Assessing Mercury Risks for the Optimization of Nutrient Benefits from Wild-harvested Fish Consumption in the Northwest Territories, Canada

by

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A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Science
in
Health Studies and Gerontology

Waterloo, Ontario, Canada, 2016

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 presents the work of Ellen Stephanie Reyes in collaboration with her advisor, Dr. Brian Laird. I would like to acknowledge the contributions of each co-author, in addition to my supervisor providing guidance throughout each stage of the project.

Chapter 4

Chapter 4 is co-authored with Juan J. Aristizabal Henao, Katherine M. Kornobis, Dr. Rhona M. Hanning, Dr. Shannon E. Majowicz, Dr. Karsten Liber, Dr. Ken D. Stark, George Low, Dr. Heidi K. Swanson, and Dr. Brian D. Laird. Juan Aristizabal Henao and Dr. Stark assisted with the technical support for the omega-3 fatty acid analysis. Katherine Kornobis assisted with the technical support for the mercury analysis. Dr. Hanning and Dr. Majowicz provided valuable guidance during the study design. Dr. Liber assisted with the technical support for the selenium analysis. George Low provided guidance with the data collection. Dr. Swanson provided guidance with the data collection, study design, and technical support for the mercury analysis. Dr. Laird provided ongoing guidance and supervision of the data collection, study design, and analysis. All co-authors reviewed the final manuscript and provided editorial advice before submission for peer-reviewed publication.

Citation: Reyes, E.S., Henao Aristizabal, J.J., Kornobis, K.M., Liber, K., Stark, K.D., Lowe, G., Swanson, H.K., & Laird, B.D. (2015). Associations Between Omega-3 fatty Acids, Selenium Content, and Mercury Levels in Wild-harvested Fish from the Dehcho Region, Northwest Territories, Canada. *Environmental Research.* (Under Review).

Chapter 5

Chapter 5 is co-authored with Dr. Brian D. Laird. Dr. Laird provided ongoing guidance and supervision of the study design and data analysis. All co-authors will review the final manuscript and provide editorial advice.

Citation: Reyes, E.S., & Laird, B.D. (2015). Maximizing Nutrient Intake and Limiting Mercury Exposure of those Consuming Wild-harvested Fish in the Dehcho Region of the Northwest Territories, Canada by Probabilistic Modelling. (Under Preparation).

Abstract

Background: Fish are often rich in essential nutrients, such as omega-3 fatty acids (*n*-3 FAs) and selenium (Se), and thus can promote health. However, methylmercury (MeHg), a contaminant found in many species in the Canadian subarctic, can pose potential health risks. Fish consumption is important to traditional diets because these foods have nutritional, social, and cultural benefits.

Objectives: The objectives for this study are to: 1) determine the concentrations of mercury (Hg), *n*-3 FAs, and Se in various freshwater fish species harvested from three lakes in the Dehcho Region, Northwest Territories (NWT); 2) evaluate the correlations between nutrient and Hg concentrations; 3) identify which fish species have the highest nutrient levels relative to their Hg content; 4) utilize a probabilistic optimization software (Crystal Ball's OptQuest) to inform dietary recommendations that mitigate risks of Hg exposure and promote human health from traditional food consumption.

Methods: Samples from seven freshwater fish species [Burbot (*Lota lota*), Cisco (*Coregonus artedi*), Lake Trout (*Salvelinus namaycush*), Lake Whitefish (*Coregonus clupeaformis*), Longnose Sucker (*Catostomus catostomus*), Northern Pike (*Esox Lucius*), and Walleye samples (*Sander vitreus*)] were harvested in August 2013 from Ekali, Sanguez, and Trout Lakes in the NWT. The laboratory analysis for Hg involved freeze drying the tissue muscle samples prior to analysis and quantifying Hg in the samples by a direct mercury analyzer; *n*-3 FAs levels were determined by a lipid extraction on pulverized fish tissue and measured by a gas chromatograph with a flame ionization detector; and Se levels were determined by a tissue digestion and measured by an inductively coupled plasma mass spectrophotometer. Thereafter, a probabilistic optimization method was assessed using OptQuest, a feature in Crystal Ball (Oracle).

Results: The average total Hg (HgT) concentrations varied among fish species according to their trophic guild, from 0.057 mg kg^{-1} for benthivorous/planktivorous species to 0.551 mg kg^{-1} for predatory species. There were also substantial differences in *n*-3 FA composition among fish species, with averages ranging from 101 mg/100 g for Burbot to 1,689 mg/100 g for Lake Trout. However, in contrast to the HgT and *n*-3 FA results, average Se concentrations were relatively similar among species, ranging from 0.140 mg kg^{-1} for Northern Pike to 0.195 mg kg^{-1} for Walleye. Strong, negative correlations were observed in HgT concentration and nutrient content in the muscle tissue in particular fish species, including Lake Trout, Northern Pike, Walleye, Lake Whitefish, and Cisco. These analyses indicated that Lake Whitefish, Cisco, and Longnose Sucker had the highest nutrient levels relative to their HgT content. For the OptQuest probabilistic model, the term best solutions refers to the optimum food choices that maximize nutrient intake, while also limiting the upper percentile of Hg exposure from the consumption of freshwater fish. The total amount of fish within the best solutions from the OptQuest model for the women of child-bearing age were 546 and 1,359 g/week to achieve nutritional adequacy for EPA+DHA and Se, respectively, while also limiting the upper percentile for the toxicological reference values (TRVs) for Hg exposure. Whereas, the total amount of fish within the best solutions for the general population age were 851 and 1,848 g/week, to achieve nutritional adequacy for EPA+DHA and Se, respectively, while also minimizing Hg exposure. The models indicated that the consumption of Burbot, Cisco, Lake Whitefish, and Longnose Suckers would most help people achieve nutritional adequacy without exceeding the TRVs for Hg.

Conclusion: Some fish species are particularly rich sources of nutrients; however, Hg concentrations occasionally exceed Health Canada's retail Hg guidelines. My research shows that people can frequently consume fish that are lower in the food chain without exceeding the Hg TRV. Larger, long-lived predatory fish species also offered nutritional benefits in terms of *n*-3 FAs and Se; however, some limits on intake are necessary to avoid the adverse effects of Hg. The nutrient:Hg ratios and OptQuest model approaches that were utilized to determine the optimal food choices that achieve nutritional sufficiency, while not exceeding the Hg TRV yielded different answers. Future research will be necessary to determine which of these approaches, if either, yield the most useful information for promoting healthy eating in ways that balance nutrient benefits and contaminant risks.

Acknowledgements

First, I would like to thank my supervisor, Dr. Brian Laird, for his guidance, support, and encouragement during my Master's degree at the University of Waterloo. I am grateful for the opportunities I had to conduct current environmental public health and toxicology research as well as the amazing opportunities I had to present my projects at conferences and to submit my manuscript for publication before I completed my degree. I would like to extend my gratitude to my committee members, Dr. Rhona Hanning, Dr. Shannon Majowicz, and Dr. Heidi Swanson, for providing insightful ideas and feedback and George Low for designing the "Return to Country Foods Project". I would also like to thank my former supervisors, Dr. Eric Liberda at Ryerson University and Dr. Leonard J.S. Tsuji at the University of Toronto for the opportunity to work with them on a health research project during my undergraduate degree at Ryerson University.

I would like to acknowledge the funding I received through the School of Public Health and Health Systems at the University of Waterloo. My research was supported by the Northern Contaminants Program, which is jointly funded by Aboriginal Affairs and Northern Development Canada and Health Canada. Further funding supporting this research was obtained through the Aboriginal Aquatic Resources and Oceans Management and Dr. Heidi Swanson's and George Low's research grant applications to Health Canada's Climate Change Adaptation Fund and the Cumulative Impacts Monitoring Program.

Lastly, I would like to thank my friends and family for their support during my graduate studies. My mom deserves a lot of credit for supporting my goal with furthering my education. I would also like to thank my immediate family, my Dad, Esther, Dan, Justin, and Lallaine for their love and encouragement. Also, my friends, Raizza, Eshan, Ellie, and Edwin, in addition to all of my friends that I made during my time at Waterloo, Jessica, Sierra, Mylène, Leah, Matt, Gobi, Sarah, Sonja, Katelyn, Sara, Diana, Mike, Kyle, and Sebastian.

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1. Introduction

Elemental mercury (Hg^0) is a naturally occurring, non-essential heavy metal that is distributed throughout the world by long-range atmospheric transport following emission from anthropogenic and as natural sources (Jæger et al., 2009; Li et al., 2009). Mercury compounds are among the most important ubiquitous, environmental contaminants that potentially cause neurotoxic effects in both humans and animals (Farina et al., 2011; Fernandes Azevedo et al., 2012; Mahaffey, 2005). Methylmercury (MeHg), an organic form of Hg, has the ability to pass through the blood-brain barrier, which means it can accumulate in the central nervous system (CNS) (Aschner & Aschner, 1990; Auger et al, 2005). Exposure to MeHg can cause neurological symptoms, such as motor disturbance (especially weakness and spasm), incoordination, auditory disturbances, and tremor (Aschner & Aschner, 1990; Auger et al, 2005). In addition, many epidemiological studies document Hg's neurodevelopmental risks for infants and children, including cerebral palsy (Harada, 1995), delayed psychomotor or mental performance, and reduced birth weight and length (Ramon et al., 2009). These adverse effects have been noted in the infants of asymptomatic mothers (Harada et al., 2011); therefore, it is widely held that developmental neurotoxicity is the most sensitive health endpoint, and thus this outcome is used as a basis for Hg guidelines and public health policies (Legrand et al., 2010; Mergler et al., 2007). It is presumed that, by protecting this most sensitive health endpoint, Hg guidelines will also protect against cardiovascular and immune system effects (Roman et al., 2011; Shenker et al., 1998).

The Arctic and subarctic locations in Canada are a sink for Hg (Ariya et al., 2004). As a result of atmospheric cycling described by Selin (2014), inorganic Hg enters the aquatic ecosystem and converts to MeHg via methylation by sulfate-reducing bacteria (Clarkson et al., 2003; King et al., 2000). Hg is a persistent, toxic pollutant, and can bioaccumulate in muscle tissues of animal species and biomagnify in aquatic ecosystems (Ariya et al., 2004; Ayotte et al., 1995). Fish consumption is the primary exposure route for MeHg in most human populations worldwide (Mergler et al., 2007). Aboriginal populations living in Arctic and subarctic locations in Canada have high exposure to MeHg because they frequently consume traditional foods from among the highest trophic levels of the Arctic food web (Ayotte et al., 1995). These foods play a critical role in the social, cultural, spiritual, economic, and nutritional well-being in many Aboriginal communities; these benefits must be considered alongside the potential health risks of Hg exposure from traditional food consumption (Oostdam et al., 2005). The social and cultural impacts of Hg pollution in Canadian Aboriginal communities include disruption of lifestyle, changes in diet and eating patterns, and severe economic damage (Wheatley & Wheatley, 2000; Wheatley, 1997). Additionally, the research of Egeland & Middaugh (1997), Mahaffey (2004), and Mahaffey et al. (2011) has demonstrated that nutrients, such as omega 3-fatty acids (*n*-3 FAs) and selenium (Se) found in fish may have a protective effects against Hg toxicity. Thus, it is imperative that public health interventions related to Hg exposure are designed to maximize the benefits of *n*-3 FA and Se intake while minimizing Hg exposure (Egeland & Middaugh, 1997).

My thesis project investigated dietary Hg exposure as well as nutrient intake among First Nation communities in the Dehcho Region of the Northwest Territories (NWT). This work will relied on a dose-reconstruction approach that integrates food consumption and composition data (Slob, 2006). The muscle portion of each fish sample was analyzed for total Hg by a direct mercury analyzer, *n*-3 FAs by a gas chromatograph with flame ionization detection (GC-FID), and total Se by inductively coupled plasma mass spectrometer (ICP-MS). Food composition and consumption data was integrated using Crystal Ball's OptQuest to identify fish species and their quantities that maximize nutrient intake while also minimizing Hg exposure.

The goal of this research is to provide data that can be used in risk communication strategies that promote traditional food use as a pathway to health equity and help improve the health and well-being of First Nations in the Dehcho, NWT. Although the data generated by this project is specific to the Dehcho Region, the research tools and participatory approaches developed during this project can provide a foundation for finding solutions to similar risk-benefit dilemmas faced by Aboriginal and non-Aboriginal Canadians alike.

2. Study Rationale

2.1. Mercury in the Freshwater Environment of the Northwest Territories, Canada

From 1999-2002, freshwater research undertaken by scientists funded through the Northern Contaminants Program (NCP) was conducted on freshwater biota of Canada's Arctic and Subarctic regions (e.g., Evans et al., 2005). The chemicals of concern studied included organic contaminants [e.g. polychlorinated biphenyls (PCBs) and toxaphene] and naturally-occurring heavy metals (e.g. Hg) (Braune et al., 1999; Evans et al., 2005). The results from this environmental monitoring research indicated that several lakes in the NWT have fish populations with mean concentrations of Hg that exceed Health Canada's Hg guideline limits (Braune et al., 1999).

The strategy to reduce potential health risks of exposure to Hg from fish consumption was first implemented in Canada in the late 1960s when Health Canada established a standard for Hg concentrations in fish (Health Canada, 2007). Since this time, the risk management approach has been periodically updated (Health Canada, 2007). The guideline limits set by Health Canada are 0.5 part per million (ppm; wet weight) total Hg in retail fish and 0.2 ppm wet weight for subsistence fish (Braune et al., 1999; Health Canada, 2007). Health Canada recommended a list of predatory fish, including shark, swordfish, and fresh/frozen, but not canned tuna, that are subjected to a 1.0 ppm total Hg standard (Health Canada, 2007).

The levels of Hg in Lake Trout (*Salvelinus namaycush*) throughout NWT and northern Quebec frequently exceeded both the commercial and subsistence fish guideline limits (Braune et al., 1999). There was also substantial intraspecific variation in Hg between lakes (Braune et al., 1999). For example, Lake Whitefish (*Coregonus clupeaformis*) from Lac-Ste.-Therese in the western NWT have Hg concentrations approximately 40 times higher than from Colville Lake in the Sahtu Region of NWT (Braune et al., 1999). Most of the data supporting NCP's freshwater research came from periodic assessments of Hg levels in fish by the Inspection Service of the Department of Fisheries and Oceans (Braune et al., 1999; Evans et al., 2005).

Since 2007, a series of health advisories have been issued by the Government of the Northwest Territories Department of Health and Social Services in response to the high Hg levels observed in some fish species in some lakes in the territory. These advisories prompted concerns among some Dehcho Region residents regarding the safety of their traditional foods. Although consumption advisories often emphasize the nutritional importance of traditional foods in general, their development does not typically include comprehensive, quantitative risk-benefit assessments that consider both nutrient benefits and contaminant risks (Oostdam et al., 2005). There is anecdotal evidence that Dene and Métis Nation in the NWT, like many other Aboriginal populations in Canada and globally, are experiencing a dietary transition, such that reliance on traditional food has decreased and reliance on primarily store-bought food has increased (Kuhnlein et al., 2004; Receveur et al., 1997). In socio-economic context of the Canadian North, accessibility of traditional foods also extends to the amount of time, energy, and costs of transportation, equipment, and fuel necessary to harvest and prepare traditional food (Kuhnlein & Receveur, 1996).

2.2. Study Contributions and Importance

In the Dehcho, NWT, Hg fluxes have increased in some lake sediments, but decreased in others even though there are few local point sources and atmospheric long-range transport from multiple regions (Chételat et al., 2014; Durnford et al., 2010). Environmental change and climate warming impacts the cycling and bioaccumulation of Hg in lakes and other aquatic ecosystems (Chételat et al., 2014). Microbial metabolism transforms inorganic Hg to organic Hg and this occurs more efficiently in warmer and more productive lakes (Chételat et al., 2014; Riget et al., 2000). Further research is needed on dietary exposures to Hg because biochemical and physical processes of MeHg may lead to increased Hg levels in fish populations in subarctic regions in Canada (Chételat et al., 2014). As the climate continues to change and become warmer, the more inorganic Hg is expected to enter the ecosystem, and thus more MeHg will bioaccumulate in fish and may increase MeHg dietary exposure to humans (Chételat et al., 2014; Stern et al., 2012).

Traditional food consumption advisories that only focus on risks (and not benefits) of eating contaminating fish may have severe consequences for human health (Patterson, 2002). For example, Harper & Harris (2008) studied fish advisories for Indigenous populations that focuses on risks to contaminant exposure, and found they may be detrimental through socio-economic impacts, cultural loss, and food insecurity. Harper & Harris (2008, p. 66) classified this dilemma as an environmental injustice: “Chemical contaminants place [communities] in a lose-lose situation: either eat the fish and suffer the health effects from contaminants, or do not eat the fish and suffer the health and cultural effects of lost fish.” Other types of traditional foods consumed among Dene/ Métis in the NWT include caribou (*Rangifer tarandus*) and moose (*Alces alces*) (Berti et al., 1998; Kuhnlein & Chan, 2000). Previous work showed that the liver and kidney of both caribou and moose often contain high levels of particular metals, including cadmium (Cd) and Hg (Berti et al., 1998). Health advisories have been issued for moose kidneys and liver from some parts of the Dehcho Region.

The important contributions of my thesis project are to quantify the biological benefits and risks of traditional food sources, to promote the health and cultural benefits of fish consumption, and to provide information for authorities in public health departments to make recommendations regarding eating certain fish species that are high in nutrients relative to their Hg levels.

2.3. Research Questions and Objectives

To promote traditional food use as a pathway to health equity and to maximize nutrient intake while minimizing contaminant exposure in the Dehcho Region, I will attempt to answer the following research questions:

i. Which fish species have the highest nutrient levels relative to the amount of Hg?
ii. To what extent can a probabilistic optimization software (Crystal Ball's OptQuest) be utilized to balance contaminant risks and nutrient benefits that promote the health of traditional food consumption?

In order to answer the research questions, the following objectives have been developed:

i. Determine the concentration of total Hg, *n*-3 FAs, and total Se in the muscle of various fish species harvested from Ekali and Sanguez near the Jean Marie River First Nation (JMRFN) and Trout Lake near Samba'a K'e First Nation.
ii. Investigate the utility of probabilistic optimization software to balance contaminant risks and nutrient benefits from wild-harvested fish in the Dehcho Region, NWT.
iii. Inform mitigation and risk communication strategies that promote health by maximizing nutrient intake while minimizing Hg exposure.

3. Literature Review

3.1. Introduction

The following literature review provides current research and background information on the toxicology of MeHg exposure and risk-benefit assessment of Hg and nutrients from dietary fish consumption. The literature review is not meant to describe the deposition, global cycling, and long-range transport mechanisms of Hg to subarctic locations. These mechanisms are thoroughly discussed in papers by Goodsite et al. (2013), Selin (2014), and Durnford et al. (2010), respectively. In addition, the review does not include a comprehensive assessment of the potential health effects of Hg as such content has been thoroughly described by Clarkson et al. (2003) and Mergler et al. (2007).

3.2. Aboriginal People in Canada

3.2.1. Demographic Characteristics of Aboriginal Populations

Aboriginal populations in Canada include First Nations, Inuit, and Métis peoples. The National Household Survey (NHS) completed in 2011 showed there to be 1.4 million people in Canada with an Aboriginal identity, representing 4.3% of the Canadian population (National Household Survey, 2014). Aboriginal people are a minority in some provinces in Canada, for example, in Manitoba and Saskatchewan, they constitute only 15% of the population (Young, 2012). However, Aboriginal people are a significant segment of the population in northern Canada – compromising 35% of the population living in the Yukon, 50% of NWT, and 85% of Nunavut (Young, 2012). In addition, 54% of Aboriginal people now live in Canadian cities. For example, in Winnipeg and Regina, Aboriginal people account for 9-10% of these cities' populations (Young, 2012). In Ontario, Aboriginal people account for only 2.4%, although the total numbers are high and Prince Edward Island is the lowest of all the provinces at only 1.6% (Statistics Canada, 2013).

