Mercury Exposure Analyses Amongst Dene and Métis Communities of the Northwest Territories

by

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

This thesis is the work of Leicester Fung in collaboration with his supervisor, Dr. Brian Laird. I would like to acknowledge the contributions of each co-author, as well as my supervisor for providing me with guidance and insight throughout each stage of the project.

Chapter 4 & Chapter 5

Chapter 4 and Chapter 5 of this thesis were co-authored with Dr. Leanne Baker, Dr. Michèle Bouchard, Dr. Brian Branfireun, Dr. Ashok Chaurasia, Dr. Mylene Ratelle, Dr. Kelly Skinner, Dr. Heidi Swanson, and Dr. Brian Laird. All of these researchers are part of a multi-institutional, interdisciplinary project - Contaminant Biomonitoring in the Northwest Territories Mackenzie Valley: Investigating the Links Between Contaminant Exposure, Nutritional Status, and Country Food Use. This project was funded by the Northern Contaminants Program, which is jointly supported by Indigenous and Northern Affairs Canada and Health Canada. Additional support was received from Global Water Futures and the University of Waterloo. In Chapter 4, Dr. Mylene Ratelle and Dr. Brian Laird designed the food frequency questionnaire. Additionally, Dr. Kelly Skinner provided valuable feedback and insight for the food frequency questionnaire. In Chapter 4, Dr. Michèle Bouchard and Dr. Brian Branfireun assisted with mercury analysis in blood and hair samples. In Chapter 4, Dr. Ashok Chaurasia and Dr. Heidi Swanson provided assistance with simple linear regression models. In Chapter 5, Dr. Heidi Swanson prepared the whitefish samples and provided laboratorial space to conduct mercury analysis for the whitefish samples. In Chapter 5, Dr. Leanne Baker assisted with mercury analysis in the whitefish samples. Dr. Brian Laird oversaw my progress in the lab, provided detailed edits to my various thesis drafts and guided me along the way.

Abstract

Background: Fish is an important food source for many Indigenous communities within the Dehcho and Sahtú regions of Northwest Territories (NWT). However, environmental toxicants, such as mercury (Hg), can bioaccumulate and biomagnify to reach detectable levels in fish, particularly among predatory fish species. From a public health perspective, Hg exposure and biomonitoring assessments are invaluable assets that can highlight potential risk factors and evaluate risks associated with Hg toxicity. With the current body of knowledge, exposure assessment models conservatively account for Hg bioaccessibility in fish to be 100% because of the limited information available. Past literature have also noted statistical differences in Hg bioaccessibility with respect to food source as well as food preparation methods. As such, there is a need for more Hg biomonitoring and Hg bioaccessibility assessments to elucidate the relationships between dietary and demographic determinants with respect to Hg exposure.

Objectives: *Hg biomonitoring component.* 1) To determine internal Hg exposure levels (blood and hair) from six Indigenous communities in the Dehcho and Sahtú regions of NWT. 2) To conduct t-test analysis for internal Hg exposure levels with respect to sex. 3) To construct simple linear regression models between internal Hg exposure levels with the following factors: i) age ii) non-piscivorous fish consumption iii) piscivorous fish consumption.

Hg bioaccessibility component. 1) To characterize Hg concentrations for uncooked and dried by smoking (dried/smoked) lake whitefish (*Coregonus clupeaformis*). 2) To determine Hg bioaccessibility in uncooked and dried/smoked lake whitefish. 3) To conduct t-test analysis to determine Hg concentration and Hg bioaccesibility differences between uncooked and dried/smoked whitefish.

Methods: *Hg biomonitoring component.* 150 Dene and Métis participants, between the ages of 6-79, were recruited from Deline, Fort Providence, Hay River Reserve, Kakisa, West Point First Nation and Jean Marie River of NWT. Blood and hair samples were collected for internal Hg exposure assessments. Participants were also asked to provide demographic information and complete a food frequency questionnaire (FFQ) for describing potential determinants of Hg exposure. T-test analysis and log-linear simple regression models were used to identify individual relationships.

Hg bioaccessibility component. Five whole lake whitefish (*Coregonus clupeaformis*) were used to investigate Hg bioaccessibility. Subsamples were subjected to two treatments: uncooked and dried/smoked. All dried/smoked and uncooked fish samples underwent a single phase (gastric only) IVBA model treatment. All Hg concentrations were expressed as wet weight and determined using an Ohio-Lumex portable mercury analyzer with pyrolyzer attachment.

Results: *Hg biomonitoring component.* Hg levels in blood and hair were highly linearly-correlated with one another ($r= 0.854$, $p<0.05$) and all of the participants had blood and hair Hg levels below 20μg/L and 6μg/g respectively. T-test analysis found no statistical differences ($p>0.05$) when comparing blood Hg levels of male and female participants (2.711 ± 2.616 μg/L and 2.667 ± 4.079 μg/L respectively) Similarly, T- test analysis found no statistical differences ($p>0.05$) when comparing hair Hg levels of male and female participants (0.868 ± 0.925 μg/L and 0.671 ± 0.866 μg/g respectively). According to the simple regression model's findings, age was the only statistically significant factor and was positively associated with blood and hair Hg levels ($B=0.020$ and $p<0.01$; $B=0.011$ and $p<0.01$). Based on simple regression model's findings, piscivorous fish consumption was not significantly associated with either Hg levels found within blood and hair ($B= 0.068$ and $p>0.05$; $B= 0.023$ and $p>0.05$). Similarly, non-piscivorous fish

consumption does not appear to be significantly associated with blood and hair Hg levels (B= 0.022 and $p>0.05$; B= 0.035 and $p>0.05$)

Hg bioaccessibility component. After culinary treatment, dried/smoked whitefish samples retained, on average, 46% of their original mass (i.e., due to moisture loss). As a result of moisture loss, the initial Hg concentration in dried/smoked whitefish samples (0.194 mg/kg) was significantly greater ($p=0.002$) relative to uncooked whitefish samples (0.080 mg/kg). When comparing Hg bioaccessibility, dried/smoked whitefish (53%) was statistically lower ($p< 0.001$) compared to uncooked whitefish (102%). Despite the reduction in Hg bioaccessibility for dried/smoked whitefish, the bioaccessible Hg concentration in dried/smoked whitefish (0.101 mg/kg) was significantly greater ($p< 0.05$) compared to uncooked whitefish samples (0.081 mg/kg). The loss of moisture mass and the increased density of Hg within dried/smoked whitefish samples were likely responsible for the increase in total and bioaccessibile mercury in dried/smoked whitefish. As such, dried/smoked whitefish samples had greater amounts of Hg digested and solubilized in aqueous solution.

Conclusion: Both simple regression and static IVBA models' findings have provided additional information for characterizing Hg exposure among participating communities in Northwest Territories. Based on the study population's demographic determinants and dietary patterns, age is a key factor for understanding and predicting Hg exposure in the Northwest Territories. Bioaccessibility findings suggest source apportionment models that incorporate differences in Hg concentration between raw and smoked/dried fish may also need to account for the corresponding difference in bioaccessibility. For further generalizability of IVBA model's findings, additional *in vitro* bioaccessibility studies using different fish species are needed. This research is important because it highlights dietary factors, such as cooking method, are

potentially important to human exposure. Inclusion of such factors to source apportionment model may help further characterize the relationship between internal Hg exposure levels detected in biomarkers and the external exposure estimates.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

Arctic Monitoring and Assessment Programme (AMAP)
Blood brain barrier (BBB)
Cadmium (Cd)
Copper (Cu)
Dimethylmercury (Me_2Hg)
Electron transport chain (ETC)
Elemental Hg (Hg^0)
Ethylmercury (EtHg)
Food Frequency Questionnaire (FFQ)
Glutathione (GSH)
Glutathione disulfide (GSSG)
Glutathione peroxidase (GPX)
Glutathione reductase (GR)
Inorganic mercury (iHg)
In vitro bioaccessibility (IVBA)
Iron (Fe)
Joint Expert Committee on Food Additives (JECFA)
Lead (Pb)
L-type neutral amino acid carrier transport-1 (LAT-1)
Magnesium (Mg)
Mercury (Hg)
Methylmercury (MeHg)
National Household Survey (NHS)
Northwest Territories (NWT)
Part per million (PPM)
Potassium (K)
Reactive oxidative species (ROS)
Red blood cell (RBC)
Selenium (Se)
Simulator of Human Intestinal Microbial Ecosystem (SHIME)
Superoxide dismutase (SOD)
Toxicological reference values (TRV)
Zinc (Zn)

1. Introduction

Mercury (Hg) is a metallic chemical element that can be found in all parts of the world (Donaldson et al., 2010; Sheehan et al., 2014). Mercury is present in the atmosphere through natural processes, such as weathering of soil and rocks, volcanic activity and forest fires, and also through anthropogenic processes, such as coal combustion, smelting operations and waste incineration (Roman et al., 2011). As such, inorganic mercury (iHg) and organic mercury, primarily of methylmercury (MeHg), can both be found circulating in the human body (Bernhoft, 2012; Hong et al., 2012; Park and Zheng, 2012). MeHg has the capability to bioaccumulate within the aquatic food chain and concentrations are biomagnified amongst predatory fish species (Cott et al., 2016; Power et al., 2002; Wagemann et al., 1998). Past literature have also shown that dietary consumption of fish was positively associated with increase levels of blood and hair Hg concentrations (Kim et al., 2016; Oken et al., 2008; Schaefer et al., 2014). Through dietary consumption of fish, Hg's toxicodynamic effects can present a great concern to health of the general public (Clarkson et al., 2003; Kim et al., 2016; Oken et al., 2008; WHO, 1991).

When it comes to toxicokinetics-related research, it is important to understand both the contaminant's source as well as its bioavailability and bioaccessibility properties (Fernández-García et al. 2009). As such, these factors can refine human exposure assessments. In general, consumption of fish consumption is one of the main dietary routes for Hg exposure in humans (Siedlikowski, 2015). As such, Hg exposure assessments typically include dietary surveys and account for fish consumption patterns (Schaefer et al., 2014; Siedlikowski et al., 2016). Exposure assessments typically assume Hg bioavailability to be 100% as a conservative default (Siedlikowski et al., 2016). As such, exposure models conservatively assume that nearly all of the dietary Hg has been absorbed into systemic circulation, despite past literature have found a

negative correlation between initial Hg concentration in seafood and Hg bioaccessibility (Siedlikowski et al., 2016). Furthermore, studies have shown that Hg bioaccessibility can vary between fish species and with different cooking methods (Costa et al., 2015; Ouédraogo and Amyot, 2011; Siedlikowski et al., 2016).

Mercury toxicity is a potential concern for the Dene and Métis communities residing in the Dehcho and Sahtú regions of Northwest Territories (NWT) because almost half of the territory's population fish for either sustenance or recreation (NWT Bureau of Statistics, 2016). In terms of nutritional composition, wild-harvested freshwater fish is an important source of protein, omega 3-fatty acids and essential micronutrients for Dene and Métis communities (Kuhnlein and Humphries, 2017; Kuhnlein and Receveur, 2007). Omega 3-fatty acids is known to possess anti-inflammatory properties as well as neuroprotective capabilities (Groeger et al., 2010; Richardson and Montgomery 2005). In contrast, fish consumption patterns can also greatly influence Hg levels detected in these communities (Van Oostdam et al. 2005; Wheatley and Paradis, 1995). Once Hg is circulating within the body, it can lead to the development of cardiovascular disease, neurocognitive and sensory impairments (Carta et al., 2003; Houston, 2011). Furthermore, MeHg can cross the placental blood barrier, affect neonate development, leading to cognitive impairment among exposed children (Axelrad et al., 2007; Carta et al., 2003).

Firstly, this Hg cross-sectional biomonitoring study is part of a larger, ongoing interdisciplinary project within NWT. This research characterized blood and hair Hg exposure for 6 Indigenous communities within the Dehcho and Sahtú regions. In addition, this thesis project specifically investigated and highlighted key information about the study population's wild-harvested freshwater fish consumption and demographic patterns. Using R 3.5.1 open-

source program, simple regression models were developed in order to characterize and predict estimates for Hg exposure in the Dehcho and Sahtú regions. This project lays the foundation for assessing the relationship between an individual's blood and hair Hg level with respect to country food intake, particularly fish consumption patterns, in the Dehcho and Sahtú regions of NWT. Beyond this project, regression models can become more refined by increasing the study population which will improve the model's accuracy for predicting and characterizing an individual's exposure to Hg. This research may serve as a tool to explore as well as identify the relationship between external Hg exposure sources found in the environment with internal exposure levels found within the individual.

Secondly, this work analyzed Hg concentrations and bioaccessibility in store bought whitefish. This research choose to analyze whitefish because it is one of the more commonly consumed wild-harvested freshwater fish in NWT (Kuhnlein et al., 1994; Morrison and Kuhnlein, 1993). Whitefish samples were subsections into two different culinary treatment methods, uncooked and dried through smoking (dried/smoked). To characterize Hg bioaccessibility, uncooked and dried/smoked whitefish samples were treated in a single phase, gastric-only, static *in vitro* bioaccessibility (IVBA) model. Mercury content in initial and bioaccessible whitefish samples were characterized using a Hg analyzer RA-915M with pyrolyzer PYRO-915+. This research will also help provide more information about Hg bioaccessibility and improve the accuracy of Hg exposure estimates. Finally, findings from the Hg biomonitoring and Hg bioaccessibility research may help promote continued consumption of country foods.

2. Study Rationale

The Dehcho and Sahtú regions are two of the five administrative regions within the NWT of Canada. Located in the western half of NWT, these regions include many different Indigenous communities. Not only do the Dene and Métis communities maintain a deep and profound connection with their environment for many generations, but they also are part of the local ecosystem through fishing, gathering, hunting and trapping (Beckford et al., 2010; Earle, 2011; Reading and Wien, 2009; Richmond and Ross, 2009). The Dene and Métis communities consume wild-harvested freshwater fish as part of their country food diets which includes species such as lake trout (*Salvelinus namaycush*), northern pike (*Esox lucius*), walleye (*Sander vitreus*) and whitefish (*Coregonus clupeaformis*) (Kuhnlein et al., 1994; Morrison and Kuhnlein, 1993; NWT Health and Services, 2002; Smith, 1982). Residents in Dene and Métis communities consume fish in a variety of ways, including boiling, drying, frying and smoking (NWT Health and Services, 2002). Consuming wild-harvested freshwater fish has many nutritional benefits as it is an important source of protein, omega 3-fatty acids and essential micronutrients (Kuhnlein and Humphries, 2017; Kuhnlein and Receveur, 2007). For example, omega 3-fatty acids possess anti-inflammatory properties as well as neuroprotective capabilities (Groeger et al., 2010; Richardson and Montgomery 2005). How fish consumption is also an external source for Hg exposure that can present a concern to the Indigenous communities in NWT (Government of Northwest Territories, 2016). Past literature have found positive associations between dietary exposure to MeHg and the development of cardiovascular disease, neurocognitive and sensory deficits (Carta et al., 2003; Houston, 2011). Studies have also shown that MeHg can cross the placental blood barrier which can lead to cognitive impairment among developing neonates and children (Axelrad et al., 2007; Carta et al., 2003).

Mercury toxicity is a potential concern for the Dene and Métis communities residing in Dehcho and Sahtú regions because of the importance of wild-harvested fish to the diets of residents (NWT Bureau of Statistics, 2016). Fish consumption patterns can influence internal Hg exposure levels detected in blood and hair biomarkers (Van Oostdam et al. 2005; Wheatley and Paradis, 1995). In a previous study, 38,571 Indigenous Canadians from 514 different Indigenous communities participated in a longitudinal Hg exposure study (Wheatley and Paradis, 1995). Research by Wheatley and Paradis (1995) noted that 23% (8,847 of 38,591) of the study population had blood Hg concentrations above Health Canada's recommended Hg guidance level (20μg/L) (Statistics Canada, 2013). Wheatley and Paradis (1995) noted that most of the individuals with elevated levels of Hg came from communities residing within the northern regions of Canada, of which the Dehcho and Sahtú are included. As such, it is important to characterize the potential relationships between dietary and demographic determinants with respect to Hg exposure found within blood and hair biomarkers.

As a potential source for dietary Hg, it is important to conduct Hg exposure assessments for various lakes within NWT and determine the levels of Hg found present within fish species. Studies have shown that various species of fish harvested from Great Slave Lake contain detectable levels of Hg (0.05-0.27 ppm) that were below 0.5ppm, the guideline values for commercial sale of fish (Cott et al., 2016, Evans et al., 2013). Mercury contaminant studies have also been conducted for the following lakes along the Mackenzie River which includes Big Island Lake, Deep Lake, Ekali Lake, Fish Lake, Gargan Lake, Little Doctor Lake, McGill Lake, Mustard Lake, Sanguez Lake, Tathlina Lake, Trout Lake, and Willow Lake (Evans et al., 2013; Laird et al., 2018; Reyes et al., 2017). Past literature have found non-piscivorous fish species, such as cisco (*Coregonus autumnalis*), lake whitefish (*Coregonus clupeaformis*) and longnose

sucker (*Catostomus catostomus*), caught from lakes along the Mackenzie River had Hg content below 0.5ppm (Evans et al., 2013; Laird et al., 2018; Reyes et al., 2017). In contrast, piscivorous fish species, such as lake trout (*Salvelinus namaycush*), loche (*Lota lota*), northern pike (*Esox lucius*), sucker (*Catostomus catostomus*) and walleye (*Sander vitreus*), can possess Hg levels well above 0.5ppm (Evans et al., 2013; Laird et al., 2018; Reyes et al., 2017). Past literature have also noticed a change in Hg content overtime as Hg levels detected in lake trout and loche from Great Slave Lake have increased during 1990-2012 (Evans et al., 2013). In response to the levels of Hg detected, there are fish consumption notices for various lakes within NWT that advise individuals to limit consumption of piscivorous fish species (Government of Northwest Territories, 2016).

In order to fully characterize Hg exposure it is important to understand internal levels (e.g., biomarkers), their demographic and dietary determinants, as well as potential constraints (e.g., bioavailability) on exposure pathways. Previous studies have noted a relationship between the internal levels of Hg found in blood and hair biomarkers with external Hg sources such as fish consumption (Kim et al., 2016; Oken et al., 2008; Schaefer et al., 2014). Currently, exposure assessment models would conservatively assume Hg bioavailability to be 100% because of the limited site-specific information about Hg's bioaccessibility. Previous studies have noted Hg bioaccessibility can vary between different foods (Laird et al., 2009; Siedlikowski et al., 2016). Past literature have found gastrointestinal Hg bioaccessibility to be independent from Hg concentration in country foods (Laird et al., 2009). In contrast, past literature have also found a negative correlation between initial Hg concentration in seafood and Hg bioaccessibility (Siedlikowski et al., 2016). Studies have also shown that cooking methods can affect Hg bioaccessibility in fish (Costa et al., 2015; Ouédraogo and Amyot, 2011). Therefore, there is a

need for more Hg bioavailability and Hg bioaccessibility findings to account for Hg bioavailability within exposure assessment models.

Previous biomonitoring studies have identified positive relationships between demographic and dietary factors associated with an individual's Hg levels. However, few of these studies have investigated these topics among Dene and Métis populations of the Northwest Territories. As such, this research project will address the current understanding about Hg exposure within the Dene and Métis communities of the Dehcho and Sahtú regions, by conducting both Hg biomonitoring and Hg bioaccessibility studies. Firstly, the biomonitoring component will characterize demographic information as well as fish consumption patterns for the participating Indigenous communities in the Dehcho and Sahtù regions. This research will then explore the relationships between demographic patterns and internal Hg exposure levels found within blood and hair samples. Similarly, this research will also attempt to identify relationships between piscivorous and non-piscivorous fish consumption patterns with respect to the community's internal Hg exposure levels. Secondly, the bioaccessibility component will provide information about Hg concentration and Hg bioaccessibility in lake whitefish which is one of the most commonly consumed fish species within the Dehcho and Sahtú regions of NWT (Kuhnlein et al., 1994; Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). This research will also lay the foundation for how drying/smoking whitefish, may potentially influence Hg bioaccessibility. Overall, the bioaccessibility research findings may be important on a larger scale and it may be necessary to factor in Hg bioavailability into exposure assessment models.

2.1 Research Question

For the Dene and Métis communities in the Dehcho and Sahtú regions, wild-harvested freshwater fish are consumed within their country food diets (Kuhnlein et al., 1994; Morrison and Kuhnlein, 1993). However, increased fish consumption is positively associated with blood and hair Hg levels (McDowell et al., 2004; Oken et al., 2008). It is important to further characterize the potential relationships between dietary and demographic determinants with respect to blood and hair Hg levels, particularly for the Dene and Métis communities in the Dehcho and Sahtú regions of NWT. As such, the following research questions were addressed in Part 1 (Chapter 4) of this two-part thesis:

1.1 Individually, which demographic factors and fish species are associated (positively or negatively) with the blood and hair Hg levels found within the Dene and Métis communities from the Dehcho and Sahtú regions of NWT?

1.2 Using the demographic dataset and FFQ, what dietary factors can account for the variances in blood and hair Hg levels found within the Dene and Métis communities from the Dehcho and Sahtú regions of NWT?

Part 2 (Chapter 5) of this thesis project evaluated how culinary methods, specifically drying, can influence Hg concentrations and bioaccessibility. Part 2 of this provides complementary information for ongoing mercury exposure characterization in the Northwest Territories. As such, the following research questions were addressed in Part 2 of this two-part thesis:

2.1 Does drying whitefish affect its initial Hg concentration, compared to uncooked?

2.2 Does drying whitefish affect Hg bioaccessibility, compared to uncooked?

2.2 Research Objectives

The following research objectives were divided up into two components, in order to answer the research questions mentioned above:

Part 1 - Hg biomonitoring component.

1.1 To determine internal Hg exposure (blood and hair) levels from six Indigenous communities in the Dehcho and Sahtú regions of NWT.

1.2 To characterize the relationship between internal Hg exposure and sex, by conducting t-test analysis.

1.3 To construct simple linear regression models and identify the relationship between internal exposure with the following factors: i) age ii) non-piscivorous fish consumption iii) piscivorous fish consumption.

Part 2- Hg bioaccessibility c omponent.

2.1 To characterize Hg concentrations for uncooked and dried/smoked lake whitefish (*Coregonus clupeaformis*).

2.2 To determine Hg bioaccessibility in uncooked and dried/smoked lake whitefish.

2.3 To conduct t-test analysis to determine Hg concentration and bioaccessibility differences between uncooked and dried/smoked whitefish.

3. Literature Review on the Bioaccessibility and Biomonitoring of Methylmercury in Indigenous Communities

3.1 Introduction

This literature review has four distinct sections. Firstly, the current socio-demographics of Canada's Indigenous populations, particularly Dene and Métis communities within NWT, are outlined and the current health patterns within these communities are described. The literature review also covers the current environmental contaminants present in the circumpolar region of Canada and mechanisms that explain the influx of local environmental toxicants. The literature review will also examine food consumption patterns and preparation methods used by Indigenous communities.

The second component of the literature review provides an overview of the existing research about anthropogenic sources, toxicokinetics and toxicodynamics of Hg. Within this section, this literature review will discuss about key concepts, which include bioavailability and bioaccessibility, as well as the properties and application of *in vitro* bioasccessibility (IVBA) models. Furthermore, this literature will review focuses on Hg's mechanism of action in order to elicit cytotoxicity and highlights Hg's ability to induce neurodegenerative symptoms, cardiovascular diseases, and immune-compromised effects from *in vitro, in vivo* and clinical studies.

The third component of the literature review examines internal and external exposure assessment models. This literature review also identified the strengths and limitations of biomarkers that are commonly used for internal exposure assessments. Demographic and external exposure factors are also examined and how they may influence internal exposure

assessments. Furthermore, this literature review describes the application of biomonitoring data and exposure assessment models.

3.2 Indigenous People of NWT and Canada

The National Household Survey (NHS) data identified 1,400,685 people as Indigenous (i.e., identifying as First Nations, Métis, or Inuit), which represents 4.3% of Canada's total population (Turner et al., 2011). During 2006 – 2011, the Indigenous population increased by 232,385 and of the 1,400,685 Indigenous individuals, 60.8% identified themselves as First Nations, 32.3% as Métis, and 4.2% as Inuit (Turner et al., 2011). About 1 in 3 Canadians living in NWT are of First Nations descent, which makes them the largest ethnicity group in the area (Turner et al., 2011). There are more than 30 different communities residing within NWT, which can be subdivided into 6 regions including Beaufort Delta, Dehcho, Sahtú, South Slave, Tilcho and Yellowknife (NWT Bureau of Statistics, 2017, Receveur et al., 1997).

