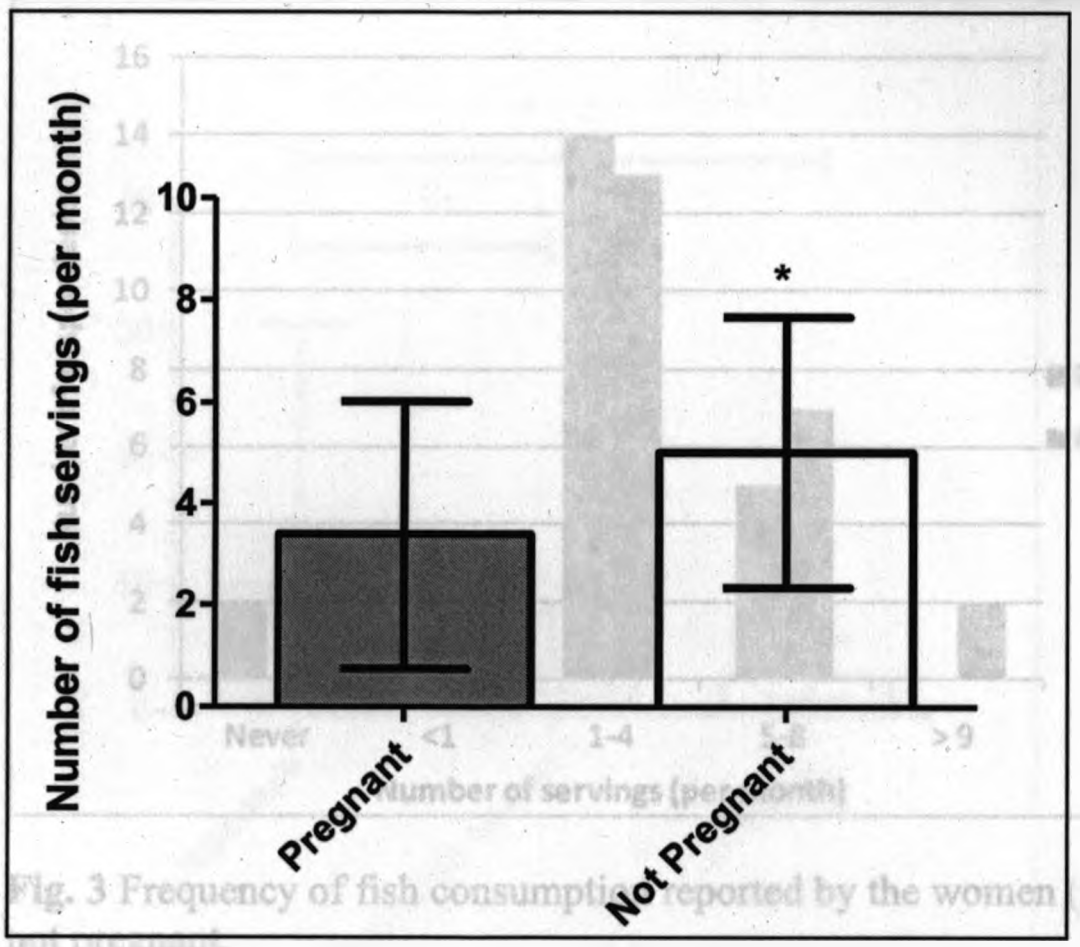

Fig. 2 Amount of fish consumed per month reported by women during pregnancy compared to when not pregnant. (Values plotted are mean ± standard deviation, n=22; Means were significantly different when compared by an unpaired t-test). (*$p<0.05$, vs. fish servings when pregnant).

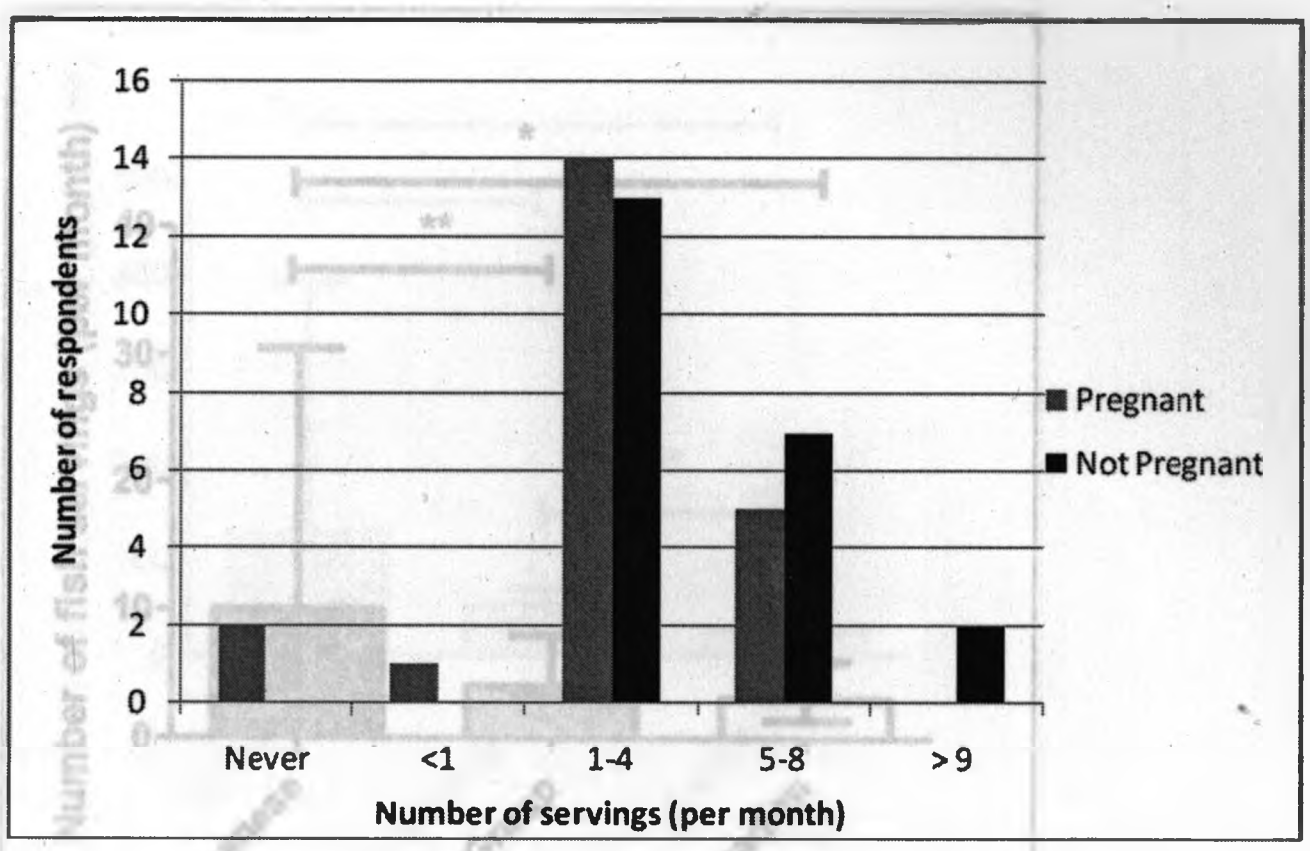

Fig. 3 Frequency of fish consumption reported by the women (n=22) when pregnant vs. not pregnant.

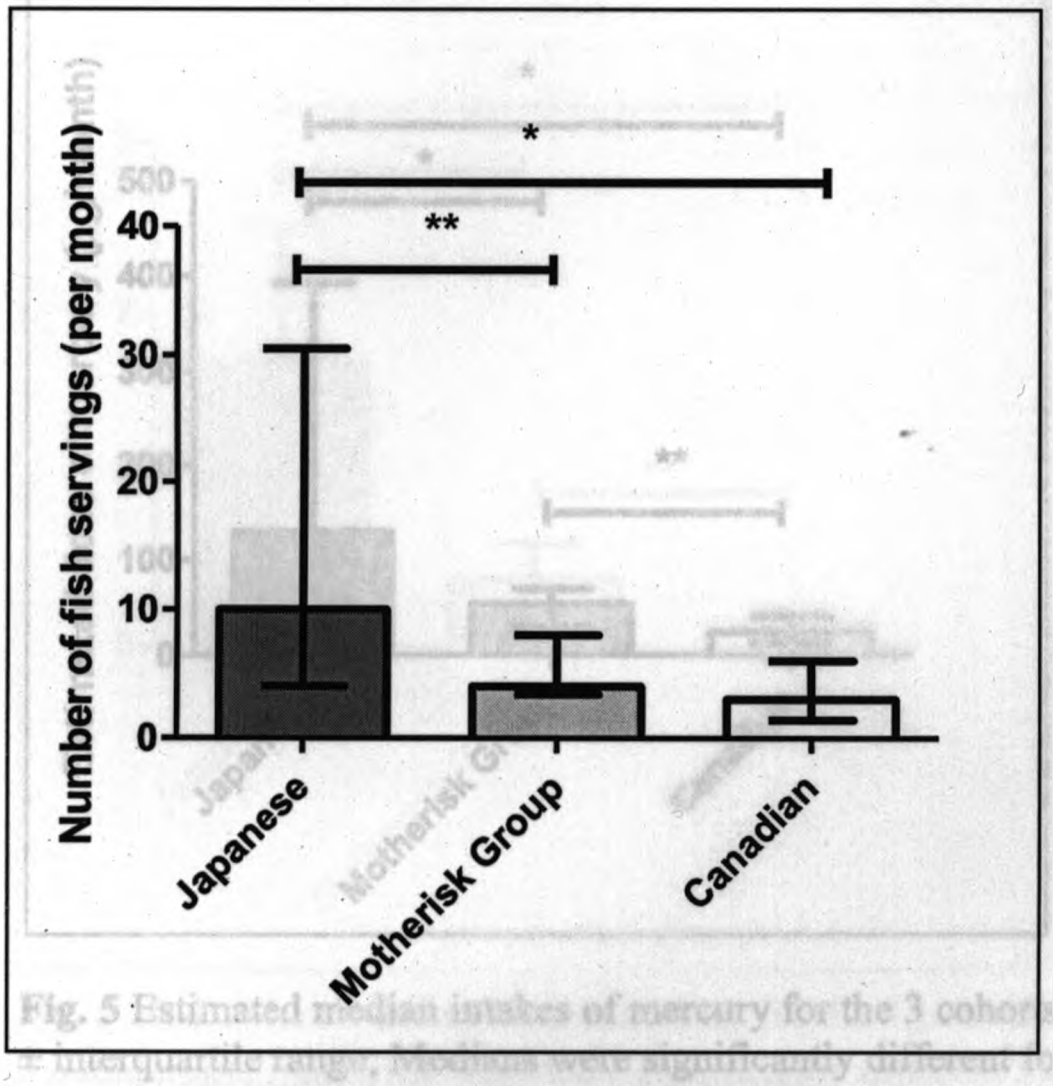

Fig. 4 Median number of fish servings reportedly eaten by the 3 cohorts (n=23, n=22 and n=20, respectively) per month, with calculated interquartile range. (Values plotted are median ± interquartile range; Medians were significantly different for the 3 cohorts when compared by a Kruskal-Wallis test followed by a Mann-Whitney U-test). (*p<0.001 vs. Motherisk Group and **p<0.0001 vs. Canadian group).

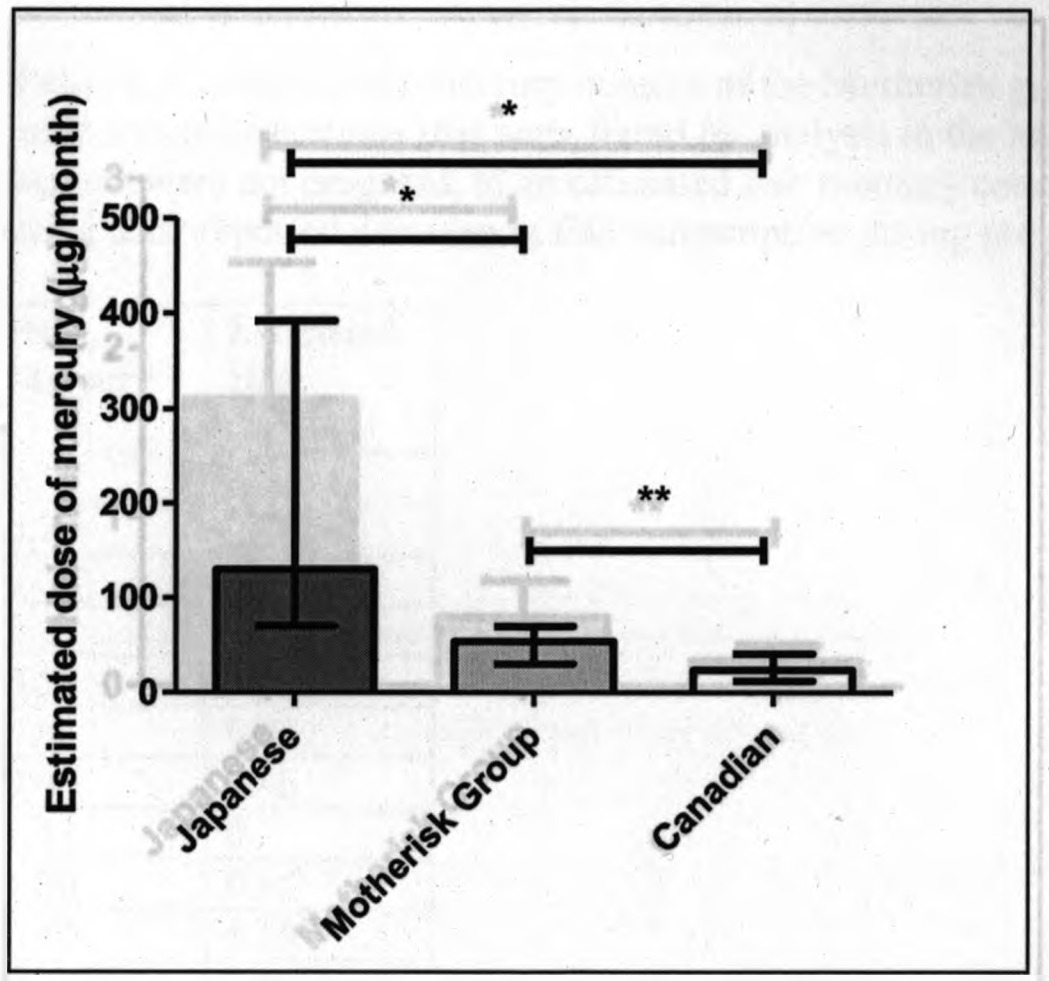

Fig. 5 Estimated median intakes of mercury for the 3 cohorts. (Values plotted are median ± interquartile range; Medians were significantly different for the 3 cohorts when compared by a Kruskal-Wallis test followed by a Mann Whitney U-test). (*$p<0.0001$;**$p<0.05$).