3.2.2. Health Characteristics and Patterns Within Aboriginal Communities

Aboriginal people in Canada face numerous health disparities such that the health of Aboriginal communities continues to lag behind that of the national population (Young, 2003). For instance, on-reserve First Nations communities in Canada experience high levels of overcrowded housing and isolation from health care services, and increasing susceptibility to infectious diseases such as tuberculosis (Clark et al., 2002). While Aboriginal people have higher incidence rates of infectious diseases, there is an additional burden of chronic diseases such as type-2 diabetes and cancer that are now taking a high detriment on health (Adelson, 2005). Although the health status of certain Aboriginal populations have improved considerably during the past 50 years, infant mortality rates, tuberculosis incidence, and life expectancy remains poor in comparison to the general population of Canada (Bjerregaard et al., 2004; Young, 2003).

3.2.3. Aboriginal Peoples Relationship with the Environment

Aboriginal communities maintain a close and intimate relationship with their environment and preserve close ties to their land due to historical and cultural traditions (Richmond & Ross, 2009). Many Aboriginal communities living in northern environments find that their traditional land has become polluted by the long-range transport of chemical contaminants as well as local sources of contamination from mining, oil, exploration, and pesticide use (Kuhnlein & Chan, 2000; Stephens et al., 2006). Contamination can adversely impact communities when pollutants enter soil, air, freshwater, and marine ecosystems (Stephens et al., 2006).

3.2.4. Traditional Food Consumption

Traditional food refers to mammals, fish, plants, berries, vegetation, and waterfowl/seabirds harvested from the local environment, whereas, store-bought foods are imported from other regions of the country/world (Oostdam et al., 2005). The nutritional benefits of the traditional Aboriginal diet are significant because these foods are an important source of fatty acids, vitamins, trace elements, protein, and other essential nutrients (Oostdam et al., 2005). In communities where traditional food is not widely consumed and has instead been largely replaced by nutrient-poor, store-bought food, the health implications include nutritional deficiencies as well as the development of obesity, type 2 diabetes and cardiovascular diseases (Donaldson et al., 2010). Just as traditional food contributes to the physical and nutritional well-being of Aboriginals, the social, cultural, and spiritual aspects of these foods are also significant. Hunting, harvesting, and using traditional food are important activities to maintain social norms and expectations, and to bring individuals and families together (Donaldson et al., 2010). Furthermore, these social activities are opportunities for transferring knowledge between generations and preserving language; therefore, the use of traditional knowledge passes on information about hunting techniques, places, and local history (Oostdam et al., 2005). Aboriginal populations are exposed to higher levels of contaminants compared to the rest of the Canadian population as some of the traditional foods that they rely upon can contain elevated concentrations of certain types of contaminants (Oostdam et al., 2005).

3.2.5. Nutrition Transition

A dietary assessment conducted by Kuhnlein et al. (2004) noted that Aboriginal people are experiencing a nutrition transition associated with increased obesity and fewer calories derived from traditional foods. The diet quality of Aboriginal peoples is often low in vitamins and minerals including, iron, folacin, calcium, and vitamin A and D as well as low in fibre, fruit and vegetables (Willows, 2005). Another measure of dietary change is the difference in traditional food consumption between older and younger subpopulations. For example, individuals over 40 years of age consumed significantly more traditional food than those under 40 (Kuhnlein et al., 2004). The results from the Canadian Total Diet Survey (TDS), a survey carried out by the Health Canada's Bureau of Chemical Safety to ensure that chemical levels found in food do not pose an unacceptable risk to human health, have thus far only surveyed large urban cities during the collection part of the study (Dabeka & Cao, 2013; Health Canada, 2009). Therefore, few dietary intake data are available for Aboriginal people (Dabeka & Cao, 2013). Since data is not collected for this survey and changes cannot be assessed over time, the shift in dietary changes and patterns among Aboriginal people is not accounted.

As a result, in 2007, the Assembly of First Nations addressed the knowledge gap that currently exists on consumption rates, nutritional composition, diet quality assessment, and environmental safety of traditional foods in First Nations reserves (Chan et al., 2011; Health Canada, 2008). This gap is targeted by the multi-year First Nations Food, Nutrition, and Environment Study (FNFNES), which is being implemented over a 10-year period to survey 100 randomly selected First Nations communities in ten provinces (Chan et al., 2011; Health Canada, 2008). The data will be used to obtain background exposures to persistent organic pollutants

(POPs) and trace metals from consumption of both traditional and market food (Health Canada, 2008).

The trend of consuming nutrient poor food is also evident in the general Canadian population. In 1997-1998, a national survey of adult and teenaged Canadians was taken to monitor whether changes in dietary and nutrient intake have occurred since the last Canadian dietary survey in 1970 (Gray-Donald et al., 2000; Phillips et al., 2004). The results from the survey showed that mean dietary energy from protein (16-18%), carbohydrate (50-56%) and fat (29-31%) were close to Health Canada's *Food Guide* recommended levels in the different age-sex groups (Gray-Donald et al., 2000). In addition, fat intake was reduced in comparison to previous dietary surveys (Gray-Donald et al., 2000). The sample of Canadian teenagers from the survey consumed low nutrient dense products, including cakes, cookies, carbonated beverages, and salty snacks (Phillips et al., 2004). Some nutrient-rich foods such as eggs, fish, and organ meats were absent or low in consumption (Phillips et al., 2004). There is a serious problem of obesity in Canada and the intake of certain nutrients is inadequate (Gray-Donald et al., 2000; Phillips et al., 2004).

3.3. Sources of Methylmercury Exposure

3.3.1. Mercury Distribution in the Environment

Both natural and anthropogenic activities release Hg into the environment (Mahaffey, 1999). Natural phenomena include volcanic activity, weathering of rocks, flooding, volatilization from the ocean, and forest fires (Mahaffey, 1999; Schuster et al., 2002). During the pre-industrial period, Hg was released into the environment by natural activities and these levels of exposure did not appear to pose significant human health risks (Mahaffey, 1999).

Currently, most Hg emissions into the environment in the NWT are due to non-point sources, such as long-rate transport and runoff from land use activities (Chételat et al., 2014). Thawing permafrost is also releasing significant amounts of long-stored inorganic Hg and organic matter into lakes (Stern et al., 2012).

As a result of global atmospheric cycling of Hg described by Selin (2014), inorganic Hg enters the aquatic ecosystem and is converted to MeHg via methylation by bacterial activity (Clarkson et al., 2003; King et al., 2000). Waste contaminated with Hg from industrial processes or from disposal of Hg-containing products can be released into the atmosphere or water (Mahaffey, 1999). In addition, combustion of fuel, especially coal, from power plants is another source of Hg into the environment (Mahaffey, 1999; Schuster et al., 2002).

3.3.2. Methylmercury Exposure from Dietary Sources

In aquatic ecosystems, the type of microorganism that methylate Hg are sulfate-reducing bacteria (SRB) (King et al., 2000). Methyl transferase enzymes are induced during complete oxidation of acetate by SRB (King et al., 2000). The MeHg produced through this microbial activity enters aquatic food webs; most human exposure to Hg comes from the consumption of fish and marine animals contaminated with MeHg (Clarkson et al., 2003; Mergler et al., 2007).

Not all fish contain similar concentrations of MeHg, Hg bioaccumulates and biomagnifies to reach highest concentrations in the muscle tissues of large, older predatory fish (Choi & Grandjean, 2008; Clarkson et al., 2003). Higher trophic-level fish can be contaminated with MeHg at concentrations greater than 1 ppm (wet weight), with highly variable concentrations between and within fish and shellfish species (Karimi et al., 2012; Mergler et al., 2007). In Canadian freshwater fish species that were collected in contaminant monitoring programs at the provincial (Ontario, Manitoba, Saskatchewan, and Quebec) and federal levels (Fisheries and Oceans Canada Inspection Branch), total Hg concentrations vary widely from below detection limit to 10 ppm (Depew et al., 2013). A study by Depew et al. (2013) found that the highest Hg levels were found in predatory species that are at higher trophic levels, such as Walleye (mean: 0.41 ppm; range: less than detection limit – 10.4 ppm), Northern Pike (mean: 0.38 ppm; range: less than detection limit – 10.9 ppm), and Lake Trout (mean: 0.28 ppm; range: less than detection limit – 10.0 ppm). In addition, MeHg binds to proteins and free amino acids that are components of muscle tissue, and therefore cannot be removed by cleaning or cooking methods of the fish (Mergler et al., 2007).

3.4. Health Effects of Methylmercury Exposure

Evidence from animal and human epidemiology studies indicates that exposure to MeHg may have adverse health effects on neurologic function in both adults and children (Harada, 1995), cardiovascular system (Roman et al., 2011; Stern, 2005), immune function (Shenker et al., 1998), and endocrine system (Tan et al., 2009). Subtle neurodevelopmental effects have been observed in populations with moderate MeHg exposures from consumption of fish or marine mammals including decrements in memory, attention, language, and visual-motor skills during childhood (Karagas et al., 2012). Given that fish is a high source of dietary protein in the world, MeHg contamination of fish may impact the health of geographically diverse populations (Karagas et al., 2012).

3.4.1. Neurological Effects

Epidemiology studies in adults

The neurotoxicity of MeHg was first recognized in Minamata, Japan during 1956 when humans consumed MeHg-contaminated fish and shellfish (Harada, 1995). The industrial waste from a chemical plant was discharged into the Minamata Bay and Agano River, which resulted in the accumulation of MeHg in fish and shellfish, subsequently caused two large epidemics due to humans consuming contaminated fish products (Clarkson et al., 2003; Harada, 1995). The main target organ of MeHg exposure is the brain; symptoms include: sensory disturbances, ataxia (lack of muscle control during voluntary movements), dysarthria (abnormal, uncoordinated movements), constriction of the visual field, auditory disturbances, and tremor (Harada, 1995). The pathological findings observed included damage to the central nervous system, particularly extensive lesions in the cerebral cortex and cerebellar cortex of the brain (Harada, 1995). The number of patients who were diagnosed at the initial stage of the "Minamata disease" decreased during 1960 as people ceased consuming fish products out of fear; however, the number of people that developed chronic onset of the disease gradually increased because symptoms manifested long after exposure or intake, or latent symptoms appeared when complicated with other diseases or old age (Harada, 1995). Of the 2252 patients who were diagnosed with "Minamata disease", 1043 have died from exposure, which yields a case fatality rate of 46.3% (Harada et al., 1976; Harada, 1995).

A long-term cohort study of low-dose MeHg exposure in a fishing village in Ooura, Japan, on the coast of the Shiranui Sea, demonstrated typical neurological symptoms including hypoesthesia (decreased pain sensitivity), ataxia, impairment of hearing, visual change, and dysarthria in comparison to a non-polluted fishing village in Ichiburi, Japan (Ninomiya et al., 1995). The neurological disorders were still detected 10 years later after the end of MeHg dispersion from Minimata with hypoesthesia showing the highest frequency in Ooura (Ninomiya et al., 1995). In another fish-eating community, a cross-sectional study conducted in the Pantanal Region of Brazil showed that hair Hg levels between 0.56 and 13.6 ppm were associated with disruptions of motor speed, dexterity, concentration, verbal learning, and memory (Yokoo et al., 2003). Elevated Hg levels in hair that ranged from 0.5 to 46 ppm were associated with tremor in adults of a fish-consuming, Aboriginal Quebec Cree community (Auger et al., 2005). The results also showed a 6 ppm increase in hair Hg was associated with increasing levels of tremor in adults under 40 years of age (Auger et al., 2005). There was little or no evidence for a correlation with other neurological outcome including motor disturbance (including weakness and spasm), incoordination, and auditory disturbances (Auger et al., 2005). The results from these epidemiological studies show that chronic low-dose dietary exposures to MeHg affect neurological functioning in adults.

A well-known MeHg poisoning example that was observed among Aboriginals relates to the consumption of locally caught fish by Grassy Narrows and Whitedog Indian residents in Northwestern Ontario during 1970 (Takaoka et al., 2014). Since the 1960s, a chlor-alkali plant had been releasing waste products contaminated with Hg into the English-Wabigoon River system in Northwestern Ontario (Takaoka et al., 2014). A study of 89 Grassy Narrow and Whitedog residents observed a range of neurological findings including numbness, tremors, hearing impairments, and pain and touch disturbances in the extremities (Harada et al., 1976; Takaoka et al., 2014). Except for the high prevalence of tandem gait abnormality and hearing loss, the neurological clinical signs and symptoms of the residents of Grassy Narrows are almost the same as those recorded for Minamata disease in Japan (Harada et al., 1976; Harada, 1995; Takaoka et al., 2014).

Epidemiological studies in fetuses and infants

"Minamata disease" also highlights the high susceptibility of the fetal CNS to Hg–induced damage (Clarkson et al., 2003; Harada, 1995). The analyses of MeHg in the umbilical cords of infants born in the Minamata area showed the highest concentrations from 1950-1965, which was the peak period of acetaldehyde production before the wastewater treatment was installed (Akagi et al., 1998; Harada, 1995). This is a particular health risk to fetuses and infants because MeHg inhibits division and migration of brain cells and disrupts the cellular arrangement of the developing brain (Clarkson et al., 2003). As a result of pregnant women consuming fish with high levels of MeHg, their children had cerebral palsy (Harada, 1995). Common symptoms observed among the infants and children were mental retardation, primitive reflex, cerebellar ataxia, disturbances in physical development and nutrition, dysarthria, and deformity of limbs (Harada, 1995). Further, a retrospective cohort study examined the effect of MeHg on the sex ratio of infants at birth and stillbirths in Minamata in the 1950s (Sakamoto et al., 2001). The study showed that fewer males were born and male stillbirths increased among fishing families in the city (Sakamoto et al., 2001). The declining male sex ratios occurred when MeHg pollution was the most severe during pregnancy (Sakamoto et al., 2001). However, further research is necessary to investigate if male infants were more vulnerable with regard to the reproductive toxicity of MeHg exposure (Sakamoto et al., 2001). The results of this study emphasize the importance of sex ratio of still births, but also stillbirths in populations near chemical sites (Sakamoto et al., 2001).

There are dietary differences between two populations that have been observed between maternal MeHg exposure and neurodevelopment. In a prospective cohort study by Davidson et al. (1998), no adverse health effects were observed with either prenatal or postnatal MeHg exposure. In the population studied, mother-child pairs living in the Republic of Seychelles, no adverse neurological outcomes were observed with either prenatal or postnatal MeHg exposure (Davidson et al., 1998). However, the findings in a large longitudinal study by Grandjean et al. (2003) showed that prenatal exposure to MeHg was associated with neurodevelopmental deficits, specifically learning and memory, which were detectable at age 7 years. These differences have been observed due to people in the Republic of Seychelles consuming mostly fish, whereas people in the Faroe Islands consume whale meat and blubber (Davidson et al., 1998; Grandjean et al., 2003; Myers & Davidson, 1998). The MeHg concentrations in the Seychelles are similar to those in commercial fish found in the United States, and the people in the Seychelles do not consume sea mammals and PCB levels are low (Davidson et al., 1998; Myers & Davidson, 1998). In contrast, dietary exposure to MeHg in the Faroe Islands primarily comes from whale meat and blubber, and these food items are not commonly consumed in most countries (Myers & Davidson, 1998). Whale meat and blubber contain high levels of PCB and other neurotoxicants, as well as the MeHg concentrations in this type of meat is several times higher than most fish species (Grandjean et al., 2003; Myers & Davidson, 1998). Of these two large epidemiological studies, the Faroe Islands formed the basis for MeHg's toxicological reference value.

Animal studies

In addition to the epidemiological literature, animal studies have demonstrated that MeHg exposure can adversely affect the central nervous system. A study conducted by Montgomery et al. (2008) demonstrated that adult mice that were exposed to low levels of MeHg during prenatal development exhibited impaired motor function, decreased memory retention, and produced permanent changes in cerebellar circuitry. This study showed that even low level exposure to Hg affected brain development and function well after acute exposure (Montgomery et al., 2008).

3.4.2. Cardiovascular Effects

There is a growing body of evidence suggesting the potential association between MeHg exposure and an increased risk of developing cardiovascular health effects (Roman et al., 2011). The cardiovascular outcomes of MeHg exposure include atherosclerosis, acute myocardial infarction, heart rate variability, and increased blood pressure or (Roman et al., 2011). The strongest evidence is the association between MeHg exposure and acute myocardial infarction (Roman et al., 2011; Stern, 2005). A case-control study by Guallar et al. (2002) and a prospective cohort study by Virtanen et al. (2005) have shown strong evidence for an association between the risk of coronary heart disease and exposure to Hg in adult men. In general, the risk of a coronary heart event from these two studies increased with the increasing levels of Hg exposures (Guallar et al., 2002; Virtanen et al., 2005).

A case-control study examined the association of toenail Hg levels and docasahexaenoic acid (DHA) levels in adipose tissue with the risk of myocardial infarction (Guallar et al., 2002). The cases were 684 men with a first diagnosis of myocardial infarction and the controls were 724 men that were representative of the same population in eight European countries and Israel (Guallar et al., 2002). After adjusting for age, location, cardiovascular risk factors, and DHA levels, the cases had Hg levels that were 15% higher than the controls (Guallar et al., 2002). Furthermore, the study showed that after adjusting for Hg levels, the DHA level was inversely associated with the risk of myocardial infarction (Guallar et al., 2002).

A retrospective-cohort study examined 42 males of the Faroese whaling society to assess the possible cardiovascular health effects from MeHg exposure that mainly originates from the consumption of pilot whale meat (Choi et al., 2009). Relative to the baseline data, the results from this study suggest that long-term MeHg exposure was significantly associated with the development of cardiovascular disease, as indicated by increased blood pressure and increased intima-media thickness (IMT) of carotid artery (Choi et al., 2009). As noted by a systematic review and meta-analysis, carotid IMT is increasingly used as a marker for the development of atherosclerosis and is a strong predictor of future cardiovascular endpoints (Lorenz et al., 2007).

A few retrospective-cohort studies examined the association between Hg levels and cardiovascular risk factors, such as heart rate variability and blood pressure, among a native population in northern Quebec, Canada (Valera et al., 2009; Valera et al., 2011; Valera et al., 2013). After controlling for *n*-3 FAs, Se, and other variables, increased Hg exposure is associated with increased blood pressure and pulse pressure among Nunavik Inuit adults (Valera et al., 2009). The two other retrospective studies found that MeHg exposure was associated with increased resting heart rate and decreased heart rate variability, even after adjusting for traditional risk factors, polyunsaturated fatty acids, Se, and other contaminants (lead and total PCBs) (Valera et al., 2011; Valera et al., 2013). It is necessary to adjust for nutrients and other contaminants when determining an association between MeHg in fish and cardiovascular health effects due to the potential of negative confounding variables (Choi et al., 2008). If the negative confounding variables are not controlled for in the design or analysis stage of a study, this may underestimate both MeHg toxicity and fish benefits (Choi et al., 2008).

3.4.3. Immune System Effects

There is also growing evidence that the immune system is a vulnerable target for Hg compounds (Shenker et al., 1998). Low exposure levels to MeHg results in reduced cell function and immunotoxic changes in lymphocytes and decreased phagocytic ability shown in monocytes using in vitro models (Shenker et al., 1998). In contrast, high exposure to MeHg causes lymphocytes and monocytes to undergo apoptosis 16-24 hours after exposure (Shenker et al., 1998). Additionally, the immunotoxic effects of MeHg compounds were studied in animal models (Ortega et al., 1997). Exposure to low exposure levels of MeHg compounds enhanced lymphocyte immune response (Ortega et al., 1997). Conversely, high exposure levels showed a four-fold increase in lymphocyte proliferative response (Ortega et al., 1997). The observations by Ortega et al. (1997) and Shenker et al. (1998) may have important implications for human health due to MeHg exposures in the environment, since the immune system plays an important role in host defense.

3.4.4. Endocrine Effects

Endocrine disrupting chemicals (EDCs) include those that mimic endogenous hormone activity as well as those that block natural hormone activity by competing for the receptors or influencing the physiological concentration of a hormone (Waring & Harris, 2005). Low exposure levels of Hg may have adverse effects in the endocrine system of animals and humans by disruption of the pituitary, thyroid, adrenal glands and pancreas (Tan et al., 2009). It is suggested that Hg accumulation impairs endocrine function via a defect in adrenal steroid biosynthesis by inhibiting the enzymatic activity of 21 alpha-hydroxylase (Veltman & Maines, 1986). The hormones that appear to be the most affected by Hg exposure are insulin, estrogen, testosterone, and adrenaline (Iavicoli et al., 2009; Tan et al., 2009; Veltman & Maines, 1986).

