Determining mercury and selenium in vitro bioaccessibility in country foods collected from Nunavik, Québec

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 Sierra Durga Palaniyandi in collaboration with her supervisor, Dr. Brian Laird. I would like to acknowledge the contributions of all the co-authors, as well as my supervisor for providing me with guidance throughout this project. The co-authors do not form the majority of the examining committee or the reading or examining of this thesis. This work is a major contribution

Section 4 & Section 5

Section 4 and Section 5 of this thesis were co-authored with Paulin J. Vanié, Dr. Mélanie Lemire, Dr. Michael Kwan, Dr. Laurie H.M. Chan, Dr. Pierre Ayotte, and Dr. Brian D. Laird. All of these researchers are contributing to a large, multi-institutional, interdisciplinary project to determine the effects of country foods on cardiometabolic diseases in Inuit adults in Nunavik of a large multidisciplinary project funded by the National Contaminants Program. Dr. Laurie H.M. Chan and Paulin J. Vanié are both from Centre for Advanced Research in Environmental Genomics (CAREG) at the University of Ottawa. Dr. Mélanie Lemire and Dr. Pierre Ayotte both work for Axe Santé des Populations et Pratiques Optimales en Santé at the Centre de Recherche du CHU de Québec. Paulin J. Vanié also used the country food samples for his research on in vitro bioaccessibility as well as microbial digestion. Paulin and I processed the samples together (i.e. homogenization, labelling and repackaging) and included some of his initial bioaccessibility work in Section 4. Dr. Mélanie Lemire, Dr. Pierre Ayotte and Dr. Laurie H.M. Chan provided valuable insights and very useful edits. Dr. Michael Kwan (of the Makivik Corporation at the Nunavik Research Centre) coordinated with Inuit communities to collect

samples and determine the total concentrations of mercury and selenium. Dr. Brian Laird supervised my progress in the lab, provided edits and invaluable guidance along the way.

Abstract

Country food is a cornerstone of cultural, social, and spiritual life for Inuit communities (Gombay, 2005; Van Oostdam et al., 2005). Country foods refer to marine mammals, fish, plants, berries, seabirds and wild game that have been hunted and gathered from the local area (Van Oostdam et al., 2005). These country foods are a significant route of exposure to many environmental contaminants, including mercury (Hg) (Donaldson et al., 2010; Van Oostdam et al., 2005). Mercury can be very detrimental to human health through adverse cardiovascular, endocrine and neurotoxic effects, especially in vulnerable populations such as the elderly or pregnant women (Clarkson and Magos, 2006; Zahir et al., 2005). Despite being a major route for Hg exposure, country foods are very nutritious and contain high levels of numerous vitamins and minerals including the essential micronutrient – selenium (Se), which can potentially mitigate Hg toxicity (Kuhnlein and Receveur, 2007, 1996). An in vitro gastrointestinal model was used to estimate the in vitro bioaccessibility (IVBA) of Hg and Se in certain country foods. We used an in vitro gastrointestinal model to determine the Hg and Se in vitro bioaccessibility for country foods collected from Nunavik, Quebec that have been digested separately and in combination (i.e. single digest and co-digest, respectively). These country food samples were collected 2008-2013 through the community-based sampling programs overseen by the Nunavik Research Centre of the Makivik Corporation.

The purpose of this thesis was to determine:

(1) Do the Inuit country foods that have high levels of Hg also have high levels of Se?

(2) Does the bioaccessibility of Hg and Se vary from one country food to another?

(3) Can the co-consumption of specific food combinations affect the solubilization of Hg within the GI tract?

For the single digest country foods, we evaluated Hg and Se in vitro bioaccessibility (IVBA) using a two-stage in vitro gastro-intestinal (GI) model for a variety of country foods harvested in Nunavik, Québec. By assessing the IVBA (i.e. the metal fraction of that would is soluble in the gut lumen), we can approximate metal bioavailability (i.e. the metal fraction o that would cross the gut-blood barrier and reach systemic circulation). The results showed a large variation in Hg (1.4 - 90%) and Se (29 - 108%) bioaccessibility for the country foods studied. The samples with the highest Hg concentration (ringed seal liver) also had the lowest Hg percent bioaccessibility. Generally, Se:Hg molar ratios for the majority of country foods increased and they were greater than one after accounting for metal bioaccessibility (i.e. Se bioaccessibility > Hg bioaccessibility). The main exceptions to this trend included the muscle and liver of ringed seals, which showed similar Se:Hg ratios before and after accounting for metal bioaccessibility.

Some foods were also co-digested (or co-consumed) meaning they were digested together in the GI model. For the co-digested country foods, we evaluated the Hg and Se in vitro bioaccessibility (IVBA) using a two-stage in vitro gastrointestinal (GI) model for selected country foods. We compared country foods with high levels of total and IVBA Hg (i.e. ringed seal liver, beluga nikku, raw beluga meat, walrus, lake trout, eider duck egg white) and pair them with country foods that may have a mitigating effect on Hg IVBA (i.e. crowberries, blueberries, seaweed, sculpin eggs and tomato paste). Overall, our results show that there are no additive or subadditive results for IVBA Hg when foods were digested together.

In the future, this research may aid in the ongoing contaminant exposure assessments in Nunavik and assist in the development of culturally relevant strategies promoting country food use while decreasing Hg body burden.

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Table of Contents

List of Figures xii

List of Tables xiii

1. Introduction 1

2. Study Rationale 4

2.1 Mercury in Inuit Country Foods from Nunavik, Quebec 4

2.2 Study Contributions and Importance 5

2.3 Research Questions and Objectives 6

3. Literature Review 7

3.1 Introduction 7

3.2 Inuit Communities in Canada 8

3.2.1 Health Patterns within Inuit Communities 8

3.2.2 Environmental Contaminants in Circumpolar region 9

3.2.3 Country Food Consumption 10

3.3 Mercury 12

3.3.1 Mercury and Human Health 13

3.3.1.1 Neurotoxic effects 14

3.3.1.2 Neurodevelopmental effects 15

3.3.1.3 Cardiovascular effects 16

3.3.1.4 Immune System effects 17

3.3.2 Determination of Mercury Exposure 18

3.3.4 Mercury in Country foods 20

3.3.5 Inuit Exposure to Mercury 22

3.4 Selenium 23

3.4.1 **Selenium and Human Health 24**

3.4.1.1 Neurological effects 25

3.4.1.2 Immune system effects 26

3.4.1.3 Impact on Thyroid function 26

3.4.1.4 Impact on Carcinogenesis 27

3.4.2 Dietary Recommendations for Selenium 28

3.4.3 Selenium in Country foods 29

3.5 Mercury and Selenium Interactions 29

4. The In Vitro Bioaccessibility of Selenium Exceeds that of Mercury in Inuit Country Foods 36

4.1 Introduction *36*

4.2 Materials and Methods *40*

4.2.1 Sample Collection 40

4.2.2 In vitro GI Model 40

4.2.3 Chemical Analysis 41

4.2.3.1 Total Metal Content in Country Foods 41

4.2.3.2 Bioaccessible Metals in Country Foods 42

4.2.4 Statistical Analysis 43

4.3 Results and Discussion *44*

4.4 Conclusion *48*

5. Additivity of Metal Bioaccessibility in Binary Mixtures of Inuit Country Foods 53

5.1 Introduction *53*

5.2 Materials and Methods *55*

5.2.1 Sample Preparation 55

5.2.2 In vitro GI Model 56

5.2.3 Chemical Analysis 58

5.2.4 Statistical Analysis for Co-digest Studies 59

5.3 Results and Discussion *60*

5.4 Conclusion *65*

References 68

Appendix 79

List of Figures

Table 1. Total Hg and Se concentrations of Country Food items in mg kg-1, w.w. 49

Table 2. Se:Hg ratios (± standard error) according to total and bioaccessible molar concentrations (µmol kg-1 Se:µmol kg-1 Hg) in 18 country foods collected in Nunavik, QC. 52

Table 3. Co-digest treatment combinations 57

Table 4. Co-digest results for Hg (Mean ± SD) and Se (Mean ±SD) with treatment interaction type (µg/L or ppb, w.w.)..... 60

List of Tables

Figure 1. Average % IVBA for Hg in the 18 Inuit country foods collected in Nunavik, QC. The error bars represent standard error of the mean. Please note that for certain country foods either the IVBA fraction (i.e. blue mussel) or both total and IVBA (i.e.Canadian geese) was below the detection limit for Hg. *50*

Figure 2.Average IVBA for Se in the 18 Inuit country foods collected in Nunavik, QC. Error bars represent standard error of the mean. Please note that the IVBA fraction for eider duck yolk was below the detection limit for Se. *51*

1. Introduction

Country foods play an integral role in cultural, social and spiritual life for Inuit communities (Gombay, 2005; Van Oostdam et al., 2005). These foods are hunted and gathered from local surroundings and include marine mammals (e.g., beluga meat, ringed seal meat), fish (e.g., Arctic char, lake trout, sculpin), wild game and birds (e.g., caribou, ptarmigan), berries (e.g., blueberries, crowberries), and marine algae (Donaldson et al., 2010; Van Oostdam et al., 2005). Not only are country foods a cornerstone of Inuit social and cultural identity but they also provide many nutritional and economic benefits (Donaldson et al., 2010; Gombay, 2005; Van Oostdam et al., 2005). These foods are often more healthy and cost-effective than relying solely on market foods (Chan et al., 2006; Kuhnlein et al., 1996; Kuhnlein and Receveur, 1996). However, even if quality market foods were available at reasonable prices, country foods would be consumed because of their large cultural importance (Donaldson et al., 2010).

The main route of exposure to environmental contaminants for Indigenous people in Canada is through the consumption of country foods (Health Canada, 2009; Laird et al., 2013b; Richmond and Ross, 2009). In the past, the circumpolar region was considered to be pristine due to its distance from sources of contaminant emissions; however, this is not the case (Barrie et al., 1992; Donaldson et al., 2010). Heavy metals and persistent organic pollutants can be transported and deposited in the Arctic *via* long-range atmospheric and oceanic currents (Barrie et al., 1992; Donaldson et al., 2010; Van Oostdam et al., 2005). Mercury (Hg), for example, can be emitted from industrial or environmental processes in the form of mercury vapor (Hg^0) and deposited anywhere in the world (Barrie et al., 1992; Clarkson and Magos, 2006). Elevated concentrations of mercury have been found in the marine mammal and fish populations of Nunavik due to bioaccumulation within the organism and biomagnification in the Arctic food chain (Anctil,

2004; Dewailly et al., 2007; Dórea, 2008; Duschesneau, 2011). Mercury can be very detrimental to human health through adverse cardiovascular, endocrine and neurotoxic effects, especially in vulnerable populations such as the elderly or pregnant women (Clarkson and Magos, 2006). Inuit populations, such as those living in Nunavik, Québec, have elevated exposure levels of Hg. For example, in 2004, biomonitoring efforts showed that Nunavik Inuit have higher blood Hg levels (10.7 µg/L) compared with the general Canadian population (0.822 µg/L) (Donaldson et al., 2010). Although there has been a significant decline in blood Hg levels observed among Inuit, 75% of pregnant woman in Nunavik still exceed the Health Canada (HC) guideline of 8.5 µg/L (Nunavik Nutrition and Health Committee, 2011). This is cause for concern since Hg has been shown to concentrate on the fetal side of placenta-blood barrier leading to the blood Hg levels being 1.5X greater on the fetal side than the maternal side (Butler Walker et al., 2006; Van Oostdam et al., 2005).

Conversely, country foods are often excellent sources of many essential nutrients. The Kuhnlein & Receveur (2007) found that even a single portion of country foods can increase nutrient intakes for vitamins and minerals like vitamin D, vitamin E, riboflavin, vitamin B-6, iron, zinc, copper, magnesium, manganese, phosphorus, and potassium. Prior studies have shown that selenium (Se), which is particularly high in some country foods, may be able to moderate some of the Hg toxicity (Donaldson et al., 2010; Zhang et al., 2014). However, the protective mechanisms between Hg and nutrients like Se in country foods are poorly understood (Ralston and Raymond, 2010). If benefits of Se can mitigate the risk of Hg toxicity through country food consumption, then it can have an effect on public health messaging, risk assessment, and mitigation options.

My project assessed the bioaccessibility of Hg and Se present in many country foods collected from communities in Nunavik, Québec for individual food items as well as food mixtures. Within this type of exposure assessment research, the term bioaccessibility refers to the fraction of a substance released into the gastrointestinal (GI) lumen (Intawongse and Dean, 2006). To characterize the bioaccessibility of Hg and Se, I used an in vitro GI model, which simulates gastric and duodenal digestion. The samples studied include: beluga meat (raw and air-dried), beluga muktuk, ringed seal liver, ringed seal meat, walrus meat, Arctic char, Atlantic salmon, brook trout, lake trout, lake whitefish, shorthorn sculpin flesh and eggs, blue mussel, caribou, ptarmigan meat, snowshoe hare meat, eider duck eggs, Canada goose eggs, berries and seaweed. These samples were collected in 2008 - 2013 through the community-based sampling programs overseen by the Nunavik Research Centre of the Makivik Corporation. This research will assist ongoing contaminant exposure assessments in Nunavik and may help promote country food use while decreasing Hg body burden.

2. Study Rationale

2.1 Mercury in Inuit Country Foods from Nunavik, Quebec

Although the Arctic used to be considered pristine and without pollution, seminal research conducted in the 1980's and 1990's demonstrated there to be high levels of several types of environmental contaminants within the Arctic ecosystem (Donaldson et al., 2010; Muir et al., 2005; Van Oostdam et al., 2005). As a result, heavy metals like Hg can be inadvertently consumed in a traditional Inuit diet. Such exposures to Hg can potentially adversely affect Inuit health and wellbeing (Donaldson et al., 2010; Dórea, 2008). For example, the Nunavik Child Development Study (NCDS) found that prenatal exposure to Hg was associated with decreased intellectual function and attention capacity in the classroom (Nunavik Nutrition and Health Committee, 2011). Although the Inuit diet is becoming westernized and country foods account for between 6-40 % and 0.4-15% of the daily energy in adults and children, respectively (Kuhnlein and Receveur, 2007), country foods are often the primary route by which Inuit are exposed to environmental contaminants like Hg (Nunavik Nutrition and Health Committee, 2011). Selenium, an essential micronutrient found at high levels in certain country foods (e.g. fish eggs and beluga fat), has a high affinity for binding Hg and may, in some circumstances, be able to mitigate some of Hg's adverse effects (Ralston and Raymond, 2010; Ralston, 2008). By quantifying metal bioaccessibility in Inuit country foods, we hope to gain insights into constraints upon Hg and Se internal doses that result from the consumption country foods in the Canadian Arctic. As very little is currently known regarding the bioaccessibility and bioavailability of Hg and Se in the country foods of the Inuit of Nunavik, QC, this research will help fill an important data gap in the literature.

2.2 Study Contributions and Importance

Country food consumption advisories have occasionally been necessary to inform Inuit communities of potential contaminant risks posed by the consumption of country foods. Hg consumption advisories are sometimes based upon studies that characterize the total Hg concentrations; given the toxicokinetic differences between mercury species, such advisories may not focus on the foods that most contribute to internal dose.

My Master's thesis is part of a large, multi-institutional, interdisciplinary project to determine the effects of country foods on cardiometabolic diseases in Inuit adults in Nunavik. Overall this large, interdisciplinary project has four main sections:

1. Mercury-nutrient interactions on biomarkers of T2D risk in human blood samples.
2. Mercury-nutrient interactions on the dose-response between in vitro Hg exposure and metabolic changes in adipose tissue.
3. Concentrations, speciation, and bioaccessibility/ bioavailability of Hg and Se in Inuit country foods.
4. Integration and mobilization of this knowledge in order to improve the health of Inuit in Nunavik.

My M.Sc. project, which was funded through the Northern Contaminants Program, focused on the third of these four sections. For this, I assessed the bioaccessibility (single and co-digested country foods) as well as the bioavailability (for a single country food) in a number of foods gathered from Nunavik. The in vitro GI model, a modified physiologically based extraction test (PBET), assesses the luminal bioaccessibility Hg and Se within Inuit country foods. The in vitro extraction procedure, which simulates the food breakdown that occurs in the

stomach and the duodenum, allows the measurement of the solubilized Hg and Se for the calculation of metal bioaccessibility. These country foods were studied separately (single digests) and also together (co-digests) to assess the IVBA of these trace metals. The single digest allows us to estimate the IVBA Hg and Se present in a single country foods type (i.e. Lake Trout v. walrus etc). By co-digesting specific country foods, we can determine whether the effect of a mixture is additive or not. Using these bioaccessibility measurements can refine and potentially improve the modeling of Inuit contaminant exposure for use in human health risk assessment.

2.3 Research Questions and Objectives

The main questions for my M.Sc. thesis research are the following:

1. Do the Inuit country foods that have high levels of Hg also have high levels of Se?
2. Which Inuit country foods contain the most Se relative to their Hg content?
3. Does the bioaccessibility of Hg and Se vary from one country food to another?
4. Can the co-consumption of specific food combinations affect the solubilization of Hg within the GI tract?

