Fish Consumption and Mercury Exposures Among Sub-populations in Canada through Targeted Biomonitoring: Results from Dene/Métis Communities of the Northwest Territories and a Maternal-Infant Cohort Study

by

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Examining Committee Membership

The following served on the Examining Committee for this thesis. The decision of the Examining Committee is by majority vote.

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Author's Declaration

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

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

Statement of Contributions

I, Sara Packull-McCormick was the sole author of Chapters 1 and Chapters 7, which were written under the supervision of Dr. Brian Laird and were not written for publication as individual manuscripts.

This thesis consists, in part, of five manuscripts written for publication. Exceptions to sole authorship of material are as follows:

Chapter 2:

Packull-McCormick, S. Cowan, A., Stark, K., Low, M., Gamberg, M., Swanson, H., & Laird, B. (2023). Mercury Bioaccessibility in Freshwater Fish Species from Northern Canada. *Science of the Total Environment*.

This chapter was prepared as a manuscript that will be submitted for publication. Co-authors on this manuscript were: Sara Packull-McCormick, Alicia Cowan, Dr. Ken Stark, Mike Low, Mary Gamberg, Dr. Heidi Swanson, and Dr. Brian Laird. This research was conducted by Sara Packull-McCormick at the University of Waterloo under the supervision of Dr. Brian Laird. Samples of Lake Whitefish, Lake Trout, Northern Pike, and Walleye, along with mercury concentration data (measured at the Biotron at Western University in the lab of Dr. Brian Branfireun), were obtained from Dr. Heidi Swanson and Mike Low via their community-based monitoring in partnership with Dehcho AAROM. Burbot muscle and liver samples were obtained from Mary Gamberg. Sara Packull-McCormick and Mallory Drysdale, and other members of the Laird and Swanson research teams prepared samples for fatty acid analysis which was then conducted by members of Dr. Ken Stark's research team at the University of Waterloo. Sara Packull-McCormick and Alicia Cowan conducted the *in vitro* gastro-intestinal bioaccessibility protocol, cooked fish samples, and prepared all samples for mercury analysis (in bioaccessibility fluids and tissue samples) which was conducted at the Biotron. Freeze-drying of tissue samples was conducted by Dr. Heidi Swanson's research team at the University of Waterloo. Sara Packull-McCormick performed the data analysis and drafted the manuscript with input from co-authors. All co-authors reviewed and provided input on the manuscript.

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the literature, reports, and spreadsheets, and combined the data into a dataset that was used for the exposure assessment models. Sara Packull-McCormick, in consultation with Dr. Glenn Ferguson, and under the supervision of Dr. Brian Laird, developed the models and conducted the statistical analyses. Sara Packull-McCormick drafted the manuscript under the supervision of Dr. Brian Laird. This manuscript was reviewed by co-authors that are part of the committee of this thesis, Dr. Brian Laird, Dr. Kelly Skinner, Dr. Glenn Ferguson, and Dr. Heidi Swanson and their input was incorporated. Further co-authors will be invited to review and contribute to the manuscript that will come out of this chapter in the future.

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Abstract

Fish contain many important nutrients including omega-3 polyunsaturated fatty acids (PUFA) (such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) and essential elements (such as selenium (Se)), and fish consumption has been associated with various health benefits. However, fish can also be a source of exposure to environmental contaminants, such as mercury (Hg). Fish consumption is considered to be a major source of Hg exposure for the Canadian general population, and this can pose a health risk for populations that consume significant amounts of fish or consume fish containing elevated levels of Hg. This thesis focused on two sub-populations in Canada that may be disproportionately impacted by exposure to Hg (either by being especially exposed to Hg or especially sensitive to the negative effects of Hg). The first was Dene/Métis communities in northern Canada, as these communities frequently consume locally harvested freshwater fish and elevated levels of Hg have been measured in some fish species from some local lakes in northern Canada. prompting concerns about Hg exposure as these populations may be especially exposed. The second sub-population examined was pregnant women and children in Canada, as early life stages are especially sensitive to the neurotoxic effects of Hg as their nervous systems are undergoing rapid development.