3.2.1 Health Patterns within Indigenous Communities

Indigenous communities are quite young in comparison when compared to the general population of Canada. In 2011, the median age for the Indigenous population was 28 years where as the median age for the rest of Canada was 41years (Adelson, 2005; Turner et al., 2011). Accordingly, 10.7% of the First Nations' population are below the age of 5 and 28% are under the age of 14 while 5.5 % of Canada's total population are below the age of 5 and 16.5% are under the age of 14 (Turner et al., 2011). Similarly, 5.5% of the First Nations' population are above the age of 65 while for the rest of Canada, 14.2% of the total population is above the age of 65 (Turner et al., 2011). The younger age of Indigenous populations in Canada is due in part to higher fertility rates and shorter lifespans relative to the Canadian general population (Adelson, 2005; Turner et al., 2011; Van Oostdam et al. 2005).

Chronic diseases, such as obesity, diabetes and cancer represent great concerns for both Indigenous as well as non-Indigenous communities (Bélanger-Ducharme, 2005; Hackett, 2005; Reading and Wien, 2009; Tjepkema, 2002). Currently, the obesity rates for youths between the ages of 12-17 are significantly higher in First Nations (26%) and Métis (28%) communities compared to non-Aboriginal youths (19%) (Gionet and Roshanafshar, 2015). Similar patterns were also noted for adulthood obesity rates when comparing First Nations (26%) and Métis (22%) communities with respect to the non-Aboriginal Canadians (16%) (Gionet and Roshanafshar, 2015). During 2007-2010, the prevalence of diabetes was higher in First Nations communities compared to non-Aboriginal population (Gionet and Roshanafshar, 2015). Previous studies have found a significant positive relationship between obesity and diabetes (Nguyen et al., 2011). The increased prevalence of obesity and diabetes may be related to a lack of exercise and a shift towards western diet, which is often high in fat and sugar (Earle, 2011; Reading and Wien, 2009). Additionally, there has been a decrease in consumption of traditional foods and reduction in traditional harvesting practices over time (Earle, 2011; Reading and Wien, 2009). Furthermore, rates of alcohol and substance increased in the later half of the twentieth century to present date (Hackett, 2005; Reading and Wien, 2009). With respect to smoking (which is associated with an increased rate of developing heart disease and lung cancer), Indigenous individuals are more than twice as likely to smoke (Pope III et al., 2011; Tjepkema, 2002). Past literature have found a positive association between smoking and neonates developing congenital abnormalities, such as neural tube defects, as well as higher infant mortality rates (Ray et al., 2004; Smylie et al., 2010; Tjepkema, 2002).

3.2.2 Environmental Contaminants in the Circumpolar Regions of Canada

The circumpolar region of Canada is defined as parts of Canada that lies between 55°N and the North Pole. This region encompasses the Arctic and subarctic regions of Canada and includes the Yukon, NWT, Nunavut and northern parts of Quebec and Labrador. During the mid 20th century, the Arctic was perceived to be a pristine environment and it was assumed that any pollutants present would be diluted to the point that risks were negligible (Hansen and Van Oostdam, 2009; Odland and Nieboer 2012). However, this assumption did not account for natural phenomena that globally transport pollutants and drive the bioaccumulation and biomagnification of contaminants in northern ecosystems (Odland and Nieboer, 2012). Scientists have been monitoring contaminants in the Arctic region since the 1970's and in 1991, the Arctic Monitoring and Assessment Programme (AMAP) was established to further characterize environmental toxicants in Arctic region (Hansen and Van Oostdam, 2009; Northern Contaminants Program, 2003). Past literature have shown that heavy metals, such as arsenic (As), cadmium (Cd), lead (Pb), and Hg, are present in the Arctic and capable of entering the local ecosystem (Evans et al., 2005; Kuhnlein and Chan, 2000; Larter and Nagy, 2000). Similar findings have also been observed for organochlorines, including dichlorodiphenyl-dichloroethylene (DDE), dichlorodiphenyl-trichloroethane (DDT) and polychlorinated-biphenyl (PCBs) (Evans et al., 2005; Kuhnlein and Chan, 2000).

Local anthropogenic sources, such as mining, smelting and industrial manufacturing have become major contributors to the release of contaminants in the surrounding Canadian Arctic regions (Donaldson et al. 2010; Hansen and Van Oostdam, 2009; Kuhnlein and Chan, 2000; Pirrone et al., 2010). However, for pollutants such as Hg, only a small fraction originates from the circumpolar regions of Canada (Hansen and Van Oostdam, 2009). In fact, natural phenomena

such as global distillation and thermohaline circulation pathways, account for most of the influx of Hg into Arctic regions of Canada (Donaldson et al. 2010; Mason et al., 1994; Hansen and Van Oostdam, 2009; Kuhnlein and Chan, 2000; Pirrone et al., 2010).

Once present in the soil and water, pollutant deposits can bioaccumulate into the local ecosystems (Lockhart et al., 1992; Muir et al., 1999). This presents a great concern to the Indigenous communities in NWT (Government of Northwest Territories, 2016). Not only have they maintained a deep and profound connection with their local environment for many generations, but they also are part of the local ecosystem by partaking in traditional practices such as fishing, gathering, hunting and trapping (Beckford et al., 2010; Earle, 2011; Reading and Wien, 2009; Richmond and Ross, 2009). Studies have shown that various species of fish harvested from Great Slave Lake contain detectable levels of Hg (0.05-0.27 ppm) that are below heath guidance values of 0.5ppm (Cott et al., 2016, Evans et al., 2013). However, the levels of Hg detected in lake trout (*Salvelinus namaycush*) and burbot (*Lota lota)* from Great Slave Lake have increased from 1990-2012 (Evans et al., 2013). Additionally, there are fish consumption notices for various lakes within NWT that advise individuals to limit consumption of predatory fish species (Government of Northwest Territories, 2016).

3.2.3 Dene and Métis Food Consumption and Preparation Patterns

In Canada, food insecurity is an ongoing issue that the country faces. For the Indigenous communities residing in NWT, as a greater proportion of their population face food insecurity (11.4%) compared to the reset of Canada (8.3%) (Statistics Canada, 2013). In general, food is far more expensive in the Canadian circumpolar region than in the southern provinces of Canada (NWT Bureau of Statistics, 2015). As such, there is a need for subsidies (Galloway, 2017). Furthermore, the food price index for smaller communities, such as those found within the

Dehcho and Sahtú regions of NWT, can be 1.16 – 1.96x more expensive compared to Yellowknife (NWT Bureau of Statistics, 2015). Local markets within these communities are also typically lacking in terms of variety in selection (Galloway, 2017). Perishable foods tend to be subpar because they are often damaged and/or spoilt prior to arriving at the local markets (Galloway, 2017). Even with subsidies, the cost of perishable foods acan put them out of reach for many in northern communities (Galloway, 2017). This results in increased sales and consumption of highly-processed, non-perishable foods that may be nutritionally lacking (Galloway, 2017; Richmond and Ross 2009). The presence of highly-processed foods have led to the disproportionally elevated levels of fat, salt and sugar consumption (Batal et al., 2004). These dietary patterns may have contributed to the increased incident rates of obesity and diabetes (Earle, 2011; Nguyen et al., 2011; Power, 2008; Richmond and Ross 2009). Although there has been an increase in consumption of store bought food, traditional food sources make up a significant portion of their diet and they are also culturally important for Indigenous communities (Batal et al., 2004). Additionally, the Dene and Métis communities continue to carry out these traditional practices, as they hold cultural importance (Donaldson et al., 2010; Van Oostdam et al. 2005).

In terms of nutritional composition, traditional foods can supply a large source of protein and omega 3-fatty acids to Dene and Métis communities (Kuhnlein and Humphries, 2017; Kuhnlein and Receveur, 2007). Furthermore, they are also a source of certain nutrients, such as vitamins A, B, D, and E, as well as Copper (Cu), Iron (Fe), Magnesium (Mg), Potassium (K), Selenium (Se) and Zinc (Zn) (Donaldson et al. 2010; Fediuk et al. 2002; Laird et al. 2013; Kuhnlein et al., 2006; Kuhnlein and Humphries, 2017; Kuhnlein and Receveur 2007). However,

the consumption of some traditional foods may also pose health concerns related to Cd and Hg exposure (Cott et al., 2016; Larter et al., 2016).

Generally, the Dene and Métis communities of the NWT rely less on agriculture and more on hunting, trapping and fishing (Food Safety Network, 2009; NWT Health and Services, 2002). Dietary patterns can vary between Indigenous communities with respect to geographic location (Van Oostdam et al. 2005). The Dene and Métis communities consume a variety of fish (Kuhnlein and Humphries, 2017). Depending on their region, they may consume cisco (*Coregonus autumnalis*), grayling (*Thymallus thymallus*), inconnu (*Stenodus nelma*), lake trout (*Salvelinus namaycush*), loche (*Lota lota*), northern pike (*Esox lucius*), sucker (*Catostomus catostomus*), walleye (*Sander vitreus*) and whitefish (*Coregonus clupeaformis*) (Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). The edible flesh portion of the fish as well as the head, liver and eggs are prepared by either boiling, drying, frying or smoking (Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). Specific traditional food preparation methods of fish can vary between communities (Kuhnlein and Humphries, 2017). Consumption of beaver (*Castor canadensis*), caribou (*Rangifer tarandus*), moose (*Alces alces*), muskrat (*Ondatra zibethicus*), and various species of rabbits are also common (Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). The various cuts of meats and offal are prepared by either baking, drying, frying, roasting or smoking (Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). In NWT, the Dene and Métis communities consume a variety of game birds, including goose, duck, ptarmigan, and grouse are commonly consumed (Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). Prior to consumption, the feathered game can be prepared by either drying, freezing or smoking (Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). Based on their region, some of the more commonly consumed

berries and wild greens include blueberries, cloudberries, cranberries, gooseberries, raspberries, Saskatoon berries, strawberries, dock, dandelion, fireweed and lamb quarters (NWT Health and Services, 2002).

3.3 Mercury

Mercury (Hg) is a metallic chemical element that can be found throughout all parts of the world (Sheehan et al., 2014). Hg and its derivatives can be differentiated into one of two categories: inorganic (iHg) and organic Hg (Nordberg and Aitio, 2008; Sheehan et al., 2014). Among iHg species, Hg can be further classified into several states, such as metallic mercury, mercury vapor, mercurous salt and mercuric salt (Nordberg and Aitio, 2008). In contrast, each of the organic Hg compounds include carbon-based substituents that are covalently attached to Hg (Nordberg and Aitio, 2008). Methylmercury (MeHg), dimethylmercury (Me_2Hg) and ethylmercury (EtHg) are some of the more common organic Hg derivatives, (Clarkson et al., 2003; Nordberg and Aitio, 2008). Although Hg toxicity is dependent on its state, dosage and route of exposure, its impact can be felt throughout the world. Due to the prevalence of Hg exposure, organizations such as the World Health Organization (WHO) considers Hg as one of the top 10 chemicals that presents a great concern to the general public (Burger et al., 2011; Clarkson et al., 2003; Sheehan et al., 2014). This is particularly true for MeHg because it is among the most common, hazardous and bioavailable derivatives of organic Hg (Burger et al., 2011; Clarkson et al., 2003; Sheehan et al., 2014).

3.3.1 Mercury Sources

There are two categories for Hg sources: anthropogenic and natural. Anthropogenic sources, such as mining, smelting and industrial manufacturing have become major contributors to the influx of contaminants in the Arctic regions of Canada (Donaldson et al. 2010; Hansen and

Van Oostdam, 2009; Kuhnlein and Chan, 2000; Lodenius and Malm, 1998; Pirrone et al., 2010; WHO, 1991). Additionally, iHg can be found worldwide in pigment dyes, food preservatives, antibacterial agents and cosmetics (Clarkson and Magos, (2006). Distant anthropogenic sources of Hg can either be circulated into the air through the process of global distillation or transported through the ocean's thermohaline circulation pathways, prior to being deposited into the Arctic regions of Canada (Donaldson et al. 2010; Mason et al., 1994; Hansen and Van Oostdam, 2009; Kuhnlein and Chan, 2000; O'Driscoll, 2005; Pirrone et al., 2010).

3.3.1.1 Mercury Exposure in Food

One of the main routes of exposure for MeHg in humans is through dietary consumption, particularly of fish and seafood (Burger et al., 2011; Golding et al., 2013; Gribble, et al., 2015; Hu et al., 2014; Kuhnlein and Chan, 2000; Laird et al., 2009; Laird and Chan, 2013; Ysart et al., 2000). In fact, more than one billion people worldwide rely on seafood as their main protein source with the global capita seafood consumption increasing from an average of 9.9 kg in the 1960's to 20 kg in 2014 (FAO State of World Fisheries and Aquaculture, 2016). There are various groups of reducing bacteria that methylate iHg into MeHg which allow for MeHg to bioaccumulate into aquatic species (Compeau and Bartha, 1985; Kerin et al., 2006). Through the process of MeHg biomagnification and bioaccumulation, fish and seafood consumption are often the primary sources of human dietary MeHg exposure (Power et al., 2002; Ysart et al., 2000). In North America, it was found that the ten most frequently consumed types of seafood were salmon, shrimp, white tuna canned in water, tilapia, light tuna canned in water, fresh tuna, cod, crab, halibut, and scallops (Afonso et al., 2015; Cabañero et al., 2007; Calatayud et al., 2012; Costa et al., 2015; Ouédrago and Amyot, 2011; Wang et al., 2013). Even though dietary Hg predominantly comes from fish that does not mean other dietary sources of MeHg are negligible

(FDA, 1999; Horvat et al., 2003; Lindenberg et al., 2004; Ysart et al., 2000). Within the western parts of NWT, Bluenose caribou are capable of carry considerable amount of Hg burden within their kidneys (10.45μg/g) (Larter and Nagy, 2000).

3.3.2 Toxicokinetics of Mercury

MeHg must first enter the body before it can elicit its toxicodynamic effects. MeHg is not only readily absorbed through the gastrointestinal tract but it can also efficiently enter the body through epidermal contact and inhalation (Aberg et al., 1969; Hong et al., 2012). In the past, it was often assumed that MeHg has a general oral bioavailability of 95%-100% and can remain in circulation by binding to the cysteine (CYS) residues' sulfhydryl substituents found on red blood cells (RBC)s (Aberg et al., 1969; Bradley et al., 2017; Kershaw et al., 1980; Miettinen, 1973). Studies have shown that MeHg's bioaccessibility in fish can vary greatly and be less than 100%, based on various *in vitro* models findings (Bradley et al., 2017; Ouédrago and Amyot, 2011; Maulvault et al., 2015). After 15 minutes of ingestion, MeHg can be found circulating in the bloodstream by being covalently bound to sulfhydryl substituents of RBC, particularly the CYS 104 of the α-subunit and the CYS 93 and 112 of the β-subunit (Aberg et al., 1969; Kershaw et al., 1980; Miettinen, 1973). Additionally, MeHg can achieve its peak blood concentration within 3-14 hr before it is deposited into the bone marrow, brain, heart, kidney, liver and spleen of the body through binding of CYS substituents (Aschner and Clarkson, 1987; Kerper et al., 1992; Yin et al., 2008). In order for MeHg to bioaccumulate into the brain, it must first form a complex with L-cysteine and L-methionine (Aschner and Clarkson, 1987; Kerper et al., 1992; Yin et al., 2008). Afterwards, the MeHg-complex can then be transported by the L-type neutral amino acid carrier transport-1 (LAT-1) system in order pass through the blood brain barrier (BBB) and bioaccumulate in the cortex (Aschner and Clarkson, 1987; Kerper et al., 1992;

Yin et al., 2008). Furthermore, MeHg can use the LAT system to cross the placental barrier to affect developing fetuses by forming a complex with L-type neutral amino acid (Kajiwara et al., 1996).In terms of oral digestion, approximately 7% to 15% of doses of iHg is absorbed in the gastrointestinal tract after ingestion making it 17- 15 times slower than MeHg absorption's rate (Abernethy et al., 2010; Hong et al., 2012; Park, and Zheng, 2012). Inorganic Hg can readily enter through the lungs; inhalation of iHg vapors greater than1–2 mg/m^3 can result in bronchiolitis and pneumonitis (Asano et al., 2000). Dermal absorption of iHg through the skin, sweat glands, sebaceous glands, and hair follicles is possible when applying ointments and medication containing inorganic mercury salts (Park, and Zheng, 2012).

MeHg has an estimated half life of 80 days, with approximately 90% of it being eliminated through fecal excretion (Cikrt and Tichý, 1974; Jo et al., 2015; Nordberg and Aitio, 2008). However, there also appears to be enterohepatic circulation present for MeHg. Additionally, MeHg in the brain can also be bio-transformed through the process of demethylation. Although the enzyme responsible for demethylation is not fully elucidated, MeHg is slowly demethylated and lead to the bioaccumulation of inorganic Hg in the brain (Davis et al., 1994; Rooney, 2014; Yin et al., 1996). Throughout an individual's entire lifetime, 80-100% of the total Hg found throughout the brain is inorganic Hg with an average half life of 27.4 years (Davis et al., 1994; Rooney, 2014; Yin et al., 1996).

3.3.2.1 Bioaccessibility and Bioavailability

In toxicokinetic-related research, it is important to understand a contaminant's bioavailability and bioaccessibility properties. Bioavailability is the fraction of a parent compound or active metabolite that crosses the gastrointestinal epithelium, absorb into the organism's circulatory system, remain chemically bioactive and reaches its target tissue or

receptor, for it to elicit its mechanism of action (Fernández-García et al., 2009). Bioaccessibility is the fraction of a compound that has been solubilized into gastrointestinal fluids during gastrointestinal digestion prior to crossing the gastrointestinal epithelium (Fernández-García et al., 2009). Bio accessibility and bioavailability play an important role in human risk assessments and Hg exposure (Bradley et al., 2017). Dietary sources and their estimated Hg concentrations are incorporated into exposure models, in order to predict exposure levels found present within the body (Bradley et al., 2017). Past literature have noted that such models may assume that nearly all of the Hg ingested is bioavailable because of how difficult it is to determine all of the quantitative factors with a high degree of certainty, albeit resulting in an overestimation of health risk associated with traditional country foods (ATSDR, 1999; Bradley et al., 2017; Laird et al., 2009).

In vitro bioaccessibility (IVBA) models are used to estimate a nutrient or toxicant's bioavailability which is required for nutritional efficiency and human exposure risk assessments (Alminger et al., 2012). In order to determine the bioavailability of a compound, it is also necessary to determine its rate of absorption, metabolism, tissue distribution and bioactivity (Fernández-García et al. 2009). One may be able to determine the maximum bioavailability of a compound by evaluating its bioaccessible fraction as the conservative estimate. Therefore, IVBA models can act as a surrogate to determine a toxicant's bioaccessibility, which may represent a theoretical maximum for bioavailability (Siedlikowski et al., 2016; Versantvoort et al., 2004).

Toxicology researchers can use several static IVBA methods to determine a toxicant's bioaccessibility (Glahn et al., 1999; Hur et al., 2011; Ouédraogo and Amyot, 2011). Static IVBA models propose certain fixed conditions, which include the quantity as well as the concentration of enzymes, nutrients and bio-activated compounds orbiting in a constant angular velocity (Hur

et al., 2011). Although there may be multiple steps to represent the oral, gastrointestinal and intestinal digestion, most of the enzymatic processes occur within a single bio-reaction chamber (Hur et al., 2011). Compared to *in vivo* bioavailability models, static IVBA models are overall less costly to operate and easier to maintain (Boisen and Eggum, 1991; Glahn et al., 1999). However, human digestion is naturally a dynamic process rather than being constant in terms of chemical and environmental parameters (Alegría et al., 2015). This can limit the effectiveness of static IVBA models and its ability to serve as preliminary bioavailability estimates (Hur et al., 2011; Boisen and Eggum, 1999).

Dynamic IVBA models such as the Simulator of Human Intestinal Microbial Ecosystem (SHIME) can provide an alternative methodology to estimating a contaminant's bioaccessibility (Laird et al., 2009). Compared to static IVBA models, the SHIME model proposes multiple compartmentalization stages for digestion as well as incorporating the activity of human gastrointestinal microbiota (Laird et al., 2009). However, obstacles such as cost, logistics and maintenance can prevent models like SHIME from becoming popular to use. Additionally, both dynamic and static IVBA cannot truly mimic certain physical processes, such as shearing and peristalsis (Alegría et al., 2015). Although both dynamic and static IVBA models can achieve a certain degree of homogenization, through shaking or agitation, to represent the oral digestion phase, it is unable produce a bolus (Alegría et al., 2015).

3.3.2.2 Factors that affect Bioaccessibility

There are many important variables to consider, when conducting *in vitro* bioaccessibility research. The composition of biological macromolecules used in IVBA models play a significant role in determining a contaminants bioaccessibility and therefore, are extensively researched (Hur et al, 2011). Past literature have found biological macromolecules such as digestive

enzymes, bile salts and mucin are important within IVBA models (Hur et al., 2011). Looking more closely, lipase, protease and amylase are the three major groups of hydrolytic enzymes that are responsible for breaking down food (Hur et al, 2011). There are other important physiochemical properties that can influence a contaminant's bioaccessibility which include solid food sample/ digestion liquid (S/L) ratio, degree of agitation, digestive-stage duration and pH (Drexler and Brattin, 2007; Hur et al., 2011; Shi et al., 2017). Finally, food composition and preparation methods can influence IVBA findings (Ouédraogo and Amyot, 2011).

Lipase can be found in the stomach and pancreas as either gastric lipase or pancreatic lipase, which break down triglyceride and diglyceride molecules into monoglyceride and free fatty acid molecules that could further be broken down (Hur et al., 2011; Kimura et al., 1982). Additionally, pancreatic lipase activity is dependent on colipase, calcium and bile salts (Erlanson-Albertsson 1983; Kimura et al., 1982). Bile salts are secreted into the duodenum during the fed state and can be found in the duodenal and jejunal fluid with a peak concentration of 40mM and an average concentration between 5- 15 mM after digestion (Zangenberg et al., 2001). As such, differences in commercially prepared lipases may lead to variances between bioaccessibility studies' findings; commercial preparations of porcine pancreatic lipase are not standardized resulting in various enantiomers of the same hydrolytic enzymes with differing catalytic properties (Segura et al., 2004).

Proteases are predominantly found in the stomach and intestine in the form of trypsin, chymotrypsin, pepsin and peptidase. At 37 °C, these enzymes are responsible for breaking down protein and peptide chains into either smaller peptide chains or amino acids (Bublin et al., 2008; Hur et al., 2011). A potential source of variance between different bioaccessibility studies is the variability in protease composition. IVBA models that have a gastric only phase and 3 types of

proteases can digest more protein (39-66%) compared to two-step digestion models containing pepsin and Pancreatin (Abdel-Aal et al., 2008).

Amylase is found in the mouth and stomach and is primarily responsible for deconstructing long-chained oligosaccharides into monosaccharides such as glucose. Out of all the forms of amylase found in the human digestive system, α-amylase is the ubiquitous and is readily added into IVBA models that include oral digestion (Hur et al., 2011). Aside from the presence or absence of α-amylase in the IVBA model, it is not fully elucidated whether or not amylase has an impact on other enzymes present and their catalytic activities (Hur et al., 2011).

IVBA models can be designed to have a single stage (gastric only digestion), two stage digestion (gastric and intestinal-pancreatic digestion) or three stage digestion (oral-gastric-intestinal and gastric-small intestine-colone digestion) (Hur et al., 2011). It is difficult to determine the how necessary it is to include oral digestion is in IVBA. The IVBA models cannot naturally produce a bolus, they utilize other physical process to homogenize food samples (Afonso et al., 2015; Afonso et al., 2016; Costa et al., 2015; He and Wang, 2011; Matos et al., 2015). Aside from hydrolysis of oligosaccharides, the mouth's α-amylase is not known to have any direct impact on the decomposition of protein and lipids (Hur et al., 2011). Similar to mucin and BSA, it is still included in IVBA models that are looking for potential interactions with other enzymes that have yet to be elucidated (Costa et al., 2015; Shim et al., 2009; Maulvault et al., 2011; Cano-Sancho et al., 2015; Wang et al., 2013). Additionally, there may be differences in a compound's bioaccessibility when comparing gastric only digestion and gastrointestinal digestion (Maulvault et al., 2011). For edible crab (*Cancer pagurus*), Cd is less bioaccessible in single step IVBA models (46%) compared to two-step IVBA (93%) (Maulvault et al., 2011). Alternatively, there are no statistical differences in Hg bioaccessibility between single-step and

two-step IVBA models, when using black scabbard fish (*Aphanopus carbo*) (Maulvault et al., 2011).