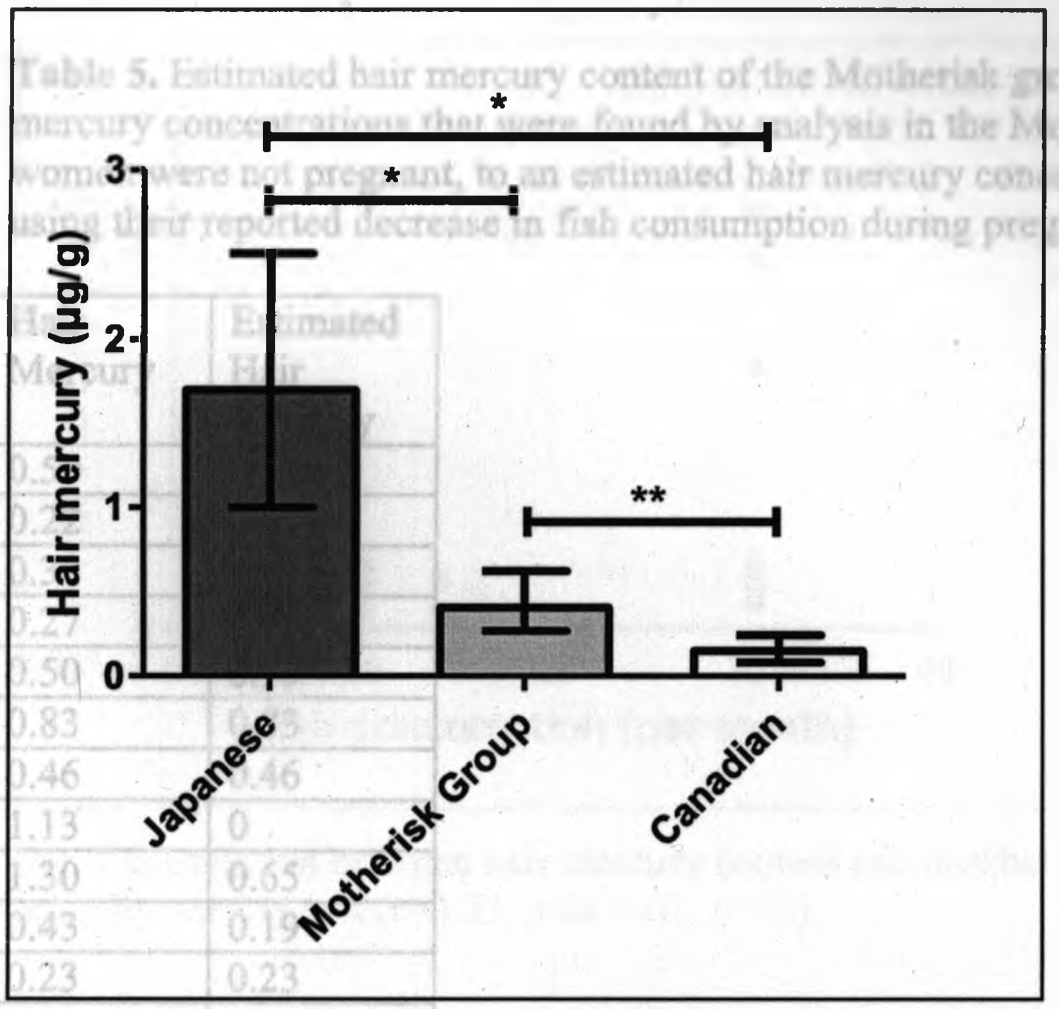

Fig. 6 Median hair mercury concentrations for the 3 cohorts. (Values plotted are median ± interquartile range; Medians were significantly different for the 3 cohorts when compared by a Kruskal-Wallis test followed by a Mann Whitney U-test). (*p<0.0001; **p<0.001).

Table 5. Estimated hair mercury content of the Motherisk group. Comparison of hair mercury concentrations that were found by analysis in the Motherisk group when the women were not pregnant, to an estimated hair mercury concentration while pregnant, using their reported decrease in fish consumption during pregnancy.

Hair Mercury	Estimated Hair Mercury
0.50	0.36
0.22	0.11
0.38	0.11
0.27	1.08
0.50	0.33
0.83	0.83
0.46	0.46
1.13	0
1.30	0.65
0.43	0.19
0.23	0.23
0.60	0.15
0.57	0
0.26	0.26
0.41	0.41
0.69	0.46
0.12	0.12
0.40	0.32
0.23	0.23
0.29	0.15
1.70	0.85
0.40	0.30

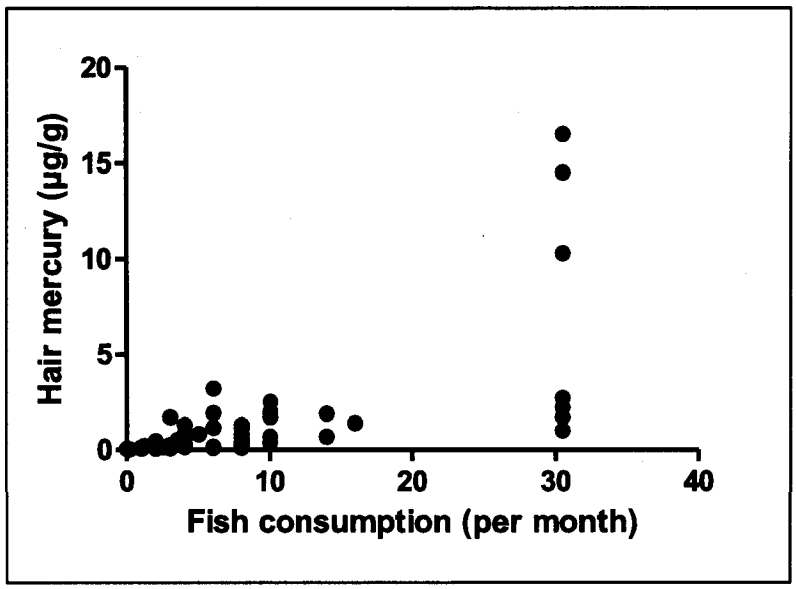

Fig. 7 Correlation between hair mercury content and number of fish servings reportedly eaten by participants ($r=0.73$, $p<0.0001$, $n=65$).

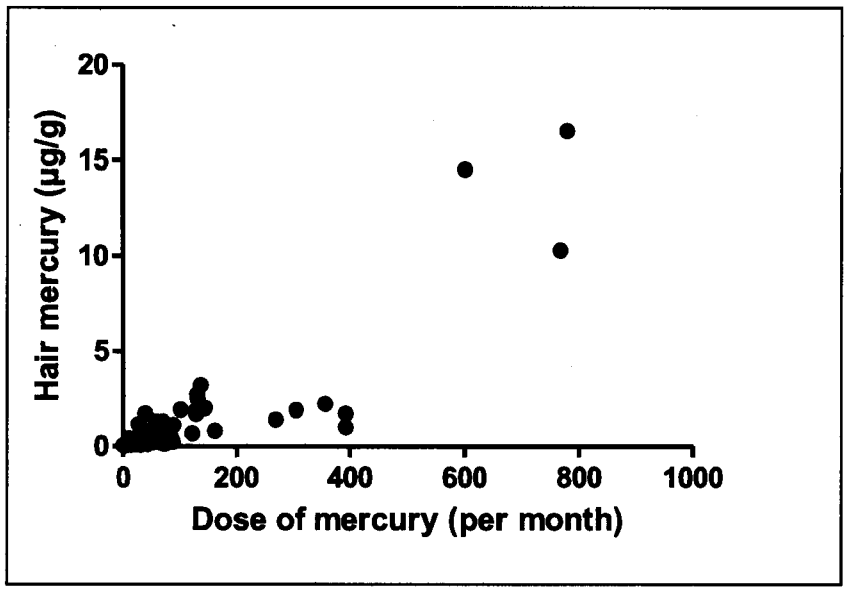

Fig. 8 Correlation between hair mercury content and estimated intake dose of mercury ($r=0.81$, $p<0.0001$, $n=65$).

4.4 Discussion

The present study examined fish consumption habits and mercury exposure in 3 cohorts. The median hair mercury content for the women of reproductive age who were concerned about consuming fish during pregnancy was 0.4 μg/g, compared with 1.7 μg/g for the Japanese population and 0.2 μg/g for the mainstream Canadian women that did not contact Motherisk. However, using the Motherisk caller's reports, their mercury hair levels during pregnancy would have a median of 0.3 μg/g (table 5). We corroborated a significant relationship between fish servings and hair mercury, a correlation that was improved when examining the association between calculated mercury intake and hair mercury content. The correlations presented here are comparable with those reported in many other studies in which hair mercury was correlated with fish consumption (Bjornberg *et al.* 2003; Daniels *et al.* 2004; Johnsson *et al.* 2004; Diez *et al.* 2008; Stern *et al.* 2001; Elhamri *et al.* 2007; Gao *et al.* 2007).

There are several limitations that may have affected the strength of these relationships in our study. First and foremost, the participants' ability to recall what they had eaten on a monthly basis is an important factor. A second source of uncertainty is the participants' knowledge of what exact species of fish they had consumed. A third limitation is that the exact size of each serving consumed by the participants was not known, but rather was estimated. Fourth, reference values of the average concentrations of methylmercury by fish species and further intraspecies variability in mercury concentrations are not well defined.

When comparing hair mercury results of the Motherisk group in the present study with other studies found in the literature similar results have been reported. Median

values for frequent fish consumers among women of childbearing age in NHANES were 0.33 μg/g (McDowell *et al.* 2004). Median hair mercury levels for 127 pregnant Swedish women were 0.35 μg/g (Bjornberg *et al.* 2003). The median hair mercury contents found in our study population were lower than those reported in studies conducted the Faroe Islands (median: 4.5 μg/g) (Grandjean *et al.* 1992) and Seychelles (median: 5.9 μg/g) (Myers *et al.* 2003). In the river basins of the Amazon, studies conducted of populations where freshwater fish forms the basis of their diets, median hair mercury levels typically range between 5 μg/g and 15 μg/g (Dorea *et al.* 2003; Dolbec *et al.* 2001; Lebel *et al.* 1997; Santos *et al.* 2000). These groups typically consume more fish than the general North American population and have higher methylmercury exposure.

We have recently defined a LOAEL for subtle fetal developmental defects caused by mercury exposure at 0.3 μg mercury/g hair, based on the systematic review of relevant longitudinal and cross-sectional studies (Schoeman *et al.* 2009). For this analysis we considered alterations in neurodevelopment in any of the studies reviewed, and adverse effects were not always observed at this threshold. While acknowledging the limitations of our defined LOAEL, in ensuring the health and development of fetuses and newborn babies, it is most reasonable to use the precautionary principle, based on measurable adverse outcomes in well designed studies. Nearly two-thirds of women in our sample who had called Motherisk had hair mercury content exceeding our LOAEL. This percentage dropped to approximately 36% when calculating their mercury exposure based on their reported fish consumption during pregnancy (table 5). All heavy fish eating Japanese participants exceeded our LOAEL, whereas only 15% of reproductive age women representing the general Canadian population did. Fish consumption habits

varied quire markedly between the Japanese population and the other two cohorts, which definitively explains the differences in hair mercury content (Knobeloch *et al.* 2005; Hightower *et al.* 2006; Mahaffey 2004; McKelvey *et al.* 2007; Vupputuri *et al.* 2005). A study conducted by Tsuchiya and colleagues found geometric mean hair mercury levels of 106 Japanese women of childbearing age, to be six-fold higher (1.2 μg/g) than the geometric mean obtained from the NHANES (0.2 μg/g) (Tsuchiya *et al.* 2008; McDowell *et al.* 2004). These results are similar to ours. Another study has shown that 73% of women of reproductive age from Japan, where more fish is consumed, have hair levels above 1.0 μg/g and 1.7% above 5 μg/g (Yasutake *et al.* 2004). The U.S. EPA has issued several proposals to the Japanese society to avoid the possible health hazards related to high mercury intake in high-risk populations such as young females and children (Mahaffey 2001).

In 2004, the U.S. FDA and EPA issued a joint warning to pregnant and nursing women, women of childbearing age and young children, to limit their fish intake to 12 ounces per week or 0.1 μg mercury/kg body weight/day (FDA 2004). Our study documents substantial overlap in the number of fish servings consumed per month, that were associated with hair mercury levels above or below our defined LOAEL.

The present study highlights the fact that fish consumption is the major source of mercury exposure for women of reproductive age in Canada. Due to the considerable uncertainty documented by us and others, specific guidelines for the number of servings of fish which are "safe for women or reproductive age" may not be sufficiently specific to practically prevent fetal risk. Analysis of hair mercury content should be suggested prior to pregnancy as dietary modification can decrease overall body content.

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Chapter 5: Maternal Fish Consumption and Mercury: Risk Perceptions of a Cohort of Women of Reproductive Age

5.1 Introduction

A well-balanced diet is important for pregnant women or those planning on becoming pregnant. Women of reproductive age are advised to consume fish because it is rich in essential nutrients such as high quality protein and omega-3 polyunsaturated fatty acids (n-3 PUFAs), which are essential for the perinatal growth of the developing brain (Neuringer *et al.* 1988). Health Canada recommends that women of reproductive age consume at least two Food Guide Servings (2 x 75 grams) of fish each week during pregnancy, as recommended in Canada's Food Guide (Health Canada 2008). They also suggest that women pay close attention to the types of fish that they eat, promoting fatty cold-water fish high in omega-3 (n-3 PUFAs) and low in mercury.

A major drawback of fish consumption is that some species of fish contain methylmercury, at sufficient amounts to cause adverse neurodevelopmental effects (Grandjean *et al.* 1994). Organic methylmercury is formed from inorganic mercury by the action of anaerobic organisms that live in aquatic environments, and it is difficult for fish to eliminate the heavy metal from their bodies, allowing methylmercury to bioaccumulate in predatory fish (Clarkson & Magos 2006). Dietary fish consumption is the major source for human methylmercury exposure (Clarkson *et al.* 2003). It is well known that both methylmercury and omega-3 concentrations vary amoung fish species, such that it is quite possible to optimize consumption of the latter while minimizing exposure to the former. Fortunately, fish with high omega-3 content do not necessarily contain high mercury (Gochfeld & Burger 2005). Of greatest concern are the predatory fish that contain the highest levels of methylmercury (Myers *et al.* 2007). Individuals who

consume fish, especially large predatory fish, on a regular basis can achieve a hair mercury level of 10 μg/g (Airey 1983), a threshold toxicological level defined by Clarkson and colleagues as being associated with adverse fetal effects (Clarkson *et al.* 2003).

The fetus is the most sensitive target to the adverse effects of methylmercury and therefore a source of major concern for pregnant women (United States Protection Agency 1997). Methylmercury crosses the placenta and is found at higher concentrations in fetal blood than in the mother's (Morrissette *et al.* 2004). Two epidemics in Japan and Iraq, in which large numbers of people were affected by methylmercury established the occurrence of fetal methylmercury poisonings, which resulted in severe neurodevelopment problems in the newborn baby (Amin-Zaki *et al.* 1974; Harada 1995). At present, accidental exposure to such high concentrations of methylmercury are rare and this has led to the general concern focusing mainly on more subtle effects that occur at much lower concentrations of methylmercury in heavy fish eating populations (Spurgeon 2006).

The two largest and most comprehensive studies that address the health effects of methylmercury in children from lower doses are the Seychelles Child Development Study (SCDS) and the Faroe Islands studies. The results from these studies are contradictory, as the Faroe Island study demonstrated dose-dependent nervous system effects (Grandjean *et al.* 1997), however the SCDS failed to find any adverse effects on child development (Davidson *et al.* 2008). The inconsistent results of these two studies are among hundreds of others, both cross-sectional and longitudinal, that have found varying conclusions reporting both adverse and beneficial outcomes.