To answer these questions, I:

(A) Evaluated the total concentrations of Hg and Se present in the country foods of the Inuit of Nunavik, QC

(B) Estimated the bioaccessibility of Hg and Se within each individual country food

(C) Estimated the bioaccessibility of Hg and Se when two country foods are digested in pairs.

3. Literature Review

3.1 Introduction

The following literature review outlines environmental health issues related to Inuit country food consumption. This review discusses the nutritional benefits of country foods, the levels of Hg and Se within country foods, and the potential implications for Hg-Se interactions on human health risk assessment. Issues related to the environmental fate of Hg in the Arctic are outside of the scope of this review. Instead, the primary aim of this literature review is to outline the impact of country food consumption on oral Hg exposure and investigate the mechanisms by which Se co-exposure may prevent or diminish these risks.

My graduate research assessed the metal bioaccessibility in country foods using a luminal in vitro extraction method. Together, this measure may provide a more accurate estimate of internal dose than existing risk assessment techniques used to determine exposure. Currently, risk assessments often make the default assumption that 100% of the ingested dose is bioavailable (Laird et al., 2009). Within my research, bioaccessibility is defined as the fraction of a substance that has been solubilized in the GI lumen and is the maximal fraction potentially available for absorption into the blood stream (Intawongse and Dean, 2006; Ruby et al., 1999). Bioavailability, on the other hand, is defined as the fraction of a chemical absorbed that reaches systemic circulation within an organism and reaches its biological target tissue (Caussy, 2003; Ruby et al., 1999). The bioaccessible concentrations of trace metals can act as an approximation for bioavailable concentrations, which tend to be more difficult to determine experimentally (Ruby et al., 1999; Torres-Escribano et al., 2011). Knowledge describing the association between

Se and Hg bioaccessibility may be able to improve risk assessment and communication regarding contaminants in Inuit country foods.

3.2 Inuit Communities in Canada

The Inuit have a population of approximately 50,000 inhabiting the Arctic regions of northern Canada (AMAP, 2009). In Canada, Inuit communities are found in Nunavut, Nunavik (in northern Quebec), Northwest Territories (NWT), and Northern Labrador (AMAP, 2009; Statistics Canada, 2003). The Inuit regions with the largest populations, Nunavut and Nunivak, have grown by 26% from 1996 to 2006 (AMAP, 2009; Makivik Corporation, 2015). Inuit communities have a relatively young population, with a large proportion under the age of 21 (Statistics Canada, 2003). One of the implications of this demographic shift has been a loss in traditional knowledge surrounding hunting and fishing, leading to an increased intake of market foods in the Inuit regions of Canada (Donaldson et al., 2010; Kuhnlein et al., 1996; Van Oostdam et al., 2005).

3.2.1 Health Patterns within Inuit Communities

Aboriginal peoples bear a disproportionate burden of illness within Canada; these health inequities are a function of economic and social conditions as well as past and present oppression and marginalization (Macmillan et al., 1996; Richmond and Ross, 2009). Inuit communities face several social challenges, including the highest suicide rate globally (Kral, 2013) as well as high substance abuse rates (including alcohol, cigarettes, and illicit substances) (Larsen et al., 2013). Smoking, which leads to lung cancer and heart disease, is 3-fold higher in Inuit communities than the rest of Canada (Adelson, 2005; Harder and Wenzel, 2012; Larsen et al., 2013). Compounded on the high rates of suicide and substance abuse, are the high rates of

unemployment (4X greater than non-aboriginal counterparts in 2006), working poor, and individuals on social assistance (Statistics Canada, 2006, 2003). Due to the high rates of financial insecurity as well as expensive market foods, that lack variety and freshness, lead to food insecurity and unhealthy diets (Egeland et al., 2011).

3.2.2 Environmental Contaminants in Circumpolar region

At one time, the Arctic was considered pristine because of its distance from industrialization (Odland and Nieboer, 2012). However, studies completed in the mid-1980s and early 1990s disproved this idea (Odland and Nieboer, 2012). Subsequent studies have confirmed elevated levels of contaminants such as heavy metals (i.e. Hg and lead or Pb) and organochlorine chemicals (i.e. (dichlorodiphenyltrichloroethane [DDT] and polychlorinated biphenyls [PCBs]) in the environment of the Arctic and subarctic regions (Berti et al., 1998). In addition to the heavy metals that occur both naturally in the environment and through anthropogenic sources worldwide can be deposited in the Arctic following long-range transport (Donaldson et al., 2010; Odland and Nieboer, 2012).

The Hg deposited in Canada, which is transported through atmospheric and oceanic currents in the form of Hg vapor, is predominantly from other countries (Pirrone et al., 2010). Mercury vapor is a monoatomic stable gas that can reside in the atmosphere for as long as one year and can be returned to the ground via rain (Pirrone et al., 2010). This transport of Hg vapor and deposition into the ecosystems is known as global cycling (Mason et al., 1994). This process of global cycling and long-range transport can result in emissions from distant countries having a profound impact on the Arctic environment (Pirrone et al., 2010).

Many programs exist to monitor and assess contaminant exposure in northern communities because environmental contamination disproportionally affects the health of

Canadian aboriginal populations (Donaldson et al., 2010). Country foods are nutrient-dense and contribute to a healthy diet; Inuit who do not consume country foods are, in general, more vulnerable to unfavorable health outcomes (Donaldson et al., 2010; Kuhnlein and Receveur, 2007). Many environmental contaminants, including Hg, are persistent, bioaccumulate within biota, and biomagnify within the food chain. As many of the country foods are high on the food chain (3^{rd} or 4^{th} trophic level), Inuit populations are exposed to higher contaminant levels.

3.2.3 Country Food Consumption

The cost of food in the North is extremely high, usually about 2-3X more expensive than foods purchased in the south (even with government subsidies) (Chan et al., 2006; Richmond and Ross, 2009). Coupled with market foods being unaffordable, the fresh food options tend to be damaged or spoilt foods that lack variety (Chan et al., 2006). Therefore, the market foods purchased are often unhealthy and highly processed leading to a diet high in fats, salt, and sugars (Fediuk et al., 2002; Kuhnlein et al., 1996; Kuhnlein and Receveur, 2007). These foods are associated with increased prevalence of obesity, type 2 diabetes, cardiovascular disease and other chronic diseases (Kuhnlein et al., 1996). On the other hand, the hunting and consumption of country foods are healthy complements to store-bought foods (Kuhnlein et al., 1996).

Country foods play an important role in the economic, cultural, and nutritional welfare of Inuit. For a variety of reasons, including cultural importance, these country foods are consumed even if market foods were affordable (Donaldson et al., 2010). Inuit practices of sharing food, which can foster a sense of community, may have started in response to the unpredictable food access (Gombay, 2005; Harder and Wenzel, 2012). More than 250 different species of plants and animals are consumed both cooked and raw as part of a traditional diet (Donaldson et al., 2010);

however, there are important regional differences that can affect the species and amounts of country foods consumed (Donaldson et al., 2010; Harder and Wenzel, 2012). The accessibility of country foods depends on a number of factors, including a) whether there is a hunter in the family, b) the costs of hunting and c) time to hunt/forage for country foods (Chan et al., 2006). Older generations have more hunting knowledge than the younger generations so, they provide country foods for the younger population (Chan et al., 2006).

Over the last couple of decades, the Inuit, like other Aboriginal populations, have been undergoing a nutrition transition associated with increasing obesity, health lifestyle changes and notably a decrease in dietary energy derived from country foods (Kuhnlein et al., 2004). Prior to colonial contact in the Americas, Indigenous people derived 100% of their dietary energy from country foods; however, currently only about 10-36% of daily calories come from these foods, with the remaining fraction coming from market foods (Kuhnlein et al., 2004). This swift dietary transition towards Western foods and away from country foods has resulted in less essential nutrient intake (Kuhnlein et al., 2004). Sheikh et al., (2011) found that recent rise in BMI is associated with declining country food consumption and increasing market food consumption. On days when country foods were consumed, there was significantly less fat, carbohydrates and sugar but more protein and essential nutrients in the diet (Kuhnlein et al., 2004). Fish eggs, raw whale skin, caribou liver, ringed seal liver, and blueberries are examples of country foods that have high levels of vitamin C (Fediuk et al., 2002). The Kuhnlein & Receveur (2007) study of 18 Inuit communities found that increased consumption of country foods was linked with increased intake of vitamins D, E and B6, riboflavin, iron, zinc, copper, magnesium and potassium. Therefore, country foods are not only culturally important but also nutritionally important as well.

3.3 Mercury

Mercury is toxic in all chemical forms; however its speciation determines its absorption, distribution, and elimination within living organisms (Kehrig et al., 2013). For mercury, speciation refers to its oxidation state as well as the presence of alkyl function groups; these toxicokinetic factors have a profound impact on Hg's bioavailability (Ruby et al., 1999). In the environment, Hg can be found in many forms, such elemental (as known as metallic mercury, Hg^0), inorganic and organic mercury (Clarkson and Magos, 2006; Clarkson, 1997). Gaseous Hg^0 is released into the environment through both natural and anthropogenic sources (Clarkson and Magos, 2006). Since Hg^0 is a monoatomic stable gas, it can reside in the atmosphere for significant periods of time before returning to the ground in rainwater (Clarkson, 1997). The volatile nature of Hg^0 allows for the emission into the atmosphere, transportation, deposition and re-emission of Hg (ATSDR, 1999).

Inorganic mercury compounds occur when Hg binds with elements such as chlorine, sulfur or oxygen (Clarkson, 1997). They have varying toxicities that depend on their solubility. For example, mercury sulphide (HgS) and mercury selenium (HgSe), which are relatively insoluble, are regarded to be non-toxic whereas mercuric chloride ($HgCl_2$) is highly soluble and very toxic (Khan and Wang, 2009). Organic Hg compounds are well researched due to their harmful effects to neurological function, as manifested in mass poisonings such as observed in Japan and Iraq (Clarkson and Magos, 2006). Although both methylmercury (MeHg) and ethylmercury (EtHg) are present in the environment, people are primarily exposed to MeHg because it is biomagnified within aquatic food chains and can reach high levels at the higher

trophic levels of the food chain (Dórea, 2008). Additionally, MeHg is thought to be particularly dangerous because it can be highly bioavailable to living organisms (Dórea, 2008).

3.3.1 Mercury and Human Health

Most human exposure to Hg is through the dietary intake of MeHg (Mergler et al., 2007; Zahir et al., 2005). The GI tract is thought to absorb approximately 95% of ingested MeHg through its complexation with the sulfhydryl group in cysteine (Cys) (Clarkson and Magos, 2006; Clarkson, 1997; Hintelmann et al., 2000). This newly formed complex (MeHg-Cys) is structurally similar to methionine (a large neutral amino acid found in the body), thus allowing the MeHg-Cys complex to gain entry into cells (Clarkson and Magos, 2006; Clarkson, 1997). Conversely, MeHg can also be demethylated into inorganic Hg by GI microbiota for excretion from the body (Clarkson and Magos, 2006; Clarkson, 1997). However, the demethylation rate in humans is only about 1% daily, which results in a long half-life for MeHg in the body (Clarkson, 1997). The MeHg remaining in the body distributed systemically to all the tissues within the body (Clarkson, 1997). Due in part to its small size, MeHg can cross the blood-brain and placental-blood barrier and subsequently biotransform and accumulate in the form of inert, less toxic inorganic Hg in the central nervous system and in a developing fetus respectively (Clarkson and Magos, 2006; Clarkson, 1997). Because of this slow conversion from MeHg to inorganic Hg, inorganic Hg can be found in tissues following prolonged MeHg exposure period. Most of the MeHg is demethylated and excreted in the inorganic form of Hg (Clarkson, 1997).

Mercury can cause many adverse health effects. For example, several large-scale epidemiological studies have shown numerous adverse health effects to be directly linked to high Hg exposures. In the early 1950s, one of the most well-known cases of mass Hg poisoning

occurred in Minimata, Japan (Ekino et al., 2007; Ninamiya et al., 1995). Residents consumed MeHg-contaminated fish and the resulting human health effects were devastating. Thousands of villagers suffered acute MeHg poisoning that manifested itself in many symptoms including but not limited to: blurred vision, hearing impairment, psychiatric symptoms and ataxia (Ekino et al., 2007). In addition, the MeHg-exposure had tragic effects on the next generation through fetal MeHg poisoning (Harada, 1995). The following sections outline the effects of Hg on the neurological, neurodevelopmental, cardiovascular, and immunological systems.

3.3.1.1 Neurotoxic effects

The most noticeable and researched effects of mercury are on the central nervous system (ATSDR, 1999). Oral exposure to inorganic mercury salts in therapeutic agents (e.g., teething powders, ointments and laxatives) can result in irritability, fretfulness, weakness and muscle twitching (Warkany and Hubbard, 1953). However, far more information is available on the neurotoxicity of organic mercury, especially MeHg. From the mass MeHg poisoning in Minimata, Japan, it was recognized that the brain and nervous system are among the most sensitive targets for MeHg (Black et al., 2011; Ekino et al., 2007). The end result of these effects includes sensory disturbances, constriction of visual fields, ataxia, tremors and dysarthria (Clarkson and Magos, 2006; Harada, 1995). Chronic low doses can result in similar neurological problems including ataxia, dysarthria and sensory impairment (Ninamiya et al., 1995). Adults in the Amazon with hair Hg levels below 50 µg/g (where the guideline for hair Hg is 6 µg/g) , which in this study represented low-level MeHg exposure, showed decreased dexterity, increased muscular fatigue and strength and visual contrast sensitivity (Lebel et al., 1998). Individuals who had similarly low Hg exposure through the consumption of fish showed poor attention, fine

motor function and verbal memory (Yokoo et al., 2003). These neurological changes stem from molecular interactions that result in increased oxidative stress, cell cytotoxicity and β-amyloid secretion (Zahir et al., 2005). β-amyloid secretion has been linked to neurodegenetive diseases like Alzeheimer's, Parkinson's, Autism and Lupus (Wilkinson and Waring, 2002). As such, exposure to either low or high doses of organic Hg can have detrimental effects on neurological functioning.

3.3.1.2 Neurodevelopmental effects

Children born in Minimata in the 1950's and 1960's showed heavy spongiosis of the cerebral cortex, thereby causing severe developmental impairment that carried through several generations (Ekino et al., 2007). This is an extreme example of the effects of Hg on the developing brain. There are vulnerable periods during brain development (including those typified by proliferation, migration, differentiation) that are especially sensitive to environmental contaminants like Hg (Zahir et al., 2005). Therefore, the developing fetus is also 2-5X more susceptible to MeHg toxicity than an adult (Zahir et al., 2005) . Mothers can pass dietary Hg to the fetus via the umbilical cord and to a lesser extent infants via breast milk (Zahir et al., 2005). Because of the sensitivity of the developing brain, adverse effects can be observed in children as a consequence of fetal exposure even when the mother is unaffected (Clarkson and Magos, 2006; Clarkson, 1997; Zahir et al., 2005). Subtle effects on language, memory and motor skills have been documented following fetal exposure (Grandjean et al., 1998). In the fish-eating communities in the Seychelle Islands in the Indian Ocean and the Faroe Islands in the North Atlantic Ocean, epidemiological studies have observed subtle neurodevelopmental effects in children as a result of MeHg exposure (Davidson et al., 1998) . They found that fine motor skills,

attention, verbal learning and memory could be affected (Davidson et al., 1998). Children that have been prenatally exposure to MeHg show structural changes and several developmental disturbances (Geelen et al., 1990).

3.3.1.3 Cardiovascular effects

The toxic effects of Hg exposure are normally associated with the central nervous system, but Hg (especially inorganic Hg) can have an impact on the cardiovascular system. Research using rat models have shown exposure to inorganic mercury can cause an increase in blood pressure and a decrease in cardiac cell contractility (Carmignani et al., 1992). Halbach et al., (1989) studied 40 cities in the Amazon basin and found that Hg concentrations in hair reached up to 150 μg/g. They found that these populations had a strong positive correlation with increased arterial blood pressure (Halbach et al., 1989). Animal studies have shown decreased in heart rate as a result to exposure to organic Hg (Arito and Takahashi, 1991). During the Iraq MeHg poisoning of 1971, during which many Iraqis consumed wheat that had been treated with MeHg-containing fungicide, cardiovascular effects were noted (ATSDR, 1999). Prenatal exposure to MeHg can affect the development of cardiovascular homeostasis, which regulates the delivery of hormones and nutrient as well as waste removal through the blood stream (Sørensen N, Murata K, Budtz-Jørgensen E, Weihe P, 1999). Further studies strengthened the preliminary literature correlating Hg and cardiovascular health by finding Hg exposure is associated with increased risk of hypertension, myocardial infarction, coronary dysfunction, and atherosclerosis (Choi et al., 2009; Salonen et al., 1995; Yoshizawa et al., 2002).