The thyroid is one of the largest endocrine glands in the human body and displays an affinity for accumulating Hg (McGregor & Mason, 1991). Concentrations of Hg in endocrine glands have been shown to exceed those in kidney, brain, and liver tissues (McGregor & Mason, 1991). Thyroid hormone production is blocked by Hg occupying iodine binding sites and inhibiting or altering hormone action (Ellingsen et al., 2000; McGregor & Mason, 1991). Thus, Hg damage causes damage in hormone balances, such as thermoregulation, hypothyroidism, thyroid inflammation, and depression (Ellingsen et al., 2000; McGregor & Mason, 1991; Wada et al., 2009).

3.4.5. Limits and Toxicological Reference Values for Methylmercury

Health Canada developed the basis for human health-risk assessments and toxicological reference values (TRVs) for exposure to MeHg (Legrand et al., 2010). It was first set in the 1970s by the Medical Services Branch following investigations of blood and hair Hg levels as well as fish consumption patterns among First Nations residents in Ontario and Quebec (Legrand et al., 2010). In 1973, a Task Force on Organic Mercury in the Environment was established to respond to unusually high Hg levels in relation to the health and well-being of residents of Grassy Narrows and Whitedog, Ontario (Legrand et al., 2010). Based on a recent re-evaluation, this limit is still considered appropriate to protect the health of Canadians from the toxic effects of MeHg (Canadian Food Inspection Agency, 2002).

In 1976, a World Health Organization (WHO) Expect Group concluded, based on data from “Minamata disease” in Japan and the Iraq mass poisoning, that the incidence of the paraesthesia (sensation of pricking or tingling on the skin) manifested in the most sensitive 5% of exposed adults when the daily MeHg intake was 3-7 µg/kg body weight (Legrand et al., 2010; World Health Organization, 1990). This level of intake corresponds with MeHg hair concentrations that would be approximately 50-125 µg/g (World Health Organization, 1990). Concerning the health risks in adults exposed to MeHg, the conclusions reached in "Environmental Health Criteria 1: Mercury" by the WHO in 1976 as well as the 1980 interim evaluation remain unchanged (World Health Organization, 1990). A daily MeHg consumption of $\leq$ 0.48 µg/kg body weight does not result in any detectable adverse health effects (World Health Organization, 1990).

The first international evaluation for the TRV of MeHg was conducted by the Joint Expert Committee on Food Additives (JECFA) (Grandjean et al., 2010). JECFA established an exposure limit of 3.3 µg/kg per week, in 2003, which was based on toxicity data in adults (Grandjean et al., 2010). In 2003, JECFA updated the provisional tolerable daily intake (pTDI) to 200 µg (1.6 µg/kg per week) (Grandjean et al., 2010). In a subsequent review by the International Programme on Chemical Safety, it was noted that fetal neurotoxicity may occur at Hg levels of 10-20 µg/g in maternal hair (Grandjean et al., 2010). For sensitive members of the general population (e.g. pregnant women, women of reproductive age, infants, and young children), Health Canada proposed a revised tolerable daily intake (TDI) of 0.2 µg/kg body weight/day for MeHg (Legrand et al., 2010; R. Wilson, 2013). The revised TDI for sensitive members was based on cohort studies in populations consuming fish in New Zealand and the Faroe Islands (Crump et al., 1998; Myers & Davidson, 1998; R. Wilson, 2013).

3.4.6. Interactions with Other Contaminants

Individuals are exposed to mixtures of contaminants and this health risk assessment may be difficult to evaluate (Oostdam et al., 2005). Multiple contaminants interacting with each other, at relevant concentrations, may have different health effects than an exposure to an individual chemical (Oostdam et al., 2005). Traditional foods may contain mixtures of contaminants that may differ from other parts of the world; the effects of these interactions are not well understood (Oostdam et al., 2005). Environmental contaminants present in fish that may interact with MeHg are organochlorine pesticides, heavy metals such as arsenic, and more recently developed industrial chemicals such as polybrominated diphenyl ethers (PBDEs) and other flame retardants (Rice, 2008).

One environmental contaminant in fish which interacts with MeHg and that has been thoroughly studied is PCBs (Rice, 2008). A prospective-cohort study in the Faroe Islands examined prenatal exposure to PCBs by analysing cord tissue from 435 children (Grandjean et al., 2001). The Faroese birth cohort study first analyzed the effects of MeHg on neurobehaviour deficits (Grandjean et al., 2001). After the cohort was formed in 1986-1987, prenatal exposure to PCBs increased due to consumption of pilot whale blubber, which was a major source of PCBs (Grandjean et al., 2001). The neuropsychological tests that examined children’s performance at age seven paid attention to fine motor function, hand-eye coordination, visual-spatial abilities, language, and recall (Grandjean et al., 2001). The results of this study suggested an interaction between these two neurotoxicants when the children were tested at seven years of age (Grandjean et al., 2001). There were no PCB health effects in children with low MeHg exposure; however, neurobehavioural effects associated with PCBs were greater at the highest tertile of MeHg exposure compared to the lowest (Grandjean et al., 2001). There is a lack of knowledge in this field because most studies conducted on prenatal exposure to PCBs have not considered MeHg as a confounder or modifier (Grandjean et al., 2001; Rice, 2008).

The temporal trends for persistent organic pollutants (POPs) [i.e., PCBs, sum of chlorobenzenes (ΣCBz), sum of chlordane related compounds (ΣCHLs), sum of DDT related compounds (ΣDDT), and toxaphene] in the Canadian Arctic are decreasing in some freshwater fish species [ie. Burbot, Lake trout, and Landlocked Arctic Char (*Salvelinus alpinus*)] (Northern Contaminants Program, 2013). However, a notable exception was Burbot, which showed an increase in concentrations of PCBs, ΣCHL, ΣDDTs, and toxaphene over the period 2001 to 2009 at Fort Good Hope on the Mackenzie River (Northern Contaminants Program, 2013). A study by Carrie et al. (2010) discovered that Burbot in Mackenzie River had increasing levels of MeHg and PCBs, a finding that is linked to climate variability and change. The explanation for the trend is increased productivity and warming temperatures stimulate MeHg production as well as bioaccumulation and biomagnification through the food web (Carrie et al., 2010; Dijkstra et al., 2013). The mean PCB concentration in the Mackenzie River for Burbot increased rapidly from 1988 to 2010 with sum of hexa-PCB increasing from 75 to 150 ppb lipid weight and sum of hepta-PCB increasing from 20 to 85 ppb lipid weight (Carrie et al., 2010).

3.5. Health Benefits of Omega-3 Fatty Acids and Selenium

3.5.1. Health Benefits of Omega-3 Fatty Acids

Brief Overview of Essential Fatty Acids

Polyunsaturated fatty acids (PUFAs) are fatty acids that contain two or more double bonds (Wall et al., 2010). These fatty acids play a significant role in regulating a wide range of functions in the body, including blood pressure, blood clotting, and development and function of the brain and nervous system (Das, 2006; Wall et al., 2010). Two classifications of PUFAs that play an important role in regulating inflammatory responses through the production of potent lipid mediators (termed eicosanoids) are omega-6 (*n*-6) and omega-3 (*n*-3) (Calder, 2004; Wall et al., 2010). Eicosanoids derived from *n*-6 PUFAs [e.g. arachidonic acid (AA)], produce excessive inflammatory and immunoreactivity functions; whereas, eicosanoids derived from *n*-3 PUFAs [e.g. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] have anti-inflammatory properties and directly or indirectly affect different stages of the immune response (Wall et al., 2010). A diet that has an *n*-6 to *n*-3 ratio of 15:1 to 16.7:1 promotes the pathogenesis of many diseases, such as cardiovascular diseases, cancer, osteoporosis, and inflammatory and autoimmune diseases, whereas increased levels of *n*-3 (a lower *n*-6/*n*-3 ratio), suppresses inflammatory effects (Simopoulos, 2002). The following section of the literature review will focus on the health benefits of *n*-3 PUFAs consumption.

Growth and Development

Long chain *n*-3 FAs are required for normal growth and development. These essential fatty acids modulate eye sight and visual signalling processes in *in vivo* models (SanGiovanni & Chew, 2005), promote sensory and neuron development in infants (SanGiovanni et al., 2000), and support optimal cognitive health in older adults (Dangour & Uauy, 2008), as well as influence gene expression, differentiation, and survival in the retina on the cellular level (SanGiovanni & Chew, 2005).

A meta-analysis conducted by SanGiovanni et al. (2000) examined DHA intakes as it relates to visual resolution acuity in healthy preterm infants. The analysis showed a difference in visual acuity at 2 and 4 months of age between DHA-supplemented formula fed infants against DHA-free formula fed infants (SanGiovanni et al., 2000). This difference indicates a positive effect of DHA-supplementation and a higher visual acuity in DHA-supplemented groups (SanGiovanni et al., 2000). These results support the health benefits of dietary essential fatty acids in early visual system development; however, the advantages of *n*-3 FAs intake in visually-based process development across the lifespan is still unknown (SanGiovanni et al., 2000).

Further, motor skills and development were assessed by infant formulas supplemented with oils containing *n*-3 FAs in a prospective, randomized controlled trial (O'Connor et al., 2001). There was evidence of improved motor function among infants that weighed ≤1250 grams at birth and randomized to the group of *n*-3 FAs derived from fish/fungal oil (O'Connor et al., 2001). A part of The Bayley Index of Infant Development measures motor abilities, such as sitting, walking, standing, stair climbing, and hand and finger fine motor skills (O'Connor et al., 2001). Infants in the *n*-3 FAs (fish/fungal) group had Bayley motor index scores that were higher than for the infants fed the control formula (O'Connor et al., 2001).

In addition to the studies conducted in babies and infants, there is evidence supporting the importance of *n*-3 FAs consumption for good cognitive health in older adults (Dangour & Uauy, 2008). A randomized-control trial evaluated the impact of *n*-3 FAs on cognitive function in older adults and recruited 174 participants with mild to moderate Alzheimer’s disease (Dangour & Uauy, 2008). The study participants were provided with 2.3g of n-3 FAs per day or placebo for 6 months (Dangour & Uauy, 2008). After six months of intervention, the decline in cognitive function did not differ between the two groups; however, the rate of cognitive decline in 32 participants with mild cognitive function loss was significantly slower than the placebo group (Dangour & Uauy, 2008). In contrast to the studies for babies where *n*-3 FAs are required for healthy neurodevelopment and cognitive abilities, in older adults *n*-3 FAs are more likely to protect and maintain health (Dangour & Uauy, 2008). There is now a growing body of research indicating that *n*-3 FAs are necessary for optimal cognitive function in later life; however, there is a lack of long-term, population-based studies in this area (Dangour & Uauy, 2008).

Cardiovascular Outcomes

A substantial body of evidence from epidemiological and clinical studies indicates that consumption of fish and *n*-3 FAs reduces the risk of cardiovascular disease (Calder, 2004). Long-chain *n*-3 FAs have been shown to modify a number of risk factors for cardiovascular disease and these include: decreased blood pressure (Hoshi et al., 2013), reduced platelet aggregation and plasma triglyceride concentrations (von Schacky et al., 1985; von Schacky & Weber, 1985), decreased ventricular arrhythmias (Wilhelm et al., 2008), reduced pulse pressure and total vascular reactivity (Nestel et al., 2002), and decreased inflammation (Calder, 2004). As a result, the consumption of *n*-3 FAs plays an important role in protecting against cardiovascular diseases (Calder, 2004).

Immune Responses

The consumption of *n*-6 FAs produces inflammatory mediators (prostaglandins, leukotrienes, and related metabolites) and through these processes regulates the activities of inflammatory cells, cytokines production, and the T-helper 1 vs T-helper 2 cells balance (Calder & Grimble, 2002). However, the consumption of *n*-3 FAs modulates the intensity and duration of inflammatory responses (Calder & Grimble, 2002). The metabolites of *n*-3 FAs influences responses in the immune system of infants, which include inducing eicosanoid production, altering gene expression, modifying lipid raft composition in biological membranes, and altering T-lymphocyte signalling (Gottrand, 2008). However, the mechanisms involved are complex and the influence of *n*-3 on the immune system in infants may vary according to dose, exposure window, and profile of the immune system (Gottrand, 2008).

3.5.2. Health Benefits of Selenium

Cancer Prevention

Several suggested mechanisms by which Se might play a critical role in the prevention of cancer include oxidative stress, inhibition of DNA synthesis, DNA repair, apoptosis, effect on endocrine and immune systems, and inhibition of angiogenesis (Letavayová et al., 2006).

In a Cochrane review of selenium and cancer prevention studies, Vinceti et al. (2014), found reduced cancer incidence and mortality with higher selenium intake. From Vinceti et al.'s (2014) meta-analysis on total cancer risk, the risk of cancer was 31% lower in the highest Se intake category of $\geq$50 μg/day and the risk of death from cancer was 36% lower. In addition, the risk of lung, gastric, or colorectal cancer was found to be reduced with higher selenium intake (Vinceti et al., 2014). No association was seen between selenium and risk of breast cancer (Vinceti et al., 2014).

Cardiovascular Outcomes

Potential cardiovascular benefits of Se are supported by evidence that selenoproteins prevent the oxidation of lipids, reduce inflammation, and prevent platelets from aggregating (Rayman, 2012). In the cardiovascular system, the production of reactive oxygen species (ROS) impairs endothelial function and decreases antioxidant capacity (Silva et al., 2012). Such damage can be prevented by the scavenger activity of Se, which is exerted through the expression of selenoproteins (Hara et al., 2001).

A meta-analysis of 25 observational studies by Flores-Mateo et al. (2007) identified a moderate, but significant inverse association between Se status and coronary heart disease outcome. A 50% increase in selenium concentrations in several tissues was associated with a 24% decreased risk of coronary heart events (Flores-Mateo et al., 2007). However, the validity of this evidence is uncertain because results from observational studies have been inconsistent in determining the cardiovascular effects of antioxidants (Flores-Mateo et al., 2007).

Thyroid Hormone Metabolism

Thyroid hormones are essential for the maintenance of metabolic and gland function in living organisms, and thus abnormal thyroid metabolism can lead to biochemical and pathological changes that are potentially injurious to the affected individual (Arthur et al., 1991). Trace elements, including Se and iodine, are essential for thyroid hormone metabolism and thyroid gland functioning (Schomburg & Köhrle, 2008). While iodine is needed to make the two key hormones produced by the thyroid gland, triodo-L-thyronine (T_3) and tetraiodo-L-thyronine (T_4), Se is essential for the biosynthesis and function of selenoproteins involved in thyroid hormone metabolism and gland function (Schomburg & Köhrle, 2008).

A large cross-sectional study by Derumeaux et al. (2003) supported the relationship between selenium intake levels and thyroid gland function. The data on 1,900 participants from the SU.VI.MAX study showed an inverse association between Se status and thyroid volume as well as a protective effect against goiter and thyroid tissue damage (Derumeaux et al. 2003). However, this relationship was only significant in woman and the goiter occurrence was not significant in men (Derumeaux et al. 2003).

3.6. Dietary Recommendations for Omega-3 Fatty Acids and Selenium

3.6.1. Dietary Recommendations for Omega-3 Fatty Acids

Dietary Reference Intake Values for Omega-3 Fatty Acids Based on Life Stage Group

In 1990, Health Canada established daily recommended intakes (DRI values) for *n*-3 FA in the form of alpha-linolenic acid (ALA) (DHA/EPA Omega-3 Institute, 2013). This was set at

0.5% of total energy and translates into DRI values for ALA of 0.5-0.9 gram/day for infants and children and 1.1-1.6 gram/day for adults (DHA/EPA Omega-3 Institute, 2013). The Food and Nutrition Board in the United States worked in co-operation with Canadian scientists to modify these recommendations (Food and Nutrition Board, Institute of Medicine, 2002). The Food and Nutrition Board recommended adequate intake (AI values) for ALA based on particular life stage group and sex (DHA/EPA Omega-3 Institute, 2013; Food and Nutrition Board, Institute of Medicine, 2002). The term AI refers to the observed average daily intake level based on a defined population or subgroup that sustains a defined nutritional status, such as growth rate, nutrient values, or other indicators of health (Food and Nutrition Board, Institute of Medicine, 2002). Also, the AI is used when a recommended daily allowance (RDA) cannot be determined (Food and Nutrition Board, Institute of Medicine, 2002). It is also important to note that the North American recommendations are currently under review (DHA/EPA Omega-3 Institute, 2013; Food and Nutrition Board, Institute of Medicine, 2002).

Dietary Recommendations for Omega-3 Fatty Acids Based on Protection Against Cardiovascular Diseases

The average intake of EPA and DHA (estimated from fish consumption) associated with cardiovascular risk reduction is 500 mg/day, which was based on evidence from five epidemiologic studies by Albert et al. (1998), Dolecek (1992), Hu et al. (2002), Mozaffarian et al. (2003), and Siscovick et al. (1995). The range of EPA and DHA intake in the studies that conferred the lowest risk was 128 mg/day to 919 mg/day (Albert et al., 1998; Dolecek, 1992; Hu et al., 2002; Mozaffarian et al., 2003; Siscovick et al., 1995). To achieve the 500 mg/day recommendation of EPA and DHA for persons without coronary heart disease (CHD), the American Heart Association recommends the consumption of two, preferably oily, fish meals per week (approximately 227 g; 8 ounces total) (Gebauer et al., 2006; Kris-Etherton et al., 2002). For the treatment of existing cardiovascular disease, The American Heart Association recommends 1 g of EPA and DHA/day (Gebauer et al., 2006; Kris-Etherton et al., 2002).

3.6.2. Dietary Recommendations for Selenium

The Institute of Medicine (IOM) and the Scientific Committee for Food (SCF) set the recommended dietary allowance (RDA) for Se at 55 µg/day for adults to achieve optimal health (Burk, 2002). The SCF also established an upper limit for Se of 300 µg/day, while the Expert Group on Vitamins and Minerals (EVM) derived a safe upper level limit for total Se of 450 µg/day over a lifetime (Expert Group on Vitamins and Minerals, 2003; Scientific Committee for Food, 2000).

The upper limit of 300 µg/day also applies to pregnant and lactating women (Scientific Committee for Food, 2000). There are no specific data indicating that children are more susceptible to the adverse effects from Se and hence, it seems appropriate to extrapolate the upper limit from adults to children on the basis of body weight (Scientific Committee for Food, 2000). This provided upper limits ranging from 60, 90, 130, 200, and 250 µg of Se/day for children 1-3, 4-6, 7-10, 11-14, and 15-17 years of age, respectively (Scientific Committee for Food, 2000).

The SCF decided to derive the safe maximal daily dietary Se intake of 850 µg/day from a study on 389 participants by Yang et al. (1989). The intake of 910 µg Se/day produced symptoms of selenosis in susceptible patients and was close to the no observed adverse effect level (NOAEL) (Yang et al., 1989). Based on this study, an uncertainty factor of 2 was applied for lowest observed adverse effect level (LOAEL) to NOAEL extrapolation (Expert Group on Vitamins and Minerals, 2003; Yang et al., 1989).

3.7. Assessing the Benefits and Risks of Fish Consumption

Evidence from epidemiological studies and animal models suggests that, under certain circumstances, *n*-3 FAs and Se may provide some degree of protection against MeHg toxicity; however the protective effects may diminish at higher Hg exposure levels (Mahaffey, 2004; Mozaffarian, 2009; Rice, 2008). Distribution of both *n*-3 FAs and MeHg can vary widely across different fish species (Mahaffey, 2004). There are fish species that have low Hg levels (e.g. <0.1 ppm) and are also rich sources of *n*-3 FAs; in contrast, there are other species that can have high concentrations of MeHg (e.g ~ 1.0 ppm and higher) but are generally poor sources of *n*-3 FAs (Mahaffey, 2004). In some circumstances, the avoidance of fish due to Hg concerns can compromise the nutritional (e.g. *n*-3 FA and Se) benefits (Mahaffey, 2004).