Fish is a good source of n-3 PUFAs, providing significant health benefits. However, balancing the rewards and possible risks of fish consumption presents a dilemma to consumers, which could lead women to avoid this food source entirely. In 2004, the U.S. EPA and FDA issued a joint warning for women of reproductive age, pregnant women, nursing mothers and young children to limit their fish intake to 12 ounces per week due to potential mercury contamination in the fish (U.S Food and Drug Administration 2004). Following this advisory it was found that women were eating less than the recommended amount of fish, out of fear of harming their babies (Oken et al. 2003). This study concluded that a broadly disseminated health advisory may substantially change dietary behaviour among pregnant women. Following this advisory, there has been mass media coverage on the topic, presenting contradictory information regarding the benefits and risks of fish consumption. Contradicting information presented simultaneously can lead to confusion in the target publics, including scepticism in the media source, anxiety and stress (Vardeman & Aldoory 2008). The objective of the present study was to investigate what motivates women of reproductive age to avoid eating fish during their pregnancy and to understand their perceptions towards consuming fish.

5.2 Methods

Upon approval by the Research Ethics Committee at the Hospital for Sick Children in Toronto, Canadian women who had been counseled by the Motherisk Program between January 2006 and 2007 about the reproductive safety of consuming fish during pregnancy were identified. The Motherisk Program is an information and counseling service that assesses maternal/fetal risks following exposure to medications,

recreational drugs and various environmental chemicals during pregnancy and lactation. Potential subjects for our study were identified using a prospectively collected database. Women were excluded if they refused verbal informed consent, could not be reached by telephone, had insufficient English language to answer the questions or made it difficult to communicate over the phone, confirmed that they did not call about "mercury" in fish or had other mercury exposures (e.g. occupational).

Verbal consent for this study was obtained from the women before the start of the telephone interview after the study was fully explained. The interview consisted of a semi-structured questionnaire to assess fish consumption habits (presented in chapter 4) and their perceptions of risk. Women were queried about their perceptions of eating fish during pregnancy using 5 open-ended questions, allowing participants to introduce other issues and concerns. Women were questioned about their general knowledge of mercury toxicity, and what provoked them to initially call the Motherisk Program for information on consuming fish during pregnancy. They were then asked about how they initially became aware of the mercury issue and about the potential toxic health implications of consuming fish to their unborn child as well as their ideas regarding the health benefits. On a scale from 0 to 10 the women were asked how worried they were about consuming fish during pregnancy, 0 being the least worried, 10 being the most. Comments and discussion of ideas and concerns regarding fish consumption were encouraged.

5.3 Results

All callers who were counseled about mercury in fish during pregnancy by the Motherisk Program between January 2006 and January 2007 were identified ($n = 253$),

and consenting mothers who were accessible by telephone were contacted (n = 100). The demographics of the sample are presented in table 6.

There were multiple reasons that provoked these women to call the Motherisk program for guidance. Some aspects of mercury toxicity were well understood by respondents while others were poorly understood. Specifically, the majority of women were aware that eating fish high in mercury during their pregnancy could be harmful to their babies (n=90). Some concerned women were also aware that fish is a healthy food choice and thus wanted to include it in their diets (n=40). These women called for a definitive answer on how much seafood was safe to eat during pregnancy and the safe types of seafood. One quarter of the women were prompted to call for information after hearing about the issue through media sources or reading material, which lead them to question their regular eating habits. Some women called for clarity on the issue as the information given to them seemed to be controversial (n=9). These women had mentioned that there were two schools of thought regarding eating fish during pregnancy. Some women had heard about the mercury issue through family or friends, making them more nervous and prompting them to call to clarify (n=17). In a minority of cases their physicians had recommended the Motherisk program if they still had questions about the safety of consuming seafood (n=5). Two women were going on a vacation to the Caribbean and wanted to know what fish was safe to eat. One woman called out of general interest as she had heard about PCBs and pesticides.

Half of the participants stated that they initially became aware of the issue of mercury in fish through electronic and printed media (figure 9) and almost all had called for clarity after what they had heard from these sources. Those who had searched the

internet had found a vast amount of information, some described by them as dramatic and overstated (n=13). After reading the controversial and varying opinions they wanted clarity. Some women had initially heard about mercury in fish through talking with friends and family (n=31). Fifteen percent had learnt about the mercury issue through prenatal books and 3 of these women had said that it was specifically reading the book "What to Expect When You're Expecting." Another 3 women had stated that it was just a well-known fact.

Most respondents were unable to describe specific toxic effects of mercury (n=66), while 21% stated that mercury could cause neurological problems or affect brain development. Most of those 66 went on to describe why they chose to avoid fish even without ever knowing the toxic consequences. Some had answered that knowing mercury was potentially harmful for their babies was sufficient for them to be scared (n=16). Some said that methylmercury was a toxin which could be "detrimental to their babies' health" (n=7). Some could recall the warnings to avoid fish but not the consequences (n=3). Some stated that mercury could cause malformations, deformities, birth defects or abnormalities (n=12). Some women believed mercury exposure produced autism (n=7). Two women quoted issues of development and memory.

While most women did not know the harmful effects of mercury, most were able to quote benefits of eating fish during pregnancy (n=89). Forty-six of them enumerated n-3 PUFAs as the source of the health benefits and some of them mentioned that these were good for brain development. Some stated that fish were a good source of protein, nutrients and was a lean form of meat (n=9). A few women mentioned that they ate salmon specifically because it had high n-3 PUFAs content (n=5), while some stated that

they got their n-3 PUFAs from other sources such as fish oil or supplements during and after pregnancy to obtain the recommended amount (n=5). Ten women stated that due to the known health benefits, they were consuming fish despite the controversy or despite its taste. Three women mentioned that although they were aware of the benefits (n-3 PUFAs) the potential harm outweighed the benefits.

When asked to rate the level of anxiety regarding eating fish on a scale from 0 to 10, the majority of women ranked themselves at 5 (figure 10). Sixteen per cent of the women were "most worried" compared to only 1% that was not worried at all. Those who ranked themselves a 10, most worried, mentioned that they completely avoided eating fish (n=7). Forty-seven women went on to justify their ranking. Of interest, women who were not concerned (<5) mentioned they minimized their risk by avoiding seafood completely during their pregnancy (n=12). Some stated that they were <5 because they had called the Motherisk program and were aware of the safe types of fish to consume and what to avoid (n=4), while one woman stopped eating fish entirely after calling the Motherisk program. Three women were worried about the consumption of tuna and other large types of fish.

Table 6. Characteristics of study participants.

Variable	**Mean (SD)**
Age (yrs)	34.7 (4.6)
Parity (offspring)	1.4 (0.7)
Dental amalgams	55
Smoked during pregnancy	1

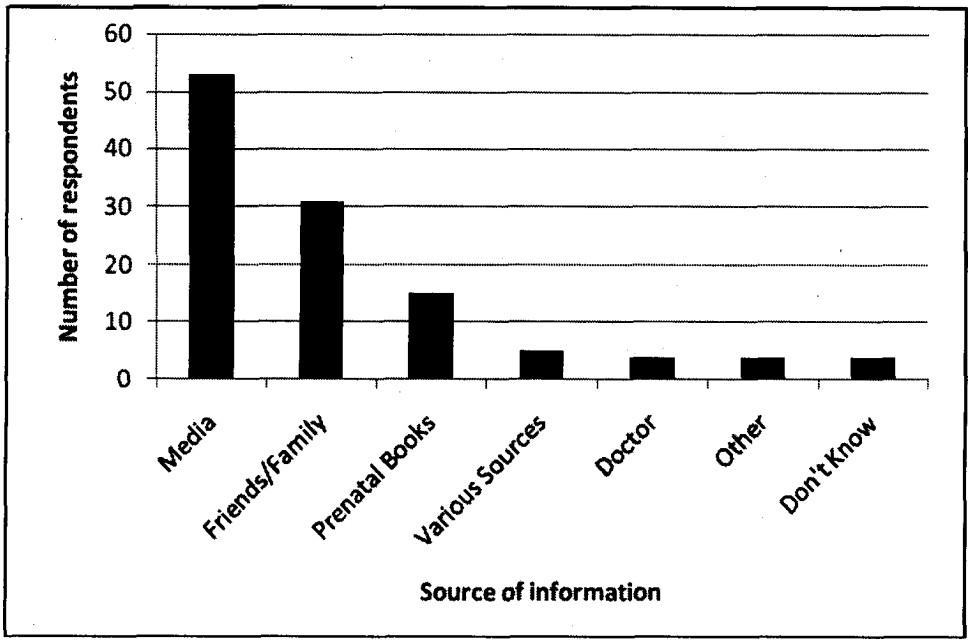

Fig. 9 Various sources of information on mercury.

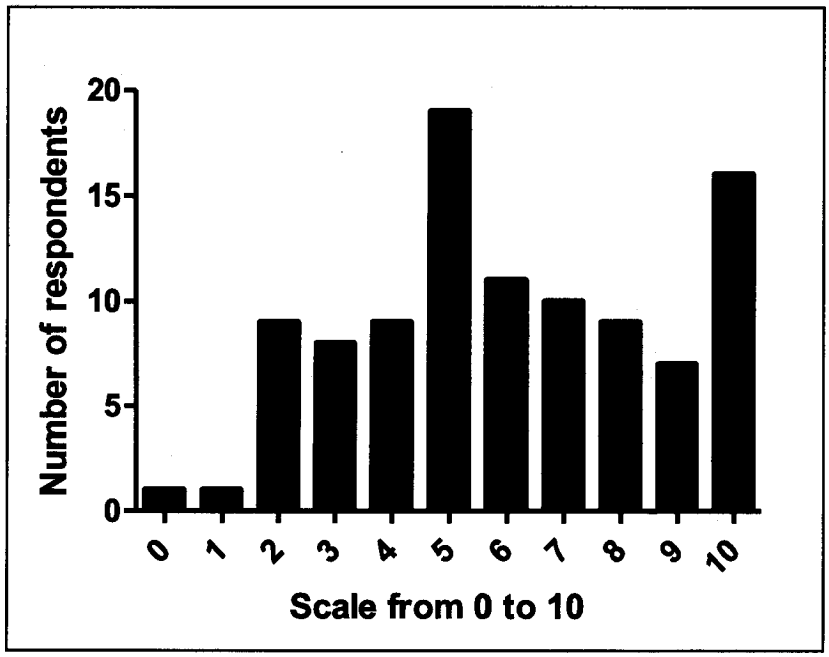

Fig. 10 Estimation of the level of concern in women towards mercury exposure through fish consumption.

5.4 Discussion

The response of these women, who were sufficiently concerned about fish consumption to warrant a call to a consultation service, shows perception of heightened teratogenic risk. We have recently completed a systematic review on the effects of methylmercury on the human fetus caused by *in utero* exposure through fish consumption in an attempt to define a LOAEL causing these effects (Schoeman *et al.* 2009). Both longitudinal and cross-sectional studies reported varying effects. We defined our LOAEL at 0.3 μg/g of mercury in maternal hair. It is acknowledged that there is considerable uncertainty with this estimation. Yet, in ensuring normal development of babies all around the world, it is most reasonable to use the precautionary principle, and set the LOAEL based on the lowest level of maternal hair mercury associated with measurable adverse outcome.

We subsequently collected hair samples from 22% of the women who completed the present questionnaire. Importantly, the results of their hair analysis showed that 64% of these women were above our LOAEL of 0.3 μg/g. With the mean mercury content of this cohort being at 0.5 μg/g. Overall these 22 women consumed a median number of 4 fish servings per month.

Our participating women are a self-selected group of concerned mothers-to-be, who had shown an initial concern regarding the safety of consuming fish and other seafood products during pregnancy. Their level of fish consumption was significantly higher than a comparison group of women who did not call Motherisk (Schoeman *et al.* 2009). Most of our participants in this study were confused over what was safe for their babies, as they had often been presented with contradictory information and called the

Motherisk program for clarity. Many women were conflicted in terms of trying to balance the benefits of fish consumption with the risks of exposure to methylmercury. It was evident from these results that even participants who were not concerned, said that they had minimized their risk by avoiding fish all together. Our data indicated that the heightened risk perception exhibited in this group is justified, based on their measured hair mercury, the most valid biological marker of long term exposure to this toxic metal.

Women of childbearing age, pregnant or breastfeeding should avoid large, top of the food web predatory fish with high levels of methylmercury, in order to avoid the harmful effects on their babies, but are not wise to entirely remove fish from their diets. Safety information on low levels of methylmercury needs to be addressed for management of a healthy diet in women of reproductive age. Health professionals can help women better understand the role fish plays in a healthy pregnancy. Given the large variability in the correlation between mercury intake and its hair levels, therapeutic monitoring using personal hair analysis and the development of precise individual dietary guidelines should be considered for women of reproductive age as a novel public health measure.

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Chapter 6: Baseline Monitoring for Mercury in Members of the Walpole Island First Nation (WIFN) to Help Assess the Health Risk from Environmental Exposure

6.1 Background

Walpole Island or Bkejwanong ("where the waters divide") is located on the large delta at the mouth of the St. Clair River which drains Lake Huron into Lake St. Clair. The St. Clair River forms part of the international boundary between Canada and the United States of America. Walpole Island is situated just downstream from Sarnia and "Chemical Valley," a site of more than 20 industrial complexes recognized to be heavy polluters into the neighbouring environment. There are approximately 60 factories and refineries located within 25 km of Sarnia and the WIFN, with 16 on the American side of the border (MacDonald & Rang 2007). Due to the close proximity of "Chemical Valley", Walpole Island has been subjected to the effects of both water and airborne pollutants for several decades (Bend *et al.* 2005). Some members of the WIFN community have developed an intense chemophobia of chemical pollutants, having serious social, cultural and psychological consequences on the community (Stephens & Darnell 2008).

Members of the WIFN have had a strong interaction with and reliance upon their surrounding environment for centuries. Water is of great importance to First Nations as it is used for nourishment of the land, creation and purification. It has provided a historical base for their economy and trade, as well as their traditional source of food. Fishing, hunting and trapping are essential parts of their culture and traditional lifestyle. Many WIFN citizens feel that both the island's water and food supply have been jeopardized by pollution (Darnell and Stephens in (Bend *et al.* 2005) pages 30-43).

Over several decades large amounts of toxic chemicals, including mercury, have been released into the St. Clair River either directly or indirectly from industrial processes. It is estimated that between 1974 and 1986, a total of 32 major spills, as well as hundreds of minor ones, were discharged into the St. Clair River, releasing at least 10 tonnes of pollutants into the river (Jacobs 1988). Methylmercury is a significant human health risk and thus is listed as 1 of the 11 targeted critical persistent pollutants of concern in the Great Lakes by the International Joint Commission (ATSDR 2008).

The WIFN community depends heavily on fish caught from this local waterway as a major food source, so the exposure levels and the effects of these contaminants on health are therefore of great concern. In 1969, mercury was discovered in the St. Clair River system which resulted in the closure of the WIFN commercial fishing industry for a decade. The ban of commercial fishing greatly affected the community, as it resulted in financial losses and unemployment that subsequently led to an economic depression for many of the community members and significant changes to their culture and traditional lifestyle (Stephens 2006). These high levels of mercury detected in Lake St. Clair were traced to huge releases from two chemical chlor-alkali plants, located near Sarnia that operated for many years (Marchand 1986). During this period approximately 400 tonnes of mercury were released into the St. Clair River. In 1986, the St. Clair River was identified as an "Area of Concern" (AOC) within the Great Lakes as a result of elevated contaminants in the water and sediments, primarily mercury (EPA 2008). Two First Nations communities: the Aamjiwnaang and Walpole Island are situated within the St. Clair River AOC. Mercury contamination is therefore of tremendous concern to community members in these areas.