Mercury also can be predictor of the levels of oxidized low-density lipoprotein (LDL) (Yoshizawa et al., 2002). Mercury exposure can cause in the inactivation of paraoxonase, the enzyme responsible for slowing the LDL oxidation process, which results in the formation of

atherosclerotic lesions that can result in atherosclerotic disease (Virtanen et al., 2014; Yoshizawa et al., 2002). The other suggested mechanism for Hg toxicity in the cardiovascular system is an increase in oxidative stress resulting in more free radicals (Virtanen et al., 2014). Chronic exposure to Hg can also impact cardiac function (Choi et al., 2009). Salonen et al., (1995) found high intake of mercury from non-fatty freshwater fish and increase in MeHg body burden resulted in an increased risk of high blood pressure, acute myocardial infarction as well as death from coronary heart disease (CHD) and cardiovascular disease (CVD) in Finnish men (Choi et al., 2008, 2009). Studies have shown that chronic exposure can induce endothelial dysfunction in blood vessels likely due to the increase in oxidative stress (Choi et al., 2009).

3.3.1.4 Immune System effects

The immune response to Hg exposure is variable, complex and dependent on the dose as well as the genetic characteristics of the exposed populations (ATSDR, 1999). For example, administering doses of inorganic Hg in animal models can decrease thymus weight and increase lymphoproliferative response, both of which indicate immunosuppression (Hultman P, 1991). In vitro studies have also shown that low doses of inorganic Hg increase lymphocyte stimulation in certain autoimmune and allergic diseases (e.g. multiple sclerosis, autoimmune thyroiditis or atopic eczema), thus intensifying and exacerbating symptoms (Karagas et al., 2012). Other studies have shown that occupational exposure to Hg was associated with changes in B lymphocytes, T-helper cells, T-suppressor cells and T-cell proliferative responses – all of which are needed for optimal immune system functioning (Miklav et al., 2011; Queiroz and Dantas, 1997).

Less literature is available on the effect of MeHg on immune function. In rodents, MeHg has been shown to alter the non-specific defense mechanisms (ATSDR, 1999). Additionally, MeHg can reduce the expression of certain activation markers on T-cells (AMAP, 2011) and affect B-cell function, thus reducing immune function (Daum et al., 1993). MeHg has also known to result in reduced natural killer cell activity in the spleen and blood (ATSDR, 1999). In general, relatively high exposures to elemental Hg is linked to a range of adverse or lessened immune functions (including markers for autoimmunity) (Karagas et al., 2012) Chronic exposure to MeHg points to lessened immune system functioning but the studies and results are inconclusive (Karagas et al., 2012).

3.3.2 Determination of Mercury Exposure

Along with dietary exposure measurements, exposure to Hg and MeHg can be assessed using biomonitoring. The kinetics of the distribution of heavy metals within the body is complex and dependent on which tissues store the metals. Hair is particularly useful as a long-term retrospective MeHg exposure biomarker and it can be easily obtained and stored (Donaldson et al., 2010; Van Oostdam et al., 2005). Additionally, Hg levels in hair can be correlated with blood and brain levels (Donaldson et al., 2010; Van Oostdam et al., 2005). Notably, Hg in maternal blood and hair is proportional to the levels of Hg exposure that accumulate in the brain tissue of the infants (Kuhnlein and Chan, 2000). Governmental agencies and health organizations, such as the World Health Organization (WHO) and Health Canada (HC) among others, set the guideline or threshold values for these biomarkers (Kuhnlein and Chan, 2000).

3.3.3 Limits and Toxicological Reference Values for Mercury

Toxicological reference values (TRVs) allow for the quantification of risk to human health when compared with exposure. These values are normally based on conservative estimates and play an important role in risk assessments, which are crucial in policy making and implementation (ATSDR, 1999; Institute of Medicine, 2000). The limits and toxicological reference values for Hg and MeHg vary between regulatory bodies. For example, HC has developed general population blood guidelines for total Hg (HgT) exposure (<20 μg/L Acceptable; 20-100 μg/L Increased Risk; 100 μg/L at Risk) (Health Canada, 2007). These HC guidelines are conservative estimates based on 200 μg/L being the lowest concentration at which a physiological change was observed from exposure to clinically adverse health effects (Legrande et al., 2010). This concentration was determined by studying the large outbreaks of exposure to organic Hg that occurred in Minimata, Japan in 1950s-60s and Iraq in 1970s (Legrande et al., 2010). The US Environmental Protection Agency (EPA) has a lower benchmark dose 58 μg/L for blood Hg levels in adults (Health Canada, 2007).

The provisional guidance values for pregnant women, women of child bearing age and children are 5.8 μg/L and 8 μg/L for the US EPA and HC, respectively (Legrand et al., 2010). These guidance values are intended to reduce the risk of neurodevelopmental effects from Hg on the developing fetus (Lye et al., 2013). These values were based on the conservative estimates described above as well as epidemiological studies conducted in the Seychelles Islands and Faroe Islands to determine the impact on Hg exposure on child development (Legrand et al., 2010).

3.3.4 Mercury in Country foods

As discussed above, Hg exposures can result in detrimental impacts on the body and, even at low chronic exposure levels, can cause adverse effects on neurological, cardiovascular, and immune function. Mercury vapour released into the atmosphere from anthropogenic sources from around the world, such as fossil-fuelled power plants and ore processing plants among others, deposits in the circumpolar region (Pirrone et al., 2010). Deposited Hg undergoes chemical reactions that transform the Hg species allowing for bioaccumulation and biomagnification within the arctic food chains (Donaldson et al., 2010). Methylmercury has a high affinity for proteins and tends to accumulate in flesh of fish and marine mammals (Wagemann et al., 1998). Thus, Hg toxicity can be a potential risk for Inuit communities in the circumpolar region.

Since Hg in traditional foods is a health concern, there is literature reporting on the total concentrations present in certain foods. In general, marine mammals have high levels of total Hg because they are high on the Arctic food chain. Lemire et al., (2015) conducted a study to assess the country food sources of MeHg, Se and omega-3 fatty acids in Nunavik. They concluded that the most commonly-consumed foods were relatively low in MeHg – except for beluga meat (1.07 μg/g), ringed seal liver (2.73 μg/g) and Lake Trout (1.03 μg/g) (Lemire et al., 2015). The Lake Trout samples were above the Health Canada guideline for seafood of 0.5 μg/g MeHg but all other fish studied were below this limit (Health Canada, 2010a, 2009, 2007). Beluga (geometric mean (GM) = 6.23 μg/g) and ringed seal kidneys (GM = 0.87 μg/g) have high HgT concentrations compared with beluga mattaaq (GM =0.38 μg/g) and ringed seal meat (GM = 0.28 μg/g)(Lemire et al., 2015). Due to Hg affinity for sulfhydryl groups found in certain proteins (i.e. found in egg whites v. egg yolk or raw beluga meat v. beluga mattaaq), it is

unsurprising that lipid-containing beluga mattaaq has lower total Hg levels (Clarkson and Magos, 2006; Raymond and Ralston, 2004). There is also a high degree of variability for HgT in marine bird eggs due to differences in the Hg in the nesting female and the egg's position in the clutch (Becker, 2009; McCloskey et al., 2013). It's important to note that the concentrations of these trace metals in animals, especially Hg, can vary greatly based on trophic level, age, and size (Becker, 2009; McCloskey et al., 2013).

Since, there is a lack of detailed information of Hg speciation often times 100% of HgT is assumed to be MeHg, in human health risk assessments which is accurate for individuals in southern Canada who are exposure to MeHg almost exclusively through fish consumption (Donaldson et al., 2010; Health Canada, 2007). However, this is not accurate for Inuit individuals because it does not account for the inorganic Hg that is present in some country foods- for example, marine mammal organs. Therefore, for risk assessments of dietary Hg to Inuit in northern Canada, it is often assumed that one third is inorganic Hg and the remaining fraction is MeHg (Laird et al., 2009). Literature shows that the bioaccessibility of Hg varies greatly from one food to another (Burger, 2012; Cabañero et al., 2007; Calatayud et al., 2012; Moreda-Piñeiro et al., 2011). Cabañero et al., (2007) found very low bioaccessibility for MeHg in both gastric and intestinal digestion in certain commerically consumed fish (i.e. tuna, sardines, and swordfish). In comparison, Laird et al., (2009) studied 16 different country foods (such as caribou, walrus, Arctic char and ringed seal) using an in vitro model found the Hg bioaccessibility widely ranged between 1 and 93%.

3.3.5 Inuit Exposure to Mercury

In the general Canadian population, exposure to Hg is primarily through the consumption of fish; the form of mercury to which people are exposed from the consumption of fish is predominately MeHg (ATSDR, 1999; Wagemann et al., 1998). However, some Aboriginal communities can also be exposed to substantial levels of inorganic Hg from the consumption of specific country foods (Laird et al., 2013a). Inuit communities are among the most at risk to Hg because of the high MeHg levels that accumulate within some country foods (Laird et al., 2013a). For example, several country foods (e.g. lake trout, beluga meat, ringed seal) relied on by Inuit, due to the processes of bioaccumulation and biomagnification, have elevated concentrations of MeHg (Donaldson et al., 2010; Dórea, 2008; Van Oostdam et al., 2005). Consequently, Inuit exposure to Hg is among the highest in the world (Donaldson et al., 2010; Van Oostdam et al., 2005).

Health Canada's Methylmercury Monitoring Program brought attention to the elevated levels of Hg exposure in Aboriginal communities and these results showed that 57% of Inuit individuals exceeded the 20 µg/L guideline for ''an increasing risk of health effects" for MeHg exposure (Wheatley and Paradis, 1995). Even though some studies show that the levels of Hg is increasing in certain country foods (AMAP, 2002; Dewailly et al., 2007), Inuit blood Hg concentrations do not mirror this increasing trend. In fact, Dewailly et al., (2007) reported a 30% decrease in Inuit blood Hg which is possibly linked to changes in dietary habits namely a decrease in country food consumption. In 1992, a health survey found that the mean blood Hg concentration of 21.8 µg/L which was higher than the levels found in southern Quebec (which was used as a control)(Dewailly et al., 2007). Although proper longitudinal studies have not been

done to confirm this, there is evidence that Hg blood concentrations in Inuit are decreasing, most likely as a result of a shift away from country foods and towards more market foods especially in younger generations (Kuhnlein et al., 2004).

3.4 Selenium

Selenium (Se) is an essential micronutrient that is important to human health (Brown and Arthur, 2001). It is a major component of several metabolic pathways and antioxidant defense systems (Brown and Arthur, 2001; Hatfield et al., 2014). Selenium is also important for proper neurological and immune function (Brown and Arthur, 2001). It is mainly acquired through diet and is particularly abundant in seafood as well as many of the foods consumed as part of a traditional Inuit diet (Van Oostdam et al., 2005).

The effects of Se on human health are dependent on dose, speciation and bioavailability (Rayman, 2000). Selenium is found in numerous inorganic and organic forms. The bioavailability of Se is dependent on the type of Se ingested and the biotransformations that occur within the body (Thiry et al., 2012; Yamashita et al., 2011). Organic forms include selenomethionine (Se-Met) and selenocysteine (Se-Cys) and the inorganic forms include selenate and selenite (Brown and Arthur, 2001; Ralston, 2008; Rayman, 2000) Before entering the relevant metabolic systems, selenium undergoes a number of chemical transformations within the body before becoming biologically active (Brown and Arthur, 2001; Rayman, 2000). Because of its structural similarity to the essential amino acid methionine, selenomethionine is readily transported complexed to hemoglobin and albumin (Brown and Arthur, 2001; Rayman, 2000). However, Se-Met does not become biologically active until it is biotransformed into the

inorganic selenite or selenate (Brown and Arthur, 2001; Forceville, 2006). These inorganic forms of selenium can be reduced to form Se-Cys, which is the species of Se that is incorporated into selenoproteins (Forceville, 2006). Under physiological conditions, the Se in Se-Cys acts as an extremely efficient biological catalyst (Brown and Arthur, 2001).

The importance of Se as an essential trace element within the body is based upon its presence in selenoproteins (Brown and Arthur, 2001). Selenoproteins require one Se atom at each active or catalytic site. The majority of functionally characterized selenoproteins are oxidoreductases because Se-Cys is more nucleophilic than cysteine (Hatfield et al., 2014). Approximately 25-30 genetically unique selenoproteins have been discovered, each of which have fundamental roles (e.g. protecting against oxidative damage, thyroid hormone metabolism) (Forceville, 2006). Selenoproteins are also critical for inhibiting proinflammatory cell responses and therefore are important in immune function (Forceville, 2006).

3.4.1 **Selenium and Human Health**

Mild Se deficiencies can have adverse health consequences in terms of disease susceptibility and maintenance of optimal health (Forceville, 2006; Rayman, 2000). Because of the role Se plays in immune function, Se is implicated in the disease aetiology, progression and outcome. Low Se levels are linked with increased occurrence, virulence and accelerated disease progression in some types of viral infections (Rayman, 2000). Selenium deficiency has also been linked reduced thyroid and anti-carcinogenic function (Brown and Arthur, 2001; Hatfield et al., 2014; Rayman, 2007; Shamberger, 1969). The following section focuses on the effects of Se on immune, thyroid, and neurological function as well as the prevention of certain types of cancer.

3.4.1.1 Neurological effects

Selenium plays a large role in maintaining optimal brain function; therefore, Se levels are often maintained in the brain at the expense of other bodily tissues in the event of reduced dietary Se (Choi et al., 2008; Rayman, 2012, 2000). Despite these stringent homeostatic controls, Se deficiency can affect neurotransmitter turnover, coordination, cognition, and increase risk of seizures, Parkinson's disease, permanent brain injury (Risher et al., 2003). Selenoprotein P (SEPP1) plays an important role in Se delivery to the brain (Forceville, 2006). SEPP1 is composed of 10 Se-Cys residues and transports Se from the liver to special receptors in the brain, testes, and kidneys via plasma (Rayman, 2000). Deficiencies in SEPP1 cause spasticity, abnormal movement and spontaneous seizures in studies using mice (Forceville, 2006; Rayman, 2000). This selenoprotein has a neuroprotective role, enhancing neuronal survival and preventing apoptotic cell death due to amyloid-β-induced oxidative damage (Forceville, 2006; Rayman, 2000). SEPP1 may also act as a heavy metal chelator thus, preventing adverse health effects related to heavy metal exposure (Forceville, 2006; Rayman, 2000). There is conflicting research on whether or not Se supplementation has a positive impact on mood and mental well-being so, the exact association is inconclusive (Rayman et al., 2006; Rayman, 2000). There is research that supports that Se supplementation as a favorable impact on mood and that Se deprivation can have the opposite impact on mood and mental wellbeing (Finley and Penland, 1998; Rayman et al., 2006; Rayman, 2000) . Overall, Se impacts the synthesis of SEPP1, which is necessary for optimal neural functioning and Se also potentially impacts mood.

3.4.1.2 Immune system effects

Selenium is important in maintaining a healthy immune system and increasing the proliferation of pathogen-fighting cells (Broome et al., 2004; Rayman, 2012, 2000). Selenium can act as both an antioxidant and anti-inflammatory agent. As the antioxidant glutathione peroxidases (GPxs), Se can reduce hydrogen peroxide as well as lipid and phospholipid hyperoxides, thereby diminishing the effects of reactive oxygen species on cells (Rayman, 2000). T-cells, lymphocytes involved in cell-mediated immunity, are sensitive to oxidative stress and remain inactive without adequate Se. Hoffmann et al. (2010) used a mouse model to show that a high-Se diet results in an increase in cell-signaling cytokines and enhanced T-cell signaling that increase pro-inflammatory immune responses. Selenium supplementation in the diets of individuals that were Se-deficient showed that there was an enhancement of not only T cells but also lymphocyte-mediated tumor cytotoxicity and natural killer cell activity (Rayman, 2012). A study conducted by Broome et al., (2004) showed that a Se enriched diet resulted in disease prevention in adults. They supplemented the participants' diet by 50 μg or 100 μg per day of sodium selenite or a placebo and challenged with an active attenuated poliovirus and found that both treatment groups cleared the virus more rapidly than the placebo group (Broome et al., 2004). As such, adequate levels of Se are vital for a healthy immune response.

3.4.1.3 Impact on Thyroid function

The thyroid is one of the largest endocrine glands in the body and plays an instrumental role in controlling energy usage, protein synthesis, as well as the body's sensitivity to other endogenous hormones (Brown and Arthur, 2001). The thyroid gland has the highest Se concentration compared to any other bodily tissue (Rayman, 2012). The two principal thyroid

hormones produced are triiodothyronine (T3) and thyroxine (T4), which are synthesized from iodine and tyrosine respectively (Brown and Arthur, 2001; Rayman, 2000). Selenium has various roles in the thyroid gland including the regulation of three Se-dependent iodothyronine deiodinases that catalyze activated T3 from its inactive precursor T4 via reductive deiodination (Brown and Arthur, 2001; Rayman, 2000). Selenium, in the form of GPx3, also plays a protective role against the oxidative effects of hydrogen peroxide produced during the synthesis of T3 and T4 (Brown and Arthur, 2001; Rayman, 2000). Consistent with Se protective effects, epidemiological studies have shown an inverse associations between Se status and thyroid volume, thyroid function and goiters in French women (Derumeaux et al., 2003).