Ginsberg & Toal (2009) developed a quantitative approach to analyze the risk-benefit of consuming individual fish species based on their MeHg and *n*-3 FAs content. Compared to other risk-benefit assessments of fish consumption, this study was the first to provide an integrated analysis that uses a dose-response relationship for MeHg and *n*-3 FAs effects on coronary heart disease and neurodevelopment (Ginsberg & Toal, 2009). The study showed that the estimated *n*-3 FAs benefits outweighed the risk for some fish species, such as salmon, herring, and trout (different species of trout than consumed in the Dehcho); however, for some species the MeHg content risk outweighed the health benefits for others, such as shark and swordfish (Ginsberg & Toal, 2009). Some of the species had a small net benefit, such as flounder, pollack, and tilapia or had a small net risk, such as canned white tuna and halibut (Ginsberg & Toal, 2009). Based on these results, fish were placed in meal frequency categories with consumption advice based on a range from “do not eat” to “unlimited” (Ginsberg & Toal, 2009). However, there are limitations of implementing such categories within food-insecure regions. The dietary recommendations for food-insecure regions are guided by robust locally-relevant risk-benefit information because “banning” certain foods can have profound nutritional, financial, social, and cultural implications.

3.8. Risk Communication

3.8.1. Balancing Risk Communication Messages

There is an extensive need to approach risk assessment and management by co-ordinating various collaborations across multiple disciplines, including nutrition, toxicology, policy development, and public health (Kuhnlein & Chan, 2000). Each discipline has their own unique perspective, and their perspectives also compete on occasion. For instance, the environmental health perspective observes the adverse health effects of MeHg on the brain and points out the negative aspects of fish consumption. In contrast, the nutrition perspective takes into account the health benefits of *n*-3 FAs and believes that people are not getting enough nutrients from fish consumption (Couzin, 2007). The most successful risk communication strategies balance the risks and benefits of traditional food consumption as well as provide a fuller picture of the specific types and quantities of food items that would be the most beneficial (Couzin, 2007; Donaldson et al., 2010).

A major difficulty of risk communication is advising people to avoid fish intake due to MeHg contamination because this viewpoint compromises the nutritional health benefits of *n*-3 FAs and Se (Cardos et al., 2013; Mahaffey, 2004). Communication that focuses only on risks may be damaging to individuals and communities by creating unnecessary fear, anxiety, and confusion (Donaldson et al., 2010). In one case, in January 2001, the US Food and Drug Administration consumer advisory recommended that pregnant women limit consumption of canned tuna, dark meat fish, shellfish, and white meat fish because of concerns about Hg contamination (Oken et al., 2003). The pregnant women followed the federal recommendations after the negative media attention to the adverse health effects of Hg exposure (Oken et al., 2003). Intake of canned tuna, dark meat fish, and white meat fish declined significantly in women who consumed more than 3 fish servings per week (Oken et al., 2003). These decreased consumption patterns resulted in a total of 1.4 fish servings per month from December 2000 to April 2001, with ongoing declines through the end of the study period (Oken et al., 2003). However, in another case during 2004, a non-profit group called National Healthy Mothers Healthy Babies Coalition (HMHB) urged pregnant women to exceed United States fish consumption guidelines, but the group's recommendation was immediately dismissed by Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) officials (Couzin, 2007). This was a concern because affordable alternative sources of the same nutrients that can replace the nutritional benefits were not promoted (Couzin, 2007). In order to understand the implications of the 2001 advisory, Frithsen & Goodnight (2009) conducted a study to determine the awareness of the fish consumption advisories and fish consumption patterns. Frithsen & Goodnight (2009) observed that women avoided consuming fish out of fear and potentially missed nutritional aspects of fish during pregnancy. In June 2014, the FDA and EPA issued advice to pregnant women by encouraging fish consumption of 8 to 12 ounces per week that are lower in Hg levels (Food and Drug Administration and Environmental Protection Agency, 2014).

The advice was issued because of the nutritional value of fish is important (Food and Drug Administration and Environmental Protection Agency, 2014).

Effective risk communication incorporates the residents' perspective of traditional food sources and integrates the importance of the traditional lifestyle to overall health and well-being (Furgal et al., 2005). Consumption advisories also need to be tailored to pregnant women in regard to making informed decisions about Hg-contaminated food items (Oostdam et al., 2005). Additionally, challenges related to food security need to be addressed when making fish consumption advisories including: how sharing and reciprocating food may contribute to food security, how families cope with food shortage, what solutions or strategies have worked (or did not work) in the past, and what new suggestions can be made by community members (Skinner et al., 2013). These communication efforts provide residents with the appropriate information to make informed decisions about traditional food choices and to avoid making the same mistakes in previous risk communication efforts related to contaminated food sources (Furgal et al., 2005; Oostdam et al., 2005).

3.8.2. Series of Health Advisories Issued by the Government of the Northwest Territories Department of Health and Social Services

From 2010 to 2012, the Department of Health and Social Services advised community members of an increase in Hg levels in Trout and Cli Lakes of the Dehcho Region as well as the Ste. Therese and Kelly Lakes in the neighboring Sahtu Region of the NWT (Healy, 2010 & 2012). The dietary recommendations to community members included consuming smaller fish, such as Whitefish or Grayling and consuming less fish that are higher in the food chain, such as Walleye, Northern Pike, or Lake Trout (Healy, 2010). The advisory indicated that the health benefits of eating fish that are high sources of nutrients far outweigh the potential risks presented by contaminant exposure (Healy, 2012). This advice applies only to those individuals who eat Northern Pike or Walleye on a regular or weekly basis for a number of months from Trout, Cli, Ste. Therese, and Kelly Lakes (Healy, 2010 & 2012). The key of risk communication is to balance fish consumption with the slow elimination process of Hg (Healy, 2010 & 2012).

3.9. Bridging the Research Gaps in Literature

Based on the current body of literature, most of the fish consumption research that has been published has only featured qualitative assessments balancing Hg risks and nutrient benefits. For example, studies by Oken et al. (2005), Oken et al. (2008), and Sakamoto et al. (2004) focused on qualitative recommendations to pregnant women by only seeking out fish varieties with lower levels of Hg. Situations among subsistence populations, including Dehcho residents of this study, who have limited alternatives to contaminated fish sources, may face this dilemma of weighing the benefits and risk (Patterson, 2002). By specifying which fish species have the highest nutrient levels in relation to the amount of Hg, the risk-benefit analysis for this study can be quantitatively assessed.

The proposed study will attempt to bridge the research gaps by focusing on a quantitative approach of balancing the Hg risks and nutrient benefits. The key strength of this study involves using optimization procedures that promote health by maximizing nutrients while minimizing Hg.

4. Associations Between Omega-3 fatty Acids, Selenium Content, and Mercury Levels in Wild-harvested Fish from the Dehcho Region, Northwest Territories, Canada

Overview

The consumption of fish, often rich in essential nutrients like omega-3 fatty acids (*n*-3 FAs) and selenium (Se), can promote health in human populations. However, methylmercury (MeHg), a common contaminant in fish, can pose potential health risks. To better understand the risks and benefits of eating fish, total mercury (HgT) and selenium (Se) content and fatty acid (FA) composition were measured in the muscle tissue of fish harvested from three lakes in the Dehcho Region, Northwest Territories, Canada. As expected, average HgT concentrations varied among fish species according to their trophic guild position, ranging from 0.057 mg kg^{-1} (Cisco) for benthivorous/planktivorous species to 0.551 mg kg^{-1} (Northern Pike) for piscivorous species. There were also substantial differences in omega-3 FA (*n*-3 FA) profiles among fish species, with averages ranging from 101 mg/100 g for Burbot to 1,689 mg/100 g for Lake Trout.

In contrast to the HgT and *n*-3 FA results, average Se concentrations were relatively similar among species, ranging from 0.140 mg kg^{-1} for Northern Pike to 0.195 mg kg^{-1} for Walleye. Consequently, species such as Lake Whitefish, Cisco, and Longnose Sucker had the highest *n*-3 FAs and Se levels relative to their HgT content. Interestingly, the HgT concentration in fish muscle tissue was occasionally inversely related to the tissue's nutrient content. For example, significant negative correlations were observed between Hg and *n*-3 FAs for Lake Trout ($\rho = -0.937$), Northern Pike ($\rho = -0.619$), and Walleye ($\rho = -0.481$) (all $P<0.001$). There were also significant negative correlations between Hg and Se observed for Lake Whitefish ($\rho = -0.818$, $P<0.001$), Cisco ($\rho = -0.685$, $P<0.05$), and Northern Pike ($\rho = -0.410$, $P<0.05$). As such, for these species, samples with the greatest nutritional content tended to have lower levels of HgT.

Although it seems plausible that trophic position, fish age, and/or growth rates are involved, the precise biological and/or ecological mechanisms behind these trends are as of yet unknown. This work provides valuable information for the design of dose reconstruction models capable of refining public health messaging related to minimizing the risks of Hg exposure and maximizing nutrient levels in wild-harvested fish in the Canadian subarctic.

4.1. Introduction

First Nations communities, which include a diverse group of cultures and languages, make up 60.8% of the Aboriginal population and 2.6% of the general population in Canada (National Household Survey, 2014). The Dehcho First Nations refers to a regional coalition of First Nations and Métis communities in the southwestern portion of Canada's Northwest Territories. In the Dehcho Region and beyond, traditional food sources, including but not limited to wild-caught fish, are instrumental to the physical, social, cultural, and spiritual well-being of First Nations people (Oostdam et al., 2005). Traditional foods, and fish in particular, provide a rich variety of important nutrients [e.g. omega-3 fatty acids (*n*-3 FAs) and selenium (Se)] (Oostdam et al., 2005). The intake of *n*-3 FAs from fish consumption promotes healthy growth and development in infants and children (SanGiovanni & Chew, 2005), supports optimal cognitive health in older adults (Dangour & Uauy, 2008), and reduces the risk of cardiovascular disease (Calder, 2004). Long chain *n*-3 FAs [e.g. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] have anti-inflammatory properties and can directly affect different stages of the immune response (Wall et al., 2010). Fish can also be an important source of the essential trace element Se, which is important to the maintenance of thyroid hormone metabolism (Arthur, 1991; Schomburg & Kohrle, 2008), potentially protects against cardiovascular disease (Rayman, 2012), and may reduce the incidence of some cancer types (Letavayova et al. , 2006; Vinceti et al., 2014).

The consumption of fish, however, can also result in elevated exposures to bioaccumulative and biomagnifying contaminants (Evans et al., 2005). One such contaminant that presents a variety of public health risks is mercury (Hg) (Ayotte et al., 1995; Evans et al., 2005). Elemental Hg is released into the atmosphere by a variety of natural and anthropogenic processes, but most notably by the burning of coal and other fossil fuels (Aboriginal Affairs and Northern Development Canada, 2012). Once in the atmosphere, Hg is distributed world-wide by long-range atmospheric transport mechanisms before being deposited in parts of the Canadian North where there are few anthropogenic point sources (Jæger et al., 2009; Li et al., 2009). Once deposited into aquatic ecosystems, the inorganic Hg can be methylated by some types of bacteria into organic forms like methylmercury (MeHg) (Clarkson et al., 2003; King et al., 2000). Since MeHg is biologically persistent, it can bioaccumulate in muscle tissues of aquatic organisms, pass through the food chain, and biomagnify in freshwater and marine ecosystems (Ayotte et al., 1995). Exposure to MeHg may have adverse health effects on neurological function (Harada, 1995), the cardiovascular system (Roman et al., 2011; Stern, 2005), immune function (Shenker et al., 1998), and the endocrine system (Tan et al., 2009). Additionally, maternal exposure to MeHg during pregnancy can lead to neurodevelopmental deficiencies in young children, with impacts on memory, attention, language, and visual-motor skills (Grandjean et al., 2003; Karagas et al., 2012). Neurodevelopmental toxicity of MeHg is the most sensitive adverse effect associated with dietary Hg exposures; therefore, this endpoint has formed the basis for Health Canada’s retail fish Hg guideline [0.5 ppm wet weight total mercury (HgT)] (HgT; Braune et al. 1999; Health Canada, 2007). The percentage of MeHg from HgT varies among species, but it is generally about 90% (Bloom, 1992). However, several predatory fish species (e.g. shark, swordfish, and tuna) were exempted from the Canadian retail fish guideline because they commonly exceed the 0.5 ppm wet weight limit (Health Canada, 2007). Instead, for these species, Health Canada set the retail Hg guideline at 1 ppm wet weight and recommended that the public, and sensitive subpopulations in particular, limit their consumption of these species by lowering total permitted serving (Health Canada, 2007).

Several lakes in the Dehcho Region contain fish populations with elevated HgT concentrations (Braune et al., 1999; Evans et al., 2005; Lockhart et al., 2005). For example, in some inland lakes, the mean Hg concentration of predatory fishes like Walleye (*Sander vitreus*), Northern Pike (*Esox lucius*), and Lake Trout (*Salvelinus namaycush*) approached or exceeded the 0.5 ppm Health Canada HgT guideline. In contrast, average HgT concentrations in other species such as Lake Whitefish (*Coregonus clupeaformis;* 0.11 ppm) and Burbot (*Lota lota;* 0.21 ppm) were generally well below 0.5 ppm (Lockhart et al., 2005). In response, the Government of the Northwest Territories Department of Health and Social Services (DHSS) issued a series of consumption advisories from 2010 to 2012 (Healy, 2010 & 2012; DHSS, 2012), recommending that people should typically consume smaller fish that feed lower on the food chain, such as Whitefish, the most frequently consumed fish in the Dehcho Region, or Arctic Grayling (*Thymallus arcticus*) rather than predatory species like Walleye, Northern Pike, or Lake Trout (Healy, 2010 & 2012). Focusing solely on the risks of Hg, however, can overlook the nutritional benefits provided by fish consumption (Mahaffey, 2004). Additionally, although their protective effects may diminish at higher Hg exposure levels, there is evidence that, under certain circumstances, *n*-3 FAs and Se provide some degree of protection against MeHg toxicity (MacDonald et al., 2015; Mahaffey, 2004; Mozaffarian, 2009; Rice, 2008). Accordingly, the

DHSS advisories did indicate that the health benefits of eating fish generally outweigh the potential risks presented by contaminant exposure (Healy, 2010 & 2012; DHSS, 2012). However, more precise statements regarding the balance between Hg risks and nutritional benefits have not yet been possible, because the levels of nutrients in wild-harvested fish in the Dehcho Region have largely gone unmeasured. Therefore, the primary objective of this research was to quantify the levels of total Hg, *n*-3 FAs, and Se in fish species harvested from three lakes in the Dehcho Region, Northwest Territories (NWT). Additionally, this work aimed to evaluate the correlations between nutrient and contaminant concentrations and identify which fish species provide the most *n*-3 FA and Se relative to their Hg content.

4.2. Materials and Methods

4.2.1. Fish Sample Collection and Preparation

In August 2013, Burbot (n=9), Cisco (n=29), Lake Trout (n=13), Lake Whitefish (n=30), Longnose Sucker (*Catostomus catostomus*) (n=6), Northern Pike (n=47), and Walleye (n=53) samples were collected from fish harvested from Ekali, Sanguez, and Trout Lakes in the Dehcho Region. Among these three lakes, dietary advisories were issued for Lake Trout over 60 cm (Trout Lake), Northern Pike (Ekali Lake, Sanguez Lake), and Walleye (Ekali Lake, Sanguez Lake) (Healy, 2010 & 2012; DHSS, 2012). Fish were collected in multi-mesh gill nets, and fork length, wet weight, sex, and maturity were determined on-site. A dorsal muscle sample was collected in the field, and frozen within 6 hours. Fish were transported on dry ice and stored at -20°C until subsequent chemical analysis.

Two subsamples were taken from each fish sample for the laboratory analyses. The skin was removed for both subsamples before the homogenization process. The first subsample was freeze dried using a LabConco Freezone lyophilizer, and ground for analysis of HgT with a mortar and pestle. The second subsample was flash-frozen in liquid nitrogen and pulverized in a Cryo-Cup Grinder (BioSpec Products, Bartlesville, OK, USA) for analyses of *n*-3 FAs and Se. The differences in sample count among measures were due to the fact that all fish samples were tested for Hg, whereas overlapping subsets were tested for *n*-3 FAs and Se.

4.2.2. Laboratory Analysis

Mercury Analysis

As described in Gantner et al. (2009), HgT concentrations in fish muscle tissues (20-25 mg tissue analyzed/fish) were determined using a direct mercury analyzer (DMA-80) according to the U.S. Environmental Protection Agency (U.S. EPA) method 7473.

Selenium Analysis

Pulverized fish muscle tissue (100 mg) was digested in 5 mL of nitric acid (HNO_3, 69%) and 1.5 mL of hydrogen peroxide (H_2O_2, 30%) by adding in 1 mL and 0.1 mL increments, respectively, to each vial, allowing sufficient time among additions for any reactions to subside. Fully digested samples were evaporated on a hot plate at 70°C and were continuously monitored and rotated to prevent unequal drying. When the samples were evaporated to a lentil sized drop, they were removed from the hot plate, capped, and cooled in the fume hood. The samples were re-diluted with 3.5 or 5 mL of 2% HNO_3 depending on the initial dry weight of the samples. Samples were filtered with a 0.45-μm polyethersulfone membrane and stored in the refrigerator until analysis. A multi-element scan on a Thermo X Series II inductively coupled plasma mass spectrometer (ICP-MS) operated in collision cell mode was used to determine the concentration of Se in the fish samples.

Omega-3 Fatty Acid Analysis

Pulverized fish muscle tissue (100 mg) was homogenized in 3 mL of chilled 2:1 chloroform:methanol (Folch et al., 1957) containing 3.33 µg mL^{-1} ethyl docosatrienoate (C 22:3n-3 ethyl ester, Nu-Chek Prep Inc., Elysian, MN, USA) as the internal standard, and 50 µg mL^{-1} butylated hydroxytoluene as an antioxidant (Metherel et al., 2015). The samples were vortexed and 500 µL of 0.2 mol L^{-1} sodium phosphate buffer were added to induce phase separation. Each sample was then inverted twice and centrifuged at 1734 *rcf* for 5 min. The lower organic phase containing lipids was collected. Total lipid extracts were dried under a stream of nitrogen gas, and fatty acid methyl esters were generated through a trans-esterification procedure by adding 300 µL hexane and 1 mL 14% boron trifluoride (BF_3) in methanol and heating for 1 hour at 95°C using a block heater (Kitson et al., 2012). Fatty acid methyl esters were analyzed on a Varian 3900 Gas Chromatograph equipped with a DB-FFAP 15 m x 0.10 mm i.d. x 0.10 µm film thickness, nitroterephthalic acid modified, polyethylene glycol, capillary column (J&W Scientific from Agilent Technologies, Mississauga, ON), and used hydrogen as the carrier gas (Masood et al., 2005). A Varian CP-8400 Autosampler was used to introduce 1-µL aliquots of each sample into the injector, which was heated to 250ºC and used a 200:1 split ratio. The initial oven temperature was 150ºC with a 0.25-minute hold, followed by a 35ºC min^{-1} ramp to 200ºC. An 8ºC min^{-1} ramp followed until 245ºC were reached, where the temperature was held for 15 minutes. The flame ionization detector temperature was set to 300ºC, air and nitrogen make-up gas flows were 300 mL min^{-1} and 45 mL min^{-1}, respectively, and the sampling frequency was 50 Hz.

4.2.3. Quality Assurance/Quality Control (QAQC)

Mercury QAQC

For the mercury analysis, blanks (an empty quartz boat), duplicates, and standard reference materials (SRMs) were analyzed in every batch of 40 samples. At least five blanks were analyzed per batch. The mean blank value ± standard deviation (S.D.) was 0.02 ± 0.06 ng Hg (n=66) and this corresponded to ~0.05% of average fish Hg concentrations. In each batch, the SRMs analyzed included National Institute Standards and Technology (NIST) 2976, NIST 2974a, DORM-3, and DOLT-4. The percent recovery ± S.D. of HgT for NIST 2976, NIST 2974a, DORM-3, and DOLT-4 were 107% ± 0.034, 105% ± 0.012, 110% ± 0.02, and 109% ± 0.012, respectively. All values were within the certified range. Duplicates were run every 10 samples and the percent difference among duplicates was 4.3%.