The primary source of contamination to the residents of Walpole Island is the zone of sediment contamination downstream of Sarnia which serves as a pool of contaminants (Bend *et al.* 2005). The level of unaccepted sediment burdens for mercury in Ontario is 2 mg/kg (2 ppm) (Ontario Ministry of the Environment 2006). At least 30% of the sites sampled in the St. Clair River exceeded this guideline. Marvin and colleagues determined the spatial distribution of mercury in sediments of the Great Lakes basin and concluded that while most of the mercury in the Great Lakes was the result of natural atmospheric loading, the waters of the St. Clair River were primarily contaminated due to industrial loading (Marvin *et al.* 2004). They also concluded that the sediments were improving because the mercury concentration was decreasing, but failed to identify that this mercury was entering the biota, becoming more available to WIFN residents to eat as methylmercury enters the food web (Bend *et al.* 2005).

A decrease in mercury content of Lake St. Clair fish, specifically in smallmouth bass and pike, between the 1970s and 1980s was found, which has been attributed to the closure of the 2 polluting chlor-alkali plants which acted as point sources of mercury (Marvin *et al.* 2004). It is important to recognize that the size of the fish was considered in this analysis and thus declining mercury content was not due to sampling of smaller fish (Weis 2004). There was a subsequent increase in mercury found in analyzed fish caught from the St. Clair River and Lake St. Clair in the 1990s, which may be due to the mobilization of mercury and/or the conversion of inorganic mercury to methylmercury by anaerobic bacteria in sediments (Marvin *et al.* 2004). A significant number of individual predatory fish caught in 2005 from waters adjacent to Walpole Island (from Lake St. Clair), specifically rock bass, largemouth bass and smallmouth bass exceeded the 2005-

06 Ontario guideline value of 0.5 ppm mercury for non-predatory fish (Ontario Ministry of the Environment 2006; Bend *et al.* 2006). One large smallmouth bass exceeded the FAO/WHO guideline for predatory fish which is 1.0 ppm (Bend *et al.* 2006).

Investigators have shown that blood serum concentrations of mercury are significantly higher in consumers of fish caught from the Great Lakes compared to those in people who do not consume fish (ATSDR 2008). This finding, in addition to the fact that approximately 400 tonnes of mercury were released into the St. Clair River from industrial sources, indicates that exposure of methylmercury is possible in the residents of Walpole Island via fish consumption. The effect these environmental pollutants are having on the health of residents of Sarnia and surrounding areas including the Aamjiwnaang First Nation and Walpole Island First Nation could be significant (MacDonald & Rang 2007). Consequently it is crucial to implement programs of biomonitoring of environmental contaminants in humans living and working in this area, while simultaneously evaluating their health status.

In trying to understand the full impact environmental mercury has on First Nations populations, such as the WIFN, research endeavours must consider the social and cultural impacts and not only focus on the clinical effects that result from exposure (Wheatley 1996). Two integral members of our interdisciplinary research team, Regna Darnell and Christianne Stephens, have been working in close collaboration with the WIFN community for almost 20 years. As cultural anthropologists on the team, they have examined community perceptions of environmental risk using narrative ethnography during this time (Stephens & Darnell 2008). They have noted that concerns of expectant

mothers, and mothers who have small children, are generally focused on the adverse effects that exposure to environmental contaminants may have on their children's health. Children's health is of utmost importance to First Nations peoples. Birth defects that might be associated with exposure to chemicals are a major cause of anxiety, in addition to abnormal neurodevelopment that can result in cognitive defects, poor gross motor function, and behavioural problems. Some women believe that chemical exposure is to blame for their inability to conceive and or complications during pregnancy. A number of women have linked the high incidence of chemical spills to peaks in the number of miscarriages and stillbirths within the community. Heightened concern over the safety of fish in some cases, has led women to stop serving fish to their families (Stephens & Darnell 2008).

Fear of mercury exposure via fish consumption, an important source of traditional food, may lead to unnecessary changes in traditional lifestyle. Consequently it is crucial to implement proper analysis and monitoring of human health effects and environmental impacts in this area, to put potential risks into an evidence-based perspective. The objective of the present study was to determine the baseline level of mercury in hair and whole blood from at least 50 WIFN volunteers to help characterize their exposure level. This will serve as background information for a potential epidemiological study of the relationships between exposure to environmental contaminants and human health within this First Nation.

6.2 Materials & Methods

Ethics Board Approval

Ethics approval for all aspects of this study including biomonitoring, administration of the health status questionnaire and personal semi-structured interviews, was obtained from the Office of Research Ethics Review Board of the University of Western Ontario (Review number 13752E) and of the London Health Sciences Centre. The Chief and Band Council of the Walpole Island First Nation also passed two resolutions supporting this research.

Sample and Data Collection

Hair (n=55; 500 mg) and whole blood samples (n=56; 5 mL) were collected for mercury analysis from a total of 57 volunteers from the WIFN at the Walpole Island Health Centre on May 27, 2008. All volunteers were 17 years of age or older and provided informed consent.

Hair was collected from the posterior vertex region of the scalp from each volunteer, as close to the scalp as possible. The sample was taped to a piece of paper using 3M Micropore surgical tape, with the root of indicated. The sample was then placed into a clean envelope for transport and storage, at room temperature for approximately 1 month prior to analysis. Blood samples were collected and stored in EDTA Becton Dickinson (BD) Vacutainer tubes and were refrigerated at 5° C until analysis.

Information about the status of health of young children was obtained by administering a health status questionnaire, adapted from the 2006 Statistics Canada

Aboriginal Children's Survey, to volunteers who gave informed consent. The health status questionnaire was administered immediately after hair and blood samples were collected at the WIFN Health Centre. The questionnaire evaluated primarily the health status of those 19 years-of-age and younger to serve as a baseline for follow-up investigations. Information regarding consumption of fish and other traditional foods was also collected in this survey.

Analytical Procedure

Laboratory analysis for mercury in hair and whole blood was performed at the London Health Science Centre (LHSC) Trace Elements Laboratory for metal analysis. Samples of blood and hair were treated and digested as follows (London Health Science Centre Trace Elements Laboratory 2008). Hair samples were washed in 0.1% Triton X and rinsed 3 times in de-ionized water to remove external contamination. The samples were then dried in an oven at 70° C for 30 min and digested with redistilled nitric acid (HNO_3) for 1 hour. RBCs were separated and digested with equal amounts of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). All samples were diluted to 10 ml with purified water for analysis. Total mercury concentrations were determined using a highly sensitive Finnigan MAT Element Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) and monitored using quality controls and commercial hair standards. ICP-MS instruments combine the strengths of two established technologies: the ion source (or ICP), a well proven analytical source and a double focusing magnetic sector mass spectrometer used to separate the elements and their isotopes for subsequent detection and measurement.

This instrumentation permits analysis of mercury in hair or blood, at low resolution (300), with a detection limit of 0.01μg/g.

6.3 Results

Study population

The median age of the 57 study participants (volunteers) was 51 and there were 24 female and 33 male participants. A majority (58%) of the study population reported that they smoked, 41% of the females and 59% of the males. Almost all of the volunteers (87%) indicated that they ate fish; 57% ate small game; 85% ate large game and 89% ate water fowl. In terms of disease burden, an unexpectedly large number (36%) of study participants reported that they had diabetes.

Analysis of mercury in hair and whole blood

A significant correlation was found between mercury concentrations in whole blood and hair (Spearman $r=0.72$; $p<0.0001$; $n=55$; figure 11). The median hair mercury concentration was 0.23 μg/g with a range of 0.05-1.45 μg/g. The median mercury concentration in whole blood was 15 nmol/L with a range of 4.00-179.0 nmol/L. A significant relationship was found between hair mercury content and the number of fish servings consumed per month determined from the survey (Spearman $r=0.40$, $p<0.05$; figure 12). However there was no significant correlation of mercury in hair or blood with respect to gender (figure 13), smoking (figure 14) or age (figure 15).

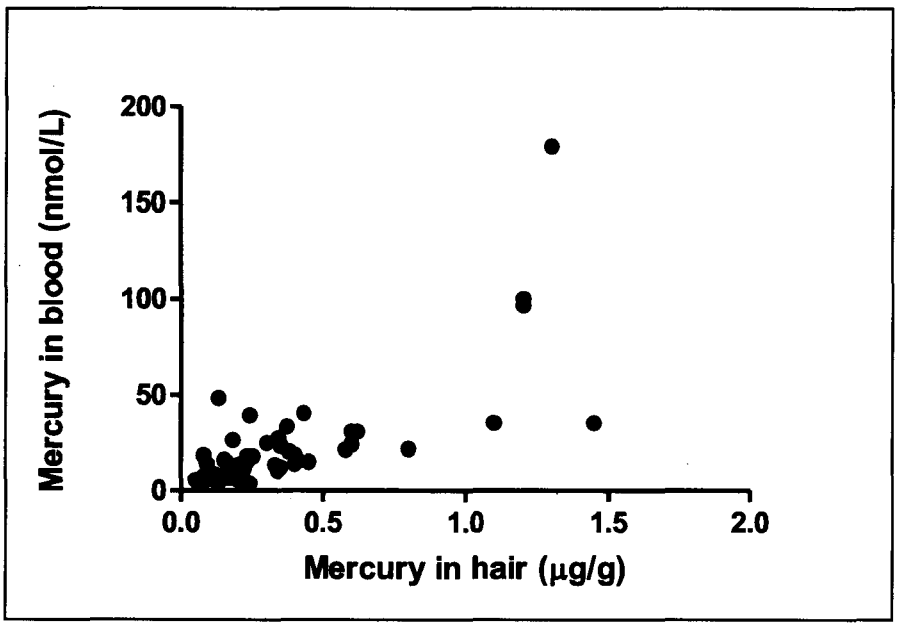

Fig. 11 Correlation between mercury in whole blood and mercury in hair (Spearman $r=0.72$; $p<0.0001$; $n=54$).

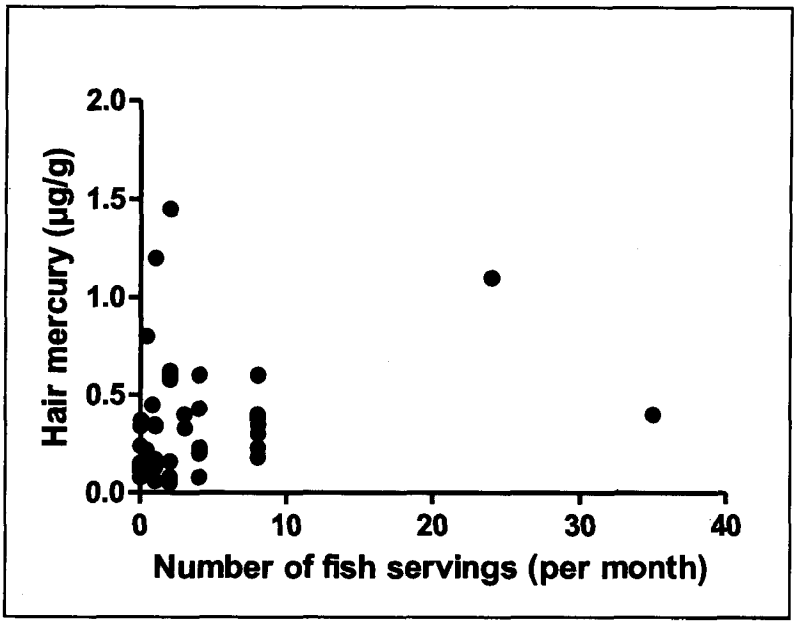

Fig. 12 Correlation between hair mercury content and number of fish servings reportedly eaten by WIFN volunteers (Spearman $r=0.40$; $p<0.05$; $n=45$).

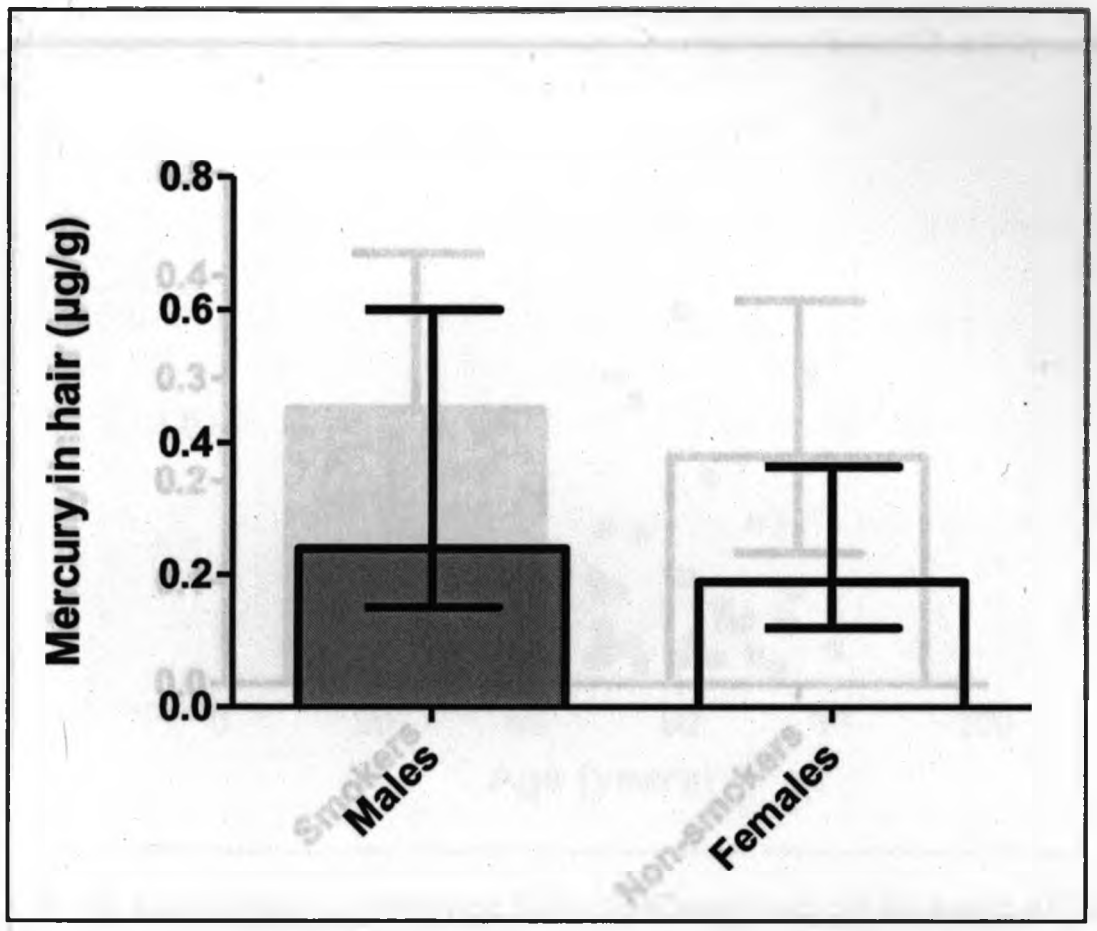

Fig. 13 Comparison of median hair mercury content in WIFN males vs. females. No significant difference was found when using a Mann-Whitney U-test. (Value plotted are median ± interquartile range, n=55). This was reflected in the blood.