3.4.1.4 Impact on Carcinogenesis

Selenium is an uncommon trace element because it has its own genetic code that specifies its insertion into selenoproteins as Se-Cys (Hatfield et al., 2014; Rayman, 2007). Selenium has also been shown to have protective effects against the formation of DNA adducts (Ravoori et al., 2010). DNA adducts, which occur when DNA covalently binds to a xenobiotic, can potentially result in carcinogenesis (Ravoori et al., 2010). Numerous in vivo studies have assessed the impact and mechanism of Se on cancer formation. Most often, such animal models use doses that are much higher than those normally observed in the environment, making direct extrapolation of results difficult. However, Waters et al., (2003) conducted a "low-dose" study with sexually intact male dogs that were biologically predisposed to spontaneous prostate cancer (Waters et al., 2005, 2003) . The Se-enriched diet consumed by the dogs reduced DNA damage and up-regulated epithelial cell apoptosis in their prostates (Waters et al., 2005, 2003). Epidemiological studies reinforce the findings from these animal studies (Rayman, 2007). There is also an inverse

relationship between dietary Se intake and a number of different cancers (Schrauzer et al., 1977; Shamberger, 1969). For example, a large longitudinal study of 1389 elderly male and females' individuals conducted by Akbaraly et al., (2005) found that inadequate plasma Se was associated with cancer-related mortality after adjusting for sociodemographic characteristics, dietary habits, health, and cognitive factors.

3.4.2 Dietary Recommendations for Selenium

Despite Se being an essential micronutrient, it is important to remember that the "dose makes the poison" and at high levels Se can be toxic and perhaps even carcinogenic (Hatfield et al., 2014; Rayman, 2000). Exceeding the daily Tolerable Upper Intake Level (UL) for adults at 400 μg/day, can lead to adverse effects including hair and nail brittleness, gastrointestinal disturbances, skin rashes, breath odor, and nervous system disorders (Institute of Medicine, 2000). The Recommended Daily Allowance (RDA) for Se is based on the amount required to maximize synthesis of glutathione peroxidase, an important selenoprotein responsible for protecting against oxidative damage (Baker et al., 1993; Institute of Medicine, 2000). The Food and Nutrition Board of the US National Academy of Sciences indicated that a safe range for daily Se intake is between 50-200 μg/day (Institute of Medicine, 2000). The Se recommendations from the Institute of Medicine (IOM) include the No Observed Adverse Effect Level (NOAEL) of 800 μg/day, the Estimated Average Requirement (EAR) of 45 μg/day and RDA of 55 μg/day for both men and women (Institute of Medicine, 2000). These recommendations apply to the total of the dietary and supplement intake of Se (Institute of Medicine, 2000).

3.4.3 Selenium in Country foods

Country foods have a high nutrient content and their consumption can substantially contribute to Se intake (Kuhnlein and Receveur, 2007). A study was conducted by Gagné et al., (2013) to determine the relationship between country food consumption and nutrient intake in preschool aged Inuit children in Nunavik. They found that despite country food intake only being 2.6% of the total energy intake, children who had country foods had statistically significant increases in vitamins and minerals, including Se (Gagné et al., 2013). Blood Se levels, as well as blood Hg levels, are elevated among Inuit due to their traditional diet (Donaldson et al., 2010). Many animals consumed have high Se levels particularly, marine mammals and fish.

Inuit communities have a Se status that is among the highest in the world (Lemire et al., 2015). Laird and Chan (2013) showed there was a correlation between the levels of essential nutrients like Se and n3 fatty acids as well as the estimated Hg intake in Inuit living in the Canadian Arctic likely, due to their traditional diets. Inuit country foods, especially marine foods, like beluga mattaaq, marine mammal organs, walrus meat, and fish eggs are exceptional sources of dietary Se (i.e. >1.0 µg/g) (Lemire et al., 2015). Previous work has shown most of the country foods of the Nunavik Inuit, including marine mammals, fish, seafood, land animals, game birds, wild berries and seaweed, are above the 0.2 µg/g level that coincides with being considered a good source of Se (Lemire et al., 2015). Country foods contain high levels of Se, and other nutrients that can potentially mitigate Hg toxicity (see Section 3.5).

3.5 Mercury and Selenium Interactions

Inuit living in Nunavik are exposed to high levels of both Hg and Se compared with the general Canadian population due to their traditional diets (Donaldson et al., 2010). Increased

dietary Se has been shown to increase MeHg concentrations in the liver, kidney and frontal lobe of the brain indicating that these are the primary regions for Hg-Se interactions and potential detoxification (Beyrouty and Chan, 2006; Donaldson et al., 2010). Studies have also shown that increasing dietary Se can reverse some of the adverse effects of Hg (Ralston and Raymond, 2010; Ralston, 2008). The mechanism by which dietary Se reverses Hg toxicity has not been completely elucidated on the molecular level. But studies show that the oxidative stress caused by MeHg that impacts neurological function because of its effects on the selenoproteins present in the neuroendocrine and nervous system (Ralston, 2008). High MeHg concentrations in the brain can lead to a diminished selenoenzyme activity in the brain (Watanabe et al., 1999). Mercury has a binding affinity for Se that is a million times higher than its affinity for sulfur (Dyrssen and Wedborg, 1991). Because of this high affinity, MeHg irreversibly binds to the active site of selenoenzymes, thus resulting in enzyme inhibition. Consequently, MeHg interferes with the homeostatic maintenance of optimal selenoenzyme function, causing a decrease in neurological functioning (Ralston and Raymond, 2010; Ralston, 2008).

The exact biological mechanism of the protective effects of Se against Hg is unclear; however, there are two predominant theories reported in the scientific literature. Briefly, the two paradigms of Hg-Se interactions are (1) Se sequesters Hg or (2) Hg sequesters Se (Ralston and Raymond, 2010). The conventional theory for Hg-Se interactions is that selenoproteins sequester Hg, forming insoluble selenide complexes (Ralston and Raymond, 2010). These complexes are then excreted, thereby reducing the potential toxic effects of Hg on the body (Ralston and Raymond, 2010). A more recent paradigm of Hg-Se interactions is that Hg sequesters free soluble Se from participating in selenoprotein synthesis, resulting in a selenoprotein deficiency that impairs neurological function (Ralston and Raymond, 2010). Due to the complexation of the

SeCys residue of the selenoprotein and MeHg, the degradation of the selenoprotein is difficult. But, eventually this complex is degraded through similar routes that dispose of MeHg-Cys or partially degraded and accumulated in lysosomes as HgSe (Ralston and Raymond, 2010). These insoluble Hg selenide complexes can be retained in the brain (Moller - Madsen and Danscher, 1991). In either paradigm, the Se is no longer available for selenoprotein synthesis.

In general, marine fish contain more Se than Hg on a molar basis; therefore, it has been postulated that marine fish intake generally provides a net health benefit rather than a health risk (Burger and Gochfeld, 2012; Ralston, 2008). Inuit communities are exposed to many contaminants that can cause oxidative stress; however, the consumption of country foods that contain high concentrations of Se and other antioxidants may reduce the effects of Hg in particular. For example, Inuit have lower incidence of cardiovascular disease despite similar exposure to MeHg because of the high antioxidant nutrient (i.e. polyunsaturated fatty acids, selenium, vitamins etc.) levels in their foods (Kuhnlein and Receveur, 2007; Kuhnlein, 1991; Laird et al., 2013b).

It is worth noting though that there is empirical evidence that appears to contradict the selenium sequestration hypothesis. For example, rodent models that shows that an enriched selenium diet alone does not prevent the adverse effects of MeHg (Beyrouty and Chan, 2006). In this work, the authors showed that diets enriched with Se, vitamin E, and phytate do not show clear protection against the neurotoxic effects of MeHg (Beyrouty and Chan, 2006). However, the rodents showed improved body weight gain and postnatal survival of offspring when their diet was enriched with both Se and vitamin E (Beyrouty and Chan, 2006). This study does not invalidate the importance of Se in mitigating Hg toxicity; but it does suggest that other mechanisms involving different nutrients may also contribute to lowering Hg toxicity.

Epidemiological studies have been utilized to further evaluate interactions between Hg and Se on human health. Unsurprisingly, there have been epidemiological studies with seemingly contradicting results in regards to the effects of dietary MeHg on neurodevelopment. In the Republic of Seychelles, many of the islanders consume a diet high in marine fish, leading to a moderately high MeHg exposure (Weihe and Joensen, 2012). Davidson et al., (1998) conducted a prospective longitudinal cohort study and found that despite a moderately high MeHg exposure through a high fish diet, no adverse prenatal or post-natal neurodevelopmental outcomes were observed. These results have been confirmed by others including Myers et al., (2003). However, the results from the Faroe Islands tell a different story. In the Faroes, the consumption of pilot whales is an important part of their culture and way of living. However, several birth cohorts have shown that the Hg from pilot whale meat consumption adversely affects the fetal development of the nervous system (Weihe and Joensen, 2012). Additionally, these effects on fetal neurodevelopment appear permanent as the impacts of Hg on health remain detectable in adolescence (Weihe and Joensen, 2012). Diet is one of the suspected factors for this inter-study discrepancy despite similar MeHg exposure. The Seychelles islander's fish diet has medium to high levels of MeHg however, the fish also contained high levels of polyunsaturated fatty acids (PUFA) and Se (Myers et al., 2003). In contrast, the pilot whale diet of the Faroe islanders had far lower PUFA and Se so; these nutrients were not able to mitigate Hg toxicity that resulted in adverse health effects (Weihe and Joensen, 2012).

3.6 Assessing the Benefits and Risks of Country food Consumption

A risk assessment refers to the evaluation the potential for adverse health or environmental effects from natural or synthetic chemical stressors (Kleinjans, 2003). A method

is needed to determine the risk-benefit relationship of Hg to Se between different country foods. A variety of methods have been explored for determining the risk-benefit relationship between Hg and Se. One such method involves the calculation of the molar concentrations of Hg and Se present in the food. Ralston (2008) showed that an excess in molar Se can mitigate the risks posed by Hg from consuming fish (Ralston, 2008). A Se:Hg molar ratio where molar Se is greater than molar Hg may indicate that the dietary Se counteracts the Hg present in the consumed fish (Burger and Gochfeld, 2012). An related method is the calculation of a Se Health Benefit Value (Se HBV) (Kaneko and Ralston, 2007). Like the previous method, the Se HBV is based on molar Se:Hg ratios; however, the Se HBV also considers the absolute amounts of Se and Hg in the food in order to provide an index (Ralston, 2008). The sign of the Se HBV value indicates whether health benefits are expected (Se HBV > 0) or health risks (SeHBV < 0). While the magnitude of index is proportional to the expected benefits or risks (Ralston, 2008). The equation used to calculate the Se HBV was revised in order to avoid "divide by zero" type errors that were common in the first iteration of this equation in situations where MeHg levels were very low (Ralston et al., 2015). With the revisions the equation may provide a better index to reflect the risks of MeHg (Ralston et al., 2015). The Se HBV has been calculated using the total molar concentrations in many species of fish and marine life (Ralston, 2008).

3.7 Assessing Bioaccessibility and Bioavailability

Bioaccessibility and bioavailability are concepts that are integral to estimating the internal dose of Hg and Se according to metal concentrations in the environment (Ruby et al., 1999; Torres-Escribano et al., 2011). When estimating exposure using external environmental concentration, measures of oral bioavailability may provide the best representation of internal

dose (Caussy, 2003; Ruby et al., 1999; Thiry et al., 2012). Oral bioavailability can be defined as the amount of contaminant ingested that is absorbed and reaches systemic circulation and eventually the biological target tissue (Caussy, 2003; Ruby et al., 1999; Thiry et al., 2012) . Because the bioavailability of Hg and Se is unknown in Inuit country foods collected from Nunavik, it increases the uncertainty of the exposure assessment. In order to more precisely determine the internal dose, a series of in vitro approaches have been developed to determine the bioaccessibility.

Bioaccessibility is often used as an estimate or proxy for metal bioavailability (Ruby et al., 1999; Torres-Escribano et al., 2011). For the purposes of my thesis, bioaccessibility represents the fraction of Hg and Se that would leach out of the solid food and into the gut lumen (Ruby et al., 1999; Torres-Escribano et al., 2011) . An in vitro gastrointestinal (GI) model can determine the Hg and Se that is bioaccessible using simulated digestive solutions that are pH-controlled and contain enzymes to aid in food breakdown (Intawongse and Dean, 2006; Torres-Escribano et al., 2010). The in vitro GI model used in my thesis research simulates the physiological conditions of the stomach and duodenum, which are primarily responsible for food breakdown. Laird et al., (2009) looked that the bioaccessibility of Hg, using an in vitro model, in country foods and found that percent bioaccessibility was independent of the total concentrations. Simply put, you cannot deduce the bioaccessible fraction of Hg from the total concentration for the country foods studied. They found high small intestinal Hg bioaccessibility in Arctic char (93.9%), ringed seal (70%) and caribou (70%) and low bioaccessibility in ringed seal liver (18.9%) and walrus (15.5%). However, given the small number of samples evaluated, these results may not be directly applicable to other country foods collected from other regions (Laird et al., 2009).

In general, Se bioaccessibility is often greater than Hg bioaccessibility. As shown by Cabañero et al., (2007) when they found Hg IVBA was consistently lower (<20%) than Se IVBA (50-83%) when using an in vitro bioaccessibility method. They also showed that the gastric bioaccessibility was about 47-70% but the intestinal bioaccessibility was about 50-83% (Cabañero et al., 2007). As shown in Calatayud et al., (2013), they found the Se to be between 35-106% and 17-125% respectively in the 16 raw seafood and shellfish samples (such as anglefish, salmon, sardine, small hale, clam, cuttlefish, mussel, and squid etc.) analyzed. In terms of Se bioaccessibility in country foods, Se bioaccessibility seems to be consistent between different marine fish species (Laird and Chan, 2013).

3.8 Bridging the Research Gaps in Literature

In the current body of scientific literature, there is limited information on Hg and Se bioaccessibility and next to no information on the bioaccessibility of these trace metals in Inuit country foods. The majority of research focuses on determining the total concentrations of Hg and Se in country foods. For example, in a recent paper by Lemire et al., (2015), they focused on total concentrations of MeHg, Se and omega-3 fatty acids in country foods collected from Nunavik, Quebec. As described in Zhang et al., (2014), there is a very large knowledge gap in country food assessments of exposure to Hg and/or Se were found including the lack of consideration for Hg-Se interactions and the bioaccessibility of these trace metals within the body. In Inuit communities, were country foods are not only crucial part of their culture and identity but also a nutritious alternative to market foods, encouraging consumption of these foods is important.

This research may assist ongoing contaminant exposure assessments in Nunavik and potentially aid in the development of culturally relevant strategies that promote these foods while reducing Hg body burden. Our research will determine the following: (1) the Inuit country foods that have high levels of Hg also have high levels of Se (2) the bioaccessibility of Hg and Se and if it varies from one country food to another and (3) the effects of co-digesting specific food combinations on the solubilization of Hg within the GI tract? The bioaccessibilities determined from this study could help give a better estimate of the internal dose of Hg and Se, which may result in more accurate risk assessments in the future.

4. The In Vitro Bioaccessibility of Selenium Exceeds that of Mercury in Inuit Country Foods

4.1 Introduction

Approximately 50,000 Inuit inhabit the Arctic regions of northern Canada. Over 10,000 of these indigenous people live in 14 coastal villages in Nunavik, the northernmost region of Québec (Makivik Corporation, 2015). Country foods harvested by Inuit from their local surroundings include marine mammals (e.g., beluga mattaaq, beluga meat, ringed seal meat), fish (e.g., arctic char, lake trout, sculpin), wild game and birds (e.g., caribou, ptarmigan, geese), berries (e.g., cloudberry, blueberries, crowberries, red berries), and certain types of seaweed. Collectively, these country foods play a critical role in the cultural, social, economic and nutritional welfare of the Inuit (AMAP, 2009; Berti et al., 1998; Chan et al., 2006). Kuhnlein et al., 1996 highlighted that country food consumption in other regions of the Arctic increased intake of iron, zinc, and vitamins A, E and D. In addition, the marine mammals, fish species, and fish eggs particularly those of marine origin, consumed in several regions of the Arctic contain from high to exceptional levels of selenium (Se) and omega-3 fatty acids (Kuhnlein, 1991;

Lemire et al., 2015). Consumption of country foods is linked with decreased the risk of obesity, cardiovascular disease and other chronic ailments (Kuhnlein and Receveur, 2007; Kuhnlein et al., 2004; Richmond and Ross, 2009; Sharma, 2010; Sheikh et al., 2011). Furthermore, country foods in Nunavik can be more affordable than nutritious store-bought foods, which are often prohibitively expensive despite subsidy programs such as Nutrition North Canada (Chan et al., 2006; Government of Canada, 2015). Research has shown than an increased country food consumption results in higher levels of Se intake, and increased Se intake is linked to many positive health outcomes including: reduced cardiovascular disease, proper immune functioning, proper reproductive function and optimal thyroid function (Hatfield et al., 2014; Rayman, 2000).