Selenium QAQC

The SRM TORT-2 and an instrumental certified reference material called 1640a (both standards from NIST) were included in each run. The percent recovery ± S.D. of Se for the reference standard and instrumental certified reference material were 103.5% ± 5.4 and 104.7% ± 5.8, respectively. The detection limit from all four runs were 0.56, 1.46, 0.65, and 0.38 ng mL^{-1}. Duplicate analyses were performed on two tissue samples and the percent difference on the first sample was 1.5% and the second sample was 4.0%.

Omega-3 Fatty Acid QAQC

An external reference standard (GLC-462, Nu-Chek Prep Inc., Elysian, MN, USA) contains a mixture of known fatty acid methyl esters, which allows identification of fatty acids in samples based on peak retention times. The detection limit was 0.151 ng of fatty acid per 1 µL of solvent (hexane) for all fatty acids. The analysis was conducted in triplicate for a pair of fish samples; the coefficients of variation between these samples and reference standard were 6.9% and 7.6%, respectively.

4.2.4. Derivation of *De Minimus* Ratios

A method proposed by Tsuchiya et al. (2008) defined a *de minimus* intake ratio of 17 mg DHA to 1 µg of Hg exposure, such that individuals consuming fish over this *de minimus* ratio would be able to meet the U.S. Dietary Reference Intake of DHA (100 mg/day) while not exceeding the U.S. EPA reference dose (RfD) for MeHg (0.1 µg/kg/d) (Kris-Etherton et al., 2009). The regulatory context for Hg differs between the United States and Canada (Legrand et al., 2010); Health Canada applies provisional tolerable daily intakes (pTDI) of 0.20 µg/kg body weight (bw)/day for women of childbearing-age and children, and 0.47 µg/kg bw/day for men over 18 years of age and women over 40 years of age (Legrand et al., 2010; Lemire et al., 2015). In contrast, the U.S. EPA's more conservative Hg RfD is typically applied for all peoples regardless of age and sex (Legrand et al., 2010; Lemire et al., 2015).

To make the *de minimus* approach more relevant to the Canadian regulatory context, we applied the method previously described by Tsuichya et al. (2008) using Health Canada's Hg toxicological reference value (TRV) for pregnant women and women of child-bearing age (Legrand et al., 2010) according to the equation:

$$\mathrm{DHA_{mr}} \times \left(\frac{1}{\mathrm{CR}}\right) : \mathrm{TRV} \times \left(\frac{1}{\mathrm{CR}}\right) \times \mathrm{BW}$$

Where $\mathrm{DHA_{mr}}$ is the daily minimum requirement of DHA (100 mg/day), CR is the daily fish consumption rate (60 g/d), TRV is the toxicological reference value (0.2 µg/kg/d), and BW refers to bodyweight (60 kg) (Legrand et al., 2010; Tsuichya et al. 2008). The resulting ratio is 8.3 mg DHA to 1 µg Hg. The equivalent *de minimus* approach was carried out for the essential trace element Se using the Recommended Daily Allowance of 55 µg/d (Burk, 2002). The resulting ratio is 34.0 µmol Se per µmol of Hg. It should be noted that these *de minimus* ratios, which are based upon input parameters specific to women of child-bearing age, are not directly applicable to other subpopulations (e.g. adults over 40 years of age, children). Generally, the *de minimus* ratios derived herein are likely overly conservative for the general adult population but may prove under-protective of children 13 years of age and under.

4.2.5. Statistical Analysis

Summary statistical analysis and correlations were conducted using SAS (Version 9.4) and Sigma Plot (Version 12.5). The correlations among fish HgT, *n*-3 FA content, Se content, vs. fork length and fish weight were assessed using linear regression analysis. The correlations among fish HgT, *n*-3 FA content, and Se content were assessed using non-parametric techniques (i.e. Spearman's rank order correlation). A series of one-way analyses of variance (ANOVAs) were conducted to determine whether Hg, Se, and *n*-3 FAs concentrations differed among lakes. Thereafter, fish Hg concentrations were compared among lakes and species with analyses of covariance (ANCOVA); lake and species were categorical variables and fork length was used as a covariate. Hg, *n*-3 FAs, and Se concentrations, fork length, and wet weight were log-transformed prior to the ANOVA and ANCOVA analyses to meet assumptions of normality. For each of these statistical tests, the results were deemed significant when $P < 0.05$.

4.3. Results and Discussion

4.3.1. Mercury Concentration

When the results were pooled across the three studied lakes, average HgT concentrations in Northern Pike (0.551 ppm) were up to 9.7-fold higher than observed in benthivorous and planktivorous fish species, such as Cisco (0.057 ppm) and Lake Whitefish (0.073 ppm; **Table 1**). The other piscivorous species also had elevated HgT concentrations. For example, average HgT concentrations in Walleye (0.415 ppm) and Lake Trout (0.330 ppm) were 7.3-fold and 5.7-fold higher than observed in Cisco, respectively (**Table 1**). The HgT concentrations from this study are similar to those previously reported for these species in the Dehcho Region (Lockhart et al., 2005).

Unsurprisingly, Hg content increased with fork length or weight for most fish species. There were positive linear relationships between log HgT and log fork length (linear regression: 1.314 – 2.154) and log fish weight (linear regression: 0.395 – 0.696) for Lake Whitefish, Northern Pike, and Walleye (all $P<0.001$; **Appendix A: Tables S1-S2**). Also, log HgT had a positive linear relationship with log fork length (but not log weight) for Cisco (linear regression: 1.596, $P<0.001$) and Burbot (linear regression: 4.883, $P<0.05$; **Appendix A: Tables S1-S2**). In contrast, a relationship between log HgT with either log fork length or log fish weight was not observed for Lake Trout and Longnose Sucker (**Appendix A: Tables S1-S2**). The absence of correlations between fish size and HgT for Lake Trout and Longnose Sucker may have been a product of the relatively small sample sizes obtained for these species. Additionally, most of the Longnose Suckers caught had similar fork lengths (51.5-53.5 cm) and fish weights (1945-2235 g). In contrast, there was considerable variation in fork length (58.7-81.0 cm) and fish weight (2745-6910 g) for Lake Trout.

For each of the species collected from more than one lake (Cisco, Lake Whitefish, Northern Pike, and Walleye), HgT concentrations measured in fish muscle differed between lakes (P<0.05; **Appendix A: Table S3**). The highest HgT levels were observed in Sanguez Lake for Cisco, Lake Whitefish, Northern Pike, and Walleye (**Appendix A: Table S3**). The lowest HgT levels were observed in Trout Lake for Lake Whitefish and Northern Pike (**Appendix A: Table S3**). In contrast, for Cisco and Walleye, there were no significant differences in HgT concentrations between Ekali and Trout Lakes (**Appendix A: Table S3**). Inter-lake differences in HgT content were not completely explained by differences in fish size. HgT concentrations differed significantly between lakes within species when fork length was treated as a covariate (ANCOVA: F≥11.7, P<0.0001, df≥ 2,15).

Table 1
Total mercury and selenium concentration in fish harvested in the Dehcho Region, Northwest Territories, Canada (2013)

		Mercury			**Selenium**	
Fish Species	**n**	**Range (ppm)**	**Mean ± SD (ppm)**	**n**	**Range (ppm)**	**Mean ± SD (ppm)**
Burbot	9	0.228 - 0.551	0.317 ± 0.101	6	0.117 - 0.155	0.141 ± 0.013
Cisco	29	0.020 - 0.194	0.057 ± 0.045	10	0.116 - 0.239	0.174 ± 0.042
Lake Trout	13	0.207 - 0.643	0.330 ± 0.153	11	0.078 - 0.140	0.140 ± 0.056
Lake Whitefish	29	0.025 - 0.150	0.073 ± 0.038	15	0.086 - 0.307	0.173 ± 0.069
Longnose Sucker	6	0.086 - 0.127	0.100 ± 0.015	5	0.170 - 0.215	0.187 ± 0.020
Northern Pike	48	0.070 - 3.121	0.551 ± 0.598	28	0.090 - 0.229	0.140 ± 0.032
Walleye	53	0.036 - 1.428	0.415 ± 0.305	22	0.089 - 0.284	0.195 ± 0.039

4.3.2. Selenium Concentration

In contrast to the Hg results, average Se concentrations were quite similar among species, ranging from 0.140 ppm to 0.195 ppm wet weight for Northern Pike and Walleye, respectively (**Appendix A: Table 1**). As seen with Hg, Se concentrations within fish species differed between lakes. However, unlike with Hg, the highest Se contents ($P<0.05$) for Cisco, Lake Whitefish, Northern Pike, and Walleye were observed in Trout Lake (**Appendix A: Table S3**). The Se content of fish in this study is similar to previous reports of freshwater fish in Canada. For example, average Se concentrations in Northern Pike ranged between 0.17-0.50 µg g^{-1} (wet weight) (Health Canada, 2012; Lemire et al., 2015; Muscatello et al., 2006), and average Se concentrations ranged between 0.22-0.45 µg g^{-1}, 0.17-0.24 µg g^{-1}, and 0.17-0.30 µg g^{-1} for Walleye, Lake Whitefish, and Lake Trout, respectively (Cappon & Smith, 1981; Health Canada, 2012; Laird et al., 2013; Lemire et al., 2015; Tamblyn, 2011). The fish listed were harvested in lakes from Canadian Arctic and subarctic regions.

Although linear relationships were not consistently observed with log Se concentrations with either log fork length or log weight of the wild-harvested Dehcho fish, there were a few exceptions. Log Se concentration had a positive linear relationship with fork length in Lake Trout (linear regression: 3.150, $P<0.01$), but had a negative linear relationship with log fork length in Lake Whitefish (linear regression: -0.820, $P<0.01$; **Appendix A: Table S1**). Additionally, log Se concentration had a negative linear relationship with log fish weight for Cisco (linear regression: -0.259, $P<0.01$) and Lake Whitefish (linear regression: -0.252, $P<0.01$; **Appendix A: Table S2**). Thus, for some freshwater species (Lake Whitefish and Cisco in this study study), larger fish are occasionally of lower nutrient density, at least in terms of Se. Selenium compounds are transferred to higher trophic levels by dietary pathways (Janz et al., 2014). Inorganic forms of Se are biotransformed by aquatic primary producers (e.g. benthic biofilm or algae) and if these populations are scattered within a lake, then there can be regions of a lake where Se levels in some fish are higher (Janz et al., 2014). The characterization of Se in the aquatic food web is dependent on biotic and abiotic factors, but not on water concentrations

(Janz et al., 2014). Interestingly, Lake Trout was the only fish species for which increasing size was associated with higher Se content, but was not associated with increased Hg.

4.3.3. Fatty Acid Composition

There were substantial differences in the fatty acid profiles among fish species (**Table 2**). For example, *n*-3 FA concentrations in Lake Trout (1,689 mg/100 g) were up to 16.7-fold higher than observed in the Burbot (101 mg/100 g), Northern Pike (212 mg/100 g), and Walleye (239 mg/100 g) (**Table 2**). Lake Whitefish had *n*-3 FAs levels (458 mg/100 g) that were up 2.1-fold higher than in some predatory fish species, such as Northern Pike and Walleye (**Table 2**). Additionally, the EPA+DHA concentrations in Lake Trout (965 mg/100 g) were up to 11.2-fold higher than Burbot (86.3 mg/100 g), Northern Pike (176 mg/100 g), and Walleye (198 mg/100 g; **Table 2**). Lastly, the polyunsaturated fatty acid (PUFA) concentrations in Lake Trout (2,269 mg/100 g) were up to 15.2-fold higher in Burbot (150 mg/100 g) relative to Walleye (275 mg/100 g), and Northern Pike (299 mg/100 g; **Table 2)**. All of the fish species analyzed had greater levels of health-promoting *n*-3 FAs relative to their pro-inflammatory *n*-6 FA content (**Tables 2** and **Appendix A: Table S5**). However, the benefits and risks of various FAs differ with dose, ratio, and total diet composition.

Of the four species where samples were collected from more than one lake, three species (i.e. Walleye, Lake Whitefish, Northern Pike) demonstrated significant differences in *n*-3 FA content between lakes (**Appendix A: Table S4**). Interestingly though, the lake with the highest *n*-3 FA levels differed by species. Specifically, the highest *n*-3 FA levels were observed in Ekali Lake for Walleye, Sanguez Lake for Lake Whitefish, and Trout Lake for Northern Pike (**Appendix A: Table S4**). The EPA+DHA levels reported here are, on average, within 10% of those reported in Walleye, Northern Pike, and Lake Whitefish harvested in the Canadian Arctic (Lemire et al., 2015). In contrast, the EPA+DHA levels reported here for Burbot were less than half of previously reported values; while, EPA+DHA levels in Lake Trout were on average 3.2-fold higher than previously reported (Lemire et al., 2015).

Here, a linear relationship was not observed between log *n*-3 FA content and log fork length, with one exception; in Lake Whitefish larger fish was associated with greater *n*-3 FA content (linear regression: 0.911, P<0.01; **Appendix A: Table S1**). Similarly, for most of the fish species harvested in the Dehcho, there were no linear relationships observed between log *n*-3 FA content and log fish weight. However, the exceptions are Lake Whitefish (linear regression: 0.264) and Walleye (linear regression: -0.224; both P<0.01; **Appendix A: Table S2**). These results indicate that, for some freshwater species, larger fish are more nutrient-dense with respect to *n*-3 FA. However, the nature of these relationships (e.g. positive vs. negative) can differ between species.

Table 2

Fatty acid composition of fish harvested in the Dehcho Region, Northwest Territories, Canada (2013)

		Total Omega-3 Fatty Acids[a]		EPA + DHA		Omega-6 to Omega-3 Ratio		Polyunsaturated Fatty Acids	
Fish Species	**n**	**Range (mg/100 g)**	**Mean ± SD (mg/100 g)**	**Range (mg/100 g)**	**Mean ± SD (mg/100 g)**	**Range**	**Average ± SD**	**Range (mg/100 g)**	**Mean ± SD (mg/100 g)**
Burbot	9	68.4 – 127	101 ± 18.2	57.4 – 110	86.3 ± 16.3	0.45 - 0.60	0.49 ± 0.04	109 – 186	150 ± 24.2
Cisco	12	187 – 551	346 ± 115	145 – 344	224 ± 58.9	0.24 - 0.41	0.30 ± 0.06	252 – 700	449 ± 148
Lake Trout	11	258 – 4,375	1,689 ± 1,294	172 – 2,332	965 ± 544	0.28 - 0.62	0.41 ± 0.15	361 – 5,699	2,269 ± 1,642
Lake Whitefish	24	215 – 2110	458 ± 401	182 – 1,048	299 ± 187	0.22 - 0.53	0.38 ± 0.09	177 – 3205	648 ± 616
Longnose Sucker	6	208 – 763	499 ± 198	160 – 372	263 ± 68.6	0.45 - 0.69	0.54 ± 0.10	301 – 1,259	784 ± 356
Northern Pike	37	131 – 836	212 ± 123	113 – 707	176 ± 98.6	0.20 - 0.48	0.34 ± 0.06	180 – 1,096	299 ± 191
Walleye	35	117 – 911	230 ± 148	87.5 – 807	198 ± 131	0.21 - 0.47	0.34 ± 0.06	167 – 360	275 ± 95.9

[a] Total omega-3 fatty acids includes: alpha-linolenic acid (C 18:3*n*-3), stearidonic acid (C 18:4*n*-3), eicosatrienoic acid (C 20:3*n*-3), eicosatetraenoic acid (C 20:4*n*-3), eicosapentaenoic acid (C 20:5*n*-3), docosapentaenoic acid (C 22:5*n*-3), and docosahexaenoic acid (C 22:6*n*-3)

4.3.4. Correlation between Mercury and Nutrient Concentrations in Dehcho Fish

Strong negative correlations were observed between HgT and *n*-3 FA content for Lake Trout (ρ = -0.937, P<0.001), Northern Pike (ρ = -0.619, P<0.001), and Walleye (ρ = -0.481, P<0.01; **Figure 1**; **Appendix A: Table S6**). There were also significant negative correlations between Hg and Se observed for Lake Whitefish (ρ = -0.818, P<0.001), Cisco (ρ = -0.685, P<0.05), and Northern Pike (ρ = -0.410, P<0.05; **Appendix A: Table S6**). For these species, samples with the greatest nutritional content tended to have lower levels of Hg. Additionally, a strong positive correlation was observed between *n*-3 FAs and Se for Northern Pike (ρ = 0.616, P<0.001) whereas a strong negative correlation was observed for Lake Whitefish (ρ = -0.600, P<0.05; **Appendix A: Table S6**). No other correlations between *n*-3 FAs and Se were detected for the other species.

This represents one of the first times that strong, negative correlations between Hg and the content of multiple nutrients have been observed in fish – that is that fish with low Hg have high nutrient levels and fish with high Hg have low nutrient levels. From a risk-benefit perspective, the presence of such negative relationships between Hg and nutrients can contribute to substantial intra-species variation in nutrient:Hg ratios. For example, among the 12 Lake Trout samples analyzed, the *n*-3 FA:Hg ratio varied from 4 to 209 mg:µg. Although it seems plausible that trophic position, fish age, and growth rates are involved, the precise biological and ecological mechanisms behind this inverse relationship between Hg and *n*-3 FA are unknown. Each of these three variables are widely regarded as important biological and ecological determinants of fish HgT content (Choi & Grandjean, 2008; Clarkson et al., 2003; Swanson et al., 2003). Therefore, the strong negative relationship documented between *n*-3 FA and Hg could be a function of higher *n*-3 FA content being associated with lower trophic position, younger fish, or faster growth rates among Lake Trout sampled in the Dehcho Region.

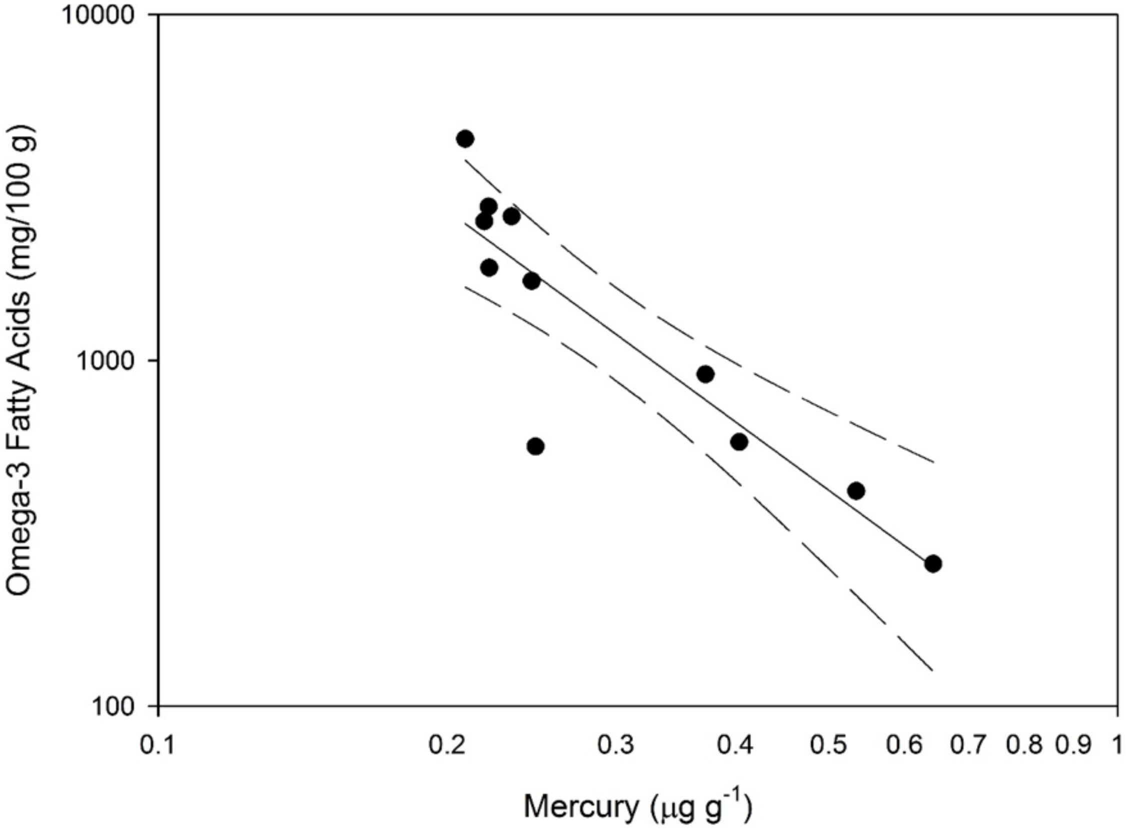

Figure 1. Relationship between total mercury concentration and omega-3 fatty acid content in Lake Trout caught in the Dehcho Region. Solid line: ANOVA regression line; Dotted line: uncertainty

4.3.5. Mercury Advisories in the Dehcho Region

Over the past five years, the DHSS has issued a series of dietary advisories regarding Hg, suggesting that people in the Dehcho should limit their consumption of predatory fish from specific lakes in the region (Healy, 2010 & 2012; DHSS, 2012). Specifically, for species exceeding, on average, Health Canada's retail HgT guideline, people were advised to limit consumption based on their susceptibility (the general population, no more than 150 grams per week; pregnant or breastfeeding mothers, no more than 150 grams per month; children 5-11 years of age, no more than 125 grams per month; and children 1-4 years of age, no more than 75 grams per month; Healy, 2010 & 2012). The specific species covered under this advisory included Northern Pike (Mean Hg: 0.62 ppm) and Walleye (Mean Hg: 0.54 ppm) from Ekali Lake and Lake Trout over 60 cm in length (Median Hg: 0. fish are likely to outweigh any potential mercury risks. As such, within these advisories58 ppm) from Trout Lake. Additionally, an advisory was put in place for Northern Pike and Walleye from Sanguez Lake. However, each of the advisories also noted that fish can be excellent sources of nutrients and that, generally speaking, the health benefits of consuming wild-harvested, the DHSS recommended that people should eat more small fish that are lower in the food chain.