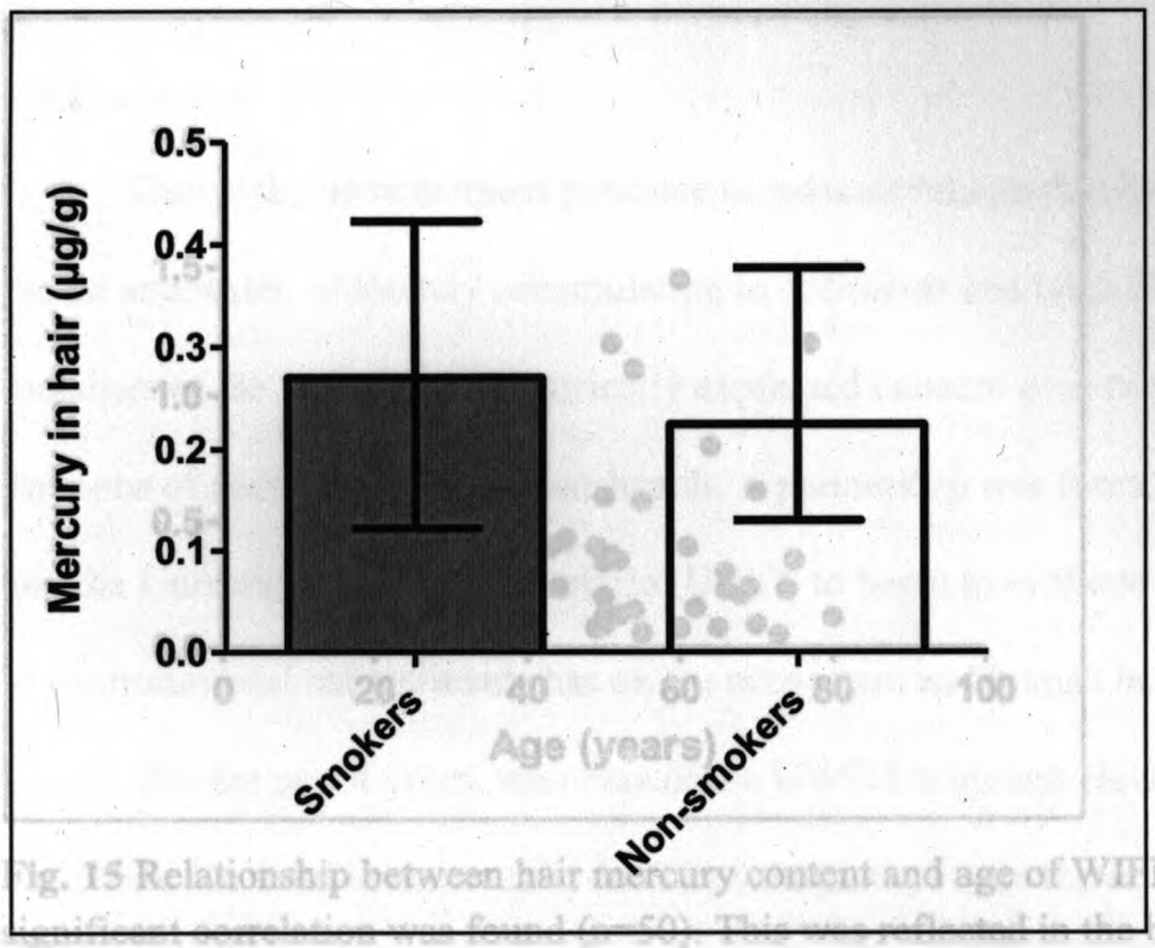

Fig. 14 Comparison of median hair mercury content in WIFN smokers vs. non-smokers. No significant difference was found between median hair mercury content in smokers vs. non-smokers when using a Mann-Whitney U-test. (Values plotted are median ± interquartile range, n=48). This was reflected in the blood.

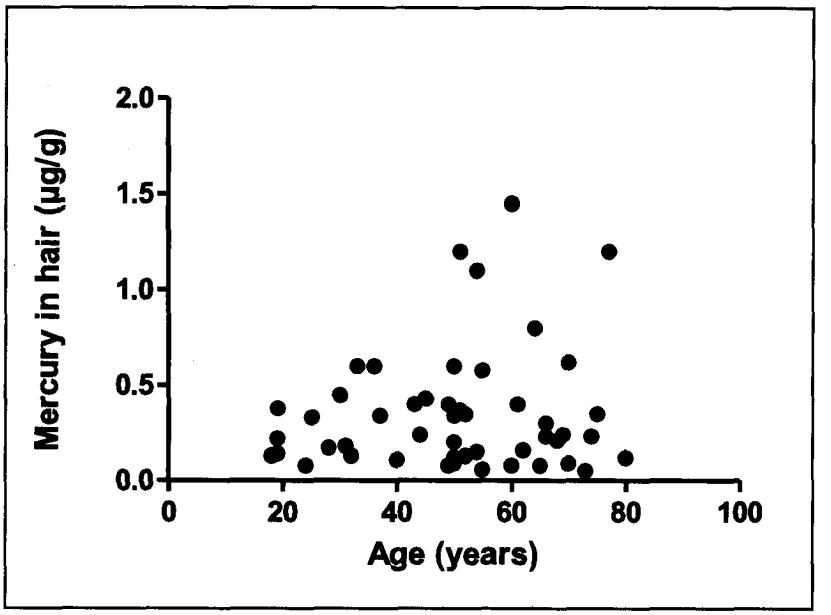

Fig. 15 Relationship between hair mercury content and age of WIFN volunteers. No significant correlation was found (n=50). This was reflected in the blood.

6.4 Discussion

Due to the demonstrated presence of toxic chemicals that have been released into the air and water, ultimately accumulating in sediments and biota of the Great Lakes, members of the WIFN have historically expressed concern over relationships between the presence of contaminants and their health. A partnership was formed between the WIFN and the University of Western Ontario (UWO) to begin to evaluate the impact exposure to environmental contaminants has on the ecosystem and human health of Walpole Island. For the past 4 years, members of the UWO Ecosystem Health Research team have been involved in community-based participatory research, which is an emerging model to enhance the relevance of biomedical research by involving community members (Westfall *et al.* 2006). In this specific case, the Director of the Walpole Island Heritage Centre invited the Schulich School of Medicine to become involved in this collaborative research project.

The first partnered collaborative community-based research project was the Health Canada financed 2004-05 Feasibility Study (Bend *et al.* 2005), which was funded through the Regional First Nations Environmental Contaminants Program (RFNECP). This preliminary research revealed that environmental degradation, industrial pollution and the threat of exposure to environmental contaminants were all major concerns of WIFN community members. Importantly, a strong willingness to participate in research investigating the health effects of environmental contaminants was found. Therefore, it was concluded that an epidemiological study with specific molecular endpoints was warranted in this community, to assess the health risk of the WIFN members to

environmental contaminants. As long as baseline monitoring was performed first to determine the current body burdens of environmental contaminants, especially mercury.

The results of the first project evolved into the second project, the Fish Consumption Study, which was also funded under the Health Canada RFNECP (Bend *et al.* 2006). Most importantly perhaps, this study revealed that 72% of the WIFN community members who participated did not consume fish at all during the 3-month survey period, further demonstrating the fear of consuming mercury-containing fish. Fourteen members (15%) had an estimated methylmercury intake, from fish only, between 0.22 and 1.44 μg mercury/ kg body weight/week, which is below the FAO/WHO provisional tolerable weekly intake (PTWI) of 1.6 μg/kg body weight/week. It is of considerable concern that 11 members (12%) had an estimated intake greater than the PTWI, particularly if these are females of reproductive age because the FAO/WHO guidelines are based on potential damage to the developing fetus.

Results from these two previous studies established the fact that WIFN community members are very concerned with environmental contamination by persistent pollutants, especially methylmercury. This "chemophobia," known as an intense fear of chemicals, has led community members to avoid eating locally-caught fish despite the positive economic and health benefits that are associated with fish consumption. The past two studies have provided the scientific foundation for the current 2007-2009 baseline biomonitoring study which is reported here.

By monitoring exposure to mercury and the selected health effects among members with diets typically characterized by fish consumption we hoped to: 1) define

strategies to limit adverse effects if exposure was high, 2) initiate remedial action in health risk assessment and preventative care if required, 3) provide information that may help to enforce new protective fish eating guidelines, and 4) decrease perceived risk through determination of current body burdens, effective dissemination of these data throughout the community, and education.

The main objective of the current study was to determine the baseline concentration of mercury in hair and whole blood samples of WIFN community volunteers. Hair and blood mercury contents are frequently used as biomarkers for estimation of mercury exposure and the related potential health risks. Blood is often used to evaluate most recent methylmercury exposures in fish eating populations, assuming that inorganic mercury concentrations in the blood are low (Grandjean *et al.* 1992; Grandjean *et al.* 1997; Schober *et al.* 2003; Weil *et al.* 2005). In blood, more than 90% of mercury is bound to haemoglobin, and therefore mercury content of RBCs is sometimes used as an estimate of exposure (Sakamoto *et al.* 2004; Skerfving 1988).

Hair has also been widely used as an indicator of mercury exposure, as mercury incorporates into the hair follicle during hair formation and over 80% of hair mercury is in the form of methylmercury (Cernichiari *et al.* 1995). Once incorporated into the hair strand, methylmercury concentrations remain stable. In terms of the best medium for estimating exposure, both provide advantages, as blood predicts most recent exposure and hair allows for a recapitulation of exposure over an extended period of time. Mercury in hair is associated with mercury in the blood, as shown in figure 11 and reported earlier

by others (Kershaw *et al.* 1980). The hair to whole blood concentration ratio is 250:1 (Cernichiari *et al.* 2007), a ratio corroborated in this study.

We tested to determine if there were statistical differences between hair or blood mercury concentrations in males vs. females (figure 13), and in smokers vs. non-smokers (figure 14) amongst the WIFN volunteers. We did not find a significant difference in either comparison. The age of the volunteer also did not correlate with hair or mercury content (figure 15). Conversely, a strong correlation was obtained between mercury content and fish consumption (figure 12), regardless of the group evaluated. This corroborates the idea that fish consumption is the most significant predictor for mercury content in hair or blood, which has been previously demonstrated by various researchers (Johnsson *et al.* 2005; Diez *et al.* 2008; Gao *et al.* 2007; McKelvey *et al.* 2007; Cole *et al.* 2004; Gerstenberger *et al.* 1997). Previous studies have also failed to show any significant effect of gender or age on methylmercury concentration in hair or blood (Barbosa *et al.* 2001; Cheng *et al.* 2009). Similarly, smoking did not have a significant effect on mercury concentrations in hair or blood in previous biomonitoring studies (Dewailly *et al.* 2001; Xue *et al.* 2007), providing recent precedents for our results.

Comparison of hair mercury contents of WIFN volunteers with data from the U.S. NHANES shows similar levels of exposure in both cases. Estimates of exposure using hair mercury concentrations were assessed among 1,730 women who were participants in the 1999 and 2000 NHANES (McDowell *et al.* 2004). The geometric mean (standard error) hair mercury content was 0.20 μg/g (0.02) in the women who participated in this survey. The geometric mean of hair mercury content found in the WIFN population was

0.25 μg/g (0.04), very similar to the NHANES value. Among frequent fish consumers in the NHANES study, geometric mean hair mercury levels were 3-fold higher for women (0.38 vs. 0.11 μg/g) compared with non-consumers. Of importance with regard to potential adverse effects on the developing fetus, the geometric mean hair mercury content of WIFN volunteers fell below that of the frequent fish consumers in the NHANES study.

Mercury exposure in the WIFN was also compared to 3 other cohorts; 1) Canadian women of reproductive age who had phoned the Motherisk program at the Hospital for Sick Children in 2006-2007 for information on the safe consumption of fish during pregnancy (n=22), 2); Canadian women of reproductive age who did not consult the Motherisk program (n=20); and 3) Japanese citizens residing in Toronto who typically consume large amounts of seafood as part of their normal diet (n=23).

Comparison of fish consumption habits among these 4 groups showed that the median number of servings of fish eaten per month differed significantly between the WIFN volunteers and Motherisk callers ($p<0.001$), and the WIFN volunteers and the Japanese population ($P<0.0001$). In these 3 groups, the lowest number of servings were reported by the WIFN population (2 servings per month), followed by the Motherisk callers (4 servings) and Japanese population (10 servings) (figure 18). The median hair mercury content followed the same trend: 0.23 μg/g, 0.41 μg/g and 1.7 μg/g, respectively (figure 19). This demonstrates that the WIFN population is consuming smaller amounts of fish than originally expected, which is responsible for their relatively low hair mercury concentrations compared to the Motherisk and Japanese groups living in Toronto.

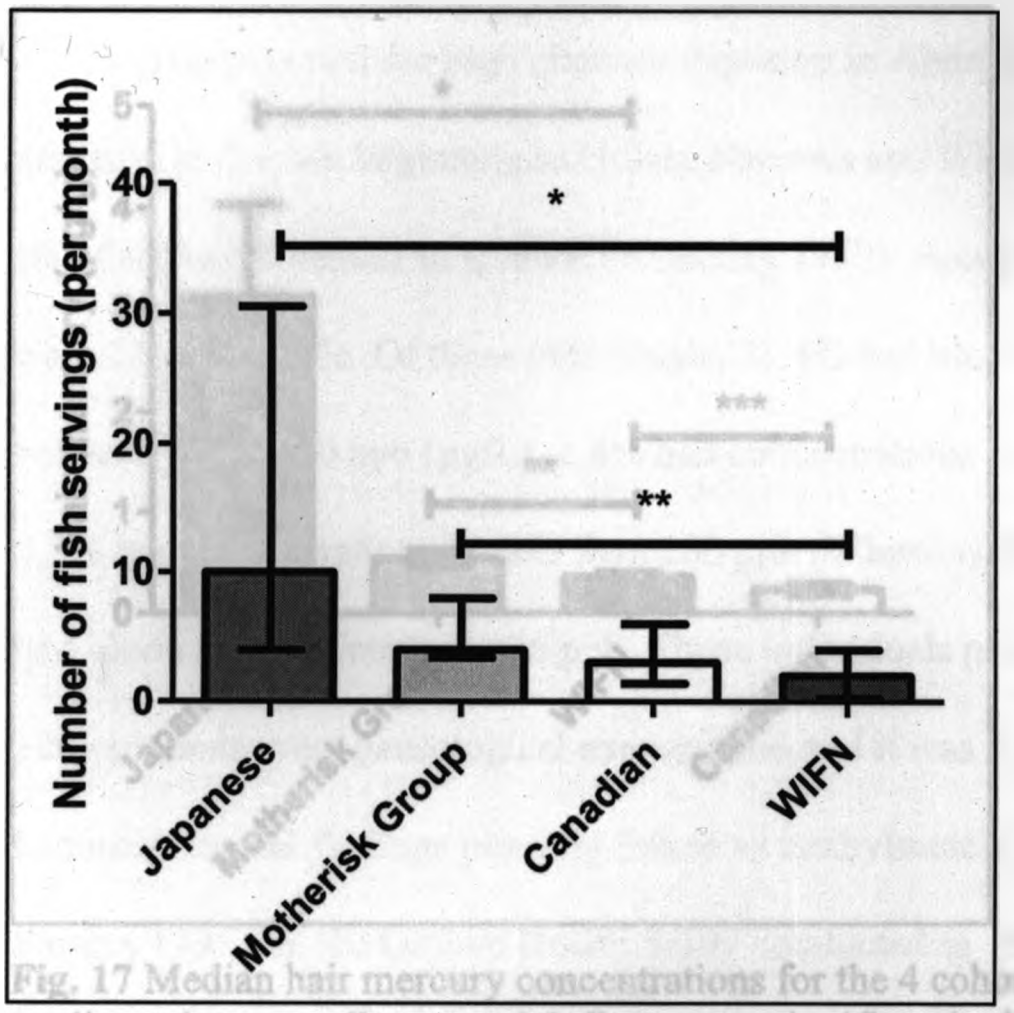

Fig. 16 Median number of fish servings reportedly eaten by the 4 cohorts (n=23, n=22, n=20 and n=45, respectively) per month, with calculated interquartile range. (Values plotted are median ± interquartile range; Medians of 2 groups were significantly different from the WIFN when compared by a Kruskal-Wallis test followed by a Mann-Whitney U-test). (*$p<0.0001$,**$p<0.001$).