To address concerns for both of these potentially disproportionately impacted sub-populations, focused biomonitoring projects can be used. In the Northwest Territories, to address concerns about exposure to environmental contaminants, including Hg from local fish, a community-based human biomonitoring project was implemented in First Nations communities in the Dehcho and Sahtú regions of the Northwest Territories. To understand chemical exposures, including Hg, in Canadian pregnant women and children, Health Canada is conducting the Maternal Infant Research on Environmental Chemicals (MIREC) pregnancy cohort study and follow-up studies, which include a biomonitoring component. This thesis draws on the results of both of these human biomonitoring projects, along with results and samples from on-going fish sampling projects and research in the Northwest Territories, to examine Hg exposures, in the context of fish consumption, among sub-populations in Canada. In order to do this, this thesis had five main objectives which were to: 1. measure Hg bioaccessibility in

commonly consumed northern freshwater fish; 2. examine and describe Hg biomarker levels in members of northern Dene/Métis communities; 3. identify potential determinants of hair Hg, blood Hg, blood Se, and plasma EPA+DHA concentrations in members of northern Dene/Métis communities; 4. construct and refine a site-specific exposure assessment model for Hg, Se, and EPA+DHA, from the consumption of local freshwater fish, for the Dehcho and Sahtú regions of the NWT; and 5. determine if there are associations between early life exposures to low levels of Hg and child IQ, and if these associations are modified by prenatal fish consumption.

The human biomonitoring project in the Northwest Territories (2016-2018) included the collection of biological samples (including hair and blood), dietary data (through the use a food frequency questionnaire (FFQ)), and demographic/anthropometric data. From the FFQ, the four most commonly consumed fish species were identified as Lake Whitefish (, Lake Trout, Northern Pike, and Walleye. Concentrations of Hg, and to a lesser extent Se and omega-3 PUFA, were available from waterbodies in the Northwest Territories for these fish species. However, to address the lack of Hg bioaccessibility data for northern freshwater fish species and fulfill objective 1 of this thesis, fish samples that were collected from waterbodies in northern Canada were analyzed for total Hg bioaccessibility and mean total Hg bioaccessibility in raw fish muscle tissue ranged between 56-96% percent for the species examined and was 39% in raw Burbot livers. Cooking increased total Hg concentrations (likely due to moisture loss) in the samples, but total Hg bioaccessibility was much lower in the cooked samples (on average 40% lower). Thus, bioaccessible concentrations of total Hg in the cooked samples were on average 32% lower.

In order to fulfill objective 2 of this thesis, human biomarker concentrations of Hg (in hair and blood), were determined and hair to blood Hg concentration ratios were calculated. The majority of hair and blood Hg concentrations were below health-based guidance values and blood Hg concentrations were similar to or below those measured in the Canadian general population. Hair Hg concentrations, especially for the Sahtú region, were higher on average than those measured in the Canadian general population, however, hair:blood Hg concentration ratios in these regions (619:1 for the Dehcho region and 1220:1 for the Sahtú region) were significantly higher than ratios typically reported in the literature for other populations. For long hair samples,

hair Hg concentrations varied over time and total reported fish consumption seemed to follow a similar seasonal pattern.

Human biomarker concentrations of Se (in blood) and EPA and DHA (in blood plasma) were also measured and potential determinants of these and the Hg biomarker levels were examined to fulfill objective 3 of this thesis. Blood Se concentrations were similar to those measured in the Canadian general population and plasma EPA and DHA concentrations were similar to levels observed in other Canadian studies. In models to explain hair Hg, blood Hg, and plasma EPA and DHA, participant age, the consumption of at least one species of fish, and the consumption of waterfowl (those that are omnivorous or piscivorous) were included as explanatory variables in the final models. The final model explaining variability in blood Se concentrations included region, and the consumption of several species of fish, burbot livers, and caribou livers and kidneys.

Results generated in addressing objectives 1-3, were used to construct and refine a probabilistic exposure assessment model for Hg, Se, and EPA and DHA, from the consumption of the four most commonly consumed fish species (to fulfill objective 4 of this thesis). The majority of Hg estimates were below tolerable daily intakes (TDIs) and the inclusion of waterbody-specific Hg concentration data and species-specific Hg bioaccessibility data resulted in lower estimated Hg intakes which was more consistent with biomarker concentrations. Model estimates for Se and EPA and DHA, indicated that the consumption of these four fish species was an important contributor to the dietary intakes of these nutrients. Overall, model estimates and biomarker levels indicate that although Hg concentrations may be elevated in some fish species, from some waterbodies in these regions, human Hg exposures in the Dehcho and Sahtú regions are relatively low.