The Hg concentrations reported here are considerably lower than those contained in the relevant DHSS advisories for Trout Lake and Ekali Lake (Healy, 2010 & 2012). For example, the median HgT concentration within Lake Trout (>60 cm) from Trout Lake was 0.25 ppm in this study, versus 0.58 ppm in the DHSS advisory (Healy 2010). Similarly, as shown in **Appendix A: Table S3**, mean HgT concentrations in Northern Pike (0.40 ppm) and Walleye (0.29 ppm) from Ekali Lake were 35% - 46% lower than reported in the DHSS advisory (Healy, 2012). Further work is necessary to determine if this apparent decline in Hg levels in Ekali Lake was related to year-to-year size differences in the harvested fish. In contrast, as described in the relevant DHSS advisory, average Hg concentrations in Northern Pike and Walleye from Sanguez Lake remain substantially above Health Canada’s guideline limit of 0.5 Hg ppm for retail fish. Community-based researchers are currently investigating whether fisheries management options are capable of lowering HgT levels in Northern Pike and Walleye in Sanguez Lake. Notably, Burbot, Cisco, Lake Trout, Lake Whitefish, Longnose Suckers, Northern Pike from Trout Lake, and Walleye from Ekali and Trout Lakes, were, on average below the 0.5 ppm guideline. Furthermore, the majority (i.e., 9 out of 11) of Lake Trout >60 cm collected in this study were below the 0.5 ppm retail Hg guideline.

4.3.6. Balancing Mercury and Nutrient Intakes from Traditional Fish Consumption

Several quantitative approaches have been used to evaluate concurrent risks and benefits of contaminants and nutrients in fish. For example, the method developed by Ginsberg & Toal (2009) estimates risks and benefits of fish consumption by identifying dose-response relationships for MeHg and *n*-3 FA on coronary heart disease and neurodevelopment. Another commonly applied method is to calculate nutrient:contaminant ratios, as has been done for Se and Hg, as well as DHA and Hg (Tsuchiya et al., 2008). Although simplistic in nature, this approach can assist efforts in identifying fish that tend to promote nutritional adequacy while also limiting contaminant exposure. However, the application of nutrient:contaminant ratios (e.g. Se:Hg molar ratio) within risk assessment is impeded by several sources of uncertainty, one of which is that, at present, it is unknown how high such ratios have to be in order to protect against adverse effects of Hg in subpopulations of varying sensitivity (Burger and Gochfeld, 2012).

One approach to address this source of uncertainty, as described by Tsuchiya et al. (2008), is to calculate *de minimus* ratios based upon Dietary Reference Intakes of nutrients and the toxicological reference values for contaminants. The consumption of fish and other seafood with nutrient:contaminant ratios above these *de minimus* benchmarks may facilitate individuals and populations to achieve nutritional adequacy while not exceeding the reference dose for a contaminant of concern.

Nutrient:Mercury Ratios

Nutrient:Hg ratios varied greatly among the fish species analyzed in this study. For instance, the mean DHA:Hg (mg:µg) ratio ± standard error for Cisco and Lake Whitefish had, respectively, DHA:Hg ratios of 32 ± 5.7 and 34 ± 2.9, considerably higher than observed in Burbot (2.3 ± 0.2) and Northern Pike (6.2 ± 1.0) (**Figure 2**). Similarly, the mean Se:Hg (µmol:µmol) ratios for Cisco (9.0 ± 1.6) and Lake Whitefish (9.6 ± 2.0) were substantially higher than observed in Burbot (1.1 ± 0.1) and Walleye (2.2 ± 0.6) (**Figure 2**). There was also large intraspecific variation in nutrient:Hg ratios. For example, DHA:Hg ratios for Walleye ranged from 0.8 to 46, Lake Trout ranged from 2.1 to 85, Cisco ranged from 5.6 to 61, and Lake Whitefish ranged from 12 to 61 (**Figure 2**). Likewise, Se:Hg molar ratios for Northern Pike, Walleye, Cisco, and Lake Whitefish ranged from, 0.1 to 6.6, 0.5 to 14, 2.2 to 18, and 1.5 to 25, respectively. Intraspecies variation in fish HgT concentration exceeded that of at least one of Se or *n*-3 FA content for each of the seven harvested species. Consequently, much of the variation in nutrient:Hg ratios is driven by intraspecies differences in fish HgT content. It is important to note that the ratios reported for this study are from the muscle tissue of the fish samples. But, some species are regularly consumed with the skin on the filet. Therefore, the ratios reported herein may not be representative of fish with the skin on, introducing a source of uncertainty that can be investigated in future studies.

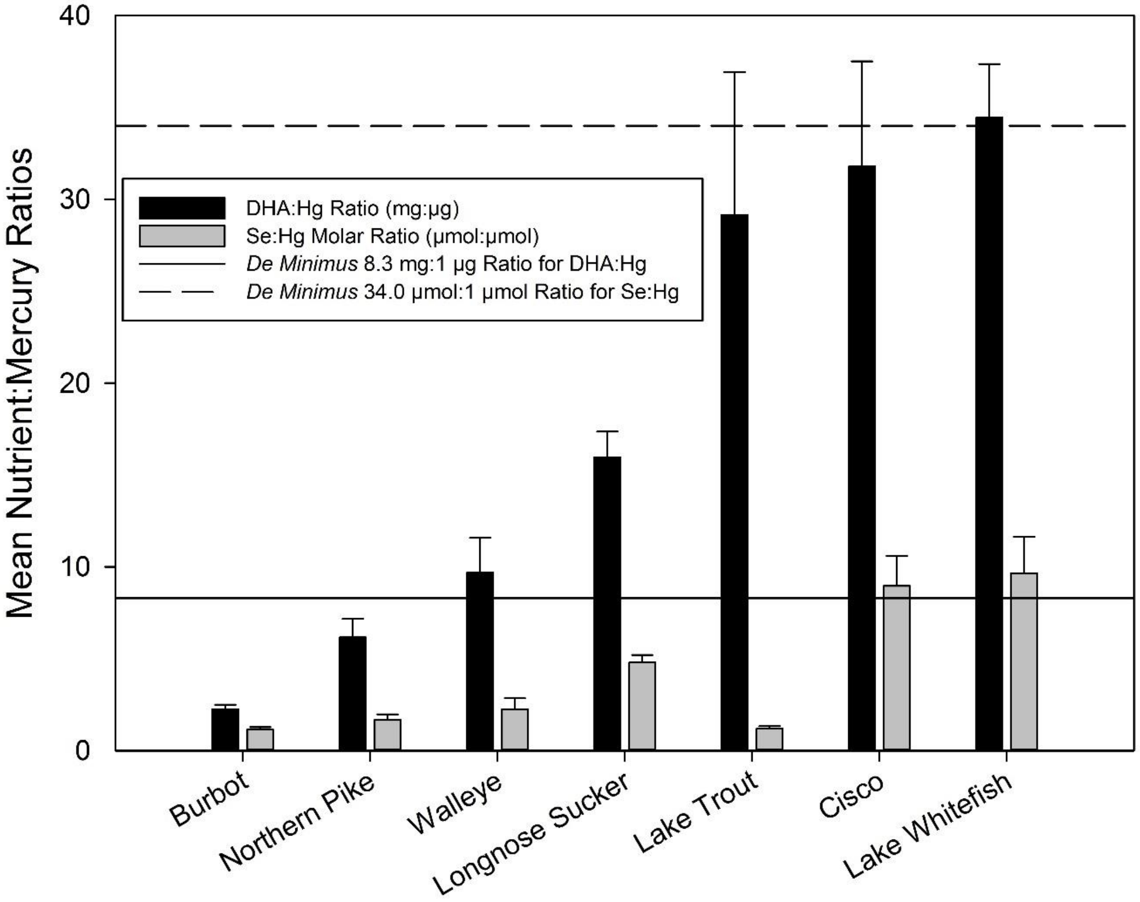

Figure 2. Mean nutrients to mercury concentration ratios ± standard error for all fish species compared to the *de minimus* ratios from Health Canada's mercury regulatory guidelines.

Both the *n*-3 FA:Hg (mg:µg) ratios and the Se:Hg molar ratios of the Dehcho fish described in this study fall within the range of nutrient:contaminant ratios reported in the literature (**Table 3**). Most of the fish results from previous literature exceed the *de minimus* ratio for DHA:Hg, while only Yellowfin Tuna, Walleye, Pacific Ocean Perch, and Whiting exceed the *de minimus* ratio for Se: Hg. Additionally, the most frequently consumed fish species in the Dehcho Region, Lake Whitefish, had the highest nutrient:contaminant ratios.

As shown in **Figure 2**, of the seven freshwater fish species collected in this study, Walleye, Longnose Suckers, Lake Trout, Cisco, and Lake Whitefish, on average, exceeded the *de minimus* ratio for DHA:Hg. In contrast, all seven of the species were below the *de minimus* molar ratio for Se:Hg. However, as noted above, it is not yet known how high the Se:Hg molar ratio has to be to protect against adverse effects of MeHg. Ralston (2008) asserted that Se:Hg molar ratios greater than 1 may be able to protect against MeHg's neurodevelopmental effects. Other researchers have used a Se:Hg molar ratio of 5 as a somewhat arbitrary risk-benefit benchmark (Burger & Gochfeld, 2012). The mechanism of this interaction is Hg binds to Se with a high affinity and inhibits selenium-dependent enzyme activity (Burger & Gochfeld, 2012). Of the seven fish freshwater fish species described herein, all on average exceeded a Se:Hg molar ratio of 1 whereas only Cisco and Lake Whitefish exceeded the 5:1 molar ratio. *De minimus* ratio methods, such as those developed by Tsuchiya et al. (2008) and expanded upon here, have several limitations. For example, they neglect to consider: i) benefits provided by other nutrients, and ii) risks posed by other contaminants within foods. This is particularly challenging because organochlorine pesticides, polychlorinated biphenyls (PCBs), and metal(loids) may interact with MeHg (Oostdam et al., 2005). Additionally, *de minimus* approaches do not account for variation in individual body weight and the role that this plays in exposure and risk. However, these methods provide a foundation for determining which fish species have higher levels of nutrients relative to Hg and developing dietary recommendations that protect fish consumers (Mahaffey et al., 2011; Tsuchiya et al., 2008).

Table 3

Mean nutrients: mercury concentration ratios from published literature

Fish Species	Mean Omega-3 Fatty Acids: Mercury Ratio[a] (mg:µg)	References	Fish Species	Mean Selenium: Mercury Molar Ratio (µmol:µmol)	References
Marlin, Blue	0.6	Dewailly et al. (2008)	Mako, Shortfin	0.3	Burger & Gochfeld (2012)
Burbot	**3.4**	**This Study**	**Burbot**	**1.1**	**This Study**
Amberjack	3.9	Dewailly et al. (2008)	**Lake Trout**	**1.2**	**This Study**
Swordfish	5.8	Mahaffey et al. (2011)	Swordfish	1.2	Kaneko & Ralston (2007)
Northern Pike	**9.3**	**This Study**	**Northern Pike**	**1.7**	**This Study**
Cod, Atlantic	14	Ginsberg & Toal (2009)	Bass, Largemouth	1.8	Burger (2012)
Walleye	14	This Study	Pickerel	1.9	Burger (2012)
Catfish	18	Mahaffey et al. (2011)	**Walleye**	**2.2**	**This Study**
Tuna, White albacore	24	Mahaffey et al. (2011)	Cod, Pacific	2.7	Burger & Gochfeld (2013)
Halibut	32	Ginsberg & Toal (2009)	Kingfish, Southern	3.2	Burger & Gochfeld (2012)
Haddock	40	Mahaffey et al. (2011)	Marlin, Blue	4.1	Kaneko & Ralston (2007)
Longnose Sucker	**52**	**This Study**	**Longnose Sucker**	**4.8**	**This Study**
Sardine	54	Domingo et al. (2007)	Albacore	5.3	Kaneko & Ralston (2007)
Lake Trout	**66**	**This Study**	Flounder, Summer	6.4	Burger & Gochfeld (2012)
Cisco	**67**	**This Study**	Sunfish, Bluegill	7.4	Burger (2012)
Lake Whitefish	**72**	**This Study**	**Cisco**	**9.0**	**This Study**
Pollack	90	Ginsberg & Toal (2009)	**Lake Whitefish**	**9.6**	**This Study**
Crayfish	127	Mahaffey et al. (2011)	Wahoo	11	Kaneko & Ralston (2007)
Cuttlefish	180	Domingo et al. (2007)	Sunfish, Red-breasted	13	Burger (2012)
Trout	342	Ginsberg & Toal (2009)	Tuna, Yellowfin	14	Kaneko & Ralston (2007)
Herring, Atlantic	504	Ginsberg & Toal (2009)	Perch, Pacific Ocean	47	Burger & Gochfeld (2013)
Salmon, Atlantic	1,537	Ginsberg & Toal (2009)	Whiting	68	Burger & Gochfeld (2013)

[a] The *n*-3 FAs:Hg ratios were calculated by dividing the average *n*-3 FA concentration by the average Hg concentration for each individual fish species.

4.4. Conclusion

The health benefits of traditional food consumption in First Nations communities include physical, social, cultural, and spiritual well-being (Oostdam et al, 2005). Therefore, public health initiatives within and beyond the Dehcho should include efforts to sustain and promote healthy, traditional food harvesting practices. These efforts may be assisted through the development of probabilistic dose reconstruction models of both nutrient and contaminant exposure. The nutrient and contaminant distributions (as well as the correlations between these distributions) described in this manuscript represent key input parameters for the development of such exposure analyses.

Funding Agencies

The authors gratefully acknowledge funding from the Aboriginal Aquatic Resources and Oceans Management (AAROM) program (George Low, Mike Low), Health Canada's Climate Change Adaptation Fund, the Northern Contaminants Program, which is jointly funded by Aboriginal Affairs and Northern Development Canada and Health Canada, and the Cumulative Impacts Monitoring Program. Juan Aristizabal Henao is the recipient of a Natural Sciences and Engineering Research Council of Canada Doctoral Scholarship. Ken Stark is supported through a Canada Research Chair in Nutritional Lipidomics. Additional funding support was provided by the School of Public Health and Health Systems at the University of Waterloo. We thank Xiaofeng Wang (University of Saskatchewan) for analytical assistance on the project, and Angus Sanguez, Laura Sanguez, and Ernest Hardisty for assistance and expertise in collecting samples.

5. Maximizing Nutrient Intake and Limiting Mercury Exposure of those Consuming Wild-harvested Fish in the Dehcho Region of the Northwest Territories, Canada by Probabilistic Modelling

Overview

Mercury (Hg) bioaccumulate in traditional food sources, such as wild-harvested freshwater fish and marine animals, in the Canadian subarctic environment. This results in a dilemma for northern Indigenous populations because some of the traditional foods with high mercury levels are also have important nutritional, social, and cultural benefits. The goal of this study was to utilize a probabilistic optimization technique using Crystal Ball's OptQuest to balance contaminant risks from Hg exposure with the nutrient benefits from eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) and selenium (Se) in wild-harvested fish from the Dehcho Region, Northwest Territories (NWT), Canada. The best solutions within the OptQuest model were the optimum food choices that maximize nutrient intake, while also limiting the upper percentile of Hg exposure from the consumption of freshwater fish. The total amount of fish within the best solutions for the women of child-bearing age were 546 and 1,359 g/week to achieve nutritional adequacy for EPA+DHA and Se, respectively, while also limiting the TRVs for Hg exposure. The total amount of fish within the best solutions for the general population age were 851 and 1,848 g/week, for EPA+DHA and Se nutrient adequacy, respectively while constraining the upper percentile of Hg exposure. The estimates of Hg exposure that were based on the single set of best solutions that maximize nutrient intake for EPA+DHA and Se, respectively, while also limiting the upper percentile of mercury exposure for Hg were 0.96 and 1.40 µg/kg/week for women of child-bearing age. Whereas, the estimates of Hg exposure from the ideal solution for the general population were 2.73 and 2.91 µg/kg/week to achieve nutritional adequacy for EPA+DHA and Se, respectively, while also limiting Hg exposure. The models indicated that the consumption of Burbot, Cisco, Lake Whitefish, and Longnose Suckers would help people achieve nutritional adequacy without exceeding the TRVs for Hg. The strengths of this OptQuest-based approach were the repeatability of the best solution from the OptQuest output, consistency with general public health messaging, and the ability to customize OptQuest parameters according to the receptor (e.g. general population, women of child-bearing age). However, the limitations encountered while attempting to use Optquest to balance contaminant risks and nutritional benefits were inconsistency with other lines of evidence (e.g. nutrient:contaminant ratios), and dramatically different best solutions between nutrients.

5.1. Introduction

Mercury compounds (Hg) are naturally-occurring pollutants that are released throughout the world by global atmospheric cycling and long-range transport mechanisms, and found in bodies of water, such as lakes and oceans (Jæger et al., 2009; Li et al., 2009). Levels of Hg in the air and water are low and therefore do not contribute a significant source of Hg exposure in human populations; people are usually exposed to Hg by dietary fish consumption (Clarkson et al., 2003). The organic form of Hg, methylmercury (MeHg) can bioaccumulate in the muscle tissues of aquatic biota, and biomagnify up the food chains of freshwater and marine ecosystems (Ariya et al., 2004; Ayotte et al., 1995). MeHg can cause a wide range of adverse health effects in humans, which are dependent on the dose and duration of exposure (Mergler et al., 2007). Some of the health effects observed following higher exposures in infants and children, include neurodevelopmental effects, such as cerebral palsy (Harada, 1995), delayed psychomotor or mental performance, and reduced birth weight and length (Ramon et al., 2009). Developmental neurotoxicity is typically used as the basis for Hg guidelines and public health policies as it is the most sensitive endpoint (Legrand et al., 2010; Mergler et al., 2007).

There are 1.4 million people living in Canada with an Aboriginal identity, representing 4.3% of the Canadian population (National Household Survey, 2014). Within the Aboriginal population, 60% were identified as First Nations people (Gionet & Roshanafshar, 2013). Data collected from Canadian Community Health Survey (CCHS) showed the health disparities facing First Nations include chronic diseases, such as obesity, type 2 diabetes, and respiratory problems, and household food insecurity (Gionet & Roshanafshar, 2013; Young, 2003).