Even though there are high levels of Hg in some country foods, these foods can also provide many nutrients and minerals. One such nutrient is Se, an essential structural component of selenoproteins (Hatfield et al., 2014; Lund, 2013). Selenoproteins play important roles in thyroid hormone metabolism, antioxidant defense systems, anti-carcinogenic mechanisms and proper neurological functioning (Brown and Arthur, 2001; Hatfield et al., 2014; Rayman, 2000). Due to the consumption of these Se-rich country foods, Se blood concentrations are considerably higher in Nunavik (geometric mean = 271 $\mu g\ L^{-1}$) relative to the general Canadian population (mean = 204 $\mu g\ L^{-1}$) (Health Canada, 2010b). Additionally, Inuit in Nunavik have blood Se levels that exceed those required for optimal selenoprotein activity (Health Canada, 2010b; Yang and Xia, 1995). There is also evidence that Hg toxicity may be mitigated in part by dietary Se (Feroci et al., 2005; Jones et al., 2013; Ralston, 2008). Recent studies have shown the presence of a new Se species present in predatory fish (i.e. tuna) called selenoneine that reacts with radicals and MeHg (Yamashita et al., 2011). In general, dietary Se is thought to protect against Hg-dependent toxicity by forming an inert Hg-Se complex that can be eliminated from the body

(Ralston and Raymond, 2010). The antagonistic relationship between Hg and Se is well known even though the exact biological mechanism is unknown (Khan and Wang, 2009). Whether this antagonist or synergistic effect occurs is dependent on the sensitivity of the organ/organism affects, and the relative Hg and Se concentrations (Khan and Wang, 2009). Consequently, it is possible that the high levels of Se present in Inuit individuals can impact and perhaps counteract some of the negative effects of Hg (Donaldson et al., 2010; Lemire et al., 2015; Valera et al., 2009).

Inuit exposure to mercury (Hg) in Nunavik is among the highest observed in the world (Donaldson et al., 2010). Despite significant declines in exposure over the past two decades, biomonitoring efforts conducted in 2004 showed that, on average, more than half of childbearing-age women and adults of 40 years old and above had blood Hg levels exceeding Canadian blood Hg guidance values (≥8 μg/L for childbearing-age women and ≥20 μg/L for other adults) (Dewailly et al., 2007; Lemire et al., 2015). The elevated Hg body burden observed among Inuit is a result of high Hg levels within some country foods, particularly some types of marine mammals and fish (Donaldson et al., 2010; Van Oostdam et al., 2005). For example, a recent deterministic exposure assessment showed beluga meat to be the largest methylmercury (MeHg) contributor for Inuit in Nunavik, and this particularly in the Hudson Strait, the region where most traditional beluga hunting takes place in Nunavik (Lemire et al., 2015). Similarly, even though it was consumed relatively infrequently, ringed seal liver was the largest contributor of dietary Hg in the Inuit regions of Nunavut, Nunatsiavut, and the Inuvialuit Settlement Region (Laird et al., 2013b). However, both Lemire et al., (2015) and Laird et al., (2013b) demonstrated that most of the country foods that are high in Hg could also provide substantial quantities of essential nutrients. All together, this work supported the creation of public health messaging that

advised Inuit populations on the general healthfulness of country foods as well as how they could limit their Hg exposures, and this until there are evidences of a decrease of Hg levels in Arctic wildlife (Government of Nunavut, 2012; Nunavik Nutrition and Health Committee, 2011).

The use of bioaccessible or bioavailable trace metal concentrations found in foods can help improve the accuracy of dietary exposure estimates and risk assessments when used instead of total concentrations (Moreda-Piñeiro et al., 2011; Torres-Escribano et al., 2010; Wang et al., 2013). Bioaccessibility, which refers to the amount of the ingested metal that is solubilized in the gut lumen, is easier to quantify (than in vivo studies, for example) and can be used as an approximation for bioavailability, i.e. the amount of the ingested metal entering into systemic circulation (Ruby et al., 1999; Torres-Escribano et al., 2010). Bioaccessibility can have interspecies and interspecies variations as a result of many factors including: food composition (i.e. amounts of protein, fats, carbohydrates, and nutrients), tissue composition, location of sample collection, cooking technique, and Hg-Se complexation (Burger et al., 2012; Ouédraogo and Amyot, 2011; Ruby et al., 1999). Research has shown that the bioaccessibility of Hg and, to a lesser extent, Se varies greatly from one food to another (Burger, 2012; Cabañero et al., 2007; Calatayud et al., 2012; Moreda-Piñeiro et al., 2011). Therefore, as shown by Moreda-Piñeiro et al., (2011) and Torres-Escribano et al., (2010), the use of total concentration for exposure modelling can introduce substantial uncertainty into assessments because it does not account for possible limitations on metal dissolution and absorption. Incorporating bioaccessibility may reduce the uncertainty within estimates of metal dietary intakes/exposures from country foods consumed in Nunavik.

The primary objective of this research was to characterize the bioaccessibility of Hg and Se in a wide variety of country foods consumed by the Inuit of Nunavik. Thereafter, we

examined whether incorporating bioaccessibility data altered the Se:Hg molar ratios of these foods. We hypothesized that due to the high concentrations of Se in certain country foods, that the total Se:Hg molar ratios would be less than the bioaccessible Se:Hg molar ratios indicating more Se than Hg is accessible when accounting for bioaccessibility and therefore, an overall nutritional benefit.

4.2 Materials and Methods

4.2.1 Sample Collection

Country foods were collected from 2008-2013 as part of the community-based sampling program overseen by the Nunavik Research Center of the Makivik Corporation. The samples collected included: beluga meat and mattaaq (Hudson Strait; East Hudson Bay), ringed seal meat and liver (Inukjuaq; Quartaq), walrus meat (Nunavik coast), Arctic char flesh (Deception Bay; Salluit), Atlantic salmon flesh (Koksoak River), brook trout flesh (Koksoak River), Lake Trout flesh (Lake Qamuttitsait), lake whitefish flesh (Koksoak River), sculpin fish flesh and eggs (Koksoak River), blue mussel flesh (Kangiqusuollujjaq), caribou muscle (Leaf River Herd). Inuit eat several parts of the caribou, including but not limited to muscle, organs, and ribs. For the purposes of my thesis however, I have referred to caribou muscle as caribou meat throughout. Other Nunavik country food samples included eider duck eggs and Canada goose eggs. In addition to analyzing fresh (i.e. raw) beluga meat, beluga nikku (traditionally made air-dried beluga meat) was also evaluated.

4.2.2 In vitro GI Model

Hg and Se in vitro bioaccessibility (IVBA) was evaluated using a two-stage in vitro gastro-intestinal (GI) model using the method previously described by Laird et al. (2013).

Specifically, simulated gastric fluid (30 mL) containing hydrochloric acid (OmniTrace®, pH 1.5), porcine pancreatin (Sigma-Aldrich®, 6 g L^{-1}), and NaCl (8.5 g L^{-1}) in MilliQ water was added to glass serum bottles containing country food samples (2 g). Subsequently, the suspension pH was adjusted to 1.5 ± 0.5 with hydrochloric acid (OmniTrace®, 0.5 M) and the extracts were then incubated at 37ºC with orbital shaking (180 rpm) for 2 h. Thereafter, $NaHCO_3$ (1 M; 5 mL) and simulated duodenal fluid (15 mL) containing $NaHCO_3$ (12.5 g L^{-1}), oxgall bile dried (EMD Millipore®, 6.0 g L^{-1}), porcine pancreatin (Sigma-Aldrich®, 3.0 g L^{-1}), and NaCl (8.5 g L^{-1}) was added to each serum bottle. The extracts were then returned to the incubator shaker for an additional 3 h of shaking at 37ºC. At the conclusion of the simulated duodenal extraction, samples were centrifuged (10.3 x 10^4 g; 15 min) and then filtered (0.45 µm; PTFE membrane) using a vacuum filtration manifold. Each batch of samples processed through the in vitro GI procedure included blanks, duplicates, and standard reference materials.

4.2.3 Chemical Analysis

4.2.3.1 Total Metal Content in Country Foods

The total metal content of the country foods were determined by Michael Kwan of the Makivik Corporation in Kuujjuaq, Nunavik. A portion of each country food underwent an acid digestion procedure prior to the measurement of HgT and Se. First, trace metal grade 70% w/v nitric acid (HNO_3) was added to each test tube and the test tube was heated at 78 ± 4ºC for 6 h. Thereafter, an aliquot of the digested sample was digested with HNO_3 acid, heating again at 78 ± 4ºC for 3 h. After cooling, concentrated hydrochloric acid (HCl) and concentrated sulphuric (H_2SO_4) acid were added to all the samples. Samples were then mixed and heated for another 3 h at 78±4ºC. After cooling, potassium dichromate in 10% v/v HCl (10% v/v) was added. Each digested sample was then measured for total Hg and Se within 24 h. Total Hg in the acid digests

was measured by Cold-Vapour Atomic Absorption Spectrometry (CVAAS) using a Model PinAAcle 900Z Atomic absorption spectrometer (Perkin Elmer) equipped with a Model AS90 auto sampler (Perkin Elmer) and a computer running the AAWinLab (version 2.3, Perkin Elmer). This technique used an electrodeless discharge Hg lamp and a Model FIAS-100 flow injection analysis system in a reducing agent containing stannous chloride (10%, w/v) in HCl (30%, v/v). For total Hg analysis, the optimal atomization and ashing temperatures were 2200ºC and 1260ºC respectively. A small number of country foods (e.g. sculpin eggs, blue mussel) with Hg concentrations below the limit of detection (0.035 µg g^{-1}) of the CVAAS, were re-analyzed using a NIC MA-3000 (Nippon Instruments) Thermal Decomposition, Amalgamation, Atomic Absorption Spectrophotometry (TDAAS). The acid digested country food samples were then analyzed for total Se concentrations using graphite furnace atomic absorption spectrometry (GFAAS) using a PinAAcle 900Z Atomic Absorption Spectrometer equipped with a Transversely-Heated Graphite Atomizer (THGA). The digested sample and a matrix modifier (Palladium (II) nitrate, 10 wt. % solution in 10 wt. % HNO_3 acid, magnesium nitrate hexahydrate, and ammonium dehydrate phosphate in MilliQ water). Total metal concentrations were determined in duplicate for each digested sample; the mean of the duplicate data was reported. The percent recovery, as determined using spiked controls was consistently greater than 98% for all metals.

4.2.3.2 Bioaccessible Metals in Country Foods

The filtered extract from the in vitro GI model (Section 2.1.1) was diluted in 2% HNO_3 and then analyzed using a MA3000 (TDAAS) and an Agilent 7700x (Inductively Coupled Plasma Mass Spectrometry or ICPMS) for Hg and Se, respectively. The metal concentration

within aliquots of the in vitro extracts of the country food samples were used to calculate Hg and Se in vitro bioaccessibility (IVBA). For each batch of bioaccessibility extracts analyzed using the MA-3000; certified reference materials (DORM-2, DORM-3, DORM-4, and DOLT-4; National Research Council of Canada) were used as instrumental controls. Percent recovery from the CRM was within 10% of its certified value for each batch; average percent recovery for these instrumental controls was 95%. The procedural duplicates for each digested sample was only accepted if the relative standard deviation was less than 10%. Instrumental controls and blanks for Se on the ICP-MS were administered by the lab technician and used spiked solutions of varying concentrations.

4.2.4 Statistical Analysis

For each country food type, descriptive analyses were used to report total Hg and Se (undigested) concentrations and bioaccessible percentages. The bioaccessible percentages represent the arithmetic mean calculated for each country food. Each country food had two or more food samples, each of which were digested in duplicate. For cases where the total or bioaccessible Hg or Se concentrations were below the detection limit of the analytical equipment, the concentration was recorded as half the detection limit. A matched pairs analysis was used to test whether incorporating IVBA significantly altered Se:Hg molar ratios. Statistical analysis were performed using JMP 11 (SAS Institute) and differences were declared statistically significant when $p < 0.05$.

4.3 Results and Discussion

Unsurprisingly, the concentrations of total Hg (HgT) and total Se (Se) varied greatly between country foods (Table 1). Due Hg's tendency to bioaccumulate and biomagnify in marine and freshwater food chains, the greatest levels of HgT were found in country foods derived from long-lived marine mammals, such as ringed seal liver (19 ± 11.1 mg kg^{-1}), beluga nikku (5.01 ± 1.15 mg kg^{--1}), and fresh beluga meat (1.16 ± 0.314 mg kg^{-1}). Beluga mattaaq, an Inuit delicacy comprised of raw whale skin and blubber, had about 2-fold less HgT than observed in fresh beluga meat and approximately 9.5-fold less HgT than seen in beluga nikku. The lower HgT levels found in the lipid-containing beluga mattaaq is in keeping with Hg's complexation affinity for sulphur groups (e.g. thiols) in muscle proteins and its lack of lipid partitioning (Clarkson and Magos, 2006; Raymond and Ralston, 2004). Air-dried beluga meat contained higher concentrations HgT (5-fold) and Se (2-fold) than fresh beluga meat. These results give the impression that air-drying disproportionately affects HgT concentrations (i.e. relative to Se). However, because the raw beluga meat and beluga nikku samples selected for the bioaccessibility analyses were not paired, and instead came from different organisms, this observation is likely an artifact of the inter-individual variability of Hg and Se in beluga. Lake Trout was the only of the analyzed fish species to exceed the 0.5 ppm HgT Canadian Federal Guideline for Human Consumption in seafood (Canadian Food Inspection Agency, 2015; Health Canada, 2007). Among the wild game and fowl studied (Table 1), HgT concentrations were typically low; the egg whites of the eider duck, a marine bird species, were the key exception to this observation (Table 1). This high HgT in egg whites may be attributed to Hg's high affinity to sulphur found in the cysteine residues of ovalbumin proteins (McCloskey et al., 2013; Van Oostdam et al., 2005).

Marine mammals also tended to have the highest Se levels, especially ringed seal liver (10.0 ± 4.4 mg kg^{-1}) and beluga mattaaq (4.6 ± 0.40 mg kg^{-1}) (see Table 1). Our results are in keeping with Lemire et al., (2015), which showed that beluga mattaaq to be the key contributor to Se dietary intake among Inuit adults. Walrus meat and sculpin fish eggs, a marine fish species, were particularly good sources of Se, especially considering the low Hg concentrations within this type of food (Table 1). A study conducted by Burger et al., (2012) in Alaska found that the Se in sculpin flesh was 0.609 ± 0.034 mg kg^{-1}, similar to the results described here (e.g. 0.44 ± 0.14 mg kg^{-1}). Among Nunavik wild game and fowl, caribou meat had the highest Se (0.16 - 0.25 mg kg^{-1}). These levels were consistent with those reported in a study conducted by Aastrup et al., (2000), who found the Se in Greenland caribou to range from 0.03 to 0.25 mg kg^{-1}.

In order to gain a clearer picture of the bioaccessibility of Hg and Se within Nunavik country foods, each of the 18 foods were processed through an *in vitro* GI model. Among the country foods studied, Hg IVBA was highest in ringed seal meat (90%), eider duck egg white (68%), and Lake Trout (57%), and lowest in eider duck egg yolk (25%), beluga mattaaq (22%), and Arctic char (9.8%) (Figure 1; Table S1). Interestingly, ringed seal meat was the only country food to approach 100% Hg IVBA. For this reason, assuming HgT to be completely bioavailable while characterizing Inuit Hg exposure from country foods may overestimate risk. Therefore, the high HgT concentrations reported within ringed seal liver may be somewhat offset by the relatively low bioaccessibility (31%) within this tissue. Laird et al., (2009) used an in vitro GI model including gut microbiota and also reported low bioaccessibility (18.9%) in ringed seal liver. The low Hg bioaccessibility reported in ringed seal liver may be a result of the formation of poorly soluble HgSe complexes during the hepatic detoxification of Hg, thus making dissolution in the simulated digestive solutions more difficult (Clarkson and Magos, 2006;

Wagemann et al., 2000). Similarly, the higher HgT concentration reported in beluga nikku (relative to raw beluga meat) may be offset by that fact that Hg was 2-fold less bioaccessible in beluga nikku. Within the fish and shellfish studied, the highest Hg IVBA was found in blue mussel (43%) and lake whitefish (42%) and the lowest was found in Arctic char (10%). The low to moderate levels of Hg IVBA in fish we studied are consistent with ranges describe in the literature (Cabañero et al., 2007; Laird et al., 2009). In the wild game studied, eider duck egg white was high in HgT (0.8ppm) and presented a particularly high HgT bioaccessibility (>60%).