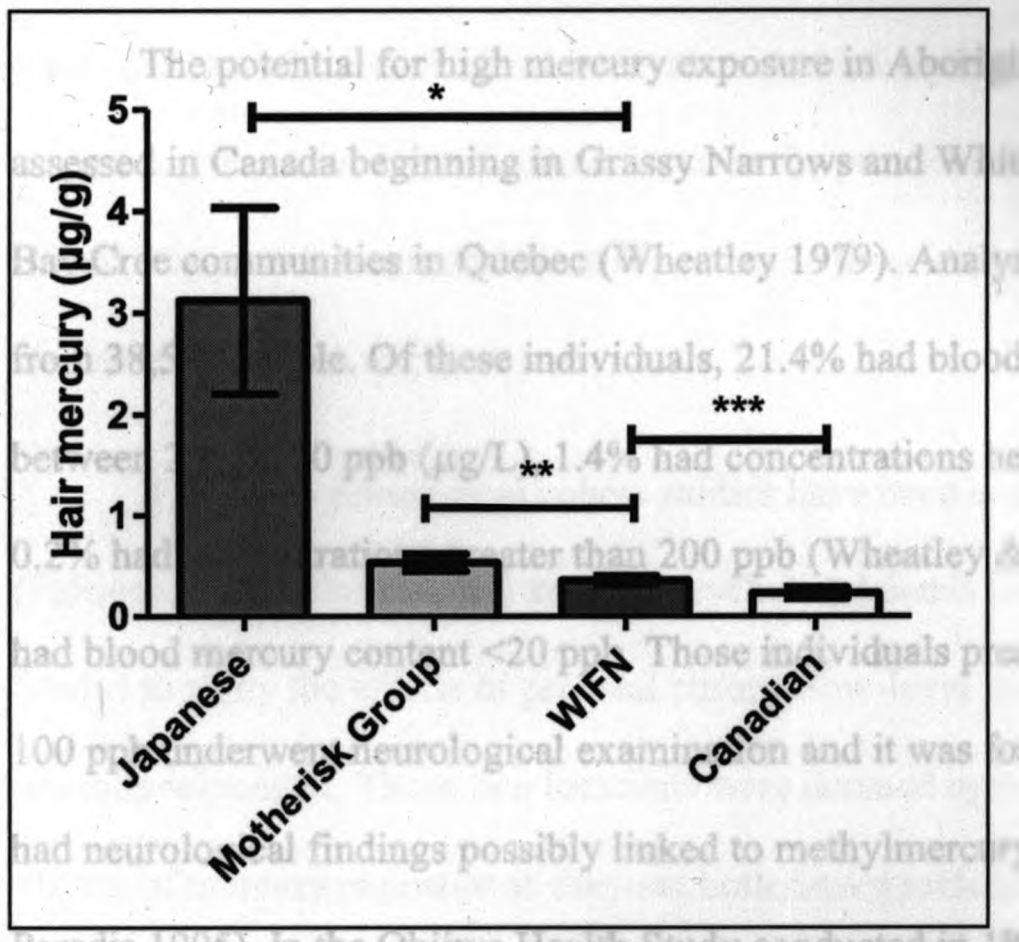

Fig. 17 Median hair mercury concentrations for the 4 cohorts. (Values plotted are median ± interquartile range; Medians were significantly different from the WIFN when compared by a Kruskal-Wallis test followed by a Mann-Whitney U-test). (*$p<0.0001$, **$p<0.01$, ***$p<0.05$).

The potential for high mercury exposure in Aboriginal populations was first assessed in Canada beginning in Grassy Narrows and Whitedog in Ontario, and James Bay Cree communities in Quebec (Wheatley 1979). Analysis was performed with blood from 38,571 people. Of these individuals, 21.4% had blood mercury concentrations between 20 and 90 ppb (μg/L), 1.4% had concentrations between 100 and 199 ppb and 0.2% had concentrations greater than 200 ppb (Wheatley & Paradis 1995). The remainder had blood mercury content <20 ppb. Those individuals presenting with levels greater than 100 ppb underwent neurological examination and it was found that approximately 10% had neurological findings possibly linked to methylmercury exposure (Wheatley & Paradis 1995). In the Objiwe Health Study conducted in 1993-2003, relatively low hair mercury concentrations (< 3 ppm) and blood mercury concentrations (< 55μg/L) were found in 89 participants (Gerstenberger *et al.* 1997). In a follow-up study, again over 90% of the participants had a total blood mercury concentration of 2.6 ppb. The overall average for all participants was 1.6 ppb (Dellinger 2004). Compared to other studies of heavy-fish eating populations, these exposures were relatively low.

High mercury concentrations in fish have also been a major problem for heavy fish-eating Inuit populations. A study of 492 participants from an Inuit population in Nunavik (Arctic Quebec) revealed geometric mean blood levels of 79.6 nmol/L (range=4-560 nmol/L) (Dewailly *et al.* 2001). When analyzing data by specific ethnic groups in the 1999-2002 NHANES survey, it was found that study subjects who were self-identified as Asian, Pacific Islander, Native American, or multiracial had a higher prevalence of elevated blood mercury than all other racial/ethnic participants in the survey. An estimated 16.59 ± 4.0% (mean ± SE) (n=140) had blood mercury levels > or

=5.8 µg/L, and 27.26 ± 4.22% had levels > or =3.5 µg/L (Hightower *et al.* 2006). When comparing these results with those found in the present study, mean blood levels of mercury are much lower and more reflective of those that were found in the Objiwe Health study.

Two large prospective cohort studies have been conducted in the Faroe Islands (Faroese Birth Cohort Study) and the Seychelles Islands (Seychelles Child Development Study) to study the effects of prenatal chronic low-level methylmercury exposure on neurodevelopment. These two locations were deemed optimal populations to study the effects of mercury exposure as they are both heavy seafood consuming communities. In the Faroe Islands, methylmercury exposure comes mainly from consumption of pilot whale meat which can be very high in mercury (up to 150 ppm) (Juhshamn *et al.* 1987). Median maternal hair mercury level in this cohort was found to be 4.27 µg/g (ppm) (Grandjean *et al.* 1997). In contrast to Faroe Islanders, the Seychellois regularly consume large quantities of ocean fish. The mean maternal hair level was 6.8 ppm (Davidson *et al.* 1998). Both the median (0.23 µg/g) and mean (0.34 µg/g) hair mercury concentrations found in the WIFN are well below (from 3.4% - 8%) the exposure values found in these two heavy fish-eating populations.

Segments of the human population who consume large amounts of fish and wildlife are purported to have higher than average risks to adverse health effects caused by mercury (Berkes 1990). There have been attempts to quantify the level of risk in populations from consuming large quantities of freshwater fish as briefly described above. After comparison of mercury exposure within the WIFN volunteers with other

First Nation, Inuit and other heavy-fish eating populations, it is evident that both fish consumption habits and mercury exposure values are lower in the WIFN. This self-imposed dietary restriction might be attributed to the chemophobia that exists in the community, or perhaps it is related to the impact mercury contamination had on the once vibrant fishing industry that was closed in the 1970s because of high mercury levels in St. Clair fish (Bend *et al.* 2006). Thus, considering that all First Nation and Inuit populations are heavy fish eaters and therefore are at greater risk to mercury exposure than the population sampled by NHANES, may be a potential bias that should be avoided.

A systematic review was conducted of all relevant research in humans focusing on the neurodevelopmental effects caused by prenatal exposure to mercury through maternal fish consumption. After inspection of the data, we defined the lowest observable adverse effect level (LOAEL) as 0.3 μg/g maternal hair mercury. No adverse outcome on child development has been observed anywhere at a lower concentration of maternal hair mercury. Comparing the results from this study cohort with the defined LOAEL, we have found that 36% of the WIFN group had hair mercury content that exceeded the conservative LOAEL and 7% were at the LOAEL. Of the 36% that exceeded the LOAEL, only 3 were women of reproductive age (18-45). Of the 7% that were at the LOAEL, one was a female of reproductive age.

Environmental contaminants, including mercury, frequently have far-reaching consequences for Aboriginal people. In Canada, as well as the medical concern of neurotoxicity, there are also a number of socio-cultural factors that impact the health and well-being of indigenous populations due to mercury (Wheatley & Paradis 1996). The

indirect negative effects of mercury exposure on Aboriginal populations include dramatic and unwanted changes in lifestyle (Wheatley & Wheatley 2000). The threat of mercury contamination to human health has led to the disruption of traditional practices and eating habits which has resulted in significant socio-economic and socio-cultural consequences (Erikson 1994). Economically, fishing skills initially learned for survival can provide employment opportunities such as guiding or outfitting sports fishermen, but not the employment for all those who are willing to work in a thriving fishery (Wheatley 1998). From the cultural aspect, the traditional knowledge involved in fishing can be transmitted to younger generations. In the 1970s, when commercial fishing was banned in Lake St. Clair, there were many negative consequences that impacted the community. Boys and young men lost the opportunity to learn the fishing trade from their fathers, and to financially benefit from bountiful natural resources on the traditional lands of the WIFN. A change in diet from high protein low in saturated fat food sources, such as fish, to a high carbohydrate diet, is now known to be detrimental to health and is associated with a change from an active social outdoor lifestyle, to one that is less-active, contributing to the risk of obesity, diabetes and cardiovascular disease (Rode & Shephard 1994). This is likely to be an important contributing factor to the very high incidence of diabetes reported in 36% of our volunteers, a disease that is widely recognized to have become a serious health problem among many Aboriginal populations in North America (Young *et al.* 2000).

6.5 Conclusion

Consumption of fish is the primary means by which humans are exposed to methylmercury. Although it is thought that First Nation communities rely heavily on fish as a source of food, making them more likely to be exposed to mercury, this did not prove to be the case in our study population. The impact of perceptions of risk may be greater than the actual risk from mercury at concentrations present in fish today. Perceptions of changes and the disruption of the relationship that Aboriginal people have with the environment may be the greatest problem (Wheatley 1996). Tension has been building within the WIFN community, as they are living in what is thought to be a "toxic" area. Anxiety has driven many community members to avoid or eat less fish and consequently, their current hair mercury levels are equivalent to NHANES data, lower than those found in two referent cohorts (analyzed by us) and also lower than other heavy fish eating populations reported in the literature. Although these low mercury exposure results are reassuring for most, some community members were found to have mercury concentrations exceeding those that have been reportedly associated with subtle neurodevelopmental deficits in other populations. These "high-risk" members, especially the females of reproductive age, will be counselled by one of the clinicians on the Ecosystem Health Research Team.

Fish consumption is a valuable source of protein and omega-3 fatty acids and thus the benefits of eating a fish diet must be continually emphasized. Community members might be counselled to eat more non-predator fish rather than predator fish; to eat small rather than large game fish; and to eat fewer fatty fish, which accumulate higher levels of

mercury. Guidelines for fish consumption specific to this First Nation community are warranted at this time to promote fish consumption, while minimizing fetal risks to mercury exposure, and will be developed as a follow-up to this baseline monitoring study.

In addition to providing a vital component of the diet, the act of fishing was traditionally an integral part of the culture, lifestyle and socio-economic well-being of the WIFN community. Our goal is to continue to monitor exposure to mercury and other persistent environmental pollutants over time, with the aim of determining whether or not environmental exposures are decreasing with time. We believe that reliable and current data and education are the best ways to reduce chemophobia and to restore the overall traditional and cultural lifestyles of WIFN community members as much as possible.

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Chapter 7: Overall Discussion. Methylmercury: A New Indication for Therapeutic Drug Monitoring

7.1 Introduction

Methylmercury, a contaminant in fish, is a known neurotoxin. The developing human brain is inherently more susceptible to injury by toxic agents, including methylmercury, than is the brain of an adult (Dobbing 1971). Evidence has accumulated over the last few decades demonstrating that methylmercury exposure can cause damage during neurodevelopment. During fetal development, the placenta is not an effective barrier against environmental pollutants (Andersen *et al.* 2000), as methylmercury readily crosses the placenta and has been found at higher concentrations in umbilical cord blood than in maternal blood (Morrissette *et al.* 2004). The blood-brain barrier does not fully develop until about 6 months after birth, potentiating the vulnerability of the fetus to many chemicals (Adinolfi 1985). Developmental neurotoxicity in children exposed to industrial chemicals or their by-products is primarily recognized by observing functional abnormalities that present following exposure to toxic concentrations (Grandjean & Landrigan 2006).

The adverse neurodevelopmental effects on the fetus caused by prenatal high-dose methylmercury exposure are well known because of two catastrophic poisoning events, one in Minimata, Japan (Harada 1995) and the other in Iraq (Amin-Zaki *et al.* 1974). In the majority of cases of women exposed to high concentrations of mercury via food, profound neurodevelopmental disorders were found in infants of asymptomatic mothers. These large scale mercury poisonings have contributed significantly to our understanding of fetal mercury toxicity. Today exposures to such high concentrations are unheard of,

which has led to an important focus on more subtle effects that occur at lower, environmentally relevant mercury concentrations, typically following chronic fish consumption. For example, A New Zealand study reported decrements in IQ in children whose mothers had mercury concentrations greater than 6μg/g hair (Kjellstrom *et al.* 1989). A second longitudinal study, conducted in the Faroe Islands found evidence for decrements in functional domains of language, attention and memory following prenatal mercury exposure (<10μg/g hair). (Grandjean *et al.* 1997). Results from several cross-sectional studies are consistent with these adverse effects caused by much lower doses of mercury (Barbone *et al.* 2004; Cordier *et al.* 2002; Foldspang & Hansen 1990; Gao *et al.* 2007; Lederman *et al.* 2008; McKeown-Eyssen *et al.* 1983; Murata *et al.* 1999; Ramirez *et al.* 2003; Saint-Amour *et al.* 2006; Stewart *et al.* 2006; Weihe *et al.* 2002; Xue *et al.* 2007). Therefore, strong evidence exists that methylmercury causes fetal neurotoxicity at varying exposure levels. Neurobehavioural damage caused by methylmercury can be prevented by controlling the amount of methylmercury consumed, mainly by eating the recommended safe types and amounts of fish low in methylmercury. Global food safety authorities have established guidelines for fish consumption during pregnancy and references doses for mercury consumption; however, these vary across different regulatory bodies and jurisdictions. These differences create confusion and uncertainty in women of reproductive age and may lead women to focus primarily on the risks of eating fish rather then any potential health benefits in moderate amounts.

Therapeutic drug monitoring (TDM) is commonly defined as the measurement of chemical, normally xenobiotic or its metabolite(s), concentrations in body matrices in an effort to provide the most effective exposure to a patient while minimizing the potential

for toxicity. TDM is most commonly applicable to pharmaceutical drugs and not environmental chemicals, as we are proposing here. However, the field of TDM also encompasses other agents not used for "therapy", for example, hair analysis of fatty acid ethyl esters in the detection of excessive drinking in the context of fetal alcohol spectrum disorders (Kulaga *et al.* 2009). TDM is also used to define toxic levels of lead or mercury that warrant chelation therapy.