First Nations people living in northern Canada are vulnerable to MeHg exposure as they consume traditional food sources that contain elevated Hg concentrations, such as wild-harvested fish and marine animals (Oostdam et al., 2005). In particular, the Dehcho First Nations, a coalition of First Nations and Métis communities in the southwestern area of the Northwest Territories (NWT) in Canada, may face elevated exposures to MeHg. A series of dietary advisories by the Government of the NWT Department of Health and Social Services (DHSS) advised residents of the increase in mercury levels in three lakes and recommended that people limit the quantity of particular fish that they eat (Healy, 2010 & 2012; DHSS, 2012). However, harvest and consumption of traditional food fish species plays a critical role in the social, cultural, spiritual, economic, and nutritional well-being in many communities; therefore, these benefits must also be considered when assessing the potential health risks posed by Hg levels in traditional foods (Oostdam et al., 2005). Nutrients found in fish, such as long chain fatty acids [e.g. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] and selenium (Se) may have protective effects against Hg exposure (Egeland & Middaugh, 1997; Mahaffey, 2004; Mahaffey et al. 2011). Therefore, it is critical to design dietary advisories that maximize the nutritional benefits of EPA+DHA and Se while also minimizing Hg exposure (Egeland & Middaugh, 1997).

Exposure assessments provide crucial information for risk characterization (Fryer et al., 2006; He et al., 2013). Probabilistic exposure models have a number of advantages over the typical, traditional deterministic approaches. For example, probabilistic models can be used to determine the full range of possible exposure scenarios, including information about variability and the distribution of exposure (Gilsenan et al., 2003; Sioen et al., 2007). Probabilistic estimates can also improve understanding of how co-associated variables within an exposure model contribute to contaminant risks (He et al., 2013). Also, probabilistic models make it unnecessary to rely on single, conservative point estimates that can overestimate contaminant risks (Sioen et al., 2007). Also, probabilistic models can facilitate optimization procedures that help characterize the ways by which populations can maximize the intake of desirable food constituents (e.g. nutrients) while limiting the exposure of potentially harmful contaminants, such as Hg (Fryer et al., 2006; Laird et al., 2013).

The primary objective for this research paper was to investigate the utility of Crystal Ball's OptQuest to balance contaminant risks and nutrient benefits from the Hg, Se, and EPA+DHA content from wild-harvested fish in the Dehcho Region, NWT, Canada. The utility of the Crystal Ball OptQuest model was judged according to the following criteria: 1) ability to simultaneously consider multiple contaminants and/or nutrients; 2) repeatability of the "best solution" from the OptQuest output; 3) consistency with other lines of evidence regarding the balance between contaminant risks and nutritional benefits (e.g. nutrient:contaminant ratios); 4) consistency with general public health messaging regarding the risks and benefits of seafood; 5) ability to customize OptQuest parameters according to receptor (e.g. general adult population, women of child-bearing age) characteristics.

5.2. Research Methods

5.2.1. Probabilistic Modelling

The use of OptQuest in Crystal Ball

The dietary probabilistic model was assessed using OptQuest in Crystal Ball (Fusion Edition; version 11.1.1.1; Oracle). Crystal Ball relies on Monte Carlo analyses, which refers to a computational method that relies on random sampling to obtain results, such as probabilistic distribution of exposure for a population (He et al., 2013; United States EPA, 1997). OptQuest is an optimization tool that runs within Crystal Ball, enhancing simulation models by searching for and finding optimal solutions to a forecast. Such forecasts represent the mathematical combination of inputted assumption (e.g. mercury concentration) and decision variables (e.g. quantity of fish consumed) within a Crystal Ball model. In OptQuest, a trial refers to a three-step process whereby Crystal Ball selects numbers for assumption and decision variables, recalculates the spreadsheet, and displays the results in the forecast chart. In contrast, a simulation refers to a set of many, usually thousands, of trials. Increasing the number of trials can improve precision whereas increasing the number of simulations can improve the ability of the model find the "best solution", however both require longer simulation times within Crystal Ball. The term "best solutions" within the OptQuest model refers to the optimum food choices that maximize nutrient intake, while also limiting the upper percentile of Hg exposure from the consumption of freshwater fish.

Each OptQuest model within Crystal Ball has one objective but can have zero, one, or more requirements; both objectives and requirements are based upon forecast variables in the model. The model does not use any solution (i.e. set of decision variables) that does not comply with the requirements. Therefore in OptQuest, the term feasible solution is a solution that satisfies any constraints imposed on the decision variables, in contrast to infeasible solutions that fail to meet at least one of the requirements. The goal of OptQuest in Crystal Ball is to find the optimal solution of the objective, while also meeting the constraints of the requirements, by selecting and improving different values for the decision variables. For additional details on the terminology used in Crystal Ball's OptQuest, see **Appendix B: Table S1**.

For this study, each Optquest model consisted of n = 10,000 simulations (i.e. sets of decision variables) each of which included 3000 trials. Additionally, each model was run three times to ensure repeatability. The two exposure assessment receptors considered within the Optquest model were women of child-bearing age and the general population (e.g. men above 18 years old and women of 40 years of age and older).

Estimation of mercury exposure and nutrient intakes

In August 2013, seven species of freshwater fish (Burbot, Cisco, Lake Trout, Lake Whitefish, Longnose Suckers, Northern Pike, and Walleye) were harvested from Ekali, Sanguez, and Trout Lakes near the Jean Marie River First Nations (JMRFN) in the NWT, Canada. The concentrations of total Hg, EPA+DHA, and total Se in the muscle tissues of the various fish species, correlation analyses between nutrient and contaminant concentrations, and the fish species that had the highest fatty acid and Se levels relative to their Hg content were obtained from previous work by Reyes et al. (Unpublished work). Input variables for the OptQuest model included decision variables, such as 1) fish (i) intake rates for each of the seven species (g

$fish_i$/wk) and assumption variables, such as 2) Hg concentration in each fish species (µg Hg/g $fish_i$); 3) Se concentration in each fish (µg Se/g $fish_i$); 4) EPA+DHA concentration in each fish (mg EPA+DHA/100 g $fish_i$); 5) body weight (*j*) for each age group (kg) (Laird et al., 2013). Body weight distribution came from the *Canadian Exposure Factors Handbook* by Richardson & Stantec (2013). **Table 4** shows the complete parameters defined in the OptQuest model.

The forecast variables in the model included the following: 6) total fish intake (g wk^{-1}) (**Eq. 1**); 7) mercury intake (µg kg^{-1} wk^{-1}) from each $fish_i$ (**Eq. 2**); 3) selenium intake (µg wk^{-1}) from each $fish_i$ (**Eq. 3**); 8) EPA+DHA intake (mg wk^{-1}) from each $fish_i$ (**Eq. 4**) (Laird et al., 2013). Each forecast represented the mathematical combination of the aforementioned assumption and decision variables:

$$Total\ Fish\ Intake = \sum_{i=1}^{7} fish_i(g \cdot wk^{-1})\ (\mathbf{Eq. 1})$$

$$Hg\ Intake_{i,j} = \frac{fish_i(g \cdot wk^{-1}) \times [Hg_i](\mu g \cdot g^{-1})}{body\ weight_j\ (kg)}\ (\mathbf{Eq. 2})$$

$$Se\ Intake_i = fish_i(g \cdot wk^{-1}) \times [Se_i](\mu g \cdot g^{-1})(\mathbf{Eq. 3})$$

$$EPA + DHA\ Intake_i = fish_i(\mathrm{g} \cdot wk^{-1}) \times [EPA + DHA_i](mg \cdot g^{-1})\ (\mathbf{Eq. 4})$$

To run the optimization in Crystal Ball’s OptQuest: 1) the objective for the upper percentile of the distribution that meets the nutrient adequacy for EPA+DHA intake is 2,100 mg/week and Se intake is 385 µg/wk; 2) the first requirement is the TRV for Hg exposure which must be $\leq$ 1.40 µg/kg/week for women of child-bearing age and $\leq$ 5.00 µg/kg/week for the general population; 3) the second requirement for total fish consumption must be < 2,100 g/wk. Therefore in total, there are four models run in OptQuest: 1) women of child-bearing age for

EPA+DHA intake; 2) women of child-bearing age for Se intake; 3) general population for EPA+DHA intake; 4) general population for Se intake.

The specific objectives were based upon the dietary reference intakes (DRIs) for EPA+DHA intake of 300 mg/day which was derived by Kris-Etherton et al. (2009) and for Se intake of 55 µg/day from the Expert Group on Vitamins and Minerals (2003) and the Scientific Committee for Food (2000). The first requirement was from Health Canada’s toxicological reference values (TRVs) for Hg exposure of 0.2 μg/kg body weight (bw)/day for women of child-bearing age and 0.47 μg/kg bw/day for the general population (Legrand et al., 2010). The second requirement was meant to constrain the decision variables such that the total fish consumption could not exceed three meals per day, seven days per week.

Table 4
Parameter distribution for maximizing nutrient intake of EPA+DHA and selenium while minimizing mercury exposure in OptQuest

Parameters	Variable	Type	Distribution
Mercury concentrations for each fish species	Assumption	log-normal	Data set in Reyes et al.*
Selenium concentrations for each fish species	Assumption	log-normal	Data set in Reyes et al.*
EPA+DHA concentrations for each fish species	Assumption	log-normal	Data set in Reyes et al.*
Women of child-bearing age body weight	Assumption	log-normal	Mean: 69.8[a]; Standard deviation: 16.3[a]; Lower limit: 34.0; Upper limit: 122.0
General population body weight	Assumption	log-normal	Mean: 76.5[a]; Standard deviation: 15.8[a]; Lower limit: 34.0; Upper limit: 122.0
Burbot intake rate	Decision	discrete	Lower limit: 0; Upper limit: 1,050; Step size: 75
Cisco intake rate	Decision	discrete	Lower limit: 0; Upper limit: 1,050; Step size: 75
Lake Trout intake rate	Decision	discrete	Lower limit: 0; Upper limit: 42; Step size: 3 for women of child-bearing age Lower limit: 0; Upper limit: 154; Step size: 11 for the general population
Lake Whitefish intake rate	Decision	discrete	Lower limit: 0; Upper limit: 1,050; Step size: 75
Longnose Sucker intake rate	Decision	discrete	Lower limit: 0; Upper limit: 1,050; Step size: 75
Northern Pike intake rate	Decision	discrete	Lower limit: 0; Upper limit: 42; Step size: 3 for women of child-bearing age Lower limit: 0; Upper limit: 154; Step size: 11 for the general population
Walleye intake rate	Decision	discrete	Lower limit: 0; Upper limit: 42; Step size: 3 for women of child-bearing age Lower limit: 0; Upper limit: 154; Step size: 11 for the general population

Reyes et al. * = unpublished data

[a] Reference for the mean body weight and standard deviation: Richardson & Stantec, 2013; U.S. EPA, 2011

5.3. Statistical Analyses

For analyzing the results, the mean, standard deviation, 10th, 50th, and 90th percentiles of the nutrient intakes and mercury exposures for both receptors were extracted from the Crystal Ball Report.

5.4. Results and Discussion

The total amount of fish within the best solutions from the OptQuest output for the general population were 851 and 1,848 g/week for EPA+DHA and Se, respectively, while also limiting the upper percentile for the TRVs for Hg exposure (**Figure 3A**). The total amount of fish within the best solutions for the women of child-bearing age were 546 and 1,359 g/week, for EPA+DHA and Se, respectively while also minimizing Hg exposure (**Figure 3B**). The total fish intake was split between the general population and women of child-bearing age (**Figures 3A** and **3B**). The nutrient intake and Hg exposure distributions associated with the best solutions from the OptQuest model are in **Table 5**.

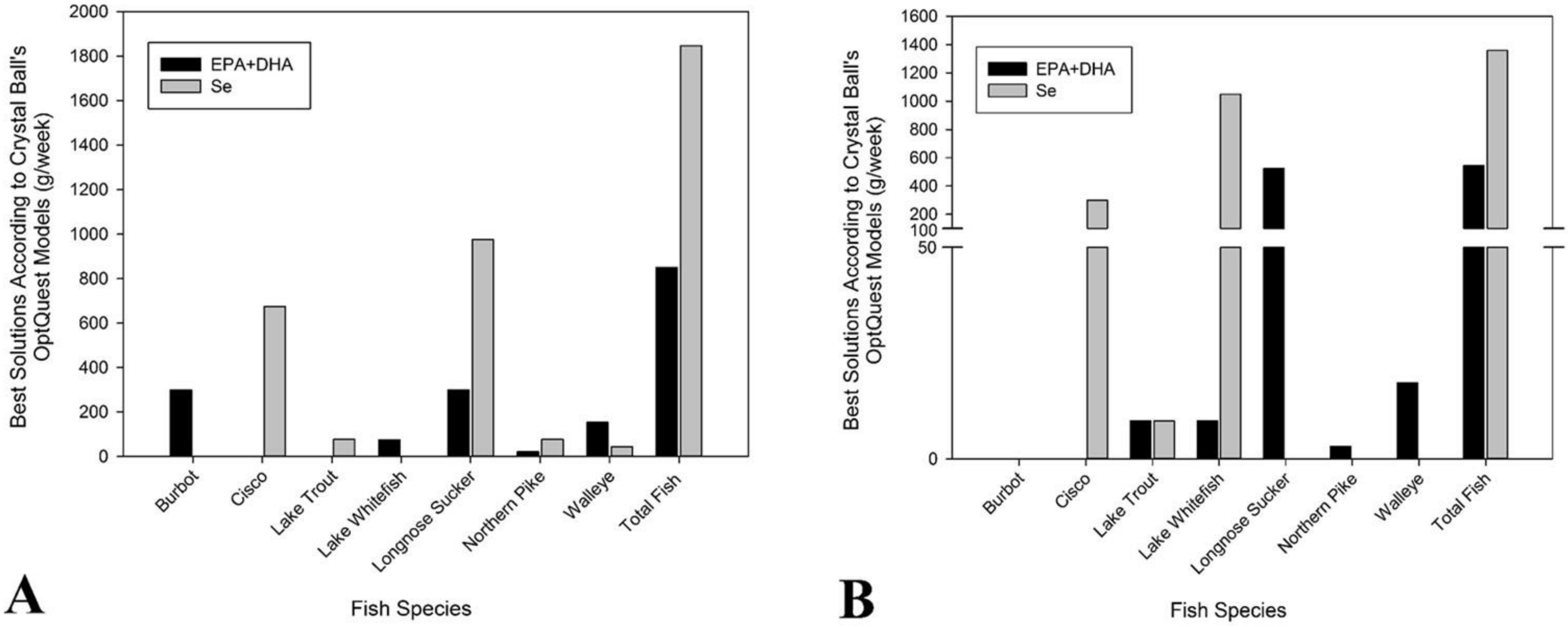

Figure 3. Best solutions according to Crystal Ball's OptQuest models that maximize nutrient intake while limiting the upper percentile of mercury exposure from the consumption of freshwater fish in the Dehcho Region for (A): the general population and (B): women of child-bearing age.

Table 5
Nutrient intake and mercury exposure distributions associated with the best solutions from the Crystal Ball's OptQuest models that maximize nutrient intake while limiting the upper percentile of mercury exposure from fish consumption in the Dehcho Region

Age Group	**Nutrient**	***n***	**Trials per *n***	**Best Solution for 95% Percentile of Nutrient Intake (Mean ± S.D.)**	**Nutrient Intake Percentiles**			**Mean Mercury Exposure ± S.D. (µg/kg/week)**	**Mercury Exposure Percentiles (µg/kg/week)**		
					10th	**50th**	**90th**		**10th**	**50th**	**90th**
Women of Child-Bearing Age	EPA+DHA[a]	10,000	3,000	2,100.10 (1,490.82 ± 336.14)	1,080.48	1,455.57	1,945.36	0.96 ± 0.26	0.67	0.93	1.30
	Se[b]	10,000	3,000	352.77 (230.65 ± 64.68)	156.70	221.28	320.17	1.40 ± 0.64	0.71	1.28	2.23
General Population	EPA+DHA[a]	10,000	3,000	2100.04 (1,582.84 ± 285.01)	1,235.62	1,561.00	1,971.35	2.73 ± 0.87	1.76	2.58	3.92
	Se[b]	10,000	3,000	384.99 (329.34 ± 32.81)	287.80	327.55	373.12	2.91 ± 0.87	1.95	2.76	4.08

[a] Units for EPA+DHA intake: mg/week
[b] Units for Se intake: µg/week

The combination of decision variables that optimized the objective remained identical when the procedure was repeated three times for each of the four models. Consequently, there are no error bars in the figures associated with the best solutions because of the lack of variability between runs (**Figures 3A** and **3B**). Future work will explore how changes in the input variables in the models influence the best solutions within Optquest. The results from this study showed that there was a higher number of infeasible solutions for women of child-bearing age compared to general population. The number of infeasible solutions for meeting the requirements for EPA+DHA and Se intake were 7,455 and 9,375, respectively, for women of child-bearing age. In contrast, the number of infeasible solutions for meeting the requirements for EPA+DHA and Se intake were 3,153 and 5,143, respectively, for the general population. Additionally, none of the infeasible solutions resulted from constraints on total fish intake. Instead, the feasibility of solutions were entirely based on the Hg TRVs exposures. As such, the total fish intakes explored within the OptQuest model appeared to fall within realistic ranges.

The best solutions for meeting the upper percentile of distribution that meets the nutrient adequacy for the women of child-bearing age was 2,100.10 mg/week for EPA+DHA intake and 352.77 µg/wk for Se intake (**Table 5**). The best solutions for meeting the nutrient adequacy for the general population was similar to the women of child-bearing age of 2,100.04 mg/week for EPA+DHA intake and 384.99 µg/wk for Se intake (**Table 5**). The estimates of Hg exposure that were based on the single set of best solutions for the women of child-bearing age that maximize nutrient intake for EPA+DHA and Se, respectively, while also limiting the upper percentile of mercury exposure for Hg were 0.96 ± 0.26 and 1.40 ± 0.64 µg/kg/week (**Table 5**). Whereas, the estimates of Hg exposure from the ideal solution for the general population were 2.73 ± 0.87 and 2.91 ± 0.87 µg/kg/week to achieve nutritional adequacy for EPA+DHA and Se, respectively, while also limiting Hg exposure (**Table 5**). The OptQuest models were also run for two additional exposure receptors: toddlers and children. However, OptQuest struggled to identify feasible solutions for these receptors. This likely a function of the higher intake to body weight ratios observed in children combined with a conservative regulatory limit based upon protecting against adverse effects to fetal neurodevelopment.

Fish intake rates for predatory fish (i.e. Lake Trout, Northern Pike, and Walleye) within the OptQuest best solution were lower for women of child-bearing than the general population (**Figures 3A** and **3B**). For example, to achieve the nutrient adequacy for EPA+DHA intake for the women of child-bearing age, the best solutions were 9, 3, and 18 g/week for Lake Trout, Northern Pike, and Walleye, respectively (**Figure 3B**). In contrast, the best solutions for the general population were 0, 22, and 154 g/week for the same species, respectively (**Figure 3A**). To achieve Se nutrient adequacy, the best solutions for the women of child-bearing age were 9, 0, and 0 g/week for Lake Trout, Northern Pike, and Walleye, respectively (**Figure 3B**); whereas, the best solutions for the general population were 44, 143, and 121 g/week for the same species, respectively (**Figure 3A**). Lastly, within each receptor, the species of fish (and their quantities) identified within the best solution from the Optquest model depended on which nutrient was being focused upon (**Figures 3A** and **3B**). For instance, with the general population, the best solutions to meet the objective for EPA+DHA intake were Burbot (300 g/week), Lake Whitefish (75 g/week), Longnose Sucker (300 g/week), Northern Pike (22 g/week), and Walleye (154 g/week) (**Figure 3A**). In contrast, the best solutions for Se intake for the same receptor included Cisco (300 g/week), Lake Trout (44 g/week), Longnose Sucker (750 g/week), Northern Pike (143 g/week), and Walleye (121 g/week) (**Figure 3A**). The sum of fish intake differ so dramatically between nutrients of interest for any particular exposure receptor because multiple objectives cannot be considered simultaneously, and thus multiple contaminants and/or nutrients cannot simultaneously considered.