Based on the current body of knowledge, there are a myriad of indirect factors that influence IVBA findings, such as digestion solid food sample/ liquid (S/L) ratio, time and pH (Hur et al., 2011; Shi et al., 2017). Firstly, Shi et al. have reported that S/L ratio has a significant negative correlation with pyrethroids bioaccessibility in apples (Shi et al., 2017). Various digestion times were reported for each stage of IVBA models but 2-3hr was the most frequent for stomach, small intestine and large intestine stage while increase to the digestion time yielded zero significant difference in the bioaccessibility fractions ((Hur et al., 2011; Shi et al., 2017). Various pHs were reported for each stage of IVBA models but pH of 2 and 6.5-7 were the most frequent reported to be used for stomach and intestine stage respectively. Gastric pH can influence bioaccessibility estimates; for pyrethroids, it is most optimal to use IVBA models with a gastric phase pH of 1.91 (Shi et al., 2017). One indirect factor that is not well understood is agitation method and the degree of agitation (Alegría et al., 2015). Studies have found physical stressors to play a necessary role in human digestion, when comparing Pb relative bioaccessibility and bioavailability (Drexler and Brattin, 2007). Finally, methods of food cooking and preparation can affect bioaccessibility; boiling and frying fish can reduce Hg bioaccessibility compared to raw (Ouédraogo and Amyot, 2011). Furthermore, co-administration of tea or coffee with raw fish can also reduce Hg bioaccessibility compared to untreated (Ouédraogo and Amyot, 2011).

3.3.3 Toxicodynamics of Mercury

Once circulating in the body, MeHg is capable of inducing cellular dysfunction as well as cytotoxicity. MeHg can cause cellular disruption to the mitochondria's electron transport chain

(ETC) which leads to the production of reactive oxidative species (ROS) such as superoxide anion and hydrogen peroxide (Cordeiro, 2014; Mori et al., 2007; Shanker and Aschner, 2003; Stohs and Bagchi, 1995). ROS are capable of binding and forming adducts with proteins, DNA and phospholipid- bilayer that will affect the integrity and functionality of the cell. As a result, oxidizing agents such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) are present within the cytoplasm to oxidize and eliminate ROS in order to prevent activation of intrinsic apoptosis pathway (Cordeiro, 2014; Stringari et al., 2008). In order for GPX to oxidize ROS, 2 glutathione (GSH) reactant molecules are required to be reduced during this redox process (Stohs and Bagchi, 1995; Stringari et al., 2008). As a result, water and glutathione disulfide (GSSG) molecules are created and GSSG must be reduced into 2 GSH molecules again by glutathione reductase (GR) which they will once again be used for reducing more ROS (Stohs and Bagchi, 1995; Stringari et al., 2008). However, not only can produce ROS, but MeHg can also covalently bind to GSH (Stohs and Bagchi, 1995; Stringari et al., 2008). By oxidizing GSH, they form MeHg-SH which is water-soluble and can be excreted out of the body (Stohs and Bagchi, 1995; Stringari et al., 2008). With decreased levels of intracellular GSH, it results in the cell having a reduced capacity to eliminate ROS and an overall increase in oxidative stress. Studies have shown that MeHg can impede GR and GPX activity and increase cellular oxidative stress (Cordeiro, 2014; Mori et al., 2007; Stringari et al., 2008).

3.3.3.1 Neurotoxic Effects

MeHg can be found within various lesions of the brain and, when present, it can induce neurotoxicity (Simmons-Willis et al., 2002). MeHg can cross the BBB and accumulates into the brain through LAT-1 and LAT-2 in order to elicit its neurotoxic mechanism (Simmons-Willis et al., 2002). ROS induced by MeHg can diffuse into adjacent neurons and induce neurotoxicity

through elevation of oxidative stress and apoptotic signals (Farina et al., 2003; Franco et al., 2007). When MeHg is present in astrocytes, it can inhibit cell migration. Additionally, MeHg can affect VEGF secretion for the inhibition of neuronogenesis, as studies have shown that MeHg can potentially attenuate cytokine production and impair neuronal cells ability to respond to such ligands (Aschner et al., 1999; Bozoyan et al., 2012; Sass et al., 2001).

3.3.3.2 Neurodevelopmental Effects

MeHg is highly associated with cognitive impairments and behavioural abnormalities as well as being significantly associated with cerebral palsy (Carta et al., 2003; Harada, 1995). Additionally, humans who consistently ingest MeHg contaminated seafood can eventually develop Minamata disease (Harada, 1995). This neurological disorder is comprised of many symptoms, which include ataxia, blurred visual, hearing deficiency, motor sensory dysfunction, speech impediment, seizures and tremors (Carta et al., 2003; Dolbec et al., 2000; Grandjean et al., 2010; Harada, 1995; Lebel et al., 1998). MeHg also plays a significant influence in early childhood neurocognitive development. Past literatures have found a negative relationship between children's intelligent quotient (IQ) and the meHg levels detected in a mother's (Axelrad et al., 2007; Cohen et al., 2005). Additionally, children with prenatal exposure to MeHg develop neuropsychological deficits in certain areas such as attention, linguistic capacity, memory and visuospatial abilities (Grandjean et al., 1997).

3.3.3.3 Cardiovascular Effects

MeHg can elevate the levels of oxidative stress of vascular endothelial and smooth muscle cells that can result in cardiovascular issues (Houston, 2011). Past literature has shown MeHg to be associated with increased risk for atherosclerosis, carotid obstruction, coronary heart disease, hypertension, ischemic events and myocardial infarction (Mozaffarian et al., 1999).

Studies have shown that individuals who are chronically exposed to Hg, such as miners and fishermen, have elevated levels of mercury in their body that is also significantly associated with hypertension and carotid intima-media thickness (Choi et al., 2009; García et al., 2007).

3.4 Internal and External Exposure Estimates

Exposure assessments provide critical information for estimating the human health risks of chemical stressors (Axelrad et al., 2007, Ye et al., 2016). Exposure assessments for toxicants can include a variety of measures, including those related to internal exposure (e.g., blood mercury in µg/L) and external exposure (e.g., mercury intake in µg/kg/d). Both measures of internal and external exposure provide critical and complementary information for risk assessment (Grandjean et al., 2010; Laird, 2017; Ou et al., 2018). For example, external exposure assessment models can help characterize Hg's exposure sources while mercury biomarkers (e.g., in blood and hair) can document differences in exposure among and within populations (Laird, 2017).

Internal exposure estimates can quantify the amount of contaminant found present within the body (Suk et al. 1996). As such, internal exposure estimates identify the level of burden associated with an environmental contaminant by assessing potential biomarkers. Blood, hair, nail and urine are all potential biomarkers that are used to determine the total Hg and MeHg exposure levels found within an individual (McDowell et al., 2004; Morrissette et al., 2004; Sakamoto et al., 2015). Furthermore, human biomonitoring studies have also observed MeHg levels of newborns through MeHg cord as the toxicant can cross the placental barrier (Morrissette et al., 2004). External exposure estimates can describe community- and population-level intake of toxicants from environmental and dietary sources (Suk et al. 1996). Environmental and dietary sources are dependent on the route of exposure, influencing how

toxicants are evaluated in their respective intake models (ATSDR, 2005). The general exposure dose equation for dietary exposure is the following:

$$EDI = \frac{C * IR * AF * EF}{BW}$$

Where EDI = Estimated dose intake, C= contaminant concentration, IR = intake rate of contaminated medium, AF = bioavailability factor, EF = exposure factor, BW = body weight of the individual (Anderson et al., 2005). When an individual faces a daily exposure to the contaminant, EF will equal to 1. For irregular exposure intervals, the exposure factor equation is used:

$$EF = \frac{F * ED}{AT}$$

Where EF = exposure factor, F = frequency of exposure (days/years), ED = exposure duration (years), AT = average time of exposure (ED x 365 days/years) (Anderson et al., 2005). For dietary exposure assessment, these equations are an essential tool to quantify a toxicant's risks associated with food consumption patterns (Kim et al., 2016; Oken et al., 2008). Dietary levels of exposure can be assessed with respect to the amount and frequency of food consumption (Health Canada 2007). Additionally, the concentration and bioavailability of the toxicant is dependent on food source (Maulvault et al., 2011). Furthermore, environmental and dietary exposure can be used to estimate internal exposure levels (McDowell et al., 2004; Kim et al., 2016). Together, both internal and external exposure assessments are capable of characterizing risk associated with exposure to environmental contaminants and can assist with the development of TRVs (Health Canada, 2010).

With each biomarker, there are strengths and limitations when determining internal exposure levels of Hg. Blood and hair biomarkers are capable of evaluating MeHg exposure in adults (Pirkle et al., 2016). In prenatal exposure, using maternal blood, cord blood and hair

biomarkers are considered to be the gold standard (Pirkle et al., 2016). Blood is considered to be the most accurate biomarker for adult and postnatal MeHg exposure (Pirkle et al., 2016). Blood biomarkers can evaluate MeHg exposure period of the past 3 months (Pirkle et al., 2016). However, infrequent or acute MeHg exposure may not result in an accurate approximation for mean MeHg blood levels over time (National Research Council, 2000). Hair is a less invasive option and can be easily preserved (National Research Council, 2000). Hair biomarkers can also be used to supplementary support blood measurements as both biomarkers are highly correlated (Pirkle et al., 2016). For hair analysis, it can evaluate Hg exposure for an additional month with each additional centimeter of hair (Pirkle et al., 2016). As such, hair analysis may be limited for individuals with short hair (Pirkle et al., 2016). Additionally, analysis may be affected by permanent hair treatments (Grandjean and Budtz-Jørgensen, 2007). There is a 20 day delay between Hg exposure and excretion of the toxicant into hair (National Research Council, 2000). This makes hair biomarkers quite useful for retrospective Hg analysis (National Research Council, 2000). For prenatal Hg exposure, cord blood is capable of looking at Hg exposure, during the past week of gestation (Pirkle et al., 2016). Upon delivery, cord blood Hg levels are about 1.5 times higher than maternal blood (Pirkle et al., 2016).

3.4.1 Factors Associated with Internal Exposure Measures

There are a myriad of demographic and external exposure variables associated with internal measures assessments of Hg as there is cross-talk between these methods (McDowell et al., 2004). Demographic factors such as age, sex, and ethnicity are very important for predicting an individual's blood or hair mercury concentration (McDowell et al., 2004; Kim et al., 2016; Oken et al., 2008). In terms of dietary patterns, past literature have shown fish and seafood consumption to be positively associated with internal Hg exposure (Kim et al., 2016; Oken et al.,

2008). Environmental factors such as, alcohol use, amalgam filling, BMI, coffee and tea consumption, as well as smoking patterns can also influence internal Hg exposure levels (Golding et al., 2013; Gustin et al., 2017). Clinical epidemiological studies have also noted potential association between internal Hg exposure levels and socio-economic status such as, employment profession, gravidity, parity, and pregnancy status (Cusack et al., 2017; Kim et al., 2016; Oken et al., 2008).

3.4.2 Applications of Exposure Assessments

Although not solely responsible, results from human biomonitoring studies and exposure assessments can assist with the development of a toxicant's TRV and human health-based guidance values (Ewers et al., 1999). Health Canada has an established guideline for blood Hg values and recommends individuals to maintain Hg blood concentration below 20 μg/L (or 6 mg/kg in hair), based on human toxicology and epidemiological studies (Health Canada, 2007; Legrand et al., 2010; Statistics Canada, 2013). Toxicological reference values are also used to identify a toxicant's risk to human health and give rise to a toxicant's provisional tolerable intake (Ewers et al., 1999). Tolerable daily intake estimates for Hg and MeHg differ between regulatory bodies (Ewers et al.,1999). For Joint Expert Committee on Food Additives (JECFA), the recommended daily provisional intake for MeHg is 0.23 μg/kg body weight (Grandjean et al., 2010). In comparison, Health Canada's recommended tolerable daily intake of MeHg for adults and children are 0.47 and 0.2 μg/kg body weight respectively (Pirkle et al., 2016).

Health-based guidance values and TRVs can play a crucial role in drafting public health legislation and policies (Suk et al. 1996). According to Health Canada, Hg levels in fish are considered low or high if concentrations are below 0.2μg/g or above 0.5μg/g respectively (Pirkle et al., 2016). Consuming more than two servings (150g) of fish high in Hg (0.5 μg/g) a week can

result in an individual exceeding their recommended weekly provisional intake of Hg and chronic exposure may lead to experiencing toxicodynamic effects (Statistics Canada, 2013). Additionally, the Canadian Food Inspection Agency (CFIA) and Health Canada have issued advisories that permits commercial distribution of seafood with Hg concentrations below 0.5mg/kg (Health Canada, 2007). Certain exceptions to this policy include escolar, orange roughy, marlin, tuna, shark, and swordfish, with the Hg threshold being 1mg/kg (Health Canada, 2007). Furthermore, other pieces of literature have leveraged Health Canada's guidelines for Hg exposure in order to draft intervention steps (Legrand et al., 2010). Publications, such as from Legrand et al., have also provided follow up recommendations with respect to an individual's blood Hg levels and recommendations were stratified by age, sex, pregnancy status or of childbearing age (Legrand et al., 2010).

3.5 Current Literature Gaps and Study Rationale

Based on the current body of literature, Hg biomonitoring studies have identified relationships between dietary and demographic determinants with internal Hg exposure levels for various study populations. Studies have shown fish consumption patterns, such as frequency, species, and method of preparation, play a role and influence an individual's Hg levels (Kim et al., 2016; Oken et al., 2008; Schaefer et al., 2014). The Dene and Métis communities consume wild-harvested freshwater fish as part of their country food diets (NWT Health and Services, 2002; Smith, 1982). However, there are limited studies that have investigated about Hg exposure sources and potential burden it has on the Dene and Métis communities residing in NWT. As such, the proposed study will attempt to establish a baseline measure of Hg exposure specifically for the Dene and Métis communities in Dehcho and Sahtú. The key strength of this study is that it explores site-specific Hg exposure data to characterize the potential relationships between

dietary and demographic determinants with respect to Hg exposure, for the participating communities of Dehcho and Sahtú regions.

Based on the current body of literature, studies have noted how important it is to characterize and account for dietary and demographic factors within their Hg exposure assessment models (Kim et al., 2016; Oken et al., 2008; Schaefer et al., 2014). Exposure assessment models would also account for Hg bioavailability within their calculations. However, there are still literature gaps with respect to Hg's bioaccessibility and bioavailability properties. Studies have shown Hg bioaccessibility can vary between different foods (Laird et al., 2009; Siedlikowski et al., 2016). Studies have also shown that cooking methods, such as frying and boiling, can lead to Hg-protein complex denaturation and affect Hg bioaccessibility in fish samples (Costa et al., 2015; Ouédraogo and Amyot, 2011). As such, exposure assessment models conservatively estimate Hg bioavailability to be 100%. Therefore, the proposed study will attempt to identify Hg bioaccessibility in uncooked and dried/smoked lake whitefish. Whitefish is one of the most commonly consumed species of fish within the Dehcho and Sahtú regions (NWT Health and Services, 2002). Secondly, traditional fish preparation methods, drying and smoking, are also part of the Dene and Métis culture and may also be a potential factor that can influence their concentration of Hg. As such it may be important to address how drying fish, may potentially have an effect on the bioaccessibility of Hg. Therefore, the proposed study will attempt to provide more information Hg bioaccessibility, in order to remove the need to assume 100% bioavailability within exposure assessment models. By incorporating bioaccessibility findings, future external exposure assessment methods may be able to provide better insight on the internal Hg exposure levels observed among the Dene and Métis communities.

4. Mercury Biomonitoring Study within the Dene and Métis Communities from the Dehcho and Sahtú regions of Northwest Territories

4.1 Introduction

Fish is an important traditional food source for the Dene and Métis communities because it is a large source of protein, omega 3-fatty acids and certain macronutrients (Kuhnlein and Humphries, 2017; Kuhnlein and Receveur, 2007). Within the geographic region, the Dene and Métis communities consume various species of fish which includes cisco, grayling, inconnu, lake trout, loche, northern pike, sucker, walleye, and whitefish (Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). As such, variance in fish consumption patterns amongst Indigenous communities may be related to geographic location (Van Oostdam et al. 2005). The Dene and Métis communities consume various parts of the fish such as the flesh, head, liver and eggs (NWT Health and Services, 2002). Fish is commonly prepared by either boiling, drying, frying or smoking (Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). Additionally, traditional food preparation methods of fish can vary between one community to another (Kuhnlein and Humphries, 2017).

Consumption of fish may also pose health concerns related to Hg exposure (Cott et al., 2016). Through the process of MeHg biomagnification and bioaccumulation, fish consumption are often the primary source of human dietary MeHg exposure (Power et al., 2002; Ysart et al., 2000). Through dietary consumption of fish, Hg's toxicodynamic effects can present a great concern (Clarkson et al., 2003; Kim et al., 2016; Oken et al., 2008; WHO, 1991). Once Hg is circulating within the body, it can lead to the development of cardiovascular disease, neurocognitive and sensory impairments (Carta et al., 2003; Houston, 2011). Furthermore, MeHg is capable of crossing the placental blood barrier and affecting development of neonates,

resulting in cognitive impairment among exposed children (Axelrad et al., 2007; Carta et al., 2003). Therefore, Hg exposure assessments can help quantify the level of risks individuals and communities may be facing. Internal exposure estimates can provide more precise quantification of circulating levels of a toxicant within the body and the risk associated with this level (Suk et al. 1996). External exposure assessments can provide additional details on the particular sources likely to be contributing to these exposures (Suk et al. 1996).

The primary objective of the present study was to characterize internal Hg exposure levels for the Dene and Métis communities of the Dehcho and Sahtú regions of NWT, by analyzing Hg in blood and hair biomarkers. In addition, this study explored the relationship between internal Hg exposure levels with respect to external Hg exposure factors, based on the demographic information and food frequency questionnaire provided by participants.

4.2 Methods

The following Hg cross-sectional biomonitoring study is part of a larger, ongoing interdisciplinary project. During 2016-2017, Dene and Métis participants were recruited from Deline, Fort Providence, Hay River Reserve, Kakisa, West Point First Nation or Jean Marie Reserve (Supplementary Figure A1). All of the participants were above the age of 6. Participants completed a “Food Frequency Questionnaire” (FFQ) survey through an iPad, in order to estimate traditional country food consumption patterns. Biological samples (hair, urine and blood) were also collected, in order to analyze internal levels of toxic metals (e.g. Hg, Pb, Cd), essential metals (e.g., Cu, Ni, Zn), fatty acids, persistent organic pollutants (POPs).

4.2.1 Study Population and Participation Recruitment

Participants of all sexes, family status and ethnicity were included. All participants were given and signed an informed consent form to be filled out, prior to participating the study. All

the participants had the opportunity to complete the FFQ survey and have their biological samples collected. Additionally, all participants were asked to provide individual demographic information (e.g., age and sex). Depending on which components of the project they participated in, participants may have spent up to 2 hours in the biomonitoring clinic. In recognition of this time commitment, each participant received a $25 gift card to a local general store (Northmart/Northern/Ehdah Cho Store). Furthermore, the participant were enter into a draw for a chance to win a $250 gift card to any Northmart Food and Retail Store.

4.2.2 Biomonitoring Project Compliance

The biomonitoring project has obtained ethics approvals from various authorities including, University of Waterloo (#20173 and #20950), the Stanton Territorial Health Authority for Human Research (29/12/2015), the Aurora Research Institute (#15560, #15775, #15966, #15977, #16021) and Health Canada (REB 2016-0022). In order to adhere to the required ethical integrity, all personal results were kept in confidentiality and anonymity. All participants were required to sign a written consent form. Participants between the ages 6-18 were asked to provide verbal consent while the parent/guardian of the minor was required to sign a consent form on their behalf. With their informed consent, participants provided biological samples of hair, blood and/or urine as well have the option to store in a biobank for up to 10 years. This allows for quantitative analysis of Hg in the present as well as the analysis of contaminants and nutrients in the future. In addition, no genetic or drug testing will be conducted on biological samples and any new findings will be provided to the community as they become available. Finally, participants had the right to decline answering any questions and withdraw from the study at any point.

4.2.3 Biological Sample Collection and Mercury Analysis Methods

For participants who agreed to provide blood samples, a registered nurse drew blood from the antecubital vein of the anterior forearm with a 21G or 23G needle and syringe collection set (BD Eclipse, Becton Dickinson, Rutherford, NJ). Blood samples were collected in a metal-free plastic 6 mL-vacutainer green tube containing sodium heparin (BD Vacutainer™, Becton Dickinson, Rutherford, NJ) for whole blood Hg analysis. Blood samples were kept at -20°C until stored at - 80°C in the biobank for further analyses.

Mercury in blood samples was analyzed by Dr. Michèle Bouchard's laboratory, from the Université de Montréal in the Department of Environmental and Occupational Health. Blood samples were sent through an argon plasma for quantification by an Agilent 7700 Series Inductively Coupled Plasma Mass Spectrometer (ICP-MS) to determine Hg concentration. Blood Hg analysis, accuracy and precision for blood Hg levels were determined using the following standard reference materials: INSPQ blood control QM-B-Q1409 and INSPQ blood control QM-B-Q1505. The standard reference materials were ran at the beginning and end of every batch of 20 samples, with no less than 5 blank runs in each sample batch. All blood samples were ran in triplicate. The limit of detection of Hg in blood samples was 0.020 ug/L.

For participants who agreed to provide a hair sample, a small lock of hair was collected by a member of the research team using sterilized scissors. For individuals with short hair, small snips of hair were evenly collected from the occipital lobe and a sheet of paper was used to catch all the falling pieces of hair. For both long and short hair, samples were stapled to a polyethylene bag in order ensure that they were secure.

Mercury in hair samples was analyzed by Dr. Brian Branfireun's laboratory (Biotron Analytical Services, University of Western Ontario). In this analysis, the 2 cm of hair (30 mg

minimum weight) most proximal to the scalp was used. Hair analysis was completed with a Milestone Direct Mercury Analyzer (DMA-80; Milestone, Sorisole, Italy) through thermal decomposition followed by atomic absorption spectroscopy, as outlined in U.S. Environmental protection Agency (US. EPA) method 7473. To validate the batch analysis, a standard reference material (IAEA-086) was run at the beginning and end of every batch of 20 samples, with no less than 5 blank runs in each sample batch. Certified reference materials were used to validate the method and percent recoveries were determined using the following: National Institute of Standards and Technology (NIST) 1566B, oyster tissue, NIST 2976, mussel tissue, NIST 2974a, freeze dried mussel tissue; National Research Council of Canada (US.NRC) DORM-3, fish protein; National Institute of Environmental Studies (NIES) No. 13, human hair; and International Atomic Energy Agency (IAEA) 086, human hair. Hair samples were ran in triplicate. The limit of detection of Hg in hair samples was 0.05 ng/g (0.05 µg/kg). Concentrations ratios between hair and blood is typically about 250-300:1 and therefore, hair may be better able to quantify low Hg exposures (US EPA, 2001; WHO, 1976).

4.2.4 Food Frequency Questionnaire Methods

Participants completed a FFQ e-survey through an iPad, in order to estimate traditional country food consumption patterns of study participants over the past year (Supplementary figure A-2). This frequency consumption window encompasses the 2-3 months Hg exposure detected in the blood and hair biomarkers. The FFQ sections were subcategorized based on food groups and cooking methods. Participants were also asked about organ consumption patterns for certain traditional foods. This master's thesis analyzed consumption patterns for the cooked section for 9 different fish species including: whitefish, inconnu, cisco, lake trout, loche, northern pike, grayling, walleye, and longnose sucker. Participants were asked to select any of the following 9

fish species they have consumed over the past year. When a fish species was selected, participants were asked to answer the consumption frequency with one of the four available responses: <1 day/week, 1-2 days/week, 3-5 days/week, or 6-7 days/week. When a fish species was not selected, participants were recorded to have consumed it 0 day/week.

4.2.5 Mercury Exposure Analysis Methods

For this project, a 2 sample T-test analysis was used to analyze the relationship between sex differences and Hg exposure levels found in blood and hair. The 2 sample T-test analysis was conducted using Excel 2017. The null hypothesis is that there is no significant differences in the dependent variables (Hg level in blood or hair) when comparing between male and female participants. While, the alternative hypothesis is that differences in the dependent variable are associated with sex differences.

Based on participants' demographic information, log-linear simple linear regression models were developed to model the relationship between age and Hg exposure levels found in blood and hair. Linear regression models were developed using the lm function in R version 3.5.0. Additional packages were also used to visualize the data, which are available on cran (car, data.table 1.10.4-3, ggplot2 2.2.1, readxl 1.0.0, scales 0.5.0, stats 3.4.4, stargazer 5.2.1). For the simple regression models, the null hypothesis is that there is no significant association between age and the dependent variable (Hg exposure level in blood or hair). While, the alternative hypothesis is that the linear regression model that the age variable was significantly associated with the dependent variable.