Finally, since early life stages are particularly susceptible to the neurotoxic effects of Hg, and as it is unclear if these effects are also seen at low Hg concentrations (like those seen in the Canadian general population and in the Dehcho and Sahtú regions), associations between prenatal and childhood Hg exposures and child intelligence quotient (IQ), were examined using data from the MIREC cohort study (2013-2015) to address objective 5 of this thesis. Higher cord

blood Hg concentrations were associated with lower Performance IQ (PIQ) (β=-3.27; 95%CI: -6.44, -0.09) in male children with the lowest prenatal fish consumption. Progressively stronger positive associations were observed with PIQ in male children for moderate (β=1.08; 95%CI: -0.10, 2.26) and high (β=3.07; 95%CI: 1.95, 4.19) prenatal fish consumption. Cord blood Hg concentrations were positively associated with female children's Full-Scale IQ (FSIQ) (β = 1.29; 95% CI: 0.77, 1.81) and PIQ (β = 2.01; 95% CI: 1.19, 2.83); however, when stratified only in the highest fish consumption subgroup. Among female children, higher child blood Hg concentrations were associated with an approximately 1-point increase in FSIQ, VIQ, and a General Language Composite (GLC). Prenatal exposure to low levels of Hg was associated with lower PIQ scores in male children with low prenatal fish intake. Findings from this analysis suggest that children exposed to low levels of Hg may be vulnerable to adverse cognitive effects especially when exposure occurs through non-fish sources of Hg. This reinforces the benefits of fish consumption (of species low in Hg) during pregnancy.

This work emphasizes that although fish consumption can be a source of Hg exposure for the Canadian general population and northern Indigenous communities, it is also a source of important nutrients. This thesis fills an important gap in the literature on the levels, determinants, and dietary intakes of Hg, Se, and omega-3 PUFA in First Nations communities in the Dehcho and Sahtú regions of the Northwest Territories. It also provides evidence of the importance of fish consumption as a modifier of the associations between early life exposures to low levels of Hg and child IQ. Future work in northern regions should include the collection of additional waterbody-specific fish Se and omega-3 fatty acid concentration and bioaccessibility data. Future exposure assessments and epidemiological studies on Hg exposure should take into account the important nutritional benefits of fish consumption. The consumption of fish, of those species that are considered low in Hg, should continue to be encouraged for populations in Canada.

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Table of Contents

Examining Committee Membership ii

Author's Declaration iii

Statement of Contributions iv

Abstract viii

Acknowledgements xii

List of Figures xxi

List of Tables xxiii

List of Abbreviations xxx

Chapter 1 : Introduction 1

1.1 Benefits of Fish Consumption 1

1.2 Risks of Fish Consumption – Mercury 3

1.3 Co-exposures to Mercury and Nutrients 4

1.4 Sub-populations Examined in this Thesis 5

1.5 Determining Mercury Exposures - Human Biomonitoring of Mercury 6

1.5.1 The Maternal Infant Research on Environmental Chemicals (MIREC) Cohort Study 9

1.5.2 Human Biomonitoring Projects in Northern Dene/Métis Communities 11

1.6 Exposure Assessments 13

1.6.1 Food Analyte Concentrations 14

1.6.2 Food Consumption/Intake Rates 15

1.6.3 Bioaccessibility Data 17

1.7 Objectives and Research Questions 19

1.7.1 Objective 1: Measure Hg Bioaccessibility in Commonly Consumed Northern Freshwater Fish 20

1.7.2 Objective 2: Examine and Describe Hg Biomarker Levels in Members of Northern Dene/Métis Communities 21

1.7.3 Identify Potential Determinants of Hair Hg, Blood Hg, Blood Se, and Plasma EPA+DHA Concentrations in Members of Northern Dene/Métis Communities 21