The strengths of utilizing Crystal Ball's OptQuest were the repeatability of the best solution from the OptQuest model, consistency with dietary recommendations as described by DHSS advisories, and the ability to customize OptQuest depending on the receptor. The best solutions from the OptQuest model predominantly showed that Burbot, Cisco, Lake Whitefish, and Longnose Suckers appear to be examples of fish that can be consumed in higher quantities without exceeding the TRV for Hg while also meeting nutrient adequacy (**Figures 3A** and **3B**). Additionally, from the ideal solutions, the fish intake rates for the predatory fish (ie. Lake Trout, Northern Pike, and Walleye) were lower than the smaller fish and fish that are lower in the food chain (**Figures 3A** and **3B**). Based on the best solutions according to the OptQuest output, these results were coherent with the general public health messages as described by the DHSS dietary advisories. OptQuest is also useful because it can be customized depending on the receptor by changing the assumption variable for body weights and the requirement for Hg exposure when entering data in the OptQuest model. For example, for women of child-bearing age, the body weight entered into the OptQuest model was 69.8 kg and the requirement for the TRV for Hg exposure was $\leq$ 1.40 µg/kg/week. In contrast, for the general population, the data entered into the OptQuest model was 76.5 kg for the body weight and the requirement for Hg exposure was $\leq$ 5.00 µg/kg/week.

However, the limitations of this OptQuest model were: incoherent results of the nutrient:mercury ratios and inconsistent sets of optimal decision variables from one nutrient to another. The mean fatty acid:Hg ratios for Cisco and Lake Whitefish were considerably higher than observed in Burbot and Northern Pike (Reyes et al., Unpublished work). In contrast with the OptQuest approach for the general population, the best solutions to meet the objective for EPA+DHA nutrient adequacy were Burbot (300 g/week), Longnose Suckers (300 g/week), and Walleye (154 g/week) (**Figure 3A**). For the women of child-bearing age, the best solutions to meet the objective for EPA+DHA nutrient adequacy were Longnose Suckers (525 g/week) and Walleye (18 g/week) (**Figure 3B**). Similarly, the mean Se:Hg molar ratios for Cisco and Lake Whitefish were substantially higher than observed in Burbot and Walleye (Reyes et al., Unpublished work). In contrast with the OptQuest approach for the general population, the best solutions to meet the objective for Se nutrient adequacy were Longnose Suckers (975 g/week) and Cisco (675 g/week) (**Figure 3A**).

Another limitation of utilizing Crystal Ball's OptQuest for balancing Hg risks and nutrient benefits was the models for each nutrient yield dramatically different sets of decision variables (**Figures 3A** and **3B**). For instance, for the women of child-bearing age, the best solution to meet the objective for EPA+DHA intake for the decision variables were Lake Trout (9 g/week), Longnose Suckers (525 g/week), Northern Pike (3 g/week), and Walleye (18 g/week) (**Figure 3B** and **Table 5**). However, for the same receptor, the best solution to meet the objective for Se intake for the decisions variables were Cisco (300 g/week), Lake Trout (9 g/week), Lake Whitefish (1,050 g/week) (**Figure 3B** and **Table 5**). A similar pattern was also observed with the general population as different species and quantities of fish are dependent on the nutrient. The next step involved to improve the OptQuest model is to simultaneously consider multiple contaminants and/or nutrients by making nutrient adequacy for EPA+DHA and Se intake requirements, while changing the objective to be the maximization of total fish intake.

5.5. Conclusion

The contaminant for risks associated with traditional food consumption are placed within the context of nutritional, social, cultural, economic, and spiritual benefits (Oostdam et al., 2005). Therefore, it is important to develop mitigation and risk communication strategies that promote health by maximizing nutrient intake while also minimizing Hg exposure. To reduce Hg exposure, it is recommended that people follow recommendations by DHSS advisories that include eating smaller fish as well as eating more fish that are lower in the food chain, such as Lake Whitefish and Grayling and eating less that are higher in the food chain such as Walleye, Northern Pike, or Lake Trout (Healy, 2010 & 2012; DHSS, 2012). The results from this study are consistent with the dietary recommendations by the DHSS consumption advisories as both the women of child-bearing age and the general population living in the Dehcho Region can consume higher quantities of smaller fish that are lower in the food chain and eat smaller amounts of predatory fish. The work from this study may inform risk communication strategies for authorities in public health departments by making recommendations of consuming certain fish species in the region that are higher in nutrients relative to their contaminant levels.

6. Conclusions

The results from this research attempts to balance contaminant risks and nutrients benefits of wild-harvested freshwater fresh in three lakes of the Dehcho Region of the NWT, Canada. The first study quantified the levels of total Hg, *n*-3 FAs, and Se in various freshwater fish species, evaluated the correlations between nutrient and contaminant concentrations, and identified which fish species provided the highest nutrient levels relative to their Hg content. The second study achieved this goal by utilizing a probabilistic modelling technique, Crystal Ball's OptQuest, to identify maximum nutrient intake while also minimizing Hg exposure to inform dietary recommendations that promote health. The literature review provided background research on sources of MeHg in the environment and the diet, adverse health effects of MeHg exposure, health benefits of *n*-3 FAs and Se, and the risk-benefit analysis of dietary fish consumption. A literature search was also used to compare the nutrient:contaminant ratios for the first study.

The concentration data from the first study showed that predatory fish [including Northern Pike (0.551 ppm), Walleye (0.415 ppm), and Lake Trout (0.330 ppm)] had higher Hg concentrations than observed in benthivorous and planktivorous fish species [e.g. as Cisco (0.057 ppm) and Lake Whitefish (0.073 ppm)]; whereas in contrast to the Hg results, average Se concentrations were quite similar between species, ranging from 0.140 to 0.195 ppm for Northern Pike and Walleye, respectively. There were substantial differences in fatty acid profiles between fish species, for instance, Lake Whitefish, the most commonly consumed fish in the Dehcho Region, had *n*-3 FA levels (458 mg/100 g) that were 2.1-fold higher than in some predatory fish species, such as Northern Pike (212 mg/100g) and Walleye (230 mg/100g). The correlation analysis showed strong negative correlations between HgT and *n*-3 FA content for

Lake Trout, Northern Pike, and Walleye. There were also significant negative correlations observed between Hg and Se observed for Lake Whitefish, Cisco, and Northern Pike. Lastly, the species that had highest *n*-3 FAs levels relative to their Hg content were Walleye, Longnose Suckers, Lake Trout, Cisco, and Lake Whitefish, whereas in contrast only Cisco and Lake Whitefish had the highest mean Se:Hg ratios.

The nutrient:Hg ratios and OptQuest model approaches that were utilized for this study to determine the optimal food choices that achieve nutritional sufficiency, while not exceeding the Hg TRV yielded different answers. Of the seven freshwater fish species collected in this study, Walleye, Longnose Suckers, Lake Trout, Cisco, and Lake Whitefish, on average, exceeded the *de minimus* ratio for DHA:Hg (8.3 mg:1 µg). However, all seven of the species were below the *de minumus* ratio for Se:Hg (34.0 µmol:1 µmol). In contrast to the OptQuest models, the consumption of Burbot, Cisco, Lake Whitefish, and Longnose Suckers would help people achieve nutritional adequacy without exceeding the TRVs for Hg. Further research will be necessary to determine which of these approaches, if either, yields the most useful information for refining public health messages and risk communication strategies that balance nutrient benefits and contaminant risks of seafood consumption. The risk-benefit balance would be helped by having a better exposure characterization through contaminant biomonitoring in humans.

Appendices

Appendix A: Statistical analyses by linear regression and analyte concentration data stratified by lake location

Supplementary Material, Table S1. Statistical analyses by linear regression for mercury, selenium, and omega-3 fatty acid content vs. fish fork length in all fish species

Fish Species	Hg vs. Fork Length	Se vs. Fork Length	*n*-3 FAs vs. Fork Length
Burbot	4.883* (n=9)	0.345 (n=6)	-1.529 (n=9)
Cisco	1.596*** (n=19)	-0.280 (n=10)	-0.462 (n=10)
Lake Trout	1.154 (n=13)	3.150** (n=11)	-3.368 (n=11)
Lake Whitefish	1.314*** (n=29)	-0.820** (n=15)	0.911** (n=24)
Longnose Sucker	7.785 (n=6)	0.863 (n=5)	-23.62 (n=6)
Northern Pike	2.154*** (n=47)	-0.083 (n=29)	-0.052 (n=37)
Walleye	1.986***(n=52)	0.251 (n=22)	-0.386 (n=35)

Level of statistical significance: * P<0.05, ** P<0.01, *** P<0.001
df = 1

Supplementary Material, Table S2. Statistical analyses by linear regression for mercury, selenium, and omega-3 fatty acid content vs. fish weight in all fish species

Fish Species	**Hg vs. Weight**	**Se vs. Weight**	***n*-3 FAs vs. Weight**
Burbot	1.062 (n=9)	0.352 (n=6)	-0.495 (n=9)
Cisco	0.188 (n=16)	-0.259** (n=9)	0.028 (n=10)
Lake Trout	-0.033 (n=6)	0.758 (n=6)	0.275 (n=6)
Lake Whitefish	0.395*** (n=29)	-0.252** (n=15)	0.264** (n=24)
Longnose Sucker	1.854 (n=6)	-0.151 (n=5)	-2.245 (n=6)
Northern Pike	0.640*** (n=47)	-0.021 (n=29)	-0.027 (n=37)
Walleye	0.696*** (n=46)	0.109 (n=19)	-0.224** (n=29)

Level of statistical significance: * $P<0.05$, ** $P<0.01$, *** $P<0.001$
df = 1

Supplementary Material, Table S3. Total mercury and selenium concentration in fish harvested in the Dehcho Region, Northwest Territories, Canada by lake location (2013)

			Mercury			**Selenium**	
Fish Species	**Location**	**n**	**Range (ppm)**	**Mean ± S.D. (ppm)**	**n**	**Range (ppm)**	**Mean ± S.D. (ppm)**
Burbot	Trout Lake	9	0.228 - 0.551	0.317 ± 0.101	6	0.117 - 0.155	0.141 ± 0.013
Cisco	Ekali Lake	13	0.020 - 0.194	0.072^{b} ± 0.060	4	0.128 - 0.173	0.151^{b} ± 0.023
	Sanguez Lake	2	0.057 - 0.127	0.092^{c} ± 0.050	1	0.116	0.116^{*} ± N/A
	Trout Lake	14	0.034 - 0.042	0.037^{ab} ± 0.002	5	0.164 - 0.239	0.204^{c} ± 0.032
Lake Trout	Trout Lake	13	0.207 - 0.643	0.330 ± 0.153	11	0.078 - 0.24	0.140 ± 0.056
Lake Whitefish	Ekali Lake	14	0.054 - 0.128	0.079^{b} ± 0.025	5	0.111 - 0.132	0.120^{b} ± 0.009
	Sanguez Lake	5	0.088 - 0.150	0.128^{c} ± 0.026	4	0.086 - 0.175	0.128^{ab} ± 0.037
	Trout Lake	10	0.025 - 0.057	0.037^{a} ± 0.009	7	0.172 - 0.307	0.237^{c} ± 0.044
Longnose Sucker	Trout Lake	6	0.086 - 0.127	0.100 ± 0.015	5	0.170 - 0.215	0.187 ± 0.020
Northern Pike	Ekali Lake	25	0.070 - 1.100	0.398^{b} ± 0.267	14	0.090 - 0.165	0.125^{a} ± 0.023
	Sanguez Lake	13	0.082 - 3.121	1.151^{c} ± 0.827	6	0.116 - 0.203	0.144^{b} ± 0.032
	Trout Lake	10	0.073 - 0.286	0.153^{a} ± 0.085	8	0.138 - 0.229	0.165^{c} ± 0.031
Walleye	Ekali Lake	26	0.097 - 0.457	0.286^{b} ± 0.093	11	0.089 - 0.224	0.177^{c} ± 0.035
	Sanguez Lake	17	0.052 - 1.428	0.708^{c} ± 0.336	5	0.170 - 0.284	0.207^{b} ± 0.045
	Trout Lake	11	0.036 - 0.848	0.293^{ab} ± 0.310	5	0.195 - 0.261	0.222^{c} ± 0.029

* n=1

Subscripted letters denote differences ($P<0.05$) by a series of one-way ANOVAs.

Supplementary Material, Table S4. Fatty acid composition of fish harvested in the Dehcho Region, Northwest Territories, Canada by lake location (2013)

			Omega-3 Fatty Acids		EPA + DHA		Omega-6 Fatty Acids		Polyunsaturated Fatty Acids	
Fish Species	**Location**	**n**	**Range (mg/100 g)**	**Mean ± SD (mg/100 g)**	**Range (mg/100 g)**	**Mean ± SD (mg/100 g)**	**Range (mg/100g)**	**Mean ± SD (mg/100g)**	**Range (mg/100 g)**	**Mean ± SD (mg/100 g)**
Burbot	Trout Lake	9	68.4 – 127	101 ± 18.2	57.4 – 110	86.3 ± 16.3	40.7 – 59.4	49.2 ± 6.20	109 – 186	150 ± 24.2
Cisco	Ekali Lake	5	187 – 512	298^{ab} ± 131	145 – 279	192 ± 50.8	61.8 – 183	106 ± 51.3	252 – 695	404 ± 182
	Sanguez Lake	1	375	375* ± N/A	215	215 ± N/A	123	123 ± N/A	498	498 ± N/A
	Trout Lake	6	300 – 551	381^{ab} ± 106	205 – 344	252 ± 60.0	75.3 – 150	101 ± 29.3	375 – 700	478 ± 136
Lake Trout	Trout Lake	11	258 – 4,375	1,689 ± 1,294	172 – 2,332	965 ± 544	95.7 – 1322	539 ± 367	361 – 5,699	2,269 ± 1,642
Lake Whitefish	Ekali Lake	11	225 – 916	401^{ab} ± 201	187 – 544	279 ± 102	73.9 – 467	176 ± 118	299 – 1384	577 ± 317
	Sanguez Lake	3	335 – 2110	$1,204^{c}$ ± 888	262 – 1,048	663 ± 393	126 – 403	239 ± 135	462 – 3205	1621 ± 1420
	Trout Lake	10	215 – 578	321^{ab} ± 119	182 – 349	240 ± 49.5	58.2 – 304	113 ± 76.5	284 – 881	434 ± 193
Longnose Sucker	Trout Lake	6	208 – 763	499 ± 198	160 – 372	263 ± 68.6	93.1 – 495	286 ± 159	301 – 1,259	784 ± 356
Northern Pike	Ekali Lake	17	131 – 216	174^{ab} ± 21	120 – 190	150 ± 18.8	49.0 – 91.1	63.0 ± 9.58	180 – 949	278 ± 182
	Sanguez Lake	9	131 – 836	228^{b} ± 215	113 – 707	192 ± 182	45.7 – 86.6	58.0 ± 12.8	177 – 1096	307 ± 279
	Trout Lake	10	201 – 438	259^{c} ± 76.7	166 – 293	205 ± 40	48.9 – 133.2	73.2 ± 30.0	251 – 571	332 ± 105
Walleye	Ekali Lake	17	147 – 911	274^{c} ± 217	132 – 807	231 ± 182	50.2 – 288	81.8 ± 60.8	202 – 321	285 ± 131
	Sanguez Lake	7	117 – 196	168^{a} ± 25.3	87.5 – 168	142 ± 26.7	49.7 – 70.6	60.0 ± 7.98	167 – 261	229 ± 31.3
	Trout Lake	11	149 – 276	230^{b} ± 36.1	124 – 238	196 ± 32.5	44.6 – 83.7	61.6 ± 11.4	201 – 360	291 ± 43.4

*n=1
Subscripted letters denote difference ($P<0.05$) by a series of one-way ANOVAs.

Supplementary Material, Table S5. Omega-6 fatty acid to omega-3 fatty acid ratio of fish harvested in the Dehcho Region, Northwest Territories, Canada (2013)

Fish Species	Lake	n	Range	Mean ± SD
Burbot	Trout Lake	9	0.45 - 0.60	0.49 ± 0.04
Cisco	Ekali Lake	5	0.29 - 0.41	0.35 ± 0.04
	Sanguez Lake	1	0.33	0.33 ± N/A
	Trout Lake	6	0.24 - 0.27	0.25 ± 0.01
Lake Trout	Trout Lake	11	0.28 - 0.62	0.41 ± 0.15
Lake Whitefish	Ekali Lake	11	0.31 - 0.53	0.42 ± 0.08
	Sanguez Lake	3	0.34 - 0.52	0.40 ± 0.08
	Trout Lake	10	0.22 - 0.53	0.33 ± 0.09
Longnose Sucker	Trout Lake	6	0.45 - 0.69	0.54 ± 0.10
Northern Pike	Ekali Lake	17	0.30 - 0.48	0.37 ± 0.05
	Sanguez Lake	9	0.27 - 0.44	0.36 ± 0.05
	Trout Lake	10	0.20 - 0.36	0.28 ± 0.05
Walleye	Ekali Lake	17	0.30 - 0.47	0.36 ± 0.05
	Sanguez Lake	7	0.31 - 0.42	0.36 ± 0.04
	Trout Lake	11	0.21 - 0.35	0.27 ± 0.05

Supplementary Material, Table S6. Statistical analyses by Spearman correlations for mercury vs. selenium, mercury vs. omega-3 fatty acid, and omega-3 fatty acid vs. selenium content in all fish species

Fish Species	**Hg vs. Se**	**Hg vs. *n*-3 FAs**	**Hg vs. EPA+DHA**	**Hg vs. PUFAs**	***n*-3 FAs vs. Se**	**EPA+DHA vs. Se**	**PUFAs vs. Se**
Burbot	-0.0286 (n=6)	-0.317 (n=9)	-0.350 (n=9)	-0.317 (n=9)	0.0857 (n=6)	0.0857 (n=6)	0.0857 (n=6)
Cisco	-0.685* (n=10)	-0.224 (n=12)	-0.420 (n=12)	-0.196 (n=12)	-0.164 (n=10)	-0.188 (n=10)	-0.248 (n=10)
Lake Trout	0.418 (n=11)	-0.937*** (n=12)	-0.951*** (n=12)	-0.951*** (n=12)	-0.573 (n=11)	-0.491 (n=11)	-0.491 (n=11)
Lake Whitefish	-0.818*** (n=15)	0.375 (n=24)	0.381 (n=24)	0.435* (n=24)	-0.600*(n=15)	-0.571* (n=15)	-0.589* (n=15)
Longnose Sucker	-0.200 (n=5)	-0.429 (n=6)	-0.0857 (n=6)	-0.314 (n=6)	0.300 (n=5)	0.000 (n=5)	0.300 (n=5)
Northern Pike	-0.410* (n=29)	-0.619*** (n=37)	-0.641*** (n=37)	-0.569*** (n=37)	0.616*** (n=29)	0.605*** (n=29)	0.538** (n=29)
Walleye	0.209 (n=22)	-0.481** (n=35)	-0.491** (n=35)	-0.473** (n=35)	0.0536 (n=22)	0.0491 (n=22)	-0.0333 (n=22)

Level of statistical significance: * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Appendix B: Terminology used in probabilistic exposure modelling

Supplementary Material, Table S1. Specific terms used in Crystal Ball's OptQuest

Term	Definition
Decision variable	Quantity the user can change and control
Bounds	Upper and lower limits for the decision variable
Type	Whether the discrete variable is discrete or continuous
Step size	Difference between successive values of a discrete variable in the defined range
Assumption variable	Estimated values that specify the uncertainty of the model data using probability distributions of exposure. Upper and lower limits and standard deviation of the estimated value are defined.
Forecast variable	Forecasts are frequency distributions of possible
Objective	A formula in terms of decision variables that gives a mathematical representation of the model's goal
Requirement	A restriction on a forecast statistic that requires the statistic to fall between specified lower and upper limits for a solution to be considered feasible

Reference for the definitions: Decisioneering, 2001

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