With respect to dietary consumption patterns, log-linear simple linear regression models were developed to model the relationship between fish consumption and Hg exposure levels found in blood and hair. Participants' individual fish consumption patterns (cisco, grayling,

inconnu, loche, lake trout, northern pike, sucker, walleye and whitefish) were aggregated into one of two categories (piscivorous and non- piscivorous fish species) due to the infrequent consumption of certain fish species (Table 4-1). The consumption patterns for inconnu, lake trout, loche, northern pike, and walleye were aggregated into piscivorous fish consumption patterns. The consumption patterns for cisco, grayling, sucker and whitefish were aggregated into non-piscivorous fish consumption patterns. Example calculation for piscivorous and non-piscivorous consumption patterns were made also available (Supplementary Table A1). The Regression models were developed using the lm function in R version 3.5.0. Additional packages were also used to visualize the data, which are available on cran (car, data.table 1.10.4-3, ggplot2 2.2.1, readxl 1.0.0, scales 0.5.0, stats 3.4.4, stargazer 5.2.1). For the simple regression models, the null hypothesis is that there is no significant association between fish consumption (piscivorous or non-piscivorous fish species) and the dependent variable (Hg exposure level in blood or hair). While, the alternative hypothesis is that the linear regression model that fish consumption (piscivorous or non-piscivorous fish species) was significantly associated with the dependent variable.

4.3 Results

4.3.1 Summary Statistics of Participants

150 participants provided demographic information, completed the FFQ, and provided blood and/or hair samples for Hg analysis. Observational findings were summarized in Table 4-1. Summary statistics show that the study population ranged from the ages of 6-79. The average age was 42.7 years old. Of the 150 participants, 46 % identified as female and 54% as male. 39 participants provided their blood for analysis; the median blood Hg level was 1.318 µg/L (IQR= 0.457-3.522 µg/L) .With respect to the health based guidance values that Canada follows, all of the participants had safe blood Hg levels that were below the 20 ug/L threshold value (Table 4-1) (Statistics Canada, 2013). 132 participants had provided their hair for analysis; the median for Hg level in hair was 0.453 µg/g (IQR= 0.230-0.967 µg/g). Consistent with the results for blood, all of the participants had safe hair Hg levels that were below the 6 ug/g threshold (Table 4-1) (Statistics Canada, 2013). The fish frequency consumption patterns for participants were also summarized in Table 4-1. The three most frequently consumed species of fish were whitefish, lake trout and northern pike, which were consumed by 92%, 61% and 57% of the study population respectively (Table 4-1). As for the remaining 6 species of fish, less than half of the study population consumed them. Overall, the average consumption for each fish species ranged from 0.77-1.63 times a week. The average overall aggregate fish consumption - the frequency consumption of all 9 species - was 4.31 (± 3.86) times a week and was statistically higher ($p<0.05$) compared to the participants' average general fish consumption (Supplementary Table A2).

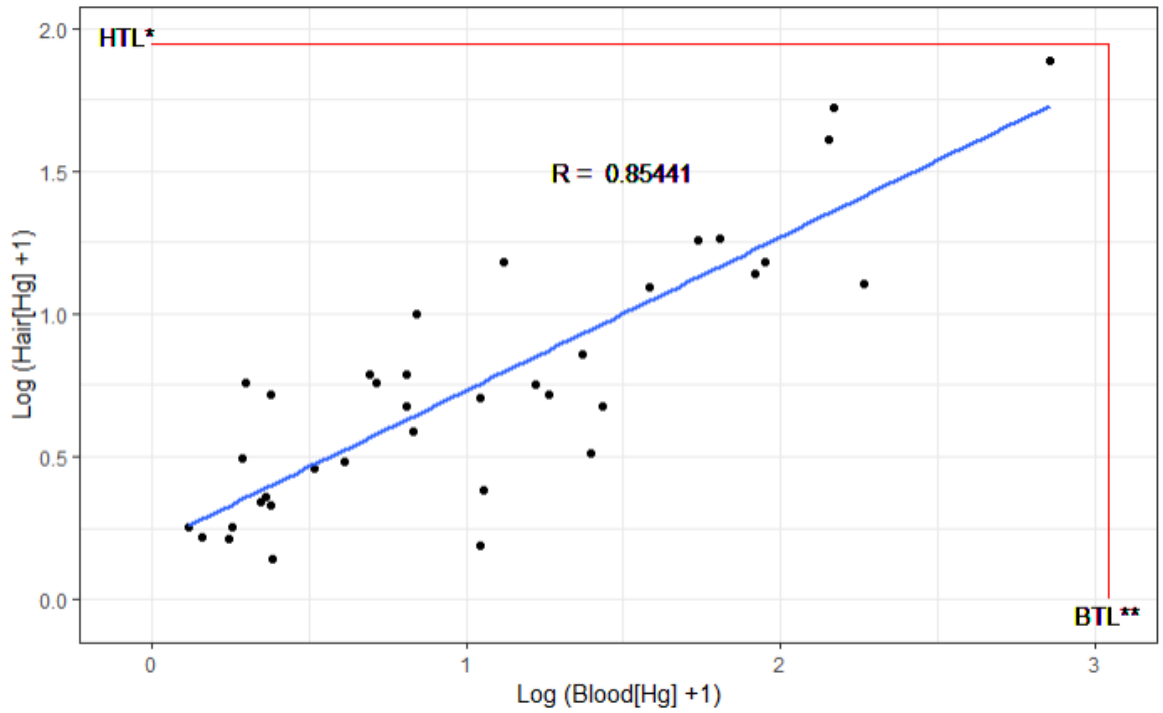
Table 4-1. Summary statistics of participants' demographic information, FFQ findings and Hg internal exposure levels

Demographic Statistic	Frequency (N)	Mean	St. Dev.	Pctl(25)	Pctl(50)	Pctl(75)
Age	150	42.733	19.665	25.5	45	59.5
Blood (μg/L)	39	2.693	3.245	0.457	1.318	3.522
Hair (μg/g)	132	0.783	0.902	0.230	0.453	0.967
FFQ Statistic	Consumer (Con.) (%)[1]	Con. Mean (times/wk)[2]	Con. St. Dev.[3]	Pctl(25)	Pctl(50)	Pctl(75)
Whitefish	92	1.63	1.62	0.50	1.50	1.50
Lake Trout	61	1.28	1.43	0.50	0.50	1.50
Northern Pike	57	1.19	0.97	0.50	0.50	1.50
Walleye	40	1.13	0.91	0.50	0.50	1.50
Inconnu	28	1.13	0.94	0.50	0.50	1.50
Grayling	22	0.77	0.45	0.50	0.50	1.50
Sucker	21	0.92	0.50	0.50	0.50	1.50
Loche	15	0.89	0.82	0.50	0.50	1.25
Cisco	7	1.25	1.09	0.50	1.00	1.50

Note: 1. Study population's consumption frequency for a particular species of fish reported as a percentage; 2. Average fish species consumption, based on participants who consume that particular species; 3. Standard deviation for fish species consumption, based on participants who consume that particular species.

4.3.2 Blood and Hair Mercury level Scatterplot

To investigate the relationship between blood and hair Hg levels, a scatter plot and Pearson's correlation coefficient was generated, based on participants who provided both blood and hair samples (Figure 4-1). Log-transformed blood and log-transformed hair Hg levels were highly linearly-correlated with one another ($r= 0.854$, $p<0.05$). All of the participants had blood and hair Hg levels below the threshold designated (Blood = 20μg/L and Hair = 6μg/g), based on the Health Canada's guidance values (Statistics Canada, 2013).



Note: *HTL denotes the hair threshold value (log (6ug/g +1)) that Health Canada's Guidance considers as safe/acceptable; **BTL denotes the blood threshold value (log (20ug/L +1)) that Health Canada's Guidance considers as safe/acceptable.

Figure 4-1. Scatterplot between blood and hair biomarkers.

4.3.3 Blood and Hair Mercury Exposure T-Test Analysis

Mercury levels in blood and hair did not differ between males and females ($p>0.05$, Table 4-2, 4-3). For female participants, the average blood Hg level was 2.667 ± 4.079 µg/L whereas male participants had an average Hg blood level of 2.711 ± 2.616 µg/L. For female participants, the average hair Hg level was 0.671 ± 0.866 µg/g whereas the average hair Hg level for male participants was 0.868 ± 0.925 µg/L (Table 4-3).

Table 4-2. T-test for sex difference in blood Hg exposure levels.

Sex	Male	Female
Mean [Blood Hg] (ug/L)	2.667	2.711
Stdandard Deviation (ug/L)	4.079	2.616
Observations	16	23
Difference between mean [Blood Hg] (ug/L)	−0.044	
df	24	
t- value	−0.037	
p- value	0.97	

Table 4-3. T-test for sex difference in hair Hg exposure levels.

Sex	Male	Female
Mean [Blood Hg] (ug/g)	0.671	0.868
Stdandard Deviation (ug/g)	0.866	0.925
Observations	57	75
Difference between mean [Blood Hg] (ug/g)	−0.197	
df	124	
t- value	−1.257	
p- value	0.21	

4.3.4 Simple Linear Regression Models for Blood and Hair Hg Exposure Levels

Based on participants' demographic information, log-linear relationships between blood and hair Hg exposure levels and age were investigated using simple regression models 1 and 2 (Table 4-4). Residual and scale-location plots for both linear regression models show homoscedasticity (Supplementary Figures A3-A4). Age was significant and positively associated with both blood and hair ($B=0.020$ and $p<0.01$; $B=0.011$ and $p<0.01$) (Table 4-3). Both simple regression models are statistically significant at accounting for Hg levels in either blood or hair ($R^2=0.22.7$ and $p<0.05$; $R^2=0.338$ and $p<0.01$) (Table 4-4).

Table 4-4. Results of simple linear regression models that were used to investigate relationships between Hg levels in blood and hair, and participant age.

	Model:	
	Model 1	Model 2
	Dependent variable:	
	Log (Blood + 1)	Log (Hair + 1)
	Regression Coefficients:	
	B-Estimate (Std.)	B-Estimate (Std.)
Age	0.020 (0.006)**	0.011 (0.001)**
B0-Intercept	−0.001 (0.331)	0.001 (0.066)
Observations	39	132
AIC	78.5	74.9
R^2	0.227	0.338
Adjusted R^2	0.206	0.333
Residual Std. Error	0.630 (df = 37)	0.316 (df = 130)
F Statistic	10.857** (df = 1; 37)	66.409** (df = 1; 130)
Note:		*p<0.05; **p<0.01

Using participants' FFQ data, log-linear relationship between non-piscivorous fish consumption and Hg levels in blood and hair were also investigated (summarized in models 3 and 4 respectively in Table 4-5). Residual plots for both linear regression models show homoscedasticity, limited skewedness and absence of influential cases (Supplementary Figures A5-A6). Non-piscivorous fish consumption does not appear to be significantly associated with either Hg levels found within blood or hair (B= 0.022 and p>0.05; B= 0.035 and p>0.05) (Table 4-5). Neither of the two regression models explain a significant amount of variation in Hg levels in blood and hair (R^2=0.003 and p>0.05; R^2=0.026 and p>0.05) (Table 4-5).

Table 4-5. Results of simple linear regression models that were used to investigate relationships between Hg levels in blood and hair, and non-piscivorous fish species frequency consumption.

	Model:	
	Model 3	Model 4
	Dependent variable:	
	Log (Blood + 1)	Log (Hair + 1)
	Regression Coefficients:	
	B-Estimate (Std.)	B-Estimate (Std.)
Non-Piscivorous	0.022 (0.066)	0.035 (0.019)
B0-Intercept	0.993 (0.172)**	0.425 (0.049)**
Observations	39	132
AIC	88.4	125.9
R^2	0.003	0.026
Adjusted R^2	-0.024	0.018
Residual Std. Error	0.715 (df = 37)	0.384 (df = 130)
F Statistic	0.114 (df = 1; 37)	3.436 (df = 1; 130)
Note:		*p<0.05; **p<0.01

Mercury levels in blood and hair were also not related to frequency of consumption of piscivorous fishes (models 5 and 6 respectively in Table 4-6). Residual plots indicated homoscedasticity, limited skewedness and absence of influential cases (Supplementary Figures A7-A8). According to the simple linear regression models 5 and 6, piscivorous fish consumption does not appear to be significantly associated with either blood or hair (B= 0.068 and p>0.05; B= 0.023 and p>0.05) (Table 4-6). Neither regression models account for a statistically significant amount of variation in blood or hair Hg levels in blood and hair (R^2=0.031 and p>0.05; R^2=0.022 and p>0.05) (Table 4-6).

Table 4-6. Results of simple linear regression models that were used to investigate relationships between Hg levels in blood and hair, and piscivorous fish species frequency consumption.

	Model:	
	Model 5	Model 6
	Dependent variable:	
	Log (Blood + 1)	Log (Hair + 1)
	Regression Coefficients:	
	B-Estimate (Std.)	B-Estimate (Std.)
Piscivorous	0.068 (0.063)	0.023 (0.013)
B0-Intercept	0.896 (0.171)**	0.438 (0.046)**
Observations	39	132
AIC	87.3	126.4
R^2	0.031	0.022
Adjusted R^2	0.005	0.014
Residual Std. Error	0.705 (df = 37)	0.385 (df = 130)
F Statistic	0.188 (df = 1; 37)	2.902 (df = 1; 130)
Note:		*p<0.05; **p<0.01

4.4 Discussions

As show in Table 4-1, the average blood Hg level in study participants was approximately 2.693 μg/L and appears to be higher compared to CHMS Cycle 1-4 reports, with mean blood Hg levels ranging from 0.26-1.1 μg/L (Statistics Canada, 2017). CHMS reported blood Hg averages increases with respect to age, which is similar to biomonitoring findings. When stratified for age groups, biomonitoring participants comparable blood Hg levels that were reported in the CHMS (Statistics Canada, 2017). It is worth noting that CHMS excluded all Canadians who live within the 3 territories from participating the survey. Furthermore, all of the biomonitoring participants had blood Hg levels were considered safe as they were all below the 20 ug/L threshold (Table 4-1) (Statistics Canada, 2013).

According to the Table 4-1, the average hair Hg level in the study population was about 0.783 μg/g. Observational findings were comparable to those presented by Schaefer et al. (2014). Schaefer et al.'s study population had a similar age and sex distribution as well as frequency consumption of seafood, when compared to this biomonitoring project (Schaefer et al., 2014). In addition, the average hair Hg levels for the 135 Florida residents who participated in Schaefer et al.'s study was 1.53 μg/g and is relatively higher than this biomonitoring's study population (Schaefer et al., 2014). Similar to the blood biomarkers findings, all of the biomonitoring participants had safe hair Hg levels that were all below the 6 ug/g threshold (Table 4-1) (Statistics Canada, 2013).

When comparing the two Hg biomarkers, both blood and hair were highly linearly correlated to one another (R=0.854). In addition, the concentration ratio between hair Hg to blood Hg was 336:1, when comparing the two exposure assessment methods. This ratio is comparable to concentration ratios set by the FDA and WHO, 250:1 and 250-300:1 respectively (US EPA, 2001; WHO, 1976). This was expected as Hg analysis in hair can be used to supplementary support Hg measurements in blood (Pirkle et al., 2016). Furthermore, biomonitoring findings suggests that the ideal hair length for Hg analysis is about 2-3 cm as the exposure windows for the two biomarkers appears to overlap.

Based on the descriptive statistics in Table 4-1, the three most frequently consumed species of fish were whitefish (92%), lake trout (61%) and walleye (57%) with their reported mean consumption being 1.63, 1.28 and 1.19 times/week respectively. As for the remaining 6 species of fish, less than half of the study population consumed them. Despite the absence of consumption for a majority of the fish species, the average consumption of all 9 fish species was 4.3 times a week (Supplementary Table A-2). It is significantly greater compared to average

Canadian who consumes less than 7 kg of fish a year (2 servings a week) (Fisheries and Oceans Canada, 2018). Prior to conducting statistical analysis with respect to fish consumption, individual fish species were aggregated into the following two groups, non-piscivorous and piscivorous fish species. It is possible to use the overall consumption of all 9 fish species. However, aggregating individual fish species consumption based on non-piscivorous and piscivorous fish species may be optimal because piscivorous fish species in the Dehcho region have significantly greater amount of total Hg content compared to non-piscivorous fish (Reyes, 2016). As previous literature have shown, higher trophic fish species are expected to have greater amounts of Hg through the process of biomagnification (Power et al., 2002). By combining the individual fish species into the two categories, this methodology may shed some light on Hg exposure with respect to non-piscivorous and piscivorous fish consumption. Although the exposure windows for FFQ and Hg biomarkers are different, it does not appear to be a limitation as there was no statistical differences in the study participants' general fish consumption patterns over the course of the year. As shown in Supplementary Table A2, study participant's overall, piscivorous and non-piscivorous fish consumption were all significantly greater when compared to their general fish consumption patterns. In addition, study participant's overall, piscivorous and non-piscivorous fish consumption patterns were also positively correlated with general fish consumption patterns. As such, over-reporting bias may have been present but it would not have influenced statistical findings.

As shown in Table 4-2 and Table 4-3, there appears to be no statistical differences between sex and Hg exposure in blood and hair respectively. Biomonitoring results appears to conflict with other literature finding as previous studies have shown to be statistical difference in both Hg exposure in blood and hair respectively (Schaefer et al., 2014; You et al., 2011). The

potential absence of association may be related to both sexes having similar fish consumption patterns. Previous studies have demonstrated that men were found to consume more fish per kg of body weight and carry higher concentrations of Hg in their hair (Schaefer et al., 2014). Conversely, the biomonitoring project did not find statistical differences in piscivorous, non-piscivorous and overall fish consumption patterns between the two sexes. As such, the contradictory findings suggests future studies to continue analyzing differences between sex.

As shown in the simple linear regression models 1 and 2, age was positively associated with blood and hair Hg exposure levels respectively (Table 4-4). This demographic factor is known to have contradictory findings as some studies have found positive association between Hg in blood or hair (Dong et al., 2015; Mahaffey et al., 2004), but not present in others (Kosatsky et al., 2000; Schaefer et al., 2014). One potential explanation is that it may be related to differences in lifestyle or fish consumption patterns as a function of age (Dong et al., 2015). In a study conducted by Dong et al. (2015), older age participants (>51 years) were noted to have eaten more local fish($p<0.05$), when compared to younger age participants (≤51 years old) (Dong et al., 2015). Furthermore, age is positively associated with Hg levels detected in the human body through chronic exposure (Laks 2009).

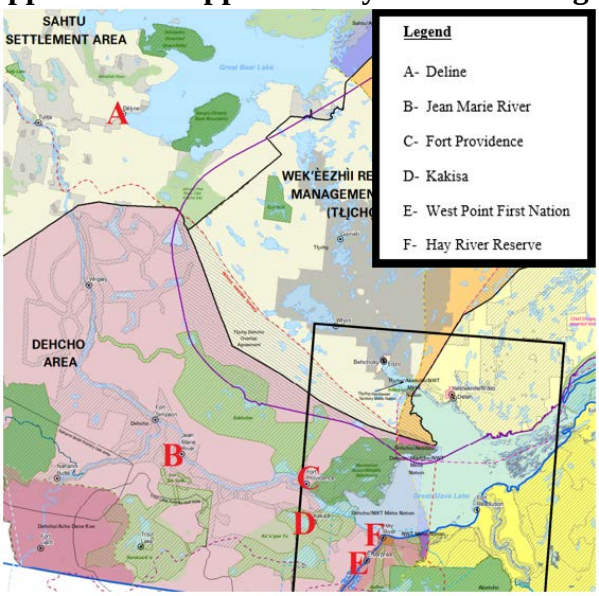
According to the simple linear regression models 3 and 4, there appears to be no significant relationship between blood and hair Hg exposure levels with respect to non-piscivorous fish consumption (Table 4-5). Similarly, simple linear regression models 5 and 6 identified that there is no significant relationship blood and hair Hg exposure levels with respect to piscivorous fish consumption (Table 4-5). Compared to the biomonitoring project's results, multiple past literatures present different findings as they found positive association with dietary fish or seafood consumption (Dong et al., 2015; Schaefer et al., 2014; You et al., 2014). Some of

the past literatures also conducted exposure models, in order to account for the specific differences in consumption patterns and Hg concentrations among food types (You et al., 2014). This allowed for identification of statistical difference in Hg exposure within the human body with respect to dietary intake. This biomonitoring study attempts to do so in a different process by breaking down the main source of dietary mercury into two parts, piscivorous and non-piscivorous consumption frequency. As such, differences in statistical findings may also be related to differences in Hg exposure assessment methodology that was implemented. With respect to non-piscivorous fish species, the absence of association with study participants' blood and hair Hg levels may be related to the relatively low levels of Hg found within them (Reyes, 2016). In a study presented by Reyes (2016), the total Hg levels for non-piscivorous fishes harvested in the Dehcho region were all below 0.1 ppm, which is well below Canada's standard permitted in retail fish (0.5ppm). This included species such as cisco, sucker and whitefish (Reyes, 2016). As such, the absence of association for non-piscivorous fish consumption may be related to negligible amounts of Hg that has bioaccumulated within these fish species, which makes it an insignificant contributing factor for dietary Hg exposure. It should be noted that all of the non-piscivorous fishes were reportedly consumed by less than a quarter of the study population, except for whitefish (92%). This may have influenced the generalizability of non-piscivorous frequency consumption findings, when interpreting simple linear regression findings. It may also be worth considering the effects of cooking fish because culinary treatments such as boiling and frying can reduce the bioaccessibility as well as limit the potential impact of Hg exposure to the human body (Ouédraogo and Amyot, 2011). As such, variances in Hg bioaccessibility due to cooking may explain for the absence of association for study population's piscivorous and non-piscivorous fish consumption with respect to internal Hg exposure levels.

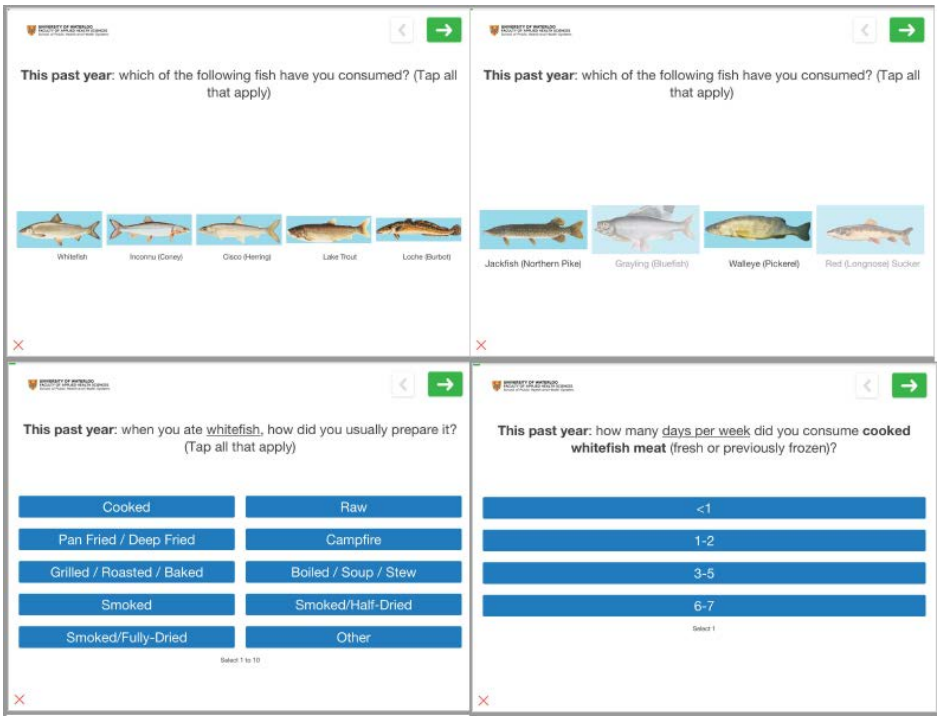
4.5 Conclusion

When used together, both FFQ and regression modelling can be effective tools for exposure and risk assessments. These techniques are capable of characterizing the relationship between external exposure factors and the various Hg biomarkers (Elhamri et al., 2007; You et al., 2014). To the author's knowledge, this multi-disciplinary biomonitoring project was the first attempt to assess the relationship between internal Hg concentrations and seafood consumption patterns among the Dehcho and Sahtú regions of NWT, through the use of FFQ and simple linear regression modelling. This Hg cross-sectional biomonitoring study determined that the age factor alone was statistically significant and has a positive relationship with blood and hair Hg exposure levels within the Dene and Métis communities from the Dehcho and Sahtú regions. Individually, sex, frequency consumption of piscivorous and non-piscivorous fish species were statistically insignificant factors. For future studies, implementing a different exposure model, based on the food types' mass and standardized Hg concentration, may also be effective in determining the relationship between dietary consumption patterns and internal Hg exposure levels. Furthermore, this biomonitoring project can allow future longitudinal studies to compare changes to dietary consumption patterns and internal Hg exposure levels over time. As such, this research can assist future studies by establishing a baseline level for internal Hg levels within the Dehcho and Sahtú regions of NWT.