1.7.4 Construct and Refine a Site-specific Exposure Assessment Model for Hg, Se, and EPA+DHA, from the Consumption of Local Freshwater Fish, for the Dehcho and Sahtú regions of the Northwest Territories 22

1.7.5 Objective 5: Determine if there are Associations between Early Life Exposures to Low Levels of Hg and Child IQ, and if these Associations are Modified by Prenatal Fish Consumption 22

Chapter 2 : Mercury Bioaccessiblity in Freshwater Fish Species from Northern Canada 23

2.1 Abstract 23

2.2 Introduction 23

2.3 Methods 26

2.3.1 Fish Samples 26

2.3.2 Sample Preparation 26

2.3.3 *In vitro* Gastrointestinal Digestion Model 27

2.3.4 Chemical Analyses 28

2.3.5 Statistical Analyses 29

2.4 Results 31

2.4.1 Bioaccessibility of Mercury in Raw Fish Samples 31

2.4.2 Cooked Fish Mercury Bioaccessibility (Gastrointestinal Model) 38

2.5 Discussion 40

2.5.1 Fish Mercury and Fatty Acid Concentrations 40

2.5.2 Total Mercury Bioaccessibility in Raw Fish Tissue 42

2.5.3 Total Mercury Bioaccessibility in Cooked Fish Tissue 44

2.5.4 Strengths, Limitations, and Recommendations 45
2.6 Conclusions 47
2.7 Supplementary Tables 48
Chapter 3 : Hair to Blood Mercury Concentration Ratios and a Retrospective Hair Segmental Mercury Analysis in the Northwest Territories, Canada 61
3.1 Abstract 61
3.2 Introduction 62
3.3 Materials and Methods 65
3.3.1 Study Design 65
3.3.2 Collection and Chemical Analyses of Human Biological Samples 65
3.3.3 Statistical Analyses 67
3.4 Results and Discussion 69
3.4.1 Mercury Concentrations in Blood Samples and Initial Hair Segments 69
3.4.2 Hair Mercury to Blood Mercury Ratios to Estimate Past Blood Mercury Concentrations from Retrospective Hair Mercury Analysis 73
3.4.3 Comparison of Hair: Blood Mercury Ratios for Estimating Blood Mercury Concentrations 74
3.4.4 Retrospective Hair Mercury Analysis 77
3.4.5 Fish Consumption Frequencies as a Predictor of Seasonal Variation in Hair Mercury Concentrations 80
3.4.6 Limitations 83
3.5 Conclusions 86
3.6 Supplementary Tables 87
3.7 Supplementary Figures 92
Chapter 4 : Determinants of human hair mercury, blood mercury, blood selenium, and plasma omega-3 fatty acid levels in Northern Canada 94

4.1 Abstract 94
4.2 Introduction 95
4.3 Methods 97
4.3.1 Study design 97
4.3.2 Collection and chemical analysis of human biological samples 98
4.3.3 Collection of dietary, demographic, and biometric data 99
4.3.4 Statistical analyses 100
4.4 Results 103
4.4.1 Participant Characteristics 103
4.4.2 Biomarker Concentrations 104
4.4.3 Bivariate associations between levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) and demographic and dietary factors 108
4.4.4 Explanatory models for levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) 113
4.5 Discussion 115
4.5.1 Determinants of Hair and Blood Mercury Concentrations 115
4.5.2 Determinants of Blood Selenium Concentrations 117
4.5.3 Determinants of EPA+DHA Concentrations 119
4.5.4 Associations between Biomarkers 120
4.5.5 Limitations and Future Directions 121
4.6 Conclusions 123
4.7 Supplemental Tables 124
Chapter 5 : Refining the Characterization of Mercury, Selenium, and Omega-3 Fatty Acid Exposure from Fish Consumption in the Northwest Territories 139
5.1 Abstract 139