The appropriateness of therapeutic drug monitoring is commonly determined on the basis of 7 criteria defined by Spector and colleagues (Spector *et al.* 1988). The objective of the present study was to assess whether therapeutic monitoring is appropriate in women of reproductive age to evaluate exposure to methylmercury. We herein present the existing evidence related to methylmercury along Spector's 7 criteria, with the understanding that the intent of monitoring in the current context is for safety rather than therapy.

7.2 Spector's 7 Criteria for TDM

1. **Analytic**

The most common methods to measure total mercury concentrations in hair with high analytical sensitivity are cold vapour atomic absorption spectrometry (CV-AAS), cold vapour atomic fluorescence spectrometry (CV-AFS) and ICP-MS (Gill *et al.* 2002). Detection limits range from approximately 0.4 mg/kg with CV-AAS to 0.04 mg/kg with CV-AFS, to 0.01 mg/kg with ICP-MS (Pellizzari *et al.* 1999; Farant *et al.* 1981).

TDM uses blood and urine as surrogate markers of a therapeutic agent. We are using hair as a surrogate marker of methylmercury exposure. Unlike blood and urine,

which reflect only the recent body burden of mercury, hair mercury avidly represents long term exposure and, hence, is optimal for correlation with long-term adverse fetal effects.

Since the brain is the critical organ for methylmercury toxicity, a biological matrix that accurately reflects brain mercury levels should be selected for analysis. Scalp hair is the tissue of choice for biological monitoring of chronic human methylmercury exposure (Clarkson *et al.* 2003; World Health Organization 1990). Hair is a stable matrix that presents advantages for human biomonitoring, such as easy collection, low cost, easy transport and storage and temporal exposure patterns by segmental analysis (Esteban & Castano 2009). Maternal hair analysis is particularly useful in assessing fetal exposure because hair grows at an average of 1 cm/month and thus can reflect various periods of gestation, providing a time record of mercury exposure throughout pregnancy (Cernichiari *et al.* 1995). Investigators have shown a significant correlation between levels of total mercury in maternal hair during late pregnancy and levels in six regions of the brain in late fetal and neonatal periods (Cerniciari *et al.* 1995).

2. Pharmacokinetic

About 95% of methylmercury ingested in fish is readily absorbed in the gastrointestinal tract (Gochfeld 2003). Once absorbed, methylmercury has a whole body elimination half-life of approximately 60-70 days in humans (Knobeloch *et al.* 2005). The mobility of methylmercury in the body is due to the formation of a thiol complex with the amino acid cysteine, which mimics the large endogenous amino acid methionine (Clarkson *et al.* 2007). Methylmercury transports rapidly throughout the body within 4

days, including across the blood-brain barrier and placenta. It is distributed to all tissues, where approximately 5% is found in the blood compartment and 10% in the brain (Clarkson *et al.* 2003). Concentrations of methylmercury in hair are proportional to concentrations in the blood but are about 250 times higher. Levels in cord blood are slightly higher than in maternal blood. Animal data indicate that methylmercury content in fetal brain may be higher than in the maternal brain (Inouye *et al.* 1986) and the concentrations of methylmercury in fetal RBCs exceed those in adults (Kuhnert *et al.* 1981).

Methylmercury transports from liver cells into bile as a cysteine complex, with reduced GSH, via adenosine triphosphate (ATP)-dependent transport proteins (Ballatori & Clarkson 1985; Ballatori *et al.* 1995). Methylmercury is slowly metabolized by microflora in the intestines and by phagocytic cells. Excretion of approximately 1% of the total body burden occurs daily, with fecal excretion accounting for 90% of the elimination (90%) and urinary excretion accounting for 10%. The process of fecal excretion commences with the secretion of methylmercury into bile, where GSH is hydrolyzed, resulting in the release of methylmercury as a complex with cysteine (Dutczak *et al.* 1991). Some is redistributed to the liver undergoing enterohepatic recycling, however a fraction is converted by microflora to inorganic mercury (Rowland *et al.* 1978). Therefore, most of the methylmercury present in the body is eliminated by demethylation and excretion of the inorganic form in the feces.

3. Large Intersubject Variability

Although most studies have found a clear association between the quantity and frequency of fish consumption and resultant mercury exposure, studies have documented

large intersubject variability in relation to levels of hair mercury (Clewell *et al.* 1999; Stern 1997). We have recently conducted a study examining fish consumption habits and hair mercury content in women of reproductive age (Schoeman *et al.* 2009), finding large variability between the reported number of fish servings and the measured hair mercury. For example, our results show that 5 women who reported eating 4 servings of fish a month had hair mercury levels less than 0.3 μg mercury/g, while another 5 women who ate less than 4 servings a month had hair mercury levels greater than 0.3 μg/g, our defined LOAEL of adverse fetal effects (Schoeman *et al.* 2009).

In addition to interindividual differences in toxicokinetics and toxicodynamics, this variability could be due to several factors (Mergler *et al.* 2007). First, the methylmercury concentration within and across species of dietary fish is variable; those consuming predatory fish have relatively higher exposure concentrations compared to those eating noncarnivorous fish. Second, the frequency of fish consumption also adds to the variability. Biomarkers reflect the average of exposure over time, and thus short-term reporting of fish consumption may not correspond with long-term average exposure. Last, infrequent consumption of fish high in methylmercury may result in bolus doses.

4. Concentration-Effect Relationship

Following the 1971 Iraqi outbreak of methylmercury poisoning, both hair and blood were collected for analysis to estimate fetal exposures that could subsequently be used for dose-response analysis (Cox *et al.* 1989). At that time, Clarkson and colleagues defined threshold toxicological levels associated with adverse effects to the fetus as low as 10 μg/g mercury in maternal hair (Clarkson *et al.* 2003). The appropriateness of these

toxic cases to the lower part of the dose response curve are dealt with in the next criterion (no.5).

5. Narrow Therapeutic Window

At present, the threshold between non-toxic fetal hair mercury concentrations and toxic concentrations is unclear. Clinical studies have reported "nonresponse ranges" and "toxic ranges" with wide overlap among studies, reporting adverse effects in the nonresponse range or exhibiting no effect in the toxic range, but these studies are complicated by the fact that methylmercury is almost never the only toxicant consumed.

To fill this gap, we recently conducted a systematic review of all relevant studies that evaluated the potential adverse effects of prenatal exposure to methylmercury through maternal fish consumption. For the purpose of defining a Lowest Observable Adverse Effect Level (LOAEL) for neurodevelopmental effects of methylmercury, we selected the lowest maternal hair mercury level associated with any adverse neurodevelopmental effect in controlled studies. Our LOAEL was found to be 0.3 μg/g mercury in maternal hair.

6. Effect over time

With sufficient exposure to high concentrations of any mercury-based toxin, damage to the CNS will occur. Toxic exposure to methylmercury results primarily in neurological damage in both adults and children, characterized by ataxia, sensory disturbances and changes in mental state (Takeuchi 1968). Well-known environmental catastrophes in which large numbers of people were poisoned by methylmercury in Minamata Bay and Iraq, confirm that consumption of high levels of methylmercury in

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diet sources by pregnant women are likely to cause severe abnormalities in their offspring (Grandjean *et al.* 1994). Mothers with mild symptoms gave birth to offspring with severe brain damage (Takeuchi 1968).

In the adult, methylmercury poisoning is characterized by damage to discrete anatomical loci of the brain, such as a loss of neurons from the visual cortex and disappearance of granule cells from the granule layer of the cerebellum (Choi *et al.* 1978). Adults present with paresthesia, visual-constriction and ataxia (Clarkson *et al.* 2003). Autopsies from the Iraqi outbreak showed that neuronal cell division, migration and organization were disrupted (Choi *et al.* 1978). Furthermore, axonal degeneration has been associated with secondary myelin disruption of the peripheral nerve (Hunter & Russel 1954). In the developing fetus, prenatal exposure to methylmercury can interfere with the growth and migration of neurons and has the potential to cause irreversible damage (Environmental Protection Agency 1997).

Subtle changes, such as small changes in intelligence or learning capacity are currently being tested in populations with low-level, chronic exposure to mercury in the diet (Davidson *et al.* 2000; Grandjean *et al.* 1997). The endpoints tested include attention, fine-motor function, language, visual-spatial ability and verbal memory, and are those that are affected at and above our defined LOAEL for methylmercury.

7. Clinical

Several large epidemiological studies have been conducted in people consuming fish and large scale studies are continuing to this day. Unfortunately these studies have not yet provided a consistent picture, reporting both deficits and beneficial outcomes from maternal fish consumption. The lowest prenatal exposure concentration that offers a

measurable risk of damage to the developing brain is not well-defined (Clarkson *et al.* 2003).

In our recent systematic review, numerous studies measured various endpoints including: status on neurological examination, age at achievement of developmental milestones, infant and preschool development, childhood development and sensory and neurophysiological functions. From this comprehensive review of the literature it is apparent that neurodevelopmental abnormalities occur in children following a range of gestational exposures, from maternal consumption of mercury contaminated fish (maternal hair: 0.3-12.7 μg/g) (Cordier *et al.* 2002; Davidson *et al.* 2000; Despres *et al.* 2005; Gao *et al.* 2007; Grandjean *et al.* 1998; Kjellstrom *et al.* 1986; Kjellstrom *et al.* 1989; McKeown-Eyssen *et al.* 1983; Murata *et al.* 1999; Murata *et al.* 2004; Myers *et al.* 1995; Myers *et al.* 2003; Oken *et al.* 2005; Ramirez *et al.* 2003; Steuerwald *et al.* 2000; Stewart *et al.* 2006; Strain *et al.* 2008; Weihe *et al.* 2002; Xue *et al.* 2007).

While there is considerable uncertainty as to the precise dose of methylmercury that will cause subtle adverse neurodevelopmental effects in children following prenatal exposure, the precautionary principle dictates setting the LOAEL at the lowest levels showing adverse cognitive effect, which has been defined by us at 0.3 μg/g (Schoeman *et al.* 2009).

7.3 Discussion

The concept of therapeutic drug monitoring of hair mercury in women who are pregnant or are planning to become pregnant appears to meet most of Spector's criteria

for TDM. The purpose of monitoring mercury exposure prior to and during pregnancy is to provide a basis for dietary adjustments, specifically addressing the amount of fish consumed. In this case, therapeutic monitoring will improve the decision so it will no longer rest on generalized regulatory guidelines but, rather, on an individual estimate of systematic exposure to methylmercury. Because even similar species of fish vary significantly in mercury content, these guidelines are not likely to be accurate enough in estimating mercury exposure. Given the importance of fetal safety during pregnancy, therapeutic monitoring of mercury represents a feasible, affordable and beneficial clinical approach to decrease potential risk to the fetus. Moreover, monitoring will allow women to consume highly valuable fish, not letting chemophobia affect their nutritional health.

In a recent study we have shown that many women calling a counselling service regarding the safety of fish consumption in pregnancy, had hair mercury above our defined LOAEL of 0.3 μg/g. For these women planning pregnancy, dietary adjustment may allow optimization of mercury body load. Prospective studies to prove the utility of using the new proposed therapeutic monitoring are warranted.

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Chapter 8: Conclusions and Future Directions

The overall objective of this work was to explore the relationship between hair mercury concentrations and fish consumption habits in various populations and most importantly, to determine what proportion of Canadian women were above our defined LOAEL for mercury-related neurodevelopmental effects.

To define a LOAEL, that would serve as a safe guideline to compare our populations to, we systematically reviewed all relevant original research written on the neurodevelopmental effects caused by *in utero* exposure to methylmercury through maternal fish consumption. Using the precautionary principle, we defined our LOAEL of fetal neurodevelopmental effects of mercury at 0.3 μg/g based on systematic review of all included longitudinal and cross-sectional studies.

To investigate the relationship between dietary fish consumption and hair mercury content, hair samples were collected from 4 cohorts with varying fish consumption habits. A significant correlation was found between number of fish servings and hair mercury content, which was further strengthened when correlated with estimated ingested dose of mercury. These data however, are subject to participant errors in species identification and portion estimation, as well as the potential error in the assignment of methylmercury concentarions by species, which may have resulted in inaccuracies in exposure assessment.

When comparing mercury exposure in our cohorts with our defined LOAEL, we found that nearly two-thirds of women in our sample that has called Motherisk had methylmercury exposures exceeding the LOAEL. All Japanese participants exceeded the LOAEL, compared with 36% of the WIFN population and only 15% of reproductive age

women representing the general Canadian population. Large epidemiological studies reporting on the potential adverse neurodevelopmental effects caused by high mercury exposure in Japanese children are warranted.

The response of women sufficiently concerned about fish consumption to warrant a call to a consultation service shows perception of heightened teratogenic risk. Our data indicate that the heightened risk perception exhibited in this group is justified, based on their measured hair mercury, the most valid biological marker of long term exposure to this toxic metal. In the WIFN group we found that the impact of perceptions was greater than the actual risk from mercury.

Due to individual variations in the toxicokinetics of mercury, we have found variations between fish consumption habits and resultant hair mercury content; meaning that even if the participants in our study ate the same amount of mercury in fish, there were differences in hair mercury content, yet there was a strong correlation between hair and blood mercury content found. Thus devising specific guidelines for the number of fish servings that are "safe" for pregnant or about to become pregnant women to consume per week may not be relevant for all, and might not be the best approach to prevent subtle neurodevelopmental defects in the unborn child. We are proposing that therapeutic monitoring of mercury using individual hair analysis be considered for selected groups of heavy fish-eating women as a novel public health measure, as dietary changes can decrease overall body load of mercury and eliminate any potential fetal risks.

SickKids **Research Ethics Board (REB)**

The Research Ethics Board for The Hospital for Sick Children is organized and operates according to the principles and practices outlined in the Tri-Council Policy Statement, the ICH Harmonized Tripartite Guideline: Good Clinical Practice, and Division 5 and the Medical Devices Regulations of the Food and Drug Act as well as the Natural Health Products Regulations of Health Canada. This signed document is in lieu of the Health Canada Research Ethics Board Attestation Form.

Approval & Terms of Agreement

Investigators: Dr. Gideon Koren, K.Schoeman

Study Title: Follow-up Fish Consumption Study Regarding Mercury Contamination During Pregnancy

REB File number: 1000012282 **Level of Continuing Review:** I B
Protocol Version Date: April 1, 2008
Consent & Assent Form Version Date(s): May 23, 2008
Investigator's Brochure Version Date: N/A
Other Approved Recruitment Document Dates: Letter of Information - May 23, 2008

I agree to carry out the proposed research involving human subjects in accordance with the above-noted guidelines and regulations (as applicable) and using only the REB-approved study protocol and consent/assent form(s). I shall notify the division/department head and the REB prior to implementing any amendments in the protocol and consent/assent forms and of any deviations or any changes in study activity. I shall also notify the REB of any unexpected adverse events as per REB guidelines. As applicable, I certify that the research contract and corresponding protocol are consistent and will inform the contract manager of any protocol amendments as required.

I agree that, in accordance with the Personal Health Information Protection Act of Ontario, I am responsible for adhering to all conditions and restrictions imposed by the REB governing the use, security, disclosure, return and disposal of the research subjects' personal health information. I am also responsible for reporting immediately any privacy breaches to the REB Chair and to Janice Campbell, the Sick Kids privacy officer. I will ensure that the personal health information is used, only as necessary, to fulfill the specific research objectives and related research questions described in this application and approved by the REB.

Signature of Principal Investigator

I approve of this research protocol, agree to share responsibility for its proper conduct, and will ensure that the REB is notified of concerns, as appropriate.