All but three of the 18 country food items studied showed Se IVBA above 50% (Figure 2). Se bioaccessibility was highest for caribou meat (108%), Atlantic salmon (106%), and ringed seal meat (104%). Relative to the HgT results described above, the bioaccessibility of Se tended to vary less between country foods. Additionally, for the majority of the 18 food types, Se IVBA tended to be equivalent or greater (1.2- to 3-fold) than Hg IVBA; the only exception to this trend was eider duck egg white (Figure 2). Similar to our results, Cabañero et al., (2007) found that Hg IVBA was consistently lower (<20%) than Se IVBA (50-83%) when using an in vitro bioaccessibility method. The Se IVBA being greater than the Hg IVBA in our research may support the assertion of the formation of insoluble complexes in the GI tract.

Researchers have used molar ratios to compare the relative quantities of HgT and Se within seafood (Burger and Gochfeld, 2012; Burger, 2012; Burger et al., 2013). Since higher levels of Se could provide some degree of protection against Hg toxicity, identifying foods with high Se:Hg molar ratios may have utility for the development of contaminant advisories and other types of risk communication materials (Rayman, 2000; Raymond and Ralston, 2004). Although this approach does not take into account all beneficial nutrients or other environmental contaminants that may also be found in these foods, we found that Se:Hg molar ratios based on

the total concentrations differed dramatically from one food to another (Table 2). For example, total Se:Hg ratios were considerably higher in shorthorn sculpin eggs (1600 ± 460), blue mussel (220 ± 1.6) and eider duck yolk (88 ± 17) than in ringed seal liver (1.8 ± 0.3), Lake Trout (0.84 ± 0.43) , and beluga nikku (0.74 ± 0.14). Similarly, Burger and Gochfield (2013) showed there to be substantial interspecific variation across commerically-available fish species (e.g. Yellow fin tuna, shrimp, swordfish, salmon) for total molar Se:Hg ratios. A Se:Hg molar ratio of less than 1 is sometimes used as an criterion signifying the food represents a net Hg risk (Ralston, 2008). However, a number of significant reservations have been raised about the utility of this criterion: (1) the exact ratio that will protect against the adverse effects of Hg is unknown, (2) the protective level is likely different between target tissues, and (3) sensitive or high risk populations may have different threshold Se:Hg molar ratios (Burger and Gochfeld, 2012; Burger, 2012; Ralston, 2008).

After accounting for metal bioaccessibility, shorthorn sculpin eggs (3300 ± 890), Arctic char (170 ± 110) and walrus meat and blubber (100 ± 40) had considerably higher Se:Hg ratios than observed in Lake Trout (1.0 ± 0.38), beluga nikku (1.8 ± 0.3) and ringed seal liver (2.4 ± 0.6). Although Canada goose eggs (2900 ± 620) and blue mussels (1900 ± 1600) had very high IVBA Se:Hg ratios, these results may have been somewhat exaggerated from the assumption that Hg concentrations less than the detection limit were equal to half the detection limit. Our results show molar Se:Hg ratios were generally higher after accounting for metal bioaccessibility (Table 2). For example, for the majority of the foods tested, the IVBA Se:Hg ratio was at least 2-fold greater than Se:Hg ratio based upon total metal concentrations. The most notable exceptions being ringed seal muscle and ringed seal liver which showed only a slight increase in molar ratios after accounting for bioaccessibility. Therefore, using total trace metal concentrations and

the total Se:Hg molar ratios may err on the conservative side with respect to the relative amount of Se and Hg that may be present in the GI tract. However, until bioaccessibility models are validated against in vivo measures of Hg and Se oral bioavailability, it remains to be seen which approach (i.e. molar ratios based on total vs. bioaccessible concentrations), if either, provides a more accurate indicator of Hg risks and Se benefits. Collectively, this work will assist ongoing efforts to better characterize the balance between Hg risks and Se benefits within the country food systems of Indigenous Peoples in general and Nunavik Inuit in particular.

4.4 Conclusion

Our results indicate that there are large differences in the bioaccessibility of Hg versus Se in the country foods of the Nunavik Inuit. These bioaccessibility estimates are to be used for the refinement of dose reconstruction models in order to determine whether accounting for metal bioaccessibility improves the association between external dose estimates and internal dose measures among Nunavik Inuit. Generally, the bioaccessibility of Se in Inuit foods exceeds that of Hg. Consequently, neglecting to account for bioaccessibility appears to systematically underestimate the Se:Hg molar ratio in foods. But, whether measures of metal bioaccessibility improve the accuracy of Se:Hg ratios as a seafood safety criteria will largely hinge on the validation of in vitro gastrointestinal models for the measurement of Hg and Se bioaccessibility.

Table 1. Total Hg and Se concentrations of Country Food items in mg kg-1, w.w.

Country Foods	n	Total Hg (Mean ± SE)	Total Se (Mean ± SE)
Marine Mammals			
Beluga nikku *(Delphinapterus leucas)*	3	5.01 ± 1.15	1.33 ± 0.0786
Beluga meat *(Delphinapterus leucas)*	7	1.16 ± 0.314	0.747 ± 0.0191
Beluga mattaq *(Delphinapterus leucas)*	15	0.529 ± 0.0958	4.58 ± 0.405
Ringed seal liver *(Pusa hispida)*	9	19 ± 11.1	10.3 ± 4.38
Ringed seal meat *(Pusa hispida)*	3	0.281 ± 0.0875	0.469 ± 0.0872
Walrus meat & blubber *(Odobenus rosmarus)*	2	0.0945 ± 0.0185	1.35 ± 0.19
Fish & Shellfish			
Arctic char *(Salvelinus alpinus)*	3	0.0593 ± 0.026	0.355 ± 0.0629
Atlantic salmon *(Salmo salar)*	6	0.0461 ± 0.00613	0.262 ± 0.0205
Brook trout *(Salvelinus fontinalis)*	3	0.0923 ± 0.0194	0.235 ± 0.0328
Lake Trout *(Salvelinus namaycush)*	2	1.01 ± 0.186	0.303 ± 0.11
Lake whitefish *(Coregonus clupeaformis)*	3	0.156 ± 0.0295	0.265 ± 0.0685
Shorthorn Sculpin *(Myoxocephalus scorpius)*	3	0.217 ± 0.0843	0.435 ± 0.14
Shorthorn Sculpin (eggs) *(Myoxocephalus scorpius)*	3	0.00257 ± 0.000644	1.4 ± 0.188
Blue mussel *(Mytilus edulis)*	3	0.00508 ± 0.0000362	0.435 ± 0
Wild Game and Fowl			
Caribou meat *(Rangifer tarandus)*	6	0.0267 ± 0.00325	0.197 ± 0.0138
Eider duck egg (yolk) *(Somateria mollissima)*	3	0.0707 ± 0.0241	2.14 ± 0.251
Eider duck egg (white) *(Somateria mollissima)*	3	0.783 ± 0.163	1.06 ± 0.186
Canada goose egg (whole) *(Branta canadensis)*	3	0.0175 ± 0	0.492 ± 0.0727

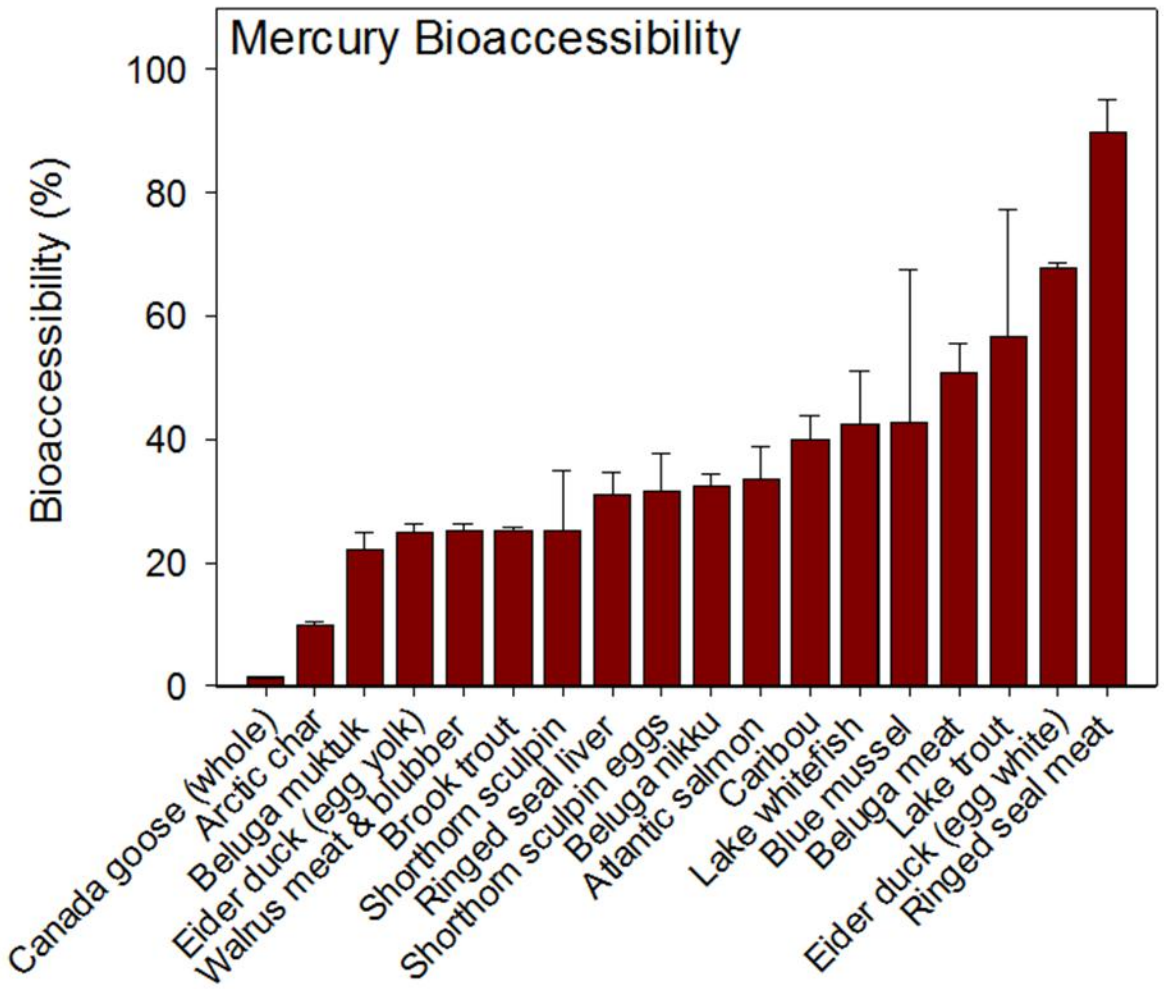

Figure 1. Average % IVBA for Hg in the 18 Inuit country foods collected in Nunavik, QC. The error bars represent standard error. Please note that for certain country foods either the IVBA fraction (i.e. blue mussel) or both total and IVBA (i.e.Canadian geese) was below the detection limit for Hg.

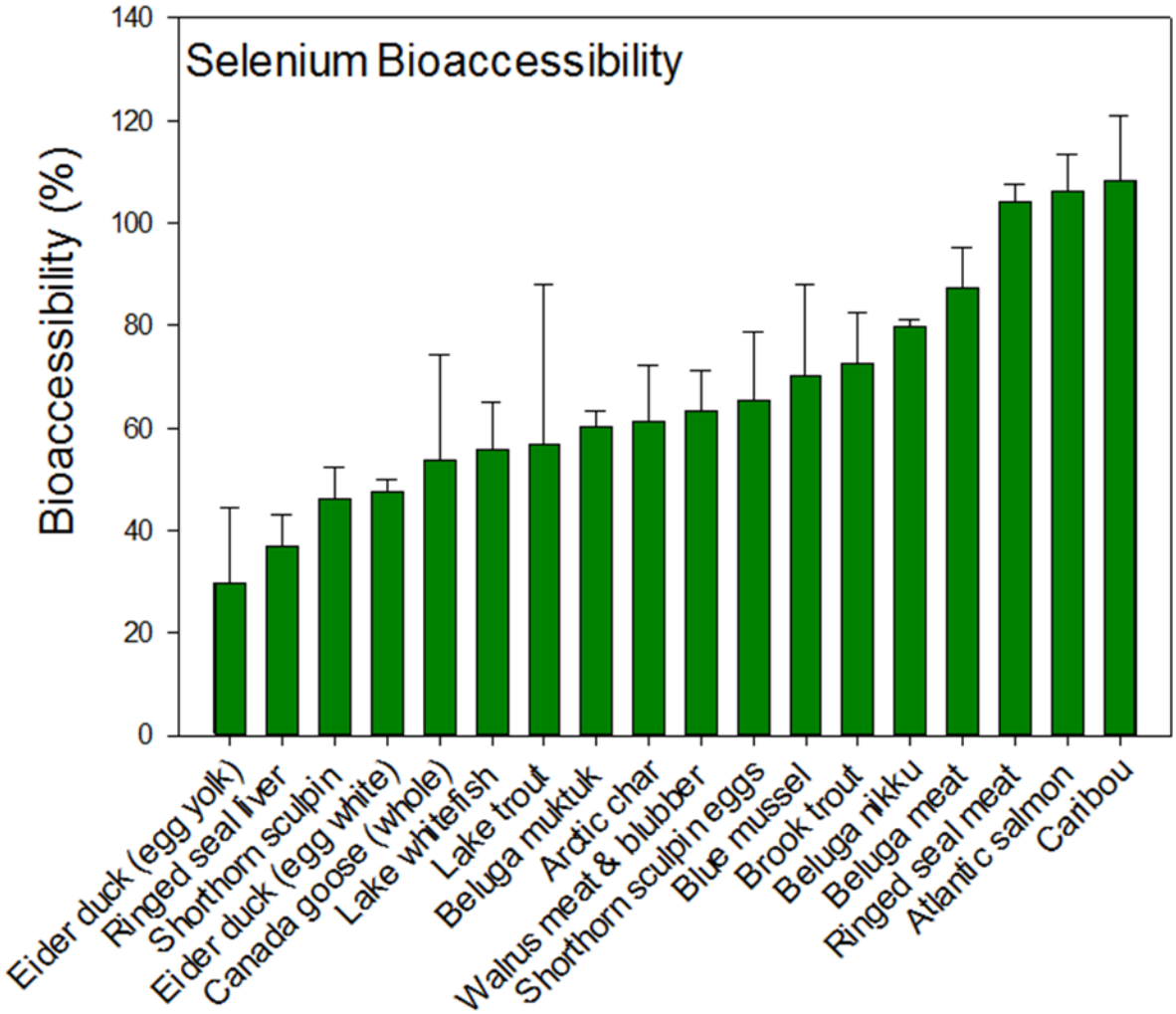

Figure 2.Average IVBA for Se in the 18 Inuit country foods collected in Nunavik, QC. Error bars represent standard error. Please note that the IVBA fraction for eider duck yolk was below the detection limit for Se.

Table 2. Se:Hg ratios (± standard error) according to total and bioaccessible molar concentrations (µmol kg-1 Se:µmol kg-1 Hg) in 18 country foods collected in Nunavik, QC.

Country Foods	n	Total Se:Hg	IVBA Se:Hg
Marine Mammals			
Beluga nikku	3	0.74 ± 0.14	1.8 ± 0.3
Beluga meat	7	2.4 ± 0.6	4.0 ± 0.9
Beluga mattaq	15	28.0 ± 3.8	98.9 ± 26.7
Ringed seal liver	9	1.8 ± 0.3	2.4 ± 0.6
Ringed seal meat	3	5.4 ± 2.1	6.7 ± 2.3
Walrus meat & blubber	2	39.4 ± 15.2	100 ± 39.6
Fish & Shellfish			
Arctic char	3	21.8 ± 9.3	167.5 ± 107.1
Atlantic salmon	6	15.9 ± 2.3	54.5 ± 10.1
Brook trout	3	7.4 ± 2.3	20.5 ± 6.5
Lake Trout	2	0.84 ± 0.43	1.0 ± 0.38
Lake whitefish	3	4.2 ± 0.3	6.8 ± 3.0
Shorthorn Sculpin	3	5.4 ± 1.0	15.4 ± 7.5
Shorthorn Sculpin (eggs)	3	1613 ± 462	3306 ± 885
Blue mussel	3	218 ± 1.56	1879 ± 1613
Wild Game and Fowl			
Caribou meat	6	19.8 ± 2.2	61.1 ± 14.8
Eider duck egg (yolk)	3	88.2 ± 16.9	216 ± 185
Eider duck egg (white)	3	3.6 ± 0.8	2.5 ± 0.7
Canada goose egg (whole)	3	71.5 ± 12.2	2876 ± 615

5. Additivity of Metal Bioaccessibility in Binary Mixtures of Inuit Country Foods

5.1 Introduction

The increase in global industrialization has had a noticeable impact on the environment. Formerly considered to be pristine, the Arctic region, which is home to many Indigenous populations, did not escape the impact of industrialization. This has resulted in high levels of environmental contaminants, like mercury (Hg) being deposited from anthropogenic point sources around the world. Mercury is a well-known toxicant that has many adverse effects on the body, most notable of which are on the central nervous system (Clarkson, 1997). Communities in the circumpolar region are exposed to Hg through their consumption of country foods (Donaldson et al., 2010; Van Oostdam et al., 2005).