Appendix A- Supplementary Tables and Figures



Supplementary Figure A1. Map of Dehcho and Sahtú regions of Northwest Territories. (Indigenous and Northern Affairs Canada, 2013)



Supplementary Figure A2. Example Images of FFQ Survey questions

Supplementary Table A1. Example Calculation for Project Participant's Piscivorous and Non-Piscivorous Consumption

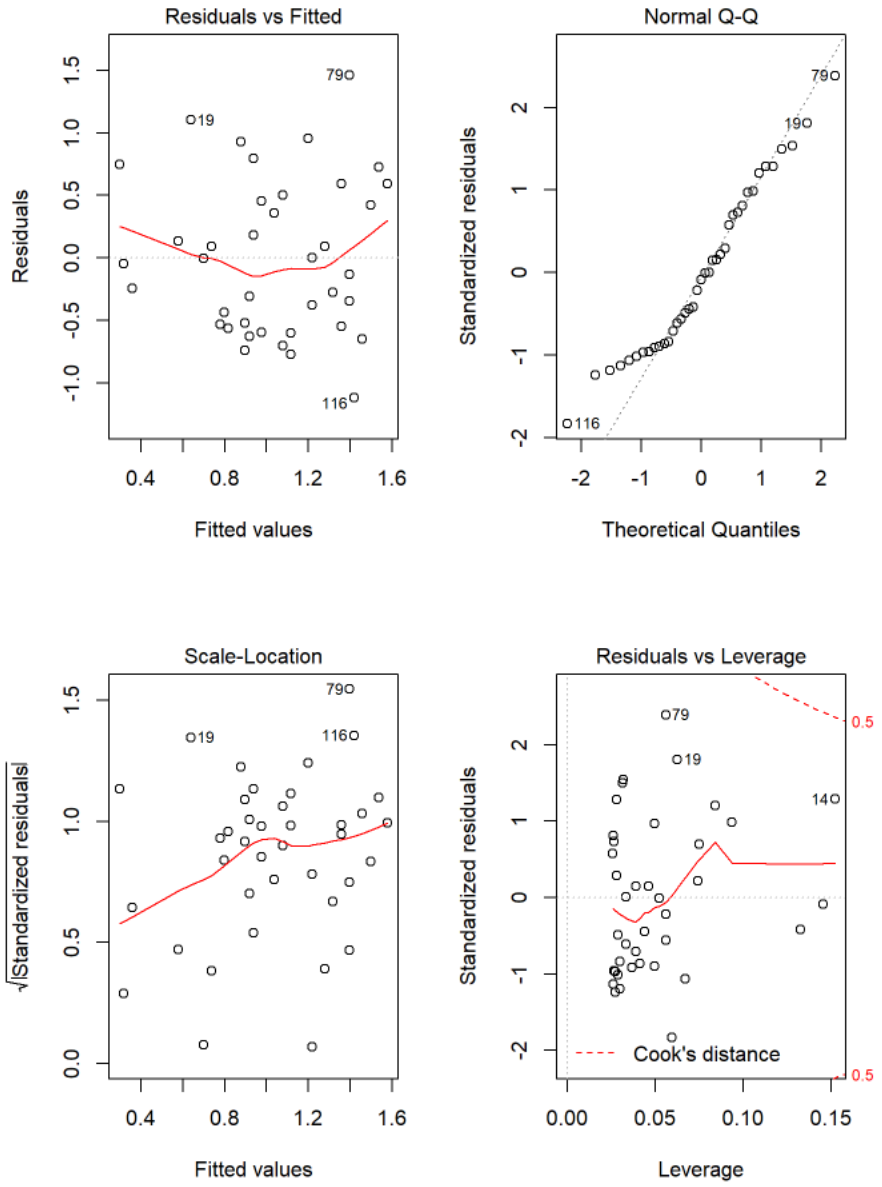
Subject	Piscivorous[1]	Inconnu	Lake Trout	Loche	N. Pike	Walleye
SUB001	3.5	0.5	1.5	0.5	0.5	0.5

Subject	Non-Piscivorous[2]	Cisco	Grayling	Sucker	Whitefish
SUB001	3	0.5	0.5	0.5	1.5

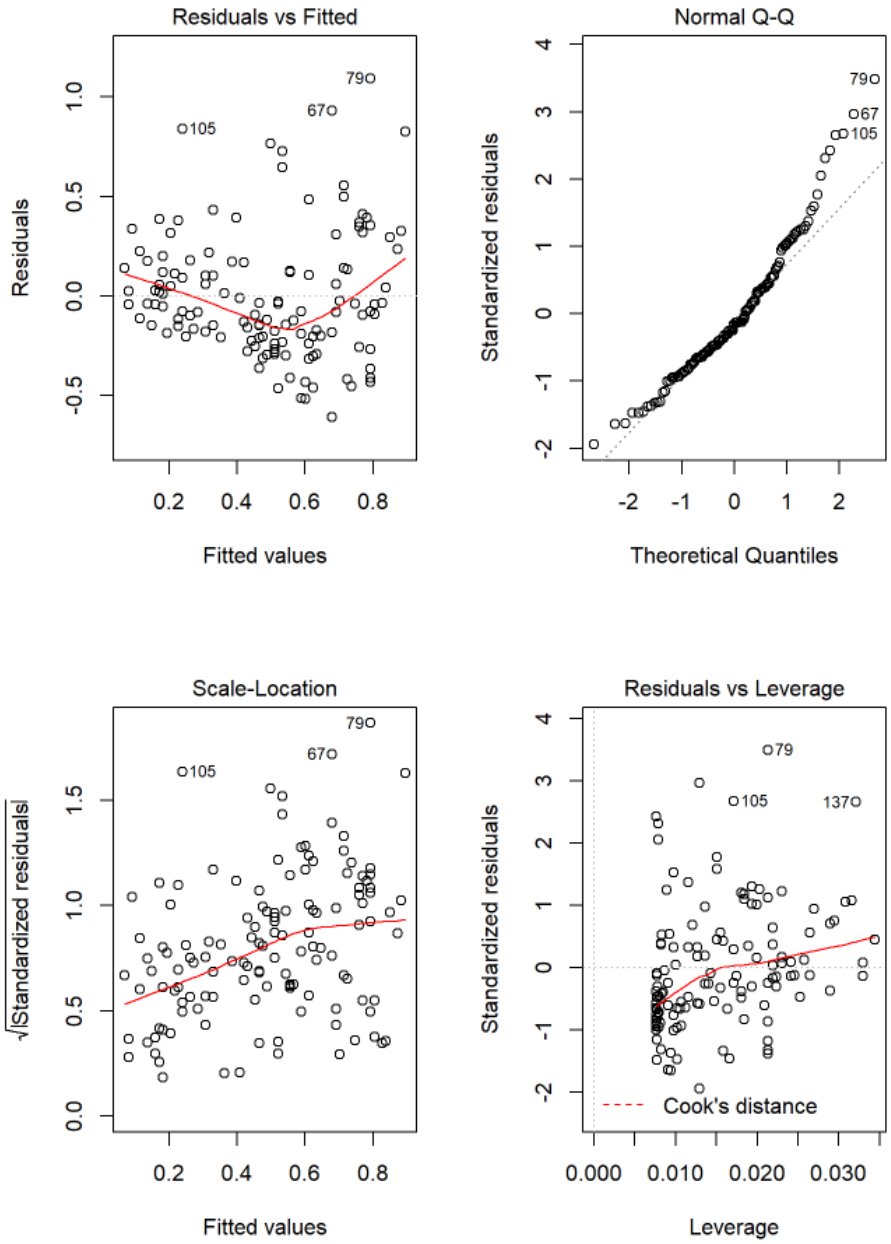
Note: 1. The aggregate consumption patterns for piscivorous fish species per week, which includes inconnu, lake trout, loche, northern pike, and walleye; 2. The aggregate consumption patterns for non-piscivorous fish species per week, which includes cisco, grayling, sucker and whitefish were aggregated into non-piscivorous fish consumption patterns

Supplementary Table A2. Summary statistics of participants' fish consumption. Overall aggregate is the sum of all the fish species specific frequency consumption per week. Predatory aggregate is the sum of lake trout, walleye, and northern pike consumption per week. Non-predatory aggregate is the sum of whitefish, inconnu, cisco, loche, grayling and sucker consumption per week.

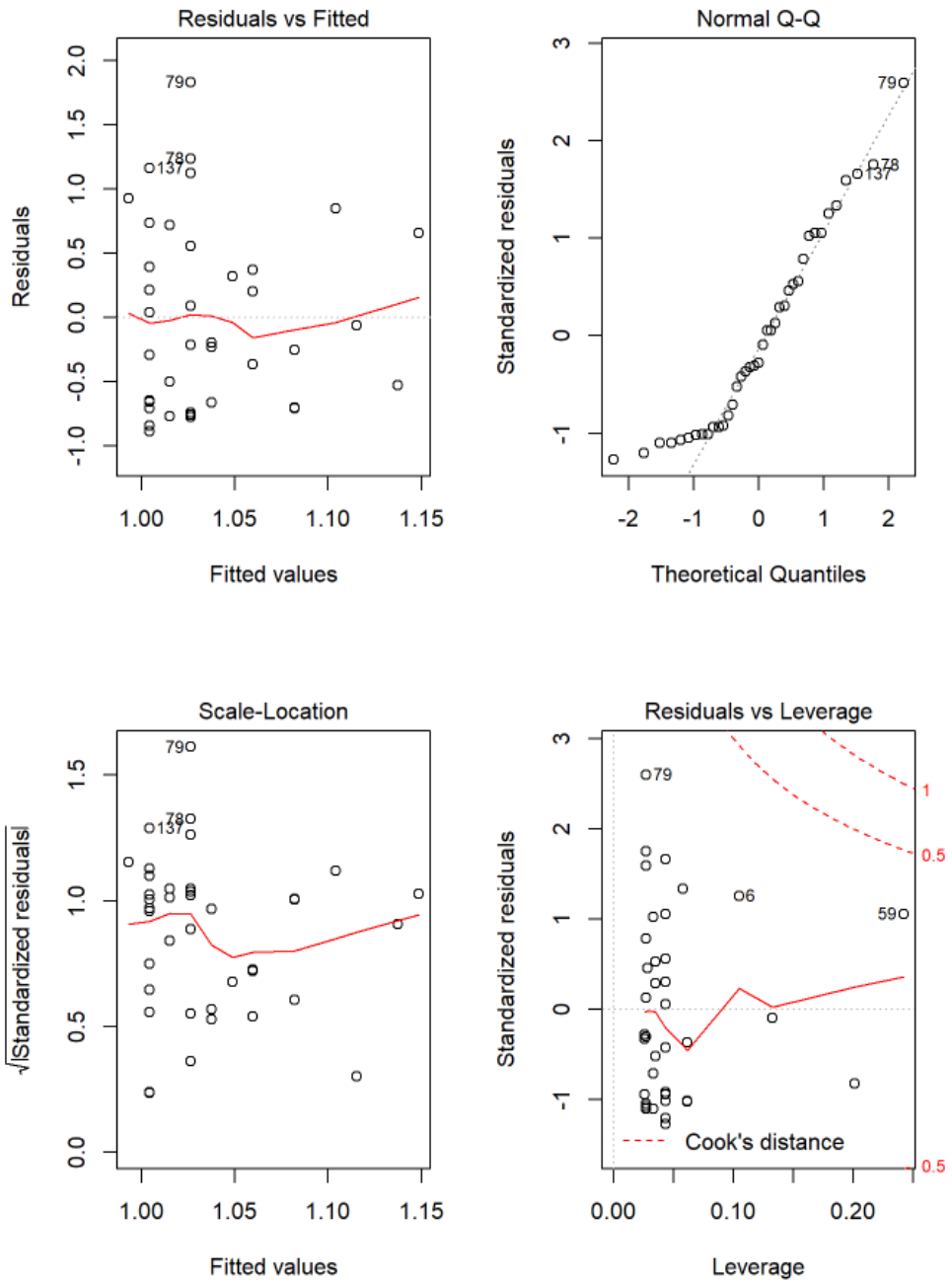
Statistic	Mean	Std.	Pctl(25)	Pctl(50)	Pctl(75)
General Fish Consumption (times/wk)	1.255	1.151	0.500	1.500	1.500
Overall Aggregate (times/wk)	4.310	3.859	1.500	3.000	6.000
Non- Piscivorous (times/wk)	1.943	1.837	0.500	1.500	3.000
Piscivorous (times/wk)	2.367	2.500	0.5	1.500	3.750



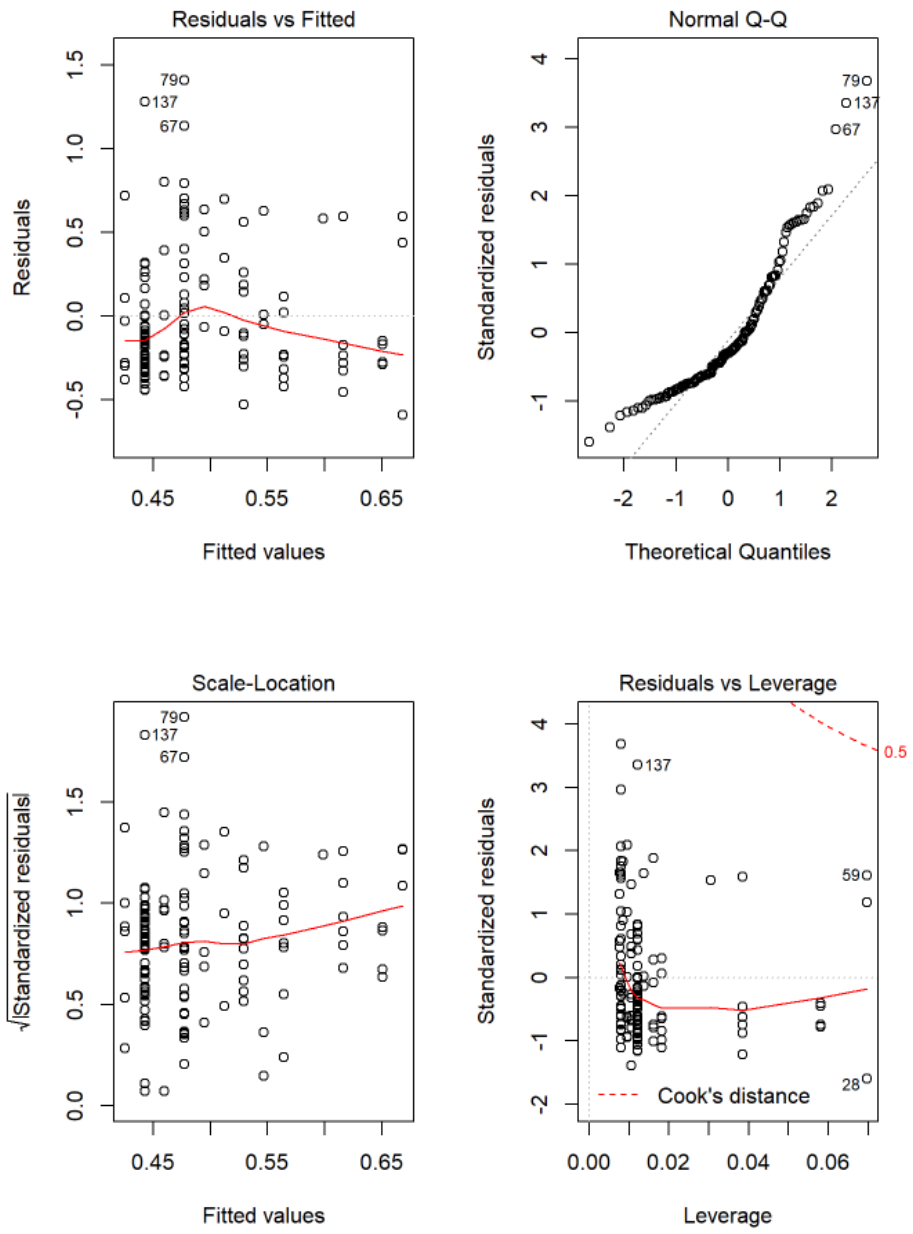
Supplementary Figure A3. Residual plots of model 1. Residual vs Fitted and Scale-location plots for model 1 show homoscedacity, as there is unequal variability and spread across the range of values. Q-Q plots indicates that sample dataset is slightly skewed left as there is the presence of a left tail. Residual vs Leverage plots indicate absence potential influential cases as all of the values lie within Cook's distance of 0.5.



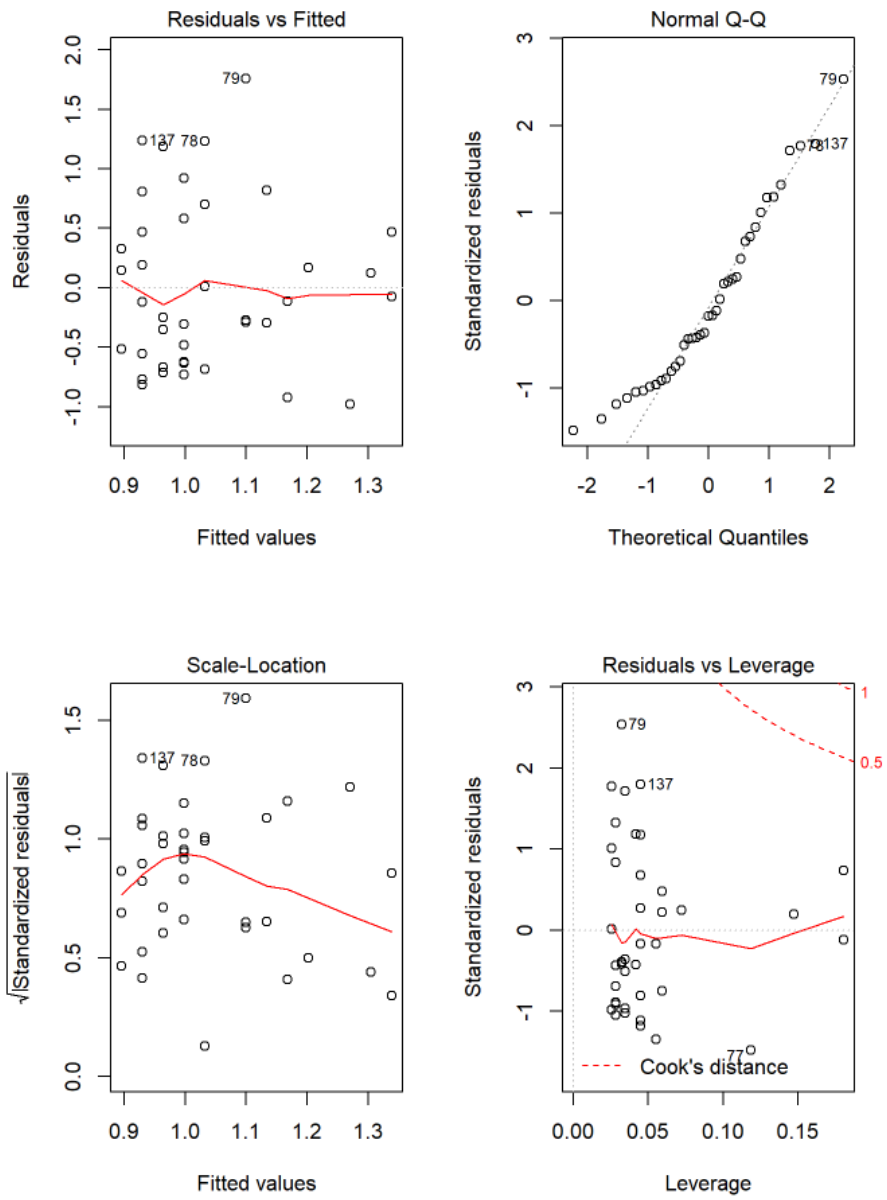
Supplementary Figure A4. Residual plots of model 2. Residual vs Fitted and Scale-location plots for model 1 show homoscedacity, as there is unequal variability and spread across the range of values. Q-Q plots indicates that sample dataset is slightly skewed right as there is the presence of a right tail. Residual vs Leverage plots indicate absence potential influential cases as all of the values lie within Cook's distance of 0.5.



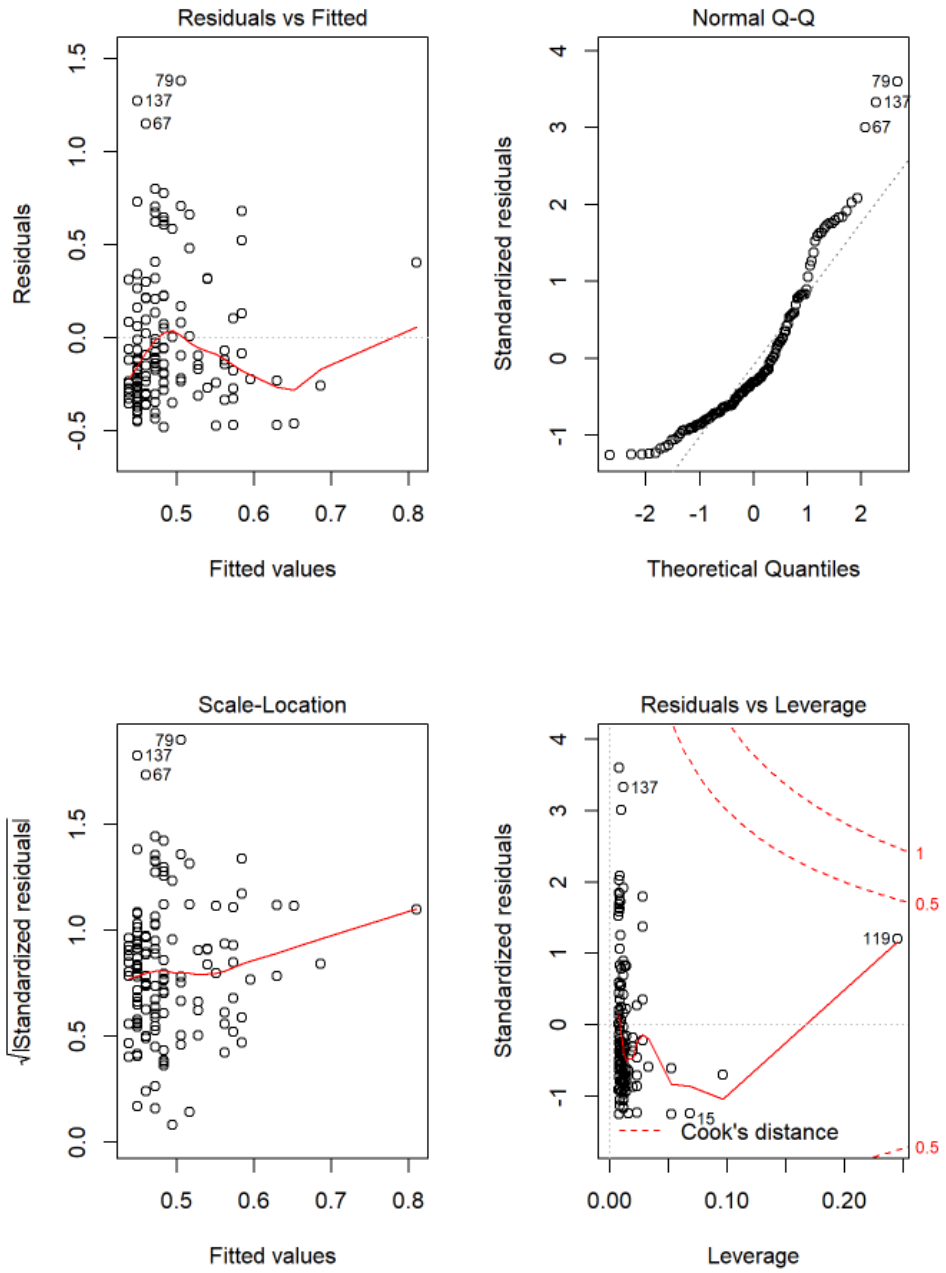
Supplementary Figure A5. Residual plots of model 3. Residual vs Fitted and Scale-location plots for model 1 show homoscedacity, as there is unequal variability and spread across the range of values. Q-Q plots indicates that sample dataset is slightly skewed left as there is the presence of a left tail. Residual vs Leverage plots indicate absence potential influential cases as all of the values lie within Cook's distance of 0.5.