Signature of Division/Department Head

The REB of the Hospital for Sick Children has reviewed and approved the above-named research study.

Mr. Richard Sugarman, REB Chair
555 University Avenue, Toronto Ontario, M5G 1X8
Tel: 416-813-6152 Fax: 416-813-5085 Email:

DATE OF APPROVAL July 8, 2008 **EXPIRY DATE** July 2009.

Office of Research Ethics

The University of Western Ontario
Room 00045 Dental Sciences Building, London, ON, Canada N6A 5C1
Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

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 .egulations.

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

Fish Consumption Study

LETTER OF INFORMATION - BIOMONITORING

Introduction

We are members of a multi-disciplinary research team that is currently conducting research with Motherisk at The Hospital for Sick Children in Toronto regarding the possible health hazards of mercury exposure. We are inviting you to participate in this study.

This letter is to inform you about our research project, funded by the University of Western Ontario. We will determine mercury exposure by analyzing hair mercury levels and linking them to the amount of fish consumed. Your opinions regarding your fish consumption patterns will be obtained by the questionnaire provided. Your name and phone number will be obtained to allow for a follow-up phone call in order to disseminate the results of the hair analysis.

You will be asked to provide informed written consent before you provide a sample hair for analysis.

Purpose of the study

The aims of this study are:

1. To determine concentrations of mercury in hair samples of volunteers that are pregnant regarding fish consumption who provide their informed consent.
2. To assess the mercury levels in canned tuna.
3. To investigate women's behaviour related to perceptions of health risks related to fish and mercury exposure

Voluntary Participation:

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

You will be asked to donate a 0.5 gram hair sample (which is a small amount) to assess the amount of mercury in the hair. Instructions on the brochure explain how to correctly cut hair strands.

Confidentiality

The information collected will be used for research purposes only. All information collected for the study will be kept confidential. Neither your name nor identifying information will be included in any publications or presentations of the study results.

Risks and Benefits

There are no direct benefits to participation in the study. Study results will lead to more extensive research in the field to develop a healthy diet, not only for pregnant women but for the general Canadian population.

The only possible physical risks are related to hair cutting.

Questions

If you have any questions about this study or your rights as a research participant you may contact the Manager, Margo Farren, Office of Research Ethics, The Hospital for Sick Children. If you have any questions about this study, please contact Katie Schoeman. You can also contact Dr. Gideon Koren, the Principal Investigator for this study. This letter is yours to keep for future reference.

Follow-up Fish Consumption Study

Katie Schoeman BSc, Gideon Koren MD FRCP

CONSENT FORM FOR HAIR SAMPLES

I have read the Letter of Information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

Name (please print):

Signature: Date:

Follow-up Fish Consumption Study

Katie Schoeman BSc, Gideon Koren MD FRCP

CONSENT FORM FOR HAIR SAMPLES

I have read the Letter of Information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

Name (please print):

Signature: Date:

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 cortisol, 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. 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 cortisol, 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 cortisol, 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 childrens' 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. At the end of this questionnaire we will be asking you about your perceived stress levels.

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.

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. Blood and hair samples will be coded immediately upon collection, by Head Nurse Rosemary Williams of the Walpole Island Health Centre, and only coded samples will be sent for analysis and for storage. However, a master list will be kept by the research

group to allow identification and removal of your coded 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.

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. If you have any questions about this study, please contact Katie Schoeman or Julie Hill. You can also contact Dr. Jack Bend, the Principal Investigator for this study. This letter is yours to keep for future reference.

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

Katie Schoeman BSc, Julie Hill BHSc, Jack Bend BSc, MSc, PhD, Regna Darnell BA, MA, PhD, Dr. Dean Jacobs, Gideon Koren MD FRCPC, Christianne, Michael Rieder, MD, PhD, Christianne Stephens BA, MA, Naomi Williams*,*

*Rosemary Williams, RN, HBScN**

* From Walpole Island First Nation

CONSENT FORM FOR BLOOD AND HAIR SAMPLES

I have read the Letter of Information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

Name (please print):

Signature: Date:

Name of Person Translating (or Reading) Document:

Signature of Person Translating (or Reading) Document:

Date:

Health Status Questionnaire to Assess the Health Perceptions of the Walpole Island First Nation Community from Exposure to Environmental Contaminants

LETTER OF INFORMATION- ENVIRONMENTAL EXPOSURES

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 cortisol, 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. This specific component of the study involves an interview to ask questions about your concerns about environmental contaminants and the impact they might be having on health of you and your children. At the end of the interview we will be asking questions about your perceived stress levels.

Purpose of the Semi-Structured Interview

The aims of this interview with one of the members of our research team are to continue our study of the psychosocial and cultural dimensions of environmental exposure in the WIFN community.

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 verbal interview conducted by a member of the research team some time before the end of our current study, in June 2008. Individuals who donate blood or hair will be asked if they wish to be interviewed when they are at the Health Centre to provide these samples. The actual interviews will take place on a day after the

samples are donated and before the end of the data collection portion of the study, in June 2008. The interview will examine your perceptions of environmental contaminants and health risks.

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 as part of the health status interview 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.

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 risks of the 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 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 community regarding the content of the interview, counselling will be provided to those individuals concerned, through the Health Centre by Rosemary Williams, or if 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. If you have any questions about this study, please contact Katie Schoeman or Julie Hill. You can also contact Dr. Jack Bend, the Principal Investigator for this study.

Health Status Questionnaire to Assess the Health Perceptions of the Walpole Island First Nation Community from Exposure to Environmental Contaminants

Katie Schoeman BSc, Julie Hill BHSc, Jack Bend BSc, MSc, PhD, Regna Darnell BA, MA, PhD, Dr. Dean Jacobs, Gideon Koren MD FRCPC, Christianne, Michael Rieder, MD, PhD, Christianne Stephens BA, MA, Naomi Williams*,*

*Rosemary Williams, RN, HBScN**

CONSENT FORM FOR HEALTH STATUS INTERVIEW

I have read the letter of information, have had the nature of the study explained to me and I agree that my child may participate in the study. All questions have been answered to my satisfaction.

_____

Name of Student

_____ _____

Student's Signature Date

_____

Printed Name of Parent/Guardian

_____ _____

Parent/Guardian's Signature Date

Name of Person Translating (or Reading) Document:

Signature of Person Translating (or Reading) Document:

Seafood Consumption Follow Up Form ID Number:_____

MERCURY STUDY Follow Up Date:_____

Telephone Log <VM>Verbal Message <MM>Machine Message <Busy>

Date/Time Result: <OS>Out of Service <W>Wrong Number <NA>No answer

_____

_____

Maternal Data

Did you smoke during pregnancy? _____

If yes, how many cigarettes a day? _____

Do you have any amalgam fillings? _____

Pregnancy History

Health Problems/Diseases Complicating Pregnancy? ↑no

↑yes:_____

G____ P____ SA____ TA_____ ectopic_____ molar_____

Stillbirth_____

Have your babies been normal and healthy in previous pregnancies □ Yes

↑No _____

Infant Data

DOB: _____ Sex: ↑M ↑F

Problems? ↑no ↑yes:_____

Birth: GA_____ weeks Weight:_____

Were there any health problems with your baby? Comments:

_____

_____

Seafood Consumption

1. How often do you consume seafood? (ie. fish, sushi, lobster, etc)

□ daily

□ weekly

□ monthly

□ yearly

□ rarely

2. On average how much seafood do you consume?

_____

3. What type of seafood do you most often consume? (if answer is canned tuna continue to question 4)

_____

Type of seafood	
Salmon	
Tuna (canned or fresh)	
Perch	
Shellfish (clams, mussels, oysters, scallops)	
Halibut	
Tilapia	
Trout	
Lobster	
Sardines	
Crab	
Sushi	
Shrimp	
Other	

4. What brand of canned tuna do you usually purchase?

_____

5. How often do you consume canned tuna?

_____

Perceptions

1. Do you remember calling us about the _____? What made you call about_____?

_____

_____

_____

_____

_____

2. Do you remember how you heard about it (ie. the news, the internet, newspaper, friends, etc)?

_____

_____

_____

3. What was it that you understood could happen to your baby from eating fish?

_____

_____

_____

_____

4. On a scale from 0 to 10, how worried were you? _____

5. Are you aware of the good things about eating fish in pregnancy?

_____

_____

_____

_____

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

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?
INTERVIEWER: 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 → b. How many times in the past 12 months?
2 ___ No
3 ___ Don't know
4 ___ Declined

__ __ __ *Times*

b. a nurse, including community health nurse, public health nurse or nurse practitioner separate from doctor's visits?	1 ___ Yes → 2 ___ No 3 ___ Don't know 4 ___ Declined	b. How many times in the past 12 months? ___ ___ ___ *Times*
c. a medical specialist such as a surgeon, allergist or orthopaedist?	1 ___ Yes → 2 ___ No 3 ___ Don't know 4 ___ Declined	b. How many times in the past 12 months? ___ ___ ___ *Times*
d. a traditional Aboriginal healer? *INTERVIEWER: By "Aboriginal", we are referring to First Nations, Métis or Inuit.*	1 ___ Yes → 2 ___ No 3 ___ Don't know 4 ___ Declined	b. How many times in the past 12 months? ___ ___ ___ *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 → 2 ___ No 3 ___ Don't know 4 ___ Declined	b. How many times in the past 12 months? ___ ___ ___ *Times*
B6. During the past 12 months, was there a time when you or your child/children wanted health care or medication and could not get it?	1 ___ Yes → 2 ___ No 3 ___ Don't know 4 ___ Declined	b. How many times in the past 12 months? ___ ___ ___ *Times*
B7. In general, is your or your child/children's physical activity limited by a health condition? *A health condition may include a disability or a long term condition.*	1 ___ Yes → 2 ___ No 3 ___ Don't know 4 ___ Declined	b. How many times in the past 12 months? ___ ___ ___ *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?	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		

B9. Do you or your child/children have any food, digestive, respiratory or other allergies? If no skip to B11.

a. Food or digestive allergies?	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		

a. Respiratory allergies (such as hay fever)?	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		

a. Any other allergies?	b. Did ___ 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		

B10. Do you or your child/children have Asthma or Chronic Bronchitis or Tuberculosis? If no skip to B11.

a. Asthma?	b. Did ___ 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		

a. Chronic Bronchitis?	b. Did ___ 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		

a. Tuberculosis?	b. Did ___ 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		

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

a. Diabetes?	b. Did ___ 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		

a. Hypoglycemia or low blood pressure?	b. Did ___ 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		

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 ___ 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
___ Declined		

a. Kidney condition or disease?	b. Did ___ 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		

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

a. Epilepsy?	b. Did ___ get a diagnosis from a doctor, nurse or	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		

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 ___ get a diagnosis from a doctor, nurse or	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 ___ Declined	4 ___ Declined	4 ___ Declined
5 ___ Refused		

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 ___ 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		

a. Attention Deficit Hyperactivity Disorder	b. Did ___ 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		

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

a. Autism?	b. Did ___ 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		

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

a. Speech or language difficulties?	b. Did ___ 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 ___ 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 week
	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 week
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?

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 week
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 week
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 week
06 ___ At least once per year
07 ___ Less than once a year
08 ___ Don't know
09 ___Declined

f. Medications for attention deficit disorder?

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 week
06 ___ At least once per year
07 ___ Less than once a year
08 ___ Don't know
09 ___ Declined

g. Other 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 week
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 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 ___ Yes → b. For how long?
2 ___ No ___ ___ months or ___ ___ years
3 ___ Don't know 1 ___ Less than one month
4 ___ Declined 2 ___ Don't know
3 ___ Declined

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

1 ___ Yes → b. For how long?
2 ___ No ___ ___ months or ___ ___ years
3 ___ Don't know 1 ___ Less than one month.
4 ___ Declined 2 ___ Don't know
3 ___ 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

____ Times →
1 ___ Never
2 ___ Don't know
3 ___ Declined

Reporting Period

1 ___ per day
2 ___ per week
3 ___ per month
4 ___ per year

---

d. Bottled water?

Number of times

____ Times →
1 ___ Never
2 ___ Don't know
3 ___ Declined

Reporting Period

1 ___ per day
2 ___ per week
3 ___ per month
4 ___ per year

---

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

Number of times

____ Times →
1 ___ Never
2 ___ Don't know
3 ___Declined

Reporting Period

1 ___ per day
2 ___ per week
3 ___ per month
4 ___ per year

---

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

Number of times

____ Times →
1 ___ Never
2 ___ Don't know
3 ___ Declined

Reporting Period

1 ___ per day
2 ___ per week
3 ___ per month
4 ___ per year

---

g. Large game animals such as deer or moose?

Number of times

____ Times →
1 ___ Never
2 ___ Don't know
3 ___ Declined

Reporting Period

1 ___ per day
2 ___ per week
3 ___ per month
4 ___ per year

h. Small game animals such as rabbit or muskrat?

Number of times

___ ___ ___ Times →
1 ___ Never
2 ___ Don't know
3 ___ Declined

Reporting Period

1 ___ per day
2 ___ per week
3 ___ per month
4 ___ per year

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

Number of times

___ ___ ___ Times →
1 ___ Never
2 ___ Don't know
3 ___ Declined

Reporting Period

1 ___ per day
2 ___ per week
3 ___ per month
4 ___ per year

j. Salt or fresh water fish?

Number of times

___ ___ ___ Times →
1 ___ Never
2 ___ Don't know
3 ___ Declined

Reporting Period

1 ___ per day
2 ___ per week
3 ___ per month
4 ___ per year

D- Developmental Milestones

For preschool children only

D1. Has your child/children ever looked for someone or something that was lost or out of sight?

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

b. At what age in months?

___ ___ ___ *Months*

D2. Has he/she/they sat up by Himself/herself?

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

b. At what age in months?

___ ___ ___ *Months*

D3. Has he/she/they 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 he/she/they ever run?

1 ___ Yes b. At what age in months?
2 ___ No
3 ___ Don't know
4 ___ Declined ___ ___ ___ *Months*

D5. Has he/she/they 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 he/she/they ever expressed his/her needs using a single word?

	b. How often?	c. At what age in months?
1 ___ Yes →	01 ___ All the time	
2 ___ No	02 ___ Most of the time	___ ___ ___ *Months*
3 ___ Don't know	03 ___ Sometimes	
4 ___Declined	04 ___ Rarely	
	05 ___ Don't know	
	06 ___ Declined	

D7. Has he/she/they 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 he/she/they ever expressed his/her needs using 2 to 3 words?

1___ Yes → b. How often?
- 01 ___ All the time
- 02 ___ Most of the time
- 03 ___ Sometimes
- 04 ___ Rarely
- 05 ___ Don't know
- 06 ___ Declined

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

D9. Has he/she/they ever counted 3 objects correctly?

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

D10. Has he/she/they ever expressed his/her needs using full sentences?

1___ Yes → b. How often?
- 01 ___ All the time
- 02 ___ Most of the time
- 03 ___ Sometimes
- 04 ___ Rarely
- 05 ___ Don't know
- 06 ___ Declined

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

E – SCHOOL – For 4 to 5 year olds only

E1. Is your child/ are your children currently Attending school
Pre- Kindergarten and kinder-. garten is to be included

1 ___ Yes
2 ___ No
3 ___ Don't know } If no go to F
4 ___ Declined

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	Don't know
6	Declined

F – CHILD CARE

F 1. Does your child/children's main child care arrangement promote First Nations, traditional and cultural values and customs?

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

G – LEARNING AND ACTIVITIES

The following are some questions about activities ____ 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

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

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

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 (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

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