Country foods play an important cultural, spiritual, nutritional and economic role in Inuit communities (Harder and Wenzel, 2012; Van Oostdam et al., 2005). Inuit country foods include marine mammals, fish, wild game, birds, berries and edible seaweed that are hunted or collected from local surrounding. Country foods have a multitude of nutritional benefits including increased intake of omega-3 fatty acid, iron, zinc, and vitamins A, E and D (Kuhnlein and Chan, 2000; Kuhnlein, 1991; Lemire et al., 2015). There are numerous benefits to country food consumption however; they are also the major route of exposure to many environmental contaminants including Hg. It follows that Canadian Inuit exposure to Hg is among the highest observed in the world despite a dietary transition towards market foods and away from country foods over the past couple of decades (Donaldson et al., 2010). Due to high levels of Hg in certain country foods, health advisories can suggest decreased consumption without fully

considering the internal concentrations of Hg in the body and the effect of consuming high Hg foods with other nutrient-rich foods (Anctil, 2004; Passi et al., 2013).

Country foods are central to Inuit culture and identity not to mention a wonderful source of many nutrients, such as selenium (Se). However due to environmental contamination, country foods are also the main source of Hg exposure in Inuit communities. As shown in the previous section, there are large differences in the bioaccessibility of Hg and Se between country foods and the bioaccessibility of Se in Inuit foods generally exceeds that of Hg. According to Laird et al., (2009), the fish, wild game and marine mammals had HgT bioaccessibility ranging from 1 to 93%. However, the current research investigating the bioaccessibility of metals in country foods is limited in that the extraction models assess foods in isolation. This design of measuring metal bioaccessibility for individual food items is convenient, if not a necessity, for typical exposure assessments. However, in reality, foods are generally eaten within mixtures (i.e. meals). Therefore, the accuracy of bioaccessibility adjustments for exposure assessments hinge on whether the bioaccessible fractions of metals in co-consumed foods are additive, less than additive, or more than additive.

The objective of this research was to characterize the impact of co-digesting binary country food mixtures on the in vitro bioaccessibility of Hg and/or Se. We focused on determining the effect of co-digestion on the in vitro bioaccessibility of two trace metals, Hg and Se, in Inuit country foods collected from Nunavik, Quebec. Each binary country food mixture tested consisted of a country food with elevated Hg concentrations (e.g. ringed seal liver, beluga nikku, raw beluga meat, walrus, lake trout, eider duck egg white) and a second food that may have a mitigating effect on Hg IVBA (e.g. crowberries, blueberries, seaweed, sculpin eggs and tomato paste). The potentially-protective foods (hereafter referred to as the "treatment country

food") included within the binary mixture treatments were selected based upon existing evidence pertaining to mercury-nutrient interactions. For example, sculpin eggs have exceptionally high Se:Hg ratios and many publications have explored the mitigating effect that Se has on Hg toxicity. Berries (i.e. crowberries and blueberries) and seaweed are high in phytochemicals which can modulate MeHg toxicity by radical scavenging and metal chelation, potentially affecting Hg absorption in the GI tract (Shim et al., 2009). Finally, the tomato paste was included because Passos et al., (2003, 2007) showed that fruit consumption can affect Hg exposure in Amazonian Indigenous communities while Gagné et al., (2013) demonstrated that tomato products were associated with lower Hg blood levels in Inuit children. We hypothesized that the presence of the treatment country food within the in vitro extraction model would decrease metal bioaccessibility in some foods. Additionally, we hypothesized that the significance and effect size of any impacts on mercury and/or selenium bioaccessibility would differ from one binary mixture to another.

5.2 Materials and Methods

5.2.1 Sample Preparation

Country foods were collected from 2008-2013 as part of the community-based sampling program overseen by the Nunavik Research Center of the Makivik Corporation. The bioaccessibility of Hg and Se was evaluated using the in vitro gastrointestinal model using previously described methods (Laird and Chan, 2013). Instead of digesting one country food at a time in the in vitro GI model, foods were digested in pairwise mixtures. The meats extracted (0.5 g) were beluga nikku, beluga meat (Hudson Strait; East Hudson Bay), ringed seal liver (Inukjuaq; Quartaq), ringed seal meat (Inukjuaq; Quartaq), walrus meat and blubber (Nunavik

coast), eider duck egg white, and Lake Trout (Lake Qamuttitsait). The meat samples were co-digested in pairwise treatments combinations with 0.25 g of crowberries (Karigiqsualujjuaq, 2012), blueberries (Karigiqsualujjuaq & Inukjuuq, 2012), seaweed (Kangiqsualujjuaq), sculpin eggs (Koksoak River), and store-bought tomato paste.

5.2.2 In vitro GI Model

Mercury and selenium in vitro bioaccessibility (IVBA) was evaluated using a two-stage in vitro GI model using previously described methods (Laird et al. 2013). The individual meat extractions were digested using the weights and volumes described in the previous section. The individual "treatment" extractions (i.e. for blueberries, crowberries, seaweed, tomato paste, sculpin eggs) are 0.5g of country food in 12.5mL of digestive solutions (i.e. 7.25 mL of gastric juice, 1.25 mL of concentrated sodium bicarbonate solution, 3.75 mL of duodenal solution) in order to test the additive effect of the metal bioaccessibility. For the co-digests, each of the meat (0.5 g) and the treatment (0.25g) country foods were added to 25 mL of simulated gastric/duodenal fluid, thus maintaining the liquid:solid ratios described in the previous section for the single digests. Specifically, simulated gastric fluid (7.5 mL) containing hydrochloric acid (OmniTrace®, pH 1.5), porcine pepsin (Sigma-Aldrich®, 6 g L^{-1}), and NaCl (8.5 g L^{-1}) in MilliQ water was added to glass serum bottles containing country foods samples and treatment foods. Subsequently, the suspension pH was adjusted to 1.5 ± 0.5 with hydrochloric acid (OmniTrace®, 0.5 M) and the extracts were then incubated at 37ºC with orbital shaking (180 rpm) for 2 h. Thereafter, $NaHCO_3$ (1 M; 1.25 mL) and simulated duodenal fluid (3.75 mL) containing NaHCO3 (12.5 g L^{-1}), dried oxgall bile (EMD Millipore®, 6.0 g L^{-1}), porcine pancreatin (Sigma-Aldrich®, 3.0 g L^{-1}), and NaCl (8.5 g L^{-1}) was added to each serum bottle. The extracts were then returned to the incubator shaker for an additional 3 h of shaking (180 rpm) at 37ºC. At the

conclusion of the simulated duodenal extraction, samples were centrifuged (10.3 x 10^4 g; 15 min) and then filtered (0.45 µm; PTFE membrane) using a vacuum filtration manifold. Each batch of samples processed through the in vitro GI procedure included blanks, duplicates, and standard reference materials. Subsamples of three replicate samples of each meat type were co-digested with the treatments – crowberries, blueberries, seaweed, tomato paste, and sculpin eggs. Each batch of co-digest samples contained blanks and DORM-4 controls to assess quality control during the in vitro extraction. In addition to analyzing fresh (i.e. raw) beluga meat, beluga nikku (traditionally made air-dried beluga meat) was also evaluated.

The co-digests were run on binary mixtures of Inuit country foods. Due to insufficient quantity for some sample types, not all co-digest treatment combinations could be completed. The binary mixtures tested using the above in vitro extraction protocol are summarized in Table

Table 3. Co-digest treatment combinations

		Treatment Country Food				
		BB	**CB**	**SW**	**SE**	**TO**
High Hg Meats	**BMAD**	**Y**	**Y**	**Y**	**N**	**Y**
	BMR	**Y**	**Y**	**Y**	**Y**	**Y**
	EDW	**Y**	**Y**	**Y**	**N**	**Y**
	LT	**Y**	**Y**	**Y**	**N**	**Y**
	RSL	**Y**	**Y**	**Y**	**Y**	**Y**
	RSM	**Y**	**Y**	**Y**	**N**	**N**
	WMB	**Y**	**Y**	**Y**	**N**	**Y**

Note: Y: we co-digested these two country foods together, N: we did not co-consume this combination of country foods together, BMAD: air dried beluga meat also known as beluga nikku; BMR: raw beluga meat; RSL: ringed seal liver; RSM: ringed seal

muscle; WMB: walrus meat and blubber; EDW: eider duck egg white; BB: blueberries, CB: crowberries, LT: lake trout; SW: seaweed; SE: sculpin eggs; TO: tomato paste

The samples were filtered using a FORTUNA® Optima glass syringes (either 30ml or 50ml) with a Millipore ® 0.45 μm PTFE syringe filter. Samples were filtered until 5-10ml of filtered sample was acquired.

5.2.3 Chemical Analysis

The filtered extract generated following the duodenal stage of the in vitro GI model (Section 4.2) was diluted in 2% HNO_3 prior to analysis for Hg and Se using a MA3000 (TDAAS) and an Agilent 7700x (Inductively Coupled Plasma Mass Spectrometry or ICPMS), respectively. Thereafter, the metal concentrations within the in vitro extracts were used to calculate the in vitro bioaccessibility of Hg and Se in the traditional foods.

For each batch of bioaccessibility extracts analyzed using the MA-3000, certified reference materials (DORM-2, DORM-3, DORM-4, and DOLT-4; National Research Council of Canada) were used as procedural and instrumental controls. The recovery of the analyte to within 10% of its certified value is used as a criterion for validation of the batch (recommended by US EPA). Cases in which the signals for the blanks were higher than the absorbance corresponding to the detection limit of the analyte invalidated the batch. The two measurements taken for the digested sample was only accepted if the relative standard deviation was less than 10%. Instrumental controls and blanks for Se on the ICP-MS were administered by the lab technician and used spiked solutions of varying concentrations.

5.2.4 Statistical Analysis for Co-digest Studies

For each country food type, descriptive analyses were used to report the bioaccessible concentration of each of the country foods and treatments and bioaccessible percentages. The bioaccessibility percentage values represent the arithmetic mean of two or more samples originating from two or more organisms. If the filtered analyte was below the detection, the concentration was recorded as half the limit of detection. For the sum of the IVBA concentrations for the individually digested meat and treatment country foods, the error was propagated. We also categorized whether the co-digested foods when compared to the sum of the foods digested separately were less than additive, additive, or more than additive.

5.3 Results and Discussion

Table 4. Co-digest results for Hg (Mean ± Stdev) and Se (Mean ± Stdev) with treatment interaction type (µg/L or ppb, w.w.)

	Description	n	Hg (ppb ± Stdev)	Interaction Type	Se (ppb ± Stdev)	Interaction Type
Beluga nikku	BMAD;BB BMAD+BB	4	61.4 ± 21.7 57.5 ± 24.8	**Additive**	36.7 ± 16.1 36.1 ± 9.63	**Additive**
	BMAD;CB BMAD+CB	4	53.1 ± 23 57.7 ± 24.8	**Additive**	34 ± 13.6 36.1 ± 9.63	**Additive**
	BMAD;SW BMAD+SW	4	62.7 ± 23.8 57.5 ± 24.8	**Additive**	34.4 ± 13.8 36.1 ± 9.63	**Additive**
	BMAD;TO BMAD+TO	3	64.2 ± 36 57.7 ± 24.8	**Additive**	23.2 ± 9.61 37.6 ± 9.72	**<Additive**
Eider duck egg white	EDW;BB EDW+BB	3	24.8 ± 7.81 22.6 ± 7.18	**Additive**	14.2 ± 10.6 21.4 ± 7.2	**<Additive**
	EDW;CB EDW+CB	3	24.6 ± 7.59 22.8 ± 7.2	**Additive**	11.5 ± 3.69 21.4 ± 7.21	**<Additive**
	EDW;SW EDW+SW	3	26 ± 9.6 22.6 ± 7.18	**Additive**	7.05 ± 4.93 21.4 ± 7.21	**<Additive**
	EDW;TO EDW+TO	3	25.7 ± 7.5 22.8 ± 7.2	**Additive**	13.2 ± 8.58 22.8 ± 7.32	**<Additive**
Lake Trout	LT;BB LT+BB	3	58.1 ± 18.2 57.1 ± 15.2	**Additive**	5.44 ± 1.37 9.74 ± 2.86	**<Additive**
	LT;CB LT+CB	3	55.1 ± 19.9 57.4 ± 15.2	**Additive**	5.89 ± 2.16 9.74 ± 2.88	**<Additive**
	LT;SW LT+SW	3	44.9 ± 16.2 57.1 ± 15.2	**Additive**	5.58 ± 2.24 9.74 ± 2.87	**<Additive**
	LT;TO LT+TO	3	34.2 ± 8.81 57.4 ± 15.2	**<Additive**	9.67 ± 3.66 11.2 ± 3.15	**Additive**
Walrus meat and blubber	WMB;BB WMB+BB	3	2.31 ± 0.615 1.49 ± 0.746	**>Additive**	31 ± 5.85 35.6 ± 4.87	**Additive**
	WMB;CB WMB+CB	3	2.03 ± 0.566 1.76 ± 0.89	**Additive**	26.2 ± 6.39 35.6 ± 4.88	**<Additive**
	WMB;SW WMB+SW	3	2.48 ± 0.707 1.49 ± 0.746	**>Additive**	26.3 ± 13.2 35.6 ± 4.88	**<Additive**
	WMB;TO WMB+TO	3	1.87 ± 0.662 1.76 ± 0.89	**Additive**	24.3 ± 1.88 37 ± 5.04	**<Additive**

Table 4. Continued

	Description	n	Hg (ppb± Stdev)	Interaction Type	Se (ppb± Stdev)	Interaction Type
Beluga meat (raw)	BMR;BB BMR+BB	3	41 ± 20.9 35 ± 15.9	**Additive**	28.6 ± 8.6 27.2 ± 4.63	**Additive**
	BMR;CB BMR+CB	3	39 ± 15.7 35.3 ± 15.9	**Additive**	27.7 ± 6.15 27.2 ± 4.64	**Additive**
	BMR;SW BMR+SW	3	35 ± 14.1 35 ± 15.9	**Additive**	27.6 ± 4.71 27.2 ± 4.64	**Additive**
	BMR;SE BMR+SE	3	42.2 ± 15.5 35.3 ± 15.9	**Additive**	43.6 ± 0.749 63.3 ± 9.89	**<Additive**
	BMR;TO BMR+TO	3	44.8 ± 18.4 35.3 ± 15.9	**>Additive**	15.9 ± 1.52 28.7 ± 4.81	**<Additive**
Ringed seal liver	RSL;BB RSL+BB	3	78.8 ± 16.1 100 ± 17.2	**Additive**	211 ± 28.1 159 ± 30.1	**>Additive**
	RSL;CB RSL+CB	3	89.2 ± 16.4 101 ± 17.2	**Additive**	197 ± 35.2 159 ± 30.1	**>Additive**
	RSL;SW RSL+SW	3	94 ± 21.9 100 ± 17.2	**Additive**	230 ± 30 159 ± 30.1	**>Additive**
	RSL;SE RSL+SE	3	67.8 ± 15.6 101 ± 17.2	**<Additive**	167 ± 15.2 195 ± 31.3	**Additive**
	RSL;TO RSL+TO	3	88.5 ± 14.2 101 ± 17.2	**Additive**	156 ± 9.06 160 ± 30.1	**Additive**
Ringed seal muscle	RSM;BB RSM+BB	3	16.2 ± 14.7 12.3 ± 10.2	**>Additive**	36 ± 4.98 23 ± 4.47	**>Additive**
	RSM;CB RSM+CB	3	18.5 ± 16.2 12.6 ± 10.2	**>Additive**	33.5 ± 5.59 23 ± 4.48	**>Additive**
	RSM;SW RSM+SW	3	18 ± 18.5 12.3 ± 10.2	**>Additive**	34.7 ± 8.24 23 ± 4.48	**>Additive**

Note 1: Below the limit of detection (these concentrations are LOD/2)
Note 2: BMAD: air dried beluga meat also known as beluga nikku; BMR: raw beluga meat; RSL: ringed seal liver; RSM: ringed seal muscle; WMB: walrus meat and blubber; EDW: eider duck egg white; BB: blueberries, CB: crowberries, LT: lake trout; SW: seaweed; SE: sculpin eggs; TO: tomato paste
Note 3: The ";" represents co-digested samples and "+" represents the sum of the country foods indicated digested separately
Note: 4: "<Additive": less than additive or "subadditive" interaction by <25% of the sum of the IVBA for the country foods individually, "Additive": the co-digested sample is ±25% of the sum of the single digest, ">Additive" ": more than additive or "superadditive" interaction OR synergistic effect by >25% of the sum of the IVBA for the country foods individually
Note 5: n refers to the number of different country food sample IDs studied
Note 6: The limit of detection was 0.5 ppb (LC ICP-MS) for Se concentrations and the 0.01 ppb (NIC Mercury Analyzer 3000) for the Hg concentration

The in vitro gastrointestinal model, which simulates the major digestion that occurs in the stomach and the duodenum, allowed us to determine the in vitro bioaccessibility of Hg and Se. This allowed us to test and quantify the effect of co-consumption of high Hg country foods with other local foods like wild berries, sculpin eggs, seaweed and tomato paste. As shown in Table 4, co-digestion may affect the bioaccessibility of Hg and Se in country foods, either increasing or decreasing metal bioaccessibility. Prior research has shown that co-digesting nutrients can have an impact on MeHg toxicity (Beyrouty and Chan, 2006).