Supplementary Figure A6. Residual plots of model 4. Residual vs Fitted and Scale-location plots for model 1 show homoscedacity, as there is unequal variability and spread across the range of values. Q-Q plots indicates that sample dataset is slightly skewed as there is the presence of tails on both ends. Residual vs Leverage plots indicate absence potential influential cases as all of the values lie within Cook's distance of 0.5.



Supplementary Figure A7. Residual plots of model 5. Residual vs Fitted and Scale-location plots for model 1 show homoscedacity, as there is unequal variability and spread across the range of values. Q-Q plots indicates that sample dataset is slightly skewed left as there is the presence of a left tail. Residual vs Leverage plots indicate absence potential influential cases as all of the values lie within Cook's distance of 0.5.



Supplementary Figure A8. Residual plots of model 6. Residual vs Fitted and Scale-location plots for model 1 show homoscedacity, as there is unequal variability and spread across the range of values. Q-Q plots indicates that sample dataset is skewed as there is the presence of tails on both ends. Residual vs Leverage plots indicate absence potential influential cases as all of the values lie within Cook's distance of 0.5.

5. The In Vitro Bioaccessibility of Mercury in Uncooked and Dried Whitefish

5.1 Introduction

Traditional foods consumption such as fish play an integral role to the Indigenous communities of the NWT, in terms of cultural importance and health benefits (Donaldson et al., 2010; Van Oostdam et al. 2005). Alongside market food consumption, Dene and Métis communities hunt, trap, fish and prepare traditional foods from their local surroundings (Food Safety Network, 2009; NWT Health and Services, 2002). Fish is an important traditional food source for Indigenous communities; edible flesh portion of the fish are often prepared by either boiling, drying, frying or smoking (Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). In addition, traditional food preparation methods of fish can vary between one community to another (Kuhnlein and Humphries, 2017). Within the Sahtú region of NWT, community members in Fort Good Hope consume raw and baked loche while communities in Tlicho have their own specific drying preparation for loche (Kuhnlein and Humphries, 2017).

Fish can supply a large source of protein, omega 3-fatty acids and they are also a source of important micronutrients (Donaldson et al. 2010; Fediuk et al. 2002; Laird et al. 2013; Kuhnlein et al., 2006; Kuhnlein and Humphries, 2017; Kuhnlein and Receveur 2007). Dietary consumption of fish can also increase an individuals' exposure to MeHg (Burger et al., 2011; Golding et al., 2013; Gribble, et al., 2015; Hu et al., 2014; Kuhnlein and Chan, 2000; Laird et al., 2009; Laird and Chan, 2013; Ysart et al., 2000). However, only the bioavailable fraction of Hg is capable of eliciting its toxicodynamic effects(Fernández-García et al., 2009).

In risk assessment, exposure models conservatively estimate dietary Hg to be completely bioaccessible because of the difficulty to determine Hg bioavailability with a high degree of certainty (Siedlikowski et al., 2016). This can result in an overestimation of risk associated with

traditional foods (ATSDR, 1999; Bradley et al., 2017; Laird et al., 2009). Studies have shown that MeHg's bioaccessibility in fish can vary greatly and be less than 100%, based on various *in vitro* models findings (Bradley et al., 2017; Ouédrago and Amyot, 2011; Maulvault et al., 2015). In a study presented by Laird et al., (2009), researchers have found Hg bioaccessibility to be independent from Hg concentration in country foods. In contrast, Siedlikowski et al. have found a negative correlation between initial Hg concentration in seafood and Hg bioaccessibility (2016). Additionally, fish cooking and preparation methods can affect bioaccessibility. For example, boiling and frying fish can reduce Hg bioaccessibility compared to uncooked (Ouédraogo and Amyot, 2011). Past literature have found Hg concentrations in dried fish jerky to be greater than 0.5 μg/g, which is above Health Canada's guideline for commercial distribution of seafood (Hightower and Brown, 2011). However, information about the process of drying fish and its affect on Hg concentration and Hg bioaccessibility remains unclear. In addition, it is unknown whether dried fish by smoking (dried/smoked), a cooking method used by communities within the Dehcho and Sahtù regions, has any influences on fish Hg concentration as well as Hg bioaccessibility (Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). Past literature have also shown co-administration of tea or coffee with raw fish samples can also reduce Hg bioaccessibility (Ouédraogo and Amyot, 2011).IVBA model design may play a role in determining Hg bioaccessibility (Hur et al., 2011). For example, a model's digestive chemical composition may influence Hg bioaccessibility (Hur et al, 2011). Other physiochemical factors include the phases of digestion, solid food sample/ digestion liquid (S/L) ratio, digestion time, and pH for each digestive phase (Hur et al., 2011; Maulvault et al., 2011; Shi et al., 2017). Furthermore, agitation methods (and the degree of agitation during each digestive phase) may indirectly influence a toxicant's solubility (Alegría et al., 2015).

The objective of the present study was to characterize Hg concentration and Hg bioaccessibility in lake whitefish (*Coregonus clupeaformis*). In terms of wild-harvested freshwater fish, whitefish is one of the most commonly consumed species of fish within the Dehcho and Sahtú regions (Kuhnlein et al., 1994; Kuhnlein and Humphries, 2017; NWT Health and Services, 2002). In addition, this study explored the differences in Hg bioaccessibility between dried/smoked and uncooked whitefish samples. Therefore, the proposed study will attempt to provide more information Hg bioaccessibility with respect to culinary methods used within the Dehcho and Sahtù regions. The goal of this research is to provide more information about Hg bioaccessibility such that it is no longer necessary to assume dietary Hg is completely bioavailable, for future exposure models.

5.2 Methods

5.2.1 Whitefish Samples

Five whole whitefish (originating from Lake Huron) were purchased from Caudle's Catch Seafood (Kitchener-Waterloo, ON). All of the whitefish samples came from the Lake Huron. The fork length for each whitefish were reported to the nearest mm (Supplementary Table B1). The project compared Hg content between whitefish caught from Lake Huron and other literature findings that determined Hg content within whitefish samples from the Dehcho region.

5.2.2 Whitefish Sample Preparation Methods

With the assistance of Dr. Heidi Swanson, the whitefish samples were prepared using a modified version of the traditional methods (Supplementary Figure B1). Using a sharp knife, the whitefish was decapitated from an inch behind its gills. On the dorsal side of the whitefish, the knife was ran down its spine, in order to separate the two fillets and the innards as well as spine

of the fish were removed. Finally, the knife was ran along the whitefish's skin to remove its edible muscle portions. The fillet was divided into the upper and lower half of the lateral line. From the upper half of the lateral line, the edible muscle was sectioned for different fish preparation methods. The anterior section of the whitefish was left uncooked. The posterior section of the whitefish was left to be dried in a wood smoker overnight, at 100 °F (37.8 °C). All samples were weighed and moisture content were reported (Table 5-1). Samples were homogenized and stored at -20°C, prior to further analysis.

5.2.3 Single Phase, Gastric-Only, Static IVBA Methods

Both uncooked and dried/smoked whitefish sample portions underwent a single phase, gastric-only, static IVBA model that was adapted from Palaniyandi (2016). In summary, 2 ± 0.2 g of homogenized fish tissue was weighed out and placed inside 50mL serum bottles. 30mL Synthetic gastric juices (pH=1.5) was inserted into the tubes (Liquid: Solid ratio of 15:1). Gastric mixture was titrated to a pH=2 ± 0.2 using HCl (0.5M). Serum bottles was placed in an orbital shaker for 3hrs at 180rpm at 37°C. Synthetic juices contained HCl (Omnitrace®, pH=1.5) porcine Pancreatin (Sigma Aldrich®, 6g/L) and NaCl (8.5g/L) in MilliQ water. Afterwards, the gastric mixture was filtered through a 0.45µm PTFE membrane filter and 50mL glass syringe. Samples were stored at -80°C prior to analysis.

5.2.4 Mercury Concentration Analysis Methods

For uncooked and dried/smoked whitefish sample portions that did not undergo gastric digestion (5.2.2), Hg concentrations were determined using a mercury analyzer RA-915M with pyrolyzer PYRO-915+ (Ohio Lumex, British Columbia, Canada). Mercury analysis was completed through thermal decomposition followed by atomic absorption spectroscopy, as outlined in US. EPA method 7473. To validate the batch analysis, a calibration standard (RPM-

043QC) and standard reference material (US.NRC DORM-4, fish protein) was run at the beginning and end of every batch of 40 samples, with no less than 3 blank runs in each sample batch. Uncooked and dried/smoked whitefish samples were ran in triplicate. The limit of detection of Hg in whitefish samples was 0.02 mg/kg. Concentrations were expressed on a wet weight basis.

5.2.5 Mercury Bioaccessibility Analysis Methods

For uncooked and dried/smoked whitefish sample portions that underwent gastric digestion (5.2.3), Hg concentrations in the aqueous solution was determined using a mercury analyzer RA-915M with pyrolyzer PYRO-915+ (Ohio Lumex, British Columbia, Canada). 50µL of aqueous solution was added to 50mg non-iodated carbon which acts as a solid matrix. Mercury analysis was completed through thermal decomposition followed by atomic absorption spectroscopy, as outlined in US. EPA method 7473. To validate the batch analysis, a calibration standard (RPM-043QC) was run at the beginning and end of every batch of 40 samples, with no less than 3 blank runs in each sample batch. Uncooked and dried/smoked whitefish samples were ran in triplicate. The limit of detection of Hg in aqueous solution was 0.5 µg/L.

Bioaccessibility was calculated with the following equation below. Excel 2017 and its statistical data analysis software package were used to generate statistical results. The null hypothesis is that there is no statistical difference in the % of Hg bioaccessibility between the raw unprepared samples and the dried/smoked whitefish samples. The alternative hypothesis is that there is a statistical difference in Hg bioaccessibility, between uncooked and dried/smoked whitefish samples.

$$Percent\ bioaccessibility = \frac{[Hg\ soluble\ in\ digested\ whitefish \quad mg/kg]}{[Hg\ in\ initial\ whitefish\ sample \quad mg/kg]} * 100$$

5.3 Results

5.3.1 Summary Statistics of Whitefish Samples

On average, the fork length of the 5 whole whitefish were 485.8 ± 13.9mm (Supplementary Table B3). The average mass for portioned uncooked and dried/smoked whitefish samples were recorded (Table 5-1). Once the drying process was completed, the average initial mass of dried/smoked whitefish samples (48.25g ± 9.13g) was reduced (22.5g ± 7.57g). As a result, dried/smoked whitefish samples only retained 45.53% ± 7.12 % of the original mass, after culinary treatment.

Table 5-1. Mass of dried and uncooked whitefish samples

Culinary Treatment	N	Mass Before Culinary Treatment(g)	Mass After Culinary Treatment(g)	Mass Retained (%)
Uncooked	5	36.87 ±6.85	NA	NA
Dried	5	48.25 ±9.13	22.5 ±7.57	45.53 ±7.12

5.3.2 Mercury Concentration of Uncooked and Dried Whitefish

Initial and bioaccesible Hg concentrations for uncooked and dried/smoked whitefish samples were reported (Table 5-2). Prior to gastric digestion phase, uncooked whitefish samples had an initial Hg concentration of 0.080 ± 0.036 mg/kg. For dried/smoked whitefish samples, samples had an initial mercury concentration of 0.194 ± 0.068 mg/kg which appears to be statistically greater than uncooked whitefish samples ($p=0.002$) (Table 5-3). Once mass/moisture loss was accounted for, initial Hg concentrations between dried/smoked and uncooked whitefish were not statistically different ($p>0.05$), prior to culinary treatment (Table 5-4).

Table 5-2. Mercury concentration and bioaccessibility in dried and uncooked whitefish.

Culinary Treatment	initial (mg/kg)	Bioaccessible (mg/kg)	Bioaccessible (%)
Uncooked	0.080 ± 0.036^{aA}	0.081 ± 0.039^{aA}	101.56 ± 6.95^{A}
Dried	0.194 ± 0.068^{aB}	0.101 ± 0.038^{aB}	52.65 ± 5.59^{B}

Note: Different lowercase letters in the same row indicate significant differences between the initial and bioaccessible fractions ($p < 0.05$); different capital letters in the same column indicate significant differences between treatments ($p < 0.05$).

Table 5-3. Paired T-test of initial Hg concentrations in dried and uncooked whitefish samples.

Culinary Treatment	Dried	Uncooked
Mean [Hg](mg/kg)	0.194	0.080
Standard Deviation (mg/kg)	0.068	0.036
Observations	5	5
Difference between mean Hg [Hg] (mg/kg)	0.114	
df	4	
t- value	7.374	
p- value	0.002	

Table 5-4. Paired T-test of initial Hg concentrations in dried and uncooked whitefish samples, when changes to mass/moisture during drying process is accounted for.

Culinary Treatment	Dried	Uncooked
Mean [Hg](mg/kg)	0.090[1]	0.080
Standard Deviation (mg/kg)	0.045	0.036
Observations	5	5
Difference between mean Hg [Hg] (mg/kg)	0.01	
df	4	
t- value	2.776	
p- value	0.061	

Note: 1. Expected initial Hg concentration of dried/smoked whitefish samples, prior to culinary treatment and reduction in expected mass due to moisture loss.

5.3.3 Mercury Bioaccessibility of Uncooked and Dried Whitefish

As stated in Table 5-2, the bioaccessible Hg concentration of uncooked whitefish samples was 0.081 ± 0.039 mg/kg and was not statistically different compared to its initial Hg concentration (p=0.701) (Table 5-5). As such, the estimated Hg bioaccessibility for uncooked whitefish was 101.56 ± 6.95% (Table 5-2). In contrast, the bioaccessible Hg concentration of dried/smoked whitefish was 0.101 ± 0.038 mg/kg and was significantly reduced compared to its initial concentration (p=0.003) (Table 5-6). Therefore, Hg bioaccessibility for dried/smoked whitefish is 52.65% ± 5.59% (Table 5-2). When comparing the two culinary treatments, Hg bioaccessibility in dried/smoked whitefish was significantly reduced in uncooked whitefish ($p<0.001$) (Table 5-7).

Table 5-5. Paired T-test of initial and bioaccessible Hg concentrations in uncooked whitefish samples.

Culinary Treatment	Bioaccessible	Initial
Mean [Hg](mg/kg)	0.081	0.080
Standard Deviation (mg/kg)	0.039	0.036
Observations	5	5
Difference between mean		
Hg [Hg] (mg/kg)	0.001	
df	4	
t- value	0.413	
p- value	0.701	

Table 5-6. Paired T-test of initial and bioaccessible Hg concentrations in dried whitefish samples.

Culinary Treatment	Bioaccessible	Initial
Mean [Hg](mg/kg)	0.101	0.194
Standard Deviation (mg/kg)	0.068	0.038
Observations	5	5
Difference between mean		
Hg [Hg] (mg/kg)	-0.093	
df	4	
t- value	-6.266	
p- value	0.003	

Table 5-7. Paired T-test of Hg bioaccessibility in dried and uncooked whitefish samples

Culinary Treatment	Dried	Uncooked
Mean Hg Bioaccessibility (%)	52.65	101.56
Standard Deviation (%)	5.59	6.95
Observations	5	5
Difference between mean		
Hg Bioaccessibility (%)	-48.90	
df	4	
t- value	-14.062	
p- value	0.0001	

5.4 Discussions

As shown in Table 5-2, uncooked whitefish samples had an initial Hg concentration of 0.080 mg/kg and were similar to whitefish caught from lakes within the Dehcho region (0.073 mg/kg) (Reyes et al., 2017). As such, subsequent statistical findings from this research may also be applicable. In comparison to uncooked whitefish samples, initial Hg concentrations in dried/smoked whitefish were not statistically different. Despite having a higher initial Hg concentration, the statistical difference was likely related to the moisture content differences and reduction in mass that occurred during the drying process. Past literature have shown that differences in culinary processes can influence the overall moisture content and mass (Ouédraogo and Amyot, 2011). In a previous study conducted by Ouédraogo and Amyot (2011), moisture content was reduced in fried tuna (*Thunnus thynnus*), cat shark (*Scyliorhinus canicular*) and Spanish mackerel (*Scomberomorus maculatus*) samples when compared to raw samples. Once accounted for, the study found were no statistical differences between raw and fried samples (Ouédraogo and Amyot, 2011). Similar findings were also observed for meagre (*Argyrosomus regius*) and salmon (*Salmo salar*), according to past literatures (Afonso et al., 2015; Costa et al., 2015). This indicates that the drying process can increase total Hg concentration in whitefish samples due to moisture loss.

According to Table 5-2, 102% of the mercury content present in uncooked whitefish was bioaccessible. When comparing to other past literature, the Hg bioaccessibility in raw whitefish was comparable to other raw cat shark (80%), meagre (87%), salmon (80%) Spanish mackerel (77%) and tuna (75%) samples (Afonso et al., 2015; Costa et al., 2015; Ouédraogo and Amyot, 2011). Despite whitefish being a lower trophic species and initial Hg concentration, past literature have noted a negative correlation between initial Hg concentration in seafood and Hg

bioaccessibility (Siedlikowski et al., 2016). As such, it appears that all of the Hg content found present uncooked whitefish have the potential to be absorbed.

In contrast, Hg bioaccessibility for dried/smoked whitefish was 53% (Table 5-2). Similar trends were also observed with various culinary treatments, which included boiling, frying, grilling, and roasting and steaming (Afonso et al., 2015; Costa et al., 2015; Ouédraogo and Amyot, 2011). For instance, less than 40% and 20% of the Hg content present in boiled and fried tuna samples were bioaccessible respectively (Ouédraogo and Amyot, 2011). This was also true for cat shark and Spanish mackerel samples treated with the same culinary treatments (Ouédraogo and Amyot, 2011). Heat and protein denaturation may be a potential mechanism that explains for the observed reduction in Hg bioaccessibility. It is likely that the drying process introduces heat and leads to the formation of modified and denatured Hg-protein complexes. As such, culinary treatments, such as grilling and roasting, can render Hg-protein complexes less available for solubilisation potentially due to reduced protease affinity and efficacy (Afonso et al., 2015; Costa et al., 2015; Ouédraogo and Amyot, 2011). Although further studies are warranted, particularly with different fish species, in order to validate and generalize the effects of drying and Hg bioaccessibility. Therefore, about half of the Hg content found in dried/smoked whitefish samples was solubilized and ready to be absorbed, upon gastric digestion. Despite the reduction in Hg bioaccessibility, the bioaccessible Hg concentration in dried/smoked whitefish was 1.26x greater compared to uncooked whitefish samples. Compared to uncooked whitefish samples, the loss of moisture mass allowed for the increased in Hg relative concentration within the dried/smoked whitefish samples but not total Hg content. As such, greater amounts of edible dried/smoked whitefish flesh and total Hg content were sampled, digested and examined. Therefore, the overall Hg content that has solubilized in aqueous solution was greater in

dried/smoked samples. Overall, whitefish consumption does not present a concern for general seafood consumption as it would require a 70kg individual to consume more than 7 servings/wk of dried/smoked whitefish to exceed their pTWI (1.6 μg/kg/wk) (Statistics Canada, 2013).

5.5 Conclusion

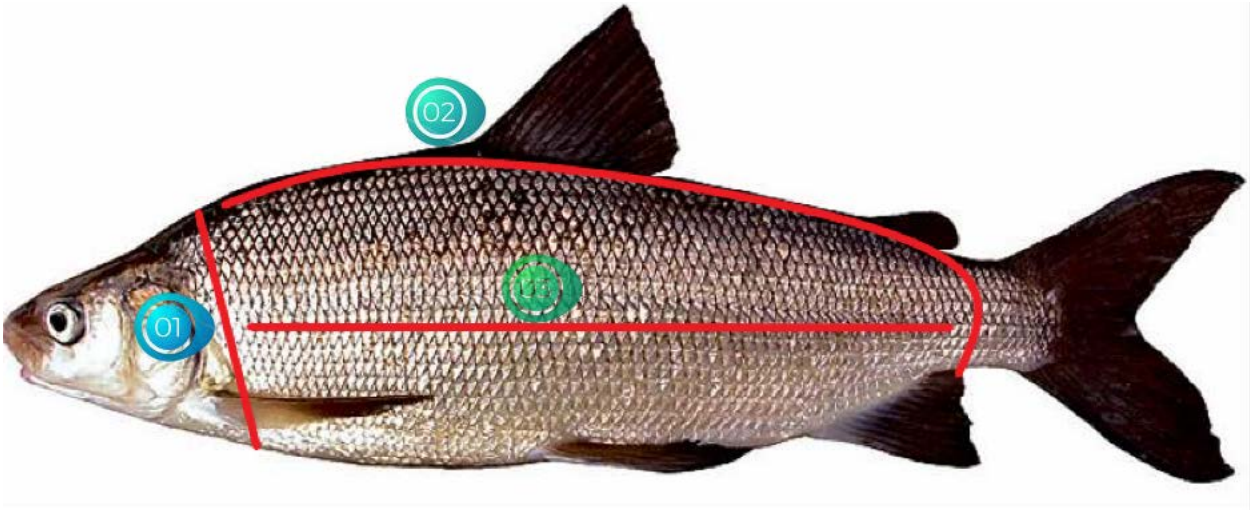
As demonstrated by this *in vitro* bioaccessibility study involving whitefish, IVBA models can be an effective tool for characterizing Hg content and bioaccessibility in food. This technique recognizes that seafood source and food preparation methods can influence Hg bioaccessibility, which could potentially play a role in identifying the relationship between dietary exposure factors and internal Hg exposure (You et al., 2014). For this particular IVBA model, this study observed no statistical differences in Hg concentrations found within uncooked and dried/smoked whitefish. Furthermore, Hg bioaccessibility in dried/smoked whitefish was significantly lower compared to uncooked whitefish samples. Reduction in Hg bioaccessibility may be the result of changes in Hg-protein complexes and protein denaturation, from applying culinary treatment. Exposure assessments that incorporate the effect of food drying on mercury content of those foods may need to also consider the changes in bioaccessibility that result from the moisture loss. As such, bioaccessibility findings suggest source apportionment models that incorporate differences in Hg concentration between raw and smoked/dried fish may also need to account for the corresponding difference in bioaccessibility. In addition, it might not be necessary to assume Hg bioaccessibility, a conservative estimate for bioavailability, to be 100% as bioaccessibility findings have shown that culinary factors can play a role in reducing Hg bioaccessibility. Further research is required to determine whether bioaccessibility findings have an impact on source apportionment models and how Hg bioaccessibility differences due to cooking methods can also be implemented within human exposure assessments. Despite the

bioaccessible Hg concentration in dried/smoked whitefish was higher compared to uncooked samples, both concentrations do not present a concern for most general seafood consumption. For future directions, both *in vitro* Hg bioaccessibility and *in vivo* Hg bioavailability studies are necessary for further generalizability of differences observed with culinary treatments by also using different fish species. When used together, bioaccessibility and bioavailability methods have the potential to bridge the gap between external Hg exposure sources and internal Hg exposure levels.

Appendix B- Supplementary Tables and Figures

Supplementary Table B1. Whitefish samples fork length.

Species	N	Average Fork Length (mm)	Minimum Fork Length (mm)	Maximum Fork Length(mm)
Whitefish	5	485.8 ±13.4	470	502



Supplementary Figure B1. Whitefish samples fork length.

6. Thesis Conclusion

In summary, this thesis project established a baseline measure for Hg exposure within the participating Indigenous communities of the Dehcho and Sahtú regions of the NWT. With the use of FFQ and simple regression models, this study found that the participants' internal Hg exposure levels were well within guidance values provided by Legrand et al. (2010). Study findings have also identified age to be positively associated with Hg exposure levels in blood and hair, for the participating Dene and Métis population in the Dehcho and Sahtú regions. Linear regression findings also found no statistical association between fish consumption with Hg exposure levels in blood and hair. This thesis project also conducted *in vitro* bioaccessibility assessments to address a potential mechanism that can link external dietary Hg exposure sources with internal Hg exposure levels seen amongst individuals and communities. Based on *in vitro* bioaccessibility findings, dried/smoked whitefish samples carry the same initial concentration of Hg as uncooked samples which were safe for the general consumption (CFIA, 2014). In addition, Hg bioaccessibility in dried/smoked whitefish was significantly reduced because of changes in Hg-protein complexes and protein denaturation, from applying culinary treatment. As such, it may be warranted to survey fish culinary preparation methods within future biomonitoring studies and integrate them within regression models, in order to characterize Hg exposure levels seen in blood and hair biomarkers. To do so, further segregation of total fish consumption by culinary preparation may be an appropriate solution. IVBA models and bioaccessibility findings can serve as a supplementary exposure assessment tools alongside with direct biomonitoring of Hg exposure. Although, there is still a need for more research to accurately determine Hg bioaccessibility. Future exposure assessments and source apportionment models should incorporate Hg bioaccessibility findings. Together, future Hg biomonitoring and bioaccessibility

studies can attempt to account for food type and cooking method into the source apportionment model's calculation and characterize its impact on human exposure. It is possible to examine the relationship between internal Hg exposure levels detected in biomarkers and the external exposure estimates that have accounted for the differences in Hg concentration and bioaccessibility due to drying. This would verify the effectiveness of incorporating Hg bioaccessibility differences due to food preparation methods within source apportionment models. Together, Hg biomonitoring and bioaccessibility studies may be useful for not only characterizing Hg exposure risks but also for promoting increased country foods consumption.

In terms of future directions for Hg biomonitoring within the Indigenous communities of NWT, there will be a need to expand the study population size and it is ideal to recruit more communities within the Dehcho and Sahtú regions. With a larger dataset, it may lead to further improvement and development of linear regression models capable of characterizing Hg exposure. Aside from obtaining a larger dataset, future studies that build upon this work should also explore other source apportionment models and how dietary Hg sources are coded within the linear regression models. Instead of surveying consumption patterns based on frequency, it may be more appropriate to inquire about fish consumption patterns based on mass and standardizing the consumed mass with respect to expected Hg concentration for each fish species. Furthermore, inclusion of fish culinary methods within regression models may improve linear regression models' ability to characterize Hg exposure patterns found in blood and hair biomarkers. This may provide a more accurate depiction of dietary Hg exposure sources and its relationship to internal Hg exposure levels.

In terms of future directions for Hg bioaccessibility in whitefish, there is a need to validate drying and other culinary preparation methods using different fish species. Although not

well established, it is also possible to explore the potential effects that co-consumption of certain country foods and beverages, such as berries or tea. This may also provide better insight for other traditional food preparation methods and can serve as a foundation for safe consumption of country food. In addition to the future *in vitro* bioaccessibility studies, it is also necessary to propose *in vivo* bioavailability studies for Hg to validate that bioaccessibility findings are not just artifacts. Both routes are potential avenues that can help improve our current understanding about dietary Hg exposure, characterize Hg exposure within biomarkers and promote continue consumption of country foods.

7. Bibliography

Abdel-Aal, E. S. (2008). Effects of baking on protein digestibility of organic spelt products determined by two in vitro digestion methods. *LWT-Food Science and Technology, 41*(7), 1282-1288.

Aberg, B., Ekman, L., Falk, R., Greitz, U., Persson, G., & Snihs, J. O. (1969). Metabolism of methyl mercury (203Hg) compounds in man: Excretion and distribution. *Archives of Environmental Health: An International Journal, 19*(4), 478-484.

Abernethy, D. R., DeStefano, A. J., Cecil, T. L., Zaidi, K., Williams, R. L., & Panel, U. M. I. A. (2010). *Metal impurities in food and drugs. Pharmaceutical research, 27*(5), 750-755.

Adelson, N. (2005). The embodiment of inequity: Health disparities in Aboriginal Canada. *Canadian Journal of Public Health/Revue Canadienne de Sante'e Publique*, S45-S61.

Afonso, C., Costa, S., Cardoso, C., Oliveira, R., Lourenco, H. M., Viula, A., ... & Nunes, M. L. (2015). Benefits and risks associated with consumption of raw, cooked, and canned tuna (Thunnus spp.) based on the bioaccessibility of selenium and methylmercury. *Environmental research, 143*, 130-137.

Afonso, C., Costa, S., Cardoso, C., Bandarra, N. M., Batista, I., Coelho, I., ... & Nunes, M. L. (2015). Evaluation of the risk/benefit associated to the consumption of raw and cooked farmed meagre based on the bioaccessibility of selenium, eicosapentaenoic acid and docosahexaenoic acid, total mercury, and methylmercury determined by an in vitro digestion model. *Food chemistry, 170*, 249-256.

Afonso, C., Costa, S., Cardoso, C., Coelho, I., Castanheira, I., Lourenço, H., ... & Bandarra, N. M. (2016). Bioaccessibility in risk-benefit analysis of raw and cooked seabream consumption. *Journal of Food Composition and Analysis*.

Alegría, A., Garcia-Llatas, G., & Cilla, A. (2015). Static digestion models: General introduction. *In The Impact of Food Bioactives on Health*, 3-12.

Alminger, M., Svelander, C., Wellner, A., Martinez-Tomas, R., Bialek, L., Larque, E., & Perez-Llamas, F. (2012). Applicability of in vitro models in predicting the in vivo bioavailability of lycopene and β-carotene from differently processed soups. *Food and Nutrition Sciences*, *3*(04), 477.

Amin-Zaki, L., Elhassani, S., Majeed, M. A., Clarkson, T. W., Doherty, R. A., & Greenwood, M. (1974). *Intra-uterine methylmercury poisoning in Iraq. Pediatrics*, *54*(5), 587-595.

Anderson, H. A., Falk, C., Hanrahan, L., Olson, J., Burse, V., & Needham, L. ATSDR public health assessment guidance manual. 2005. *Environ Res, 80*(2), 183-88.

Asano, S., Eto, K., Kurisaki, E., Gunji, H., Hiraiwa, K., Sato, M., ... & Wakasa, H. (2000). Acute inorganic mercury vapor inhalation poisoning. *Pathology International*, *50*(3), 169-174.

Aschner, M., & Clarkson, T. W. (1987). Mercury 203 distribution in pregnant and nonpregnant rats following systemic infusions with thiol-containing amino acids. *Teratology, 36*(3), 321-328.

Aschner, M., Allen, J. W., Kimelberg, H. K., LoPachin, R. M., & Streit, W. J. (1999). Glial cells in neurotoxicity development. *Annual Review of Pharmacology and Toxicology, 39*(1), 151-173

Aschner, M., Yao, C. P., Allen, J. W., & Tan, K. H. (2000). Methylmercury alters glutamate transport in astrocytes. *Neurochemistry international, 37*(2), 199-206.

Aschner, M., Syversen, T., Souza, D., Rocha, J., & Farina, M. (2007). Involvement of glutamate and reactive oxygen species in methylmercury neurotoxicity. *Brazilian Journal of Medical and Biological Research, 40*(3), 285-291.

ATSDR. (1999). Toxicological profile for mercury. *Atlanta: Division of Toxicology/Toxicology. U.S. Department of Health and Human Services, Public Health Services*

ATSDR. (2005). Appendix G: Calculating Exposure Doses. *Public Health Assessment Guidance Manual (2005 Update)*

Atwell, L., Hobson, K. A., & Welch, H. E. (1998). Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences, 55*(5), 1114-1121.

Axelrad, D. A., Bellinger, D. C., Ryan, L. M., & Woodruff, T. J. (2007). Dose-response relationship of prenatal mercury exposure and IQ: an integrative analysis of epidemiologic data. *Environmental health perspectives*, 609-615.

Batal, M., Gray-Donald, K., Kuhnlein, H. V., & Receveur, O. (2005). Estimation of traditional food intake in indigenous communities in Denendeh and the Yukon. *International Journal of Circumpolar Health, 64*(1), 46-54.

Beckford, C. L., Jacobs, C., Williams, N., & Nahdee, R. (2010). Aboriginal environmental wisdom, stewardship, and sustainability: lessons from the Walpole Island First Nations, Ontario, Canada. *The journal of environmental education, 41*(4), 239-248.

Bernhoft, R. A. (2012). Mercury toxicity and treatment: a review of the literature. *Journal of environmental and public health,* 2012.

Berti, P. R., Receveur, O., Chan, H. M., & Kuhnlein, H. V. (1998). Dietary exposure to chemical contaminants from traditional food among adult Dene/Métis in the western Northwest Territories, Canada. *Environmental Research, 76*(2), 131-142.

Boisen, S., & Eggum, B. O. (1991). Critical evaluation of in vitro methods for estimating digestibility in simple-stomach animals. *Nutrition research reviews, 4*(1), 141-162.

Bozoyan, L., Khlghatyan, J., & Saghatelyan, A. (2012). Astrocytes control the development of the migration-promoting vasculature scaffold in the postnatal brain via VEGF signaling. *Journal of Neuroscience, 32*(5), 1687-1704.

Bradley, M. A., Barst, B. D., & Basu, N. (2017). A Review of Mercury Bioavailability in Humans and Fish. *International journal of environmental research and public health, 14*(2), 169.

Bublin, M., Radauer, C., Knulst, A., Wagner, S., Scheiner, O., Mackie, A. R., ... & Breiteneder, H. (2008). Effects of gastrointestinal digestion and heating on the allergenicity of the kiwi allergens Act d 1, actinidin, and Act d 2, a thaumatin-like protein. *Molecular nutrition & food research, 52*(10), 1130-1139.

Burger, J., Jeitner, C., & Gochfeld, M. (2011). Locational Differences in Mercury and Selenium Levels in 19 Species of Saltwater Fish from New Jersey. *Journal of Toxicology and Environmental Health, Part A, 74*(13), 863-874.

Cabañero, A. I., Madrid, Y., & Cámara, C. (2007). Mercury–selenium species ratio in representative fish samples and their bioaccessibility by an in vitro digestion method. *Biological Trace Element Research, 119*(3), 195-211.

Calatayud, M., Devesa, V., Virseda, J. R., Barberá, R., Montoro, R., & Vélez, D. (2012). Mercury and selenium in fish and shellfish: Occurrence, bioaccessibility and uptake by Caco-2 cells. *Food and chemical toxicology, 50*(8), 2696-2702.

Cano-Sancho, G., Perelló, G., Maulvault, A. L., Marques, A., Nadal, M., & Domingo, J. L. (2015). Oral bioaccessibility of arsenic, mercury and methylmercury in marine species commercialized in Catalonia (Spain) and health risks for the consumers. *Food and Chemical Toxicology, 86*, 34-40.

Carta, P., Flore, C., Alinovi, R., Ibba, A., Tocco, M. G., Aru, G., ... & Randaccio, F. S. (2003). Sub-clinical neurobehavioral abnormalities associated with low level of mercury exposure through fish consumption. *Neurotoxicology, 24*(4), 617-623.

Carvalho, C. M., Chew, E. H., Hashemy, S. I., Lu, J., & Holmgren, A. (2008). Inhibition of the human thioredoxin system a molecular mechanism of mercury toxicity. *Journal of Biological Chemistry, 283*(18), 11913-11923.

CFIA. (2014). *APPENDIX 3 – CANADIAN GUIDELINES FOR CHEMICIAL CONTAMINANTS AND TOXINS IN FISH AND FISH PRODUCTS.* Retrieved from http://www.inspection.gc.ca/DAM/DAM-food-aliments/STAGING/text-texte/fish_man_standardsmethods_appendix3_1406403090196_eng.pdf

Choi, A. L., Weihe, P., Budtz-Jørgensen, E., Jørgensen, P. J., Salonen, J. T., Tuomainen, T. P., ... & Grandjean, P. (2009). Methylmercury exposure and adverse cardiovascular effects in Faroese whaling men. *Environmental health perspectives, 117*(3), 367.

Cikrt, M., & Tichý, M. (1974). Biliary excretion of phenyl-and methyl mercury chlorides and their enterohepatic circulation in rats. *Environmental research, 8*(1), 71-81.

Clarkson, T. W., Magos, L., & Myers, G. J. (2003). The Toxicology of Mercury — Current Exposures and Clinical Manifestations. *New England Journal of Medicine, 349*(18), 1731-1737.

Clarkson, T. W., & Magos, L. (2006). The toxicology of mercury and its chemical compounds. *Critical reviews in toxicology, 36*(8), 609-662.

Cohen, J. T., Bellinger, D. C., & Shaywitz, B. A. (2005). A quantitative analysis of prenatal methyl mercury exposure and cognitive development. *American journal of preventive medicine, 29*(4), 353-353.

Compeau, G., & Bartha, R. (1985). Sulfate-Reducing Bacteria: Principal Methylators of Mercury in Anoxic Estuarine Sediment. *Appl Environ Microbiol., 50*(2), 498-502.

Cordeiro, R. M. (2014). Reactive oxygen species at phospholipid bilayers: distribution, mobility and permeation. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1838*(1), 438-444.

Costa, S., Afonso, C., Cardoso, C., Batista, I., Chaveiro, N., Nunes, M. L., & Bandarra, N. M. (2015). Fatty acids, mercury, and methylmercury bioaccessibility in salmon (Salmo salar) using an in vitro model: Effect of culinary treatment. *Food chemistry, 185*, 268-276.

Cott, P. A., Zajdlik, B. A., Palmer, M. J., & McPherson, M. D. (2016). Arsenic and mercury in lake whitefish and burbot near the abandoned Giant Mine on Great Slave Lake. *Journal of Great Lakes Research, 42*(2), 223-232.

Cusack, L. K., Smit, E., Kile, M. L., & Harding, A. K. (2017). Regional and temporal trends in blood mercury concentrations and fish consumption in women of child bearing Age in the united states using NHANES data from 1999–2010. *Environmental Health, 16*(1), 10.

Dave, V., Mullaney, K. J., Goderie, S., Kimelberg, H. K., & Aschner, M. (1994). Astrocytes as mediators of methylmercury neurotoxicity: effects on D-aspartate and serotonin uptake. *Developmental neuroscience, 16*(3-4), 222-231.

Davis, L. E., Kornfeld, M., Mooney, H. S., Fiedler, K. J., Haaland, K. Y., Orrison, W. W., ... & Clarkson, T. W. (1994). Methylmercury poisoning: Long-term clinical, radiological,

toxicological, and pathological studies of an affected family. *Annals of neurology*, *35*(6), 680-688.

Dolbec, J., Mergler, D., Sousa Passos, C. J., Sousa de Morais, S., & Lebel, J. (2000). Methylmercury exposure affects motor performance of a riverine population of the Tapajos river, Brazilian Amazon. *International Archives of Occupational and Environmental Health*, *73*(3), 195-203.

Donaldson, S. G., Van Oostdam, J., Tikhonov, C., Feeley, M., Armstrong, B., Ayotte, P., ... & Dallaire, R. (2010). Environmental contaminants and human health in the Canadian Arctic. *Science of the Total Environment*, *408*(22), 5165-5234.

Dong, Z., Jim, R. C., Hatley, E. L., Backus, A. S., Shine, J. P., Spengler, J. D., & Schaider, L. A. (2015). A longitudinal study of mercury exposure associated with consumption of freshwater fish from a reservoir in rural south central USA. *Environmental research*, *136*, 155-162.

Drexler, J. W., & Brattin, W. J. (2007). An in vitro procedure for estimation of lead relative bioavailability: with validation. *Human and Ecological Risk Assessment*, *13*(2), 383-401.

Earle, L. (2011). Traditional aboriginal diets and health. *National Collaborating Centre for Aboriginal Health/Centre de collaboration nationale de la santé autochtone*.

Environment and Climate Change Canada (ECCC) (2016). Canadian Mercury Science Assessment: Summary of Key Results.

Elhamri, H., Idrissi, L., Coquery, M., Azemard, S., Abidi, A. E., Benlemlih, M., ... & Cubadda, F. (2007). Hair mercury levels in relation to fish consumption in a community of the Moroccan Mediterranean coast. *Food additives and contaminants*, *24*(11), 1236-1246.

Erlanson-Albertsson, C. (1983). The interaction between pancreatic lipase and colipase: a protein-protein interaction regulated by a lipid. *FEBS letters, 162*(2), 225-229.

Evans, M. S., Muir, D., Lockhart, W. L., Stern, G., Ryan, M., & Roach, P. (2005). Persistent organic pollutants and metals in the freshwater biota of the Canadian Subarctic and Arctic: an overview. *Science of the Total Environment, 351*, 94-147.

Evans, M. S., Lockhart, W. L., Doetzel, L., Low, G., Muir, D., Kidd, K., ... & Delaronde, J. (2005). Elevated mercury concentrations in fish in lakes in the Mackenzie River Basin: the role of physical, chemical, and biological factors. *Science of the Total Environment, 351*, 479-500.

Evans, M., Muir, D., Brua, R. B., Keating, J., & Wang, X. (2013). Mercury trends in predatory fish in Great Slave Lake: the influence of temperature and other climate drivers. *Environmental science & technology, 47*(22), 12793-12801.

Evans, M., Low, G., Muir, D., Keating, J., Wang, X., Low, M., Giroux, D., Tollis, M., & Buckley S. (2013) *Spatial and temporal variability in mercury concentrations in predatory fish in lakes along the Mackenzie River and in Great Slave Lake.* Northern Contaminants Program.

Ewers, U., Krause, C., Schulz, C., & Wilhelm, M. (1999). Reference values and human biological monitoring values for environmental toxins. *International archives of occupational and environmental health, 72*(4), 255-260.

FAO. (2016). The state of world fisheries and aquaculture 2016: contributing to food security and nutrition for all. (2016). *Rome: Food and Agriculture Organization of the United Nations.*

Farina, M., Frizzo, M. E., Soares, F. A., Schwalm, F. D., Dietrich, M. O., Zeni, G., ... & Souza, D. O. (2003). Ebselen protects against methylmercury-induced inhibition of glutamate uptake by cortical slices from adult mice. *Toxicology letters, 144*(3), 351-357.

Fediuk, K., Hidiroglou, N., Madère, R., & Kuhnlein, H. V. (2002). Vitamin C in Inuit traditional food and women's diets. *Journal of food Composition and Analysis, 15*(3), 221-235.

Feng, S., Xu, Z., Wang, F., Yang, T., Liu, W., Deng, Y., & Xu, B. (2017). Sulforaphane prevents methylmercury-induced oxidative damage and excitotoxicity through activation of the Nrf2-ARE pathway. *Molecular neurobiology, 54*(1), 375-391.

Fernández-García, E., Carvajal-Lérida, I., & Pérez-Gálvez, A. (2009). In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research, 29*(11), 751-760.

Fisheries and Oceans Canada. (2018*) Fisheries – Statistics – Commericial Fisheries – Consumption*. Retrieved from http://www.dfo-mpo.gc.ca/stats/commercial/consumption-eng.htm

Fonfría, E., Vilaró, M. T., Babot, Z., Rodríguez-Farré, E., & Sunol, C. (2005). Mercury compounds disrupt neuronal glutamate transport in cultured mouse cerebellar granule cells. *Journal of neuroscience research, 79*(4), 545-553.

Food Drug Administration (1999). Mercury Compounds in Drugs and Food. *Department of Health and Human Services: Food and Drug Administration, 64*(82), 23083-23086.

Food Safety Network. (2009). Safe Preparation and Storage of Aboriginal Traditional/Country Foods: A Review. *NATIONAL COLLABORATING CENTRE FOR ENVIRONMENTAL HEALTH*, 1-119.

Franco, J. L., Braga, H. C., Stringari, J., Missau, F. C., Posser, T., Mendes, B. G., ... & Farina, M. (2007). Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. *Chemical research in toxicology, 20*(12), 1919-1926.

Galloway, T. (2017). Canada's northern food subsidy Nutrition North Canada: a comprehensive program evaluation. *International journal of circumpolar health, 76*(1), 1279451.

García, G. M., Boffetta, P., Caballero, K. J., Español, S., & Gómez, Q. J. (2007). Cardiovascular mortality in mercury miners. *Medicina clínica, 128*(20), 766-771.

Gionet, L., & Roshanafshar, S. (2015). Health at a Glance- Select health indicators of First Nations people living off reserve, Métis and Inuit. *Statistics Canada, 82*(624), X.

Glahn, R. P., Lee, O. A., & Miller, D. D. (1999). In vitro digestion/caco-2 cell culture model to determine optimal ascorbic acid to Fe ratio in rice cereal. *Journal of food science*, *64*(5), 925-928.

Golding, J., Steer, C. D., Hibbeln, J. R., Emmett, P. M., Lowery, T., & Jones, R. (2013). Dietary predictors of maternal prenatal blood mercury levels in the ALSPAC birth cohort study. *Environmental health perspectives, 121*(10), 1214.

Government of Northwest Territories (2016). *Fish Consumption Guidance – Site Specific Fish Consumption Advice.* Health and Social Services, Government of Northwest Territories.

Grandjean, P., Weihe, P., White, R. F., Debes, F., Araki, S., Yokoyama, K., ... & Jørgensen, P. J. (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicology and teratology, 19*(6), 417-428.

Grandjean, P., & Budtz-Jørgensen, E. (2007). Total imprecision of exposure biomarkers: implications for calculating exposure limits. *American journal of industrial medicine, 50*(10), 712-719.

Grandjean, P., Satoh, H., Murata, K., & Eto, K. (2010). Adverse effects of methylmercury: environmental health research implications. *Environmental health perspectives*, 1137-1145.

Gribble, M. O., Karimi, R., Feingold, B. J., Nyland, J. F., O'hara, T. M., Gladyshev, M. I., & Chen, C. Y. (2015). Mercury, selenium and fish oils in marine food webs and implications for human health. *Journal of the Marine Biological Association of the United Kingdom, 96*(01), 43-59.

Groeger, A. L., Cipollina, C., Cole, M. P., Woodcock, S. R., Bonacci, G., Rudolph, T. K., ... & Schopfer, F. J. (2010). Cyclooxygenase-2 generates anti-inflammatory mediators from omega-3 fatty acids. *Nature chemical biology, 6*(6), 433-441.

Gustin, K., Tofail, F., Mehrin, F., Levi, M., Vahter, M., & Kippler, M. (2017). Methylmercury exposure and cognitive abilities and behavior at 10years of age. *Environment International, 102*, 97-105.

Hackett, P. (2005). From past to present: Understanding First Nations health patterns in a historical context. *Canadian Journal of Public Health/Revue Canadienne de Sante'e Publique*, S17-S21.

Hansen, J. C., & Van Oostdam, J. (2009). AMAP Assessment 2009: Human Health in the Arctic.

Harada, M. (1995). Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *Critical reviews in toxicology, 25*(1), 1-24.

He, M., & Wang, W. X. (2011). Factors affecting the bioaccessibility of methylmercury in several marine fish species. *Journal of agricultural and food chemistry, 59*(13), 7155-7162.

Health Canada. (2007). Human health risk assessment of mercury in fish and health benefits of fish consumption. *Ottawa: Health Canada, Bureau of Chemical Safety*

Health Canada. (2010). *Health Canda Toxicological Reference Values (TRVs) and Chemical-Specific Factors, Version 2.0.* Retrieved from http://publications.gc.ca/collections/collection_2012/sc-hc/H128-1-11-638-eng.pdf

Hightower, J. M., & Brown, D. L. (2011). Mercury concentrations in fish jerky snack food: marlin, ahi, and salmon. *Environmental Health*, *10*(1), 90.

Hong, Y. S., Kim, Y. M., & Lee, K. E. (2012). Methylmercury exposure and health effects. *Journal of Preventive Medicine and Public Health*, *45*(6), 353.

Horvat, M., Nolde, N., Fajon, V., Jereb, V., Logar, M., Lojen, S., ... & Drobne, D. (2003). Total mercury, methylmercury and selenium in mercury polluted areas in the province Guizhou, China. *Science of the Total Environment*, *304*(1), 231-256.

Houston, M. C. (2011). Role of mercury toxicity in hypertension, cardiovascular disease, and stroke. *The Journal of Clinical Hypertension*, *13*(8), 621-627.

Hu, Q. H., Kang, H., Li, Z., Wang, Y. S., Ye, P. P., Zhang, L. L., ... & Xie, Z. Q. (2014). Characterization of atmospheric mercury at a suburban site of central China from wintertime to springtime. *Atmospheric Pollution Research*, *5*(4), 769-778.

Hur, S. J., Lim, B. O., Decker, E. A., & McClements, D. J. (2011). In vitro human digestion models for food applications. *Food Chemistry*, *125*(1), 1-12.

Indigenous and Northern Affairs Canada. (2013) *Evaluation of the Advancing Conservation Interests in the Northwest Territories Initiative (Protected Areas Strategy).* Retrieved from https://www.aadnc-aandc.gc.ca/eng/1394198848113/1394198995182

Jayawardene, I., Saper, R., Lupoli, N., Sehgal, A., Wright, R. O., & Amarasiriwardena, C. (2010). Determination of in vitro bioaccessibility of Pb, As, Cd and Hg in selected traditional Indian medicines. *Journal of analytical atomic spectrometry*, *25*(8), 1275-1282.