Table 4 characterizes the effect of co-digests on the bioaccessibility of Hg and Se from Inuit country foods. As shown in Table 4, although bioaccessible Hg concentrations in each of the food modifiers were below the limit of detection (Table S2), the addition of the treatment country food in the co-digest appeared to occasionally increase the solubilization of mercury from some country foods (e.g. walrus meat/blubber; beluga meat; ringed seal meat). For example, the addition of berries and seaweed each appeared to increase the solubilization of mercury from ringed seal meat (Table 4). Similarly, the bioaccessible fractions of: beluga meat and tomato paste as well as blueberries and walrus meat/blubber both appeared greater than additive. In contrast, the co-digest of sculpin eggs appeared to decrease the solubilization of Hg from ringed seal liver while tomato paste may have decreased the solubilization of Hg from lake trout. Notably, none of the food modifiers consistently lowered Hg bioaccessibility from all of the tested country foods. Of the pair of mixtures (Lake Trout + tomato paste; ringed seal meat + sculpin eggs) that demonstrated sub- additivity in mercury bioaccessibility, neither demonstrated sub-additivity in Se bioaccessibility. Therefore, the prospect of decreasing mercury bioaccessibility in the gastrointestinal tract through selenium complexation following the co-digestion of selenium-rich traditional foods appears unlikely.

Unexpectedly, the plurality of binary mixtures showed less than additivity in terms of Se bioaccessibility. Accordingly, more often than not, the presence of a food modifier within the in vitro extraction fluid decreased the Se bioaccessibility of country foods (Table 4). For example, food modifiers decreased Se bioaccessibility between 1.4- (tomato paste) and 3-fold (seaweed). The effect seaweed has on some country foods is not surprising since Wang et al., (2009) showed that the phytochemicals found in seaweed can contribute to metal chelation. In contrast, although bioaccessible Se concentrations in berries and seaweed were below the limit of detection (Table S2), the addition of blueberries, crowberries, and seaweed increased the solubilization of Se from both ringed seal meat and ringed seal liver. For ringed seal meat (but not ringed seal liver), the addition of berries and seaweed both increased Se bioaccessibility and Hg bioaccessibility.

Sculpin eggs have high levels of total and bioaccessible selenium (Se), an essential nutrient. Recent scientific publications have shown that nutrients, like Se, can mitigate the toxicity of Hg. For our study, because of insufficient sculpin egg quantities in the sample archive, only beluga meat and ringed seal liver were co-digested with sculpin eggs in the in vitro extraction model. For these treatments, the bioaccessible fractions of Hg were less than additive for ringed seal liver while the bioaccessible fraction of Se were less than additive for beluga meat.

Although Se levels in blueberries and crowberries are below detection limits (Table S2), they are rich in phytochemicals that may be able to provide some degree of protection against the adverse effects of MeHg. For example, in vitro studies conducted by Black et al., (2011) have shown that teas rich in phytochemicals are able to modulate some of MeHg's effects on molecular endpoints (such as liver cytochrome activation and lipid peroxidation due to oxidative stress). However, these berries did not affect Hg or Se IVBA in any consistent manner.

Therefore, there does not seem to be a potential presence on mercury – nutrient interactions from berries.

Experimental and epidemiological research has highlighted the potentially protective effect of fruit consumption on Hg exposure in the Brazilian Amazon (Passos et al., 2003, 2007). This work suggested that the soluble dietary content as well as prebiotic nutrients may interfere with MeHg absorption in the intestine (Gagné et al., 2013; Passos et al., 2003, 2007). Similarly, Gagné et al., (2013) found that consumption of tomato products is associated with lower blood Hg levels in Inuit preschool aged children. They also found that the annual consumption of seal meat and tomato products were significant predictors (among others) of blood Hg levels compared with beluga muktuk, walrus, Arctic char and caribou meat were not (Gagné et al., 2013). Our results show that tomato paste may decrease the bioaccessibility of Hg from country foods; however, the magnitude of this effect appears to differ between dietary mercury sources (e.g. Lake Trout vs. ringed seal liver). Future work will be necessary to determine the underlying basis between these differences in the effect of tomato constituents between dietary mercury sources. Also, further research is necessary to determine whether these effects on bioaccessibility translate to differences in mercury bioavailability.

5.4 Conclusion

Our results indicate there is some differences to IVBA Hg and Se bioaccessibility when co-digesting country foods with different potentially nutritious treatments compared with digesting the country foods separately. The country food treatments affected the Se IVBA more than the Hg IVBA in these high Hg country foods. None of these food modifiers appear to be particularly effective at decreasing Hg bioaccessibility but, this doesn't necessarily mean that there are not other factors that alter metal bioaccessibility. In fact, the food modifiers seem to have more of an impact on lowering Se bioaccessibility. Differences in bioaccessibility do not necessarily mean that bioavailability would be impacted. The use of in vivo models may provide a more robust indication of nutrient-mercury interactions as well as confirm the results of the in vitro GI model. Although food modifiers occasionally appeared to alter metal bioaccessibility, the effect sizes were relatively small. This suggests that using the Hg and Se bioaccessibilities determined from single digests is appropriate for risk assessments. This is valuable information because it shoes that attempting to use mixture-based bioaccessibilities, which would be challenging to incorporate into risk assessments, would have limited impact on exposure characterizations.

6. Thesis Conclusions

As mentioned before, this thesis project is part of a much larger interdisciplinary project investigating the effects of country foods on cardiometabolic disease in Inuit adults from Nunavik. My thesis project focuses on determining the Se and Hg concentrations and in vitro bioaccessibility (i.e. single and co-digested) as part of this larger project. The results of my project provide more information on the internal dose of Hg and Se from these country foods. Through the generation of bioaccessibility data, my research provides information that may help improve the accuracy of dose reconstruction, exposure modeling and more pragmatic applications in risk messaging (i.e., encouraging co-digestion of high Se and high Hg foods) by providing bioaccessibility data. The in vitro model described in this thesis is a fast and economical procedure. Our research has utilized a pre-existing sample archive and shown that these archives can facilitate research projects that assist in future public health decision making. The participatory nature of the sample collection phase helps ground my research within the principles of ownership, control, access, and possession (OCAP). Building ties with the community is vital for continuing this type of research.

This thesis focuses on the bioaccessibility of Hg and Se for country foods that have been digested separately and co-digested. Section 4 showed that the total concentrations for HgT and SeT varied quite a bit between different country foods. The results also showed a large variation in IVBA Hg (1.4 - 90%) and IVBA Se (29 - 108%). We also found that after accounting for bioaccessibility, the Se:Hg molar ratios for the majority of country foods were greater than one. Consequently, neglecting to account for bioaccessibility appears to systematically underestimate

the Se:Hg molar ratio in foods. Section 5 focused on the effect of the co-digestion on IVBA Hg and IVBA Se. Interestingly, there are greater differences in the Se IVBA compared with Hg IBVA when co-digested. None of these food modifiers seem to be particularly effective at decreasing Hg bioaccessibility. This may indicate that it is not a feasible avenue to pursue co-digestion studies such as this one for other country foods. It seems as though single digest provide enough information to refine exposure assessments. However, further results will allow for more accuracy in exposure modeling and risk assessments, which in turn could improve Hg risk management and communication among Inuit.

The in vitro model used for this project has numerous benefits however it also has some limitations. This model focuses on the food breakdown that occurs in the stomach and duodenum and does not account for digestion that occurs before or after. Although, saliva contains amylase to break down carbohydrates, the whole process is short and significant compound dissolution from food samples is not expected at this stage (Intawongse and Dean, 2006). Nor does it account for the digestion that occurs via the gut microbiota. Also, in vitro GI extraction has not been validated with an in vivo model to confirm the bioaccessibility results. Using an animal model will allow us to determine if our in vitro model estimates biological conditions with accuracy and precision. By looking at more high mercury country foods and their potential interactions, we can confirm our current results and increase the statistical power of our study.

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Appendix

Table S1. Average % IVBA for Hg and Se in the 18 Inuit country foods collected in Nunavik, QC

Traditional Foods	n	IVBA Hg [a]	IVBA Se [b,c]
Marine Mammals			
Beluga nikku	3	32.3 ± 2.05	79.7 ± 1.62
Beluga meat	7	50.7 ± 4.74	87.3 ± 8.08
Beluga mattaaq	15	22.2 ± 2.65	60.3 ± 3.15
Ringed seal liver	9	31.1 ± 3.65	37 ± 6.07
Ringed seal meat	3	89.7 ± 5.47	104 ± 3.4
Walrus meat & blubber	2	25.1 ± 1.18	63.4 ± 7.65
Fish & Shellfish			
Arctic char	3	9.85 ± 0.706	61.2 ± 11.1
Atlantic salmon	6	33.4 ± 5.32	106 ± 7.42
Brook trout	3	25.2 ± 0.546	72.7 ± 9.95
Lake Trout	2	56.7 ± 20.5	56.7 ± 31.5
Lake whitefish	3	42.4 ± 8.62	55.8 ± 9.26
Shorthorn Sculpin	3	25.2 ± 9.72	46.2 ± 6.06
Shorthorn Sculpin (eggs)	3	31.5 ± 6.07	65.5 ± 13.2
Blue mussel	3	42.8 ± 24.8	70.2 ± 17.8
Wild Game			
Caribou meat	6	39.9 ± 4	108 ± 12.9
Eider duck egg (yolk)	3	24.8 ± 1.53	29.6 ± 14.9
Eider duck egg (white)	3	67.8 ± 0.773	47.5 ± 2.48
Canada goose egg (whole)	3	1.36 ± 0.0162	53.8 ± 20.7

[a] The Hg IVBA fraction for blue mussel, and total Hg and the Hg IVBA fraction for Canada goose eggs and sculpin eggs were below the detection limit.

[b] The Se IVBA fraction for eider duck egg yolk was below the detection limit.

Note: The limit of detection was 0.5 ppb (LC ICP-MS) for Se concentrations and the 0.01 ppb (NIC Mercury Analyzer 3000) for the Hg concentration

Table S1A. Average % IVBA for Hg and Se (with Stdev) in the 18 Inuit country foods collected in Nunavik, QC

		IVBA Hg [a]		IVBA Se [b,c]	
Country Foods	**n**	**Avg**	**Stdev**	**Avg**	**Stdev**
Arctic char	3	9.85	1.22	61.16	19.27
Atlantic salmon	6	33.40	13.03	106.14	18.18
Beluga (air-dried)	3	32.31	3.55	79.69	2.80
Beluga meat	7	50.71	12.53	87.25	21.38
Beluga muktuk	15	22.15	10.27	60.34	12.21
Blue mussel	3	42.85	43.02	70.17	30.89
Brook trout	3	25.20	0.95	72.66	17.23
Caribou	6	39.95	9.79	108.10	31.48
Lake trout	2	56.68	29.01	56.68	44.50
Lake whitefish	3	42.43	14.92	55.82	16.04
Ringed seal liver	9	31.07	10.94	37.01	18.20
Ringed seal meat	6	89.74	13.40	104.13	8.32
Shorthorn sculpin	3	25.22	16.83	46.23	10.50
Shorthorn sculpin (eggs)	3	31.51	10.52	65.46	22.82
Walrus meat & blubber	3	25.11	1.68	63.41	2.95
Eider duck (egg yolk)	3	24.80	2.64	29.63	22.52
Eider duck (egg white)	3	67.82	1.34	47.46	1.93
Canada goose (whole)	3	1.36	0.03	53.75	2.42

[a] The Hg IVBA fraction for blue mussel, and total Hg and the Hg IVBA fraction for Canada goose eggs and sculpin eggs were below the detection limit.

[b] The Se IVBA fraction for eider duck egg yolk was below the detection limit.

Note: The limit of detection was 0.5 ppb (LC ICP-MS) for Se concentrations and the 0.01 ppb (NIC Mercury Analyzer 3000) for the Hg concentration

Table S2. Bioaccessible Hg and Se concentrations of country food items digested separately (in µg kg^{-1},w.w.)

Country food	n	Subsamples	Hg (ppb)	Se (ppb)
BB	1	3	0 ± 0	0.0075 ± 0.142
CB	1	3	0.277 ± 0.479	0.0075 ± 0.313
SW	1	3	0 ± 0	0.0075 ± 0.286
SE	1	3	0.277 ± 0.479	36.1 ± 8.74
TO	1	3	0.277 ± 0.479	1.45 ± 1.31
BMAD	4	11	57.5 ± 24.8	36.1 ± 9.63
BMR	3	6	35 ± 15.9	27.2 ± 4.63
RSL	3	6	100 ± 17.2	159 ± 30.1
RSM	3	6	12.3 ± 10.2	23 ± 4.47
WMB	3	9	1.49 ± 0.746	35.6 ± 4.87
EDW	3	9	22.6 ± 7.18	21.4 ± 7.2
LT	3	9	57.1 ± 15.2	9.73 ± 2.86

Note 1: Below the limit of detection (these concentrations are LOD/2)

Note 2: BMAD: air dried beluga meat also known as beluga nikku; BMR: raw beluga meat; RSL: ringed seal liver; RSM: ringed seal muscle; WMB: walrus meat and blubber; EDW: eider duck egg white; BB: blueberries, CB: crowberries, LT: lake trout; SW: seaweed; SE: sculpin eggs; TO: tomato paste

Note 3: The limit of detection was 0.5 ppb (LC ICP-MS) for Se concentrations and the 0.01 ppb (NIC Mercury Analyzer 3000) for the Hg concentration

Table S3. Bioaccessible Hg and Se concentrations of country food items co-consumed with treatment country foods (in µg kg-1,w.w.)

Treatment Group	n	Subsamples	Hg (ppb)	Se (ppb)
BMAD + BB	4	6	61.4 ± 21.7	36.7 ± 16.1
BMAD + CB	4	6	53.1 ± 23	34 ± 13.6
BMAD + SW	4	6	62.7 ± 23.8	34.4 ± 13.8
BMAD + TO	3	3	64.2 ± 36	23.2 ± 9.61
BMR + BB	3	7	41 ± 20.9	28.6 ± 8.6
BMR + CB	3	7	39 ± 15.7	27.7 ± 6.15
BMR + SW	3	7	35 ± 14.1	27.6 ± 4.71
BMR + SE	3	3	42.2 ± 15.5	43.6 ± 0.749
BMR + TO	3	3	44.8 ± 18.4	15.9 ± 1.52
RSL + BB	3	6	78.8 ± 16.1	211 ± 28.1
RSL + CB	3	6	89.2 ± 16.4	197 ± 35.2
RSL + SW	3	6	94 ± 21.9	230 ± 30
RSL + SE	3	3	67.8 ± 15.6	167 ± 15.2
RSL + TO	3	3	88.5 ± 14.2	156 ± 9.06
RSM + BB	3	6	16.2 ± 14.7	36 ± 4.98
RSM + CB	3	6	18.5 ± 16.2	33.5 ± 5.59
RSM + SW	3	6	18 ± 18.5	34.7 ± 8.24
WMB + BB	3	3	2.31 ± 0.615	31 ± 5.85
WMB + CB	3	3	2.03 ± 0.566	26.2 ± 6.39
WMB + SW	3	3	2.48 ± 0.707	26.3 ± 13.2
WMB + TO	3	3	1.87 ± 0.662	24.3 ± 1.88
EDW + BB	3	3	24.8 ± 7.81	14.2 ± 10.6
EDW + CB	3	3	24.6 ± 7.59	11.5 ± 3.69
EDW + SW	3	3	26 ± 9.6	7.05 ± 4.93
EDW + TO	3	3	25.7 ± 7.5	13.2 ± 8.58
LT + BB	3	3	58.1 ± 18.2	5.44 ± 1.37
LT + CB	3	3	55.1 ± 19.9	5.89 ± 2.16
LT + SW	3	3	44.9 ± 16.2	5.58 ± 2.24
LT + TO	3	3	34.2 ± 8.81	9.67 ± 3.66

Note 1: Below the limit of detection (these concentrations are LOD/2)

Note 2: BMAD: air dried beluga meat also known as beluga nikku; BMR: raw beluga meat; RSL: ringed seal liver; RSM: ringed seal muscle; WMB: walrus meat and blubber; EDW: eider duck egg white; BB: blueberries, CB: crowberries, LT: lake trout; SW: seaweed; SE: sculpin eggs; TO: tomato paste

Note 3: The limit of detection was 0.5 ppb (LC ICP-MS) for Se concentrations and the 0.01 ppb (NIC Mercury Analyzer 3000) for the Hg concentration