Jo, S., Woo, H. D., Kwon, H. J., Oh, S. Y., Park, J. D., Hong, Y. S., ... & Sohn, S. J. (2015). Estimation of the biological half-Life of methylmercury using a population toxicokinetic model. *International journal of environmental research and public health, 12*(8), 9054-9067.

Juhasz, A. L., Smith, E., Weber, J., Rees, M., Rofe, A., Kuchel, T., ... & Naidu, R. (2007). Comparison of in vivo and in vitro methodologies for the assessment of arsenic bioavailability in contaminated soils. *Chemosphere, 69*(6), 961-966.

Kajiwara, Y., Yasutake, A., Adachi, T., & Hirayama, K. (1996). Methylmercury transport across the placenta via neutral amino acid carrier. *Archives of toxicology, 70*(5), 310-314.

Kerin, E. J., Gilmour, C. C., Roden, E., Suzuki, M. T., Coates, J. D., & Mason, R. P. (2006). Mercury methylation by dissimilatory iron-reducing bacteria. *Applied and environmental microbiology, 72*(12), 7919-7921.

Kerper, L. E., Ballatori, N. A. Z. Z. A. R. E. N. O., & Clarkson, T. W. (1992). Methylmercury transport across the blood-brain barrier by an amino acid carrier. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 262*(5), R761-R765.

Kershaw, T. G., Dhahir, P. H., & Clarkson, T. W. (1980). The relationship between blood levels and dose of methylmercury in man. *Archives of Environmental Health: An International Journal, 35*(1), 28-36.

Kim, S. A., Kwon, Y., Kim, S., & Joung, H. (2016). Assessment of Dietary Mercury Intake and Blood Mercury Levels in the Korean Population: Results from the Korean National Environmental Health Survey 2012–2014. *International journal of environmental research and public health, 13*(9), 877.

Kimura, H., Futami, Y., Tarui, S. I., & Shinomiya, T. (1982). Activation of human pancreatic lipase activity by calcium and bile salts. *The Journal of Biochemistry, 92*(1), 243-251.

Kuhnlein, H. V., Appavoo, D., Morrison, N., Soueida, R., & Pierrot, P. (1994). Use and nutrient composition of traditional Sahtú (Hareskin) Dene/Metis foods. *Journal of Food Composition and Analysis, 7*(3), 144-157.

Kosatsky, T., Przybysz, R., & Armstrong, B. (2000). Mercury exposure in Montrealers who eat St. Lawrence River sportfish. *Environmental Research, 84*(1), 36-43.

Kuhnlein, H. V., & Chan, H. M. (2000). Environment and contaminants in traditional food systems of northern indigenous peoples. *Annual Review of Nutrition, 20*(1), 595-626.

Kuhnlein, H. V., Barthet, V., Farren, A., Falahi, E., Leggee, D., Receveur, O., & Berti, P. (2006). Vitamins A, D, and E in Canadian Arctic traditional food and adult diets. *Journal of food composition and analysis, 19*(6), 495-506.

Kuhnlein, H. V., & Humphries, M. M. (2017). Traditional Animal Foods of Indigenous Peoples of Northern North America. Retrieved from http://traditionalanimalfoods.org/

Kuhnlein, H. V., & Receveur, O. (2007). Local cultural animal food contributes high levels of nutrients for Arctic Canadian Indigenous adults and children. *The Journal of nutrition, 137*(4), 1110-1114.

Laird, B. D., Shade, C., Gantner, N., Chan, H. M., & Siciliano, S. D. (2009). Bioaccessibility of mercury from traditional northern country foods measured using an in vitro gastrointestinal model is independent of mercury concentration. *Science of The Total Environment, 407*(23), 6003-6008.

Laird, B. D., & Chan, H. M. (2013). Bioaccessibility of metals in fish, shellfish, wild game, and seaweed harvested in British Columbia, Canada. *Food and chemical toxicology, 58*, 381-387.

Laird, B. D., Goncharov, A. B., Egeland, G. M., & Chan, H. M. (2013). Dietary advice on Inuit traditional food use needs to balance benefits and risks of mercury, selenium, and n3 fatty acids. *The Journal of nutrition*, *143*(6), 923-930.

Laird, M. (2017). Dietary Exposure Assessment and Contaminants Biomonitoring in the Dehcho Region, Northwest Territories: Exploring the Relationship Between Mercury Exposure, Omega-3 Fatty Acid Status, and Fish Consumption *(Master's thesis, University of Waterloo).*

Laird, M. J., Henao, J. J. A., Reyes, E. S., Stark, K. D., Low, G., Swanson, H. K., & Laird, B. D. (2018). Mercury and omega-3 fatty acid profiles in freshwater fish of the Dehcho Region, Northwest Territories: Informing risk benefit assessments. *Science of The Total Environment*, *637*, 1508-1517.

Laks, D. R. (2009). Assessment of chronic mercury exposure within the US population, National Health and Nutrition Examination Survey, 1999–2006. *Biometals*, *22*(6), 1103-1114.

Larter, N. C., Macdonald, C. R., Elkin, B. T., Wang, X., Harms, N. J., Gamberg, M., & Muir, D. C. G. (2016). Cadmium and other elements in tissues from four ungulate species from the Mackenzie Mountain region of the Northwest Territories, Canada. *Ecotoxicology and environmental safety*, *132*, 9-17.

Larter, N. C., & Nagy, J. A. (2000). A comparison of heavy metal levels in the kidneys of High Arctic and mainland caribou populations in the Northwest Territories of Canada. *Science of the total environment*, *246*(2-3), 109-119.

Lebel, J., Mergler, D., Branches, F., Lucotte, M., Amorim, M., Larribe, F., & Dolbec, J. (1998). Neurotoxic effects of low-level methylmercury contamination in the Amazonian Basin. *Environmental research*, *79*(1), 20-32.

Legrand, M., Feeley, M., Tikhonov, C., Schoen, D., & Li-Muller, A. (2010). Methylmercury blood guidance values for Canada. *Canadian Journal of Public Health/Revue Canadienne de Sante'e Publique*, 28-31.

Lindberg, A., Björnberg, K. A., Vahter, M., & Berglund, M. (2004). Exposure to methylmercury in non-fish-eating people in Sweden. *Environmental Research, 96*(1), 28-33.

Lockhart, W. L., Wagemann, R., Tracey, B., Sutherland, D., & Thomas, D. J. (1992). Presence and implications of chemical contaminants in the freshwaters of the Canadian Arctic. *Science of the Total Environment, 122*(1-2), 165-243.

Lodenius, M., & Malm, O. (1998). Mercury in the Amazon. *Reviews of environmental contamination and toxicology*, 25-52.

Mahaffey, K. R., Clickner, R. P., & Bodurow, C. C. (2004). Blood organic mercury and dietary mercury intake: National Health and Nutrition Examination Survey, 1999 and 2000. *Environmental health perspectives, 112*(5), 562.

Mason, R. P., Fitzgerald, W. F., & Morel, F. M. (1994). The biogeochemical cycling of elemental mercury: anthropogenic influences. *Geochimica et Cosmochimica Acta, 58*(15), 3191-3198.

Matos, J., Lourenço, H. M., Brito, P., Maulvault, A. L., Martins, L. L., & Afonso, C. (2015). Influence of bioaccessibility of total mercury, methyl-mercury and selenium on the risk/benefit associated to the consumption of raw and cooked blue shark (Prionace glauca). *Environmental research, 143*, 123-129.

Morrison, N., & Kuhnlein, H. V. (1993). Retinol content of wild foods consumed by the Sahtú (Hareskin) Dene/Métis. *Journal of Food Composition and Analysis*, *6*(1), 10-23.

Maulvault, A. L., Machado, R., Afonso, C., Lourenço, H. M., Nunes, M. L., Coelho, I., ... & Marques, A. (2011). Bioaccessibility of Hg, Cd and As in cooked black scabbard fish and edible crab. *Food and Chemical Toxicology*, *49*(11), 2808-2815.

McDowell, M. A., Dillon, C. F., Osterloh, J., Bolger, P. M., Pellizzari, E., Fernando, R., ... & Mahaffey, K. R. (2004). Hair mercury levels in US children and women of childbearing age: reference range data from NHANES 1999–2000. *Environmental health perspectives*, *112*(11), 1165.

Miettinen, J. K. (1973). Absorption and elimination of dietary (Hg++) and methylmercury in man. *Mercury, Mercurials, and Mercaptans*, 233-246.

Mori, N., Yasutake, A., & Hirayama, K. (2007). Comparative study of activities in reactive oxygen species production/defense system in mitochondria of rat brain and liver, and their susceptibility to methylmercury toxicity. *Archives of toxicology*, *81*(11), 769-776.

Morris, M. C., Brockman, J., Schneider, J. A., Wang, Y., Bennett, D. A., Tangney, C. C., & van de Rest, O. (2016). Association of seafood consumption, brain mercury level, and APOE ε4 status with brain neuropathology in older adults. *Jama*, *315*(5).

Morrissette, J., Takser, L., St-Amour, G., Smargiassi, A., Lafond, J., & Mergler, D. (2004). Temporal variation of blood and hair mercury levels in pregnancy in relation to fish consumption history in a population living along the St. Lawrence River. *Environmental research*, *95*(3), 363-374.

Mozaffarian, D., Shi, P., Morris, J. S., Spiegelman, D., Grandjean, P., Siscovick, D. S., ... & Rimm, E. B. (2011). Mercury exposure and risk of cardiovascular disease in two US cohorts. *New England Journal of Medicine*, *364*(12), 1116-1125.

Muir, D., Braune, B., DeMarch, B., Norstrom, R., Wagemann, R., Lockhart, L., ... & Reimer, K. (1999). Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem: a review. *Science of the Total Environment, 230*(1), 83-144.

National Research Council. (2000). *Toxicological effects of methylmercury*. National Academies Press.

Nguyen, N. T., Nguyen, X. M. T., Lane, J., & Wang, P. (2011). Relationship between obesity and diabetes in a US adult population: findings from the National Health and Nutrition Examination Survey, 1999–2006. *Obesity surgery, 21*(3), 351-355.

Nordberg, G. F., & Aitio, A. (2008). Handbook on the toxicology of metals. Amsterdam: Academic Press/Elsevier.

Northern Contaminants Program (2003*). Canadian Arctic Contaminants Assessment Report II*. Retrieved from http://caid.ca/CanArtCon3.2003.pdf

NWT Bureau of Statistics. (2008). Aboriginal People — 2006 Census —. *Government of the Northwest Territories,* 1-4.

NWT Bureau of Statistics. (2015). Community Price Indexes. *Government of the Northwest Territories.*

NWT Bureau of Statistics. (2016). Northwest Territories - Statistical Profile. *Government of the Northwest Territories,* 1-9.

NWT Bureau of Statistics. (2017). Northwest Territories – Population Estimates by Communites. *Government of the Northwest Territories*

NWT Health and Services. (2002). Northwest Territories Traditional Food Fact Sheet Series. Northwest Territories Health and Social Servicces. (2002). *Northwest Territories Traditional Food Fact Sheet Series,* 1-106.

Odland, J. Ø., & Nieboer, E. (2012). Human biomonitoring in the Arctic. Special challenges in a sparsely populated area. *International journal of hygiene and environmental health, 215*(2), 159-167.

O'Driscoll, N. J., Rencz, A., & Lean, D. R. (2005). The biogeochemistry and fate of mercury in the environment. *Metal ions in biological systems, 43*, 221-238.

Oken, E., Radesky, J. S., Wright, R. O., Bellinger, D. C., Amarasiriwardena, C. J., Kleinman, K. P., ... & Gillman, M. W. (2008). Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. *American journal of epidemiology, 167*(10), 1171-1181.

Ouédraogo, O., & Amyot, M. (2011). Effects of various cooking methods and food components on bioaccessibility of mercury from fish. *Environmental Research, 111*(8), 1064-1069.

Palaniyandi, S. (2016). *Determining mercury and selenium in vitro bioaccessibility in country foods collected from Nunavik, Québec* (Master's thesis, University of Waterloo).

Park, J. D., & Zheng, W. (2012). Human exposure and health effects of inorganic and elemental mercury. *Journal of Preventive Medicine and Public Health, 45*(6), 344.

Pirkle, C. M., Muckle, G., & Lemire, M. (2016). Managing mercury exposure in Northern Canadian communities. *Canadian Medical Association Journal, 188*(14), 1015-1023.

Pirrone, N., Cinnirella, S., Feng, X., Finkelman, R. B., Friedli, H. R., Leaner, J., ... & Telmer, K. (2010). Global mercury emissions to the atmosphere from anthropogenic and natural sources. *Atmospheric Chemistry and Physics, 10*(13), 5951-5964.

Pope III, C. A., Burnett, R. T., Turner, M. C., Cohen, A., Krewski, D., Jerrett, M., ... & Thun, M. J. (2011). Lung cancer and cardiovascular disease mortality associated with ambient

air pollution and cigarette smoke: shape of the exposure–response relationships. *Environmental health perspectives, 119*(11), 1616.

Power, M., Klein, G. M., Guiguer, K. R. R. A., & Kwan, M. K. H. (2002). Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. *Journal of Applied Ecology, 39*(5), 819-830.

Power, E. M. (2008). Conceptualizing food security for Aboriginal people in Canada. *Canadian journal of public health/Revue Canadienne de sante'e publique*, 95-97.

Ray, J. G., Vermeulen, M. J., Meier, C., Cole, D. E., & Wyatt, P. R. (2004). Maternal ethnicity and risk of neural tube defects: a population-based study. *Canadian Medical Association Journal, 171*(4), 343-345.

Reading, C. L., & Wien, F. (2009). Health inequalities and the social determinants of Aboriginal peoples' health. *Prince George, BC: National Collaborating Centre for Aboriginal Health.*

Receveur, O., Boulay, M., & Kuhnlein, H. V. (1997). Decreasing traditional food use affects diet quality for adult Dene/Métis in 16 communities of the Canadian Northwest Territories. *The Journal of nutrition, 127*(11), 2179-2186.

Reyes, E. S. (2016). *Assessing Mercury Risks for the Optimization of Nutrient Benefits from Wild-harvested Fish Consumption in the Northwest Territories, Canada* (Master's thesis, University of Waterloo).

Reyes, E. S., Aristizabal Henao, J. J., Kornobis, K. M., Hanning, R. M., Majowicz, S. E., Liber, K., ... & Laird, B. D. (2017). Associations between omega-3 fatty acids, selenium content, and mercury levels in wild-harvested fish from the Dehcho Region, Northwest Territories, Canada. *Journal of Toxicology and Environmental Health, Part A, 80*(1), 18-31.

Richardson, A. J., & Montgomery, P. (2005). The Oxford-Durham study: a randomized, controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. *Pediatrics, 115*(5), 1360-1366.

Richmond, C. A., & Ross, N. A. (2009). The determinants of First Nation and Inuit health: a critical population health approach. *Health & place, 15*(2), 403-411.

Rooney, J. P. (2014). The retention time of inorganic mercury in the brain—a systematic review of the evidence. *Toxicology and applied pharmacology, 274*(3), 425-435.

Sakamoto, M., Chan, H. M., Domingo, J. L., Oliveira, R. B., Kawakami, S., & Murata, K. (2015). Significance of fingernail and toenail mercury concentrations as biomarkers for prenatal methylmercury exposure in relation to segmental hair mercury concentrations. *Environmental research, 136,* 289-294.

Sass, J. B., Haselow, D. T., & Silbergeld, E. K. (2001). Methylmercury-induced decrement in neuronal migration may involve cytokine-dependent mechanisms: a novel method to assess neuronal movement in vitro. *Toxicological Sciences, 63*(1), 74-81.

Schaefer, A. M., Jensen, E. L., Bossart, G. D., & Reif, J. S. (2014). Hair mercury concentrations and fish consumption patterns in Florida residents. *International journal of environmental research and public health, 11*(7), 6709-6726.

Segura, R. L., Palomo, J. M., Mateo, C., Cortes, A., Terreni, M., Fernández-Lafuente, R., & Guisan, J. M. (2004). Different properties of the lipases contained in porcine pancreatic lipase extracts as enantioselective biocatalysts. *Biotechnology progress, 20*(3), 825-829.

Senécal, S., & O'Sullivan, E. (2006). The well-being of Inuit communities in Canada. *Ottawa: Strategic Research and Analysis Directorate, Indian and Northern Affairs Canada.*

Shanker, G., & Aschner, M. (2003). Methylmercury-induced reactive oxygen species formation in neonatal cerebral astrocytic cultures is attenuated by antioxidants. *Molecular Brain Research, 110*(1), 85-91.

Sheehan, M. C., Burke, T. A., Navas-Acien, A., Breysse, P. N., Mcgready, J., & Fox, M. A. (2014). Global methylmercury exposure from seafood consumption and risk of developmental neurotoxicity: a systematic review. *Bulletin of the World Health Organization, 92*(4).

Shi, Y. H., Xiao, J. J., Feng, R. P., Liu, Y. Y., Liao, M., Wu, X. W., ... & Cao, H. Q. (2017). In-vitro bioaccessibility of five pyrethroids after human ingestion and the corresponding gastrointestinal digestion parameters: A contribution for human exposure assessments. *Chemosphere, 182*, 517-524.

Shim, S. M., Ferruzzi, M. G., Kim, Y. C., Janle, E. M., & Santerre, C. R. (2009). Impact of phytochemical-rich foods on bioaccessibility of mercury from fish. *Food Chemistry, 112*(1), 46-50.

Siedlikowski, M. (2015). *Investigation of the bioaccessibility and bioavailability of methylmercury from seafood commonly consumed in North America* (Doctoral dissertation, McGill University).

Siedlikowski, M., Bradley, M., Kubow, S., Goodrich, J. M., Franzblau, A., & Basu, N. (2016). Bioaccessibility and bioavailability of methylmercury from seafood commonly consumed in North America: In vitro and epidemiological studies. *Environmental research, 149*, 266-273.

Simmons-Willis, T. A., CLARKSON, T. W., & BALLATORI, N. (2002). Transport of a neurotoxicant by molecular mimicry: the methylmercury–L-cysteine complex is a substrate for

human L-type large neutral amino acid transporter (LAT) 1 and LAT2. *Biochemical Journal, 367*(1), 239-246.

Smith, D. M. (1982). Moose-deer island house people: A history of the native people of Fort Resolution. *Musée National de l'Homme. Collection Mercure. Division d'Ethnologie. Service Canadien d'Ethnologie. Dossier Ottawa*, (81), 1-185.

Smylie, J., Fell, D., Ohlsson, A., & Joint Working Group on First Nations, Indian, Inuit, and Métis Infant Mortality of the Canadian Perinatal Surveillance System. (2010). A review of Aboriginal infant mortality rates in Canada: striking and persistent Aboriginal/non-Aboriginal inequities. *Canadian Journal of Public Health/Revue Canadienne de Sante'e Publique*, 143-148.

Statistics Canada (2013). *Lead, mercury and cadmium concentrations in Canadians, 2012 and 2013*. Retrieved from https://www150.statcan.gc.ca/n1/pub/82-625-x/2015001/article/14209-eng.htm

Statistics Canada (2013). *Household food insecurity, 2011-2012*. Retrieved from https://www150.statcan.gc.ca/n1/pub/82-625-x/2013001/article/11889-eng.htm

Statistics Canada. (2017). *The Canadian Health Measures Survey, Cycle 4.* Retrieved from https://www.canada.ca/en/health-canada/services/environmental-workplace-health/environmental-contaminants/human-biomonitoring-environmental-chemicals/canadian-health-measures-survey.html

Stohs, S. J., & Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free radical biology and medicine, 18*(2), 321-336.

Stringari, J., Nunes, A. K., Franco, J. L., Bohrer, D., Garcia, S. C., Dafre, A. L., ... & Farina, M. (2008). Prenatal methylmercury exposure hampers glutathione antioxidant system

ontogenesis and causes long-lasting oxidative stress in the mouse brain. *Toxicology and applied pharmacology*, *227*(1), 147-154.

Suk, W. A., Collman, G., & Damstra, T. (1996). Human biomonitoring: research goals and needs. *Environmental health perspectives*, *104*(Suppl 3), 479.

Swain, E. B., Jakus, P. M., Rice, G., Lupi, F., Maxson, P. A., Pacyna, J. M., ... & Veiga, M. M. (2007). Socioeconomic consequences of mercury use and pollution. *AMBIO: A Journal of the Human Environment*, *36*(1), 45-61.

Swedish Expert Committee. (1971). Methyl mercury in fish, a toxicologic-epidemiologic evaluation of risks. Report from an expert group. *Nord. Hyg. Tidskr., Suppl*, *4*, 19-364.

Tjepkema, M. (2002). The health of the off-reserve Aboriginal population [Canadian Community Health Survey-2002 Annual Report]. *Health Reports*, *13*, 73.

Turner, A., Crompton, S., & Langlois, S. (2011). Aboriginal peoples in Canada: first nations people, Métis and Inuit. *National household survey*.

US EPA. (2001). Water quality criterion for the protection of human health: methylmercury. *Washington. D.C.: US EPA.*

Van de Wiele, T. R., Oomen, A. G., Wragg, J., Cave, M., Minekus, M., Hack, A., ... & Van Wijnen, J. (2007). Comparison of five in vitro digestion models to in vivo experimental results: lead bioaccessibility in the human gastrointestinal tract. *Journal of Environmental Science and Health Part A*, *42*(9), 1203-1211.

Van Oostdam, J., Donaldson, S. G., Feeley, M., Arnold, D., Ayotte, P., Bondy, G., ... & Loring, E. (2005). Human health implications of environmental contaminants in Arctic Canada: a review. *Science of the total environment*, *351*, 165-246.

Versantvoort, C. H. M., Van de Kamp, E., & Rompelberg, C. J. M. (2004). Development and applicability of an in vitro digestion model in assessing the bioaccessibility of contaminants from food. *RIVM rapport 320102002*.

Versantvoort, C. H., Oomen, A. G., Van de Kamp, E., Rompelberg, C. J., & Sips, A. J. (2005). Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food and Chemical Toxicology, 43*(1), 31-40.

Wagemann, R., Trebacz, E., Boila, G., & Lockhart, W. L. (1998). Methylmercury and total mercury in tissues of arctic marine mammals. *Science of the Total Environment, 218*(1), 19-31.

Wang, H. S., Xu, W. F., Chen, Z. J., Cheng, Z., Ge, L. C., Man, Y. B., ... & Wong, M. H. (2013). In vitro estimation of exposure of Hong Kong residents to mercury and methylmercury via consumption of market fishes. *Journal of hazardous materials, 248*, 387-393.

Wheatley, B., & Paradis, S. (1995). Exposure of Canadian aboriginal peoples to methylmercury. *Water, Air, and Soil Pollution, 80*, 3-11.

World Health Organization. (1976) Environmental health criteria 1: mercury. *Geneva: World Health Organization*, 94-131

World Health Organization: International Program on Chemical Safety. (1991). Inorganic mercury: environmental health criteria 118. *Geneva: World Health Organization, 107*.

Yin, Z., Jiang, H., Syversen, T., Rocha, J. B., Farina, M., & Aschner, M. (2008). The methylmercury-l-cysteine conjugate is a substrate for the L-type large neutral amino acid transporter. *Journal of neurochemistry, 107*(4), 1083-1090.

Ye, B. J., Kim, B. G., Jeon, M. J., Kim, S. Y., Kim, H. C., Jang, T. W., ... & Hong, Y. S. (2016). Evaluation of mercury exposure level, clinical diagnosis and treatment for mercury intoxication. *Annals of occupational and environmental medicine, 28*(1), 5.

You, C. H., Kim, B. G., Kim, J. M., Yu, S. D., Kim, Y. M., Kim, R. B., & Hong, Y. S. (2011). Relationship between blood mercury concentration and waist-to-hip ratio in elderly Korean individuals living in coastal areas. *Journal of Preventive Medicine and Public Health*, *44*(5), 218.

You, C. H., Kim, B. G., Kim, Y. M., Lee, S. A., Kim, R. B., Seo, J. W., & Hong, Y. S. (2014). Relationship between dietary mercury intake and blood mercury level in Korea. *Journal of Korean medical science, 29*(2), 176-182.

Ysart, G., Miller, P., Croasdale, M., Crews, H., Robb, P., Baxter, M., . . . Harrison, N. (2000). 1997 UK Total Diet Study dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. *Food Additives and Contaminants, 17*(9), 775-786.

Zangenberg, N. H., Müllertz, A., Kristensen, H. G., & Hovgaard, L. (2001). A dynamic in vitro lipolysis model: I. Controlling the rate of lipolysis by continuous addition of calcium. *European Journal of Pharmaceutical Sciences, 14*(2), 115-122.