5.2 Introduction 140
5.3 Methods 143
5.3.1 Dietary, Demographic, and Anthropometric Data Included in the Models 144
5.3.2 Fish Analyte Data Included in the Models 145
5.3.3 Probabilistic Models 146
5.3.4 Statistical Analyses 149
5.4 Results 149
5.4.1 Exposure Estimates for Mercury Intake 149
5.4.2 Exposure Estimates for Selenium Intake 155
5.4.3 Exposure Estimates for EPA+DHA Intake 156
5.5 Discussion 162
5.5.1 Estimated Intakes in the Dehcho and Sahtú Regions 162
5.5.2 Limitations and Future Directions 169
5.6 Conclusions 171
5.7 Supplementary Figures 172
5.8 Supplementary Tables 182
Chapter 6 : Prenatal and concurrent blood mercury concentrations and associations with IQ in Canadian preschool children 187
6.1 Abstract 187
6.2 Introduction 189
6.3 Methods 190
6.3.1 Study Design and Population 190
6.3.2 Hg and Other Analyte Concentrations 191
6.3.3 Child IQ 191

6.3.4 Covariates 192
6.3.5 Statistical Analysis 193
6.3.6 Sensitivity Analysis 194
6.4 Results 194
6.4.1 Descriptive results 194
6.4.2 Prospective associations between first trimester blood Hg concentrations and child IQ 197
6.4.3 Prospective associations between cord blood Hg concentrations and child IQ 197
6.4.4 Concurrent associations between child blood Hg concentrations and child IQ 201
6.5 Discussion 201
6.5.1 Associations between prenatal Hg concentrations and child IQ 202
6.5.2 Associations between Child Blood Mercury Concentrations and IQ 205
6.5.3 Strengths and Limitations 205
6.6 Conclusions 207
6.7 Supplementary Figures and Tables 209
Chapter 7 : Conclusions 232
7.1 Overview 232
7.2 Key Findings 234
7.2.1 Key Findings – Chapter 2 234
7.2.2 Key Findings – Chapter 3 235
7.2.3 Key Findings – Chapter 4 235
7.2.4 Key Findings – Chapter 5 236
7.2.5 Key Findings – Chapter 6 236
7.3 Implications for Public Health Sciences 237
7.4 Contributions to the Literature 245

7.5 Limitations 247
7.5.1 Sample Size 247
7.5.2 Use of Food Frequency Questionnaire Data 249
7.5.3 Use of Total Hg Concentrations/Lack of Hg Speciation Data 251
7.5.4 Availability of Fish Analyte Data 252
7.5.5 Availability of Bioaccessibility Data 253
7.5.6 Consequences of the Covid-19 Pandemic 254
7.6 Recommendations and Future Directions 256
7.6.1 Recommendations Concerning Fish Consumption 256
7.6.2 Recommendations to Fill Remaining Data Gaps 257
7.6.3 Recommendations for Future Biomonitoring Studies 258
7.7 Summary 258
References 260
Appendix 301

List of Figures

Figure 1.1: Visual summary of the MIREC Research Platform including the MIREC study and the follow-up studies. This figure is from the MIREC Research Platform Website (MIREC, 2023)……. 10

Figure 1.2: Depiction of the connections between the research projects and manuscripts/chapters in this manuscript-based thesis ……..... 20

Figure 2.1: Boxplot of Ln-transformed Total Mercury Concentrations by Lake and Species for Raw Fish Muscle and Liver Samples (Plot indicates the median, interquartile range, and min and max values) ……..... 34

Figure 2.2: Boxplot of Percent Total Mercury Bioaccessibility by Lake and Species for Raw Fish Muscle and Liver Samples (Plot indicates the median, interquartile range, and min and max values) ……..... 34

Figure 2.3: Boxplot of Ln-transformed Bioaccessible Total Mercury Concentrations by Lake and Species for Raw Fish Muscle and Liver Samples (Plot indicates the median, interquartile range, and min and max values) ……..…….... 35

Figure 3.1: Mean Hair Mercury Concentrations (Subset 2 versus Subset 3) and Mean Fish Consumption Frequencies by Time …...……..... 81

Figure 4.1: Heatmap of the Spearman's rank correlation coefficients for the correlations between biomarker concentrations ……...……... 108

Figure 5.1: Formula for Calculating Estimated Fish Species-Specific Intake Rates ……...… 146

Figure 5.2: Equations to Estimate Fish Species-specific Participant Methylmercury, Total Selenium, and EPA+DHA Intake ……...……..... 148

Figure 5.3: Equation to Estimate Fish Species-specific Participant Methylmercury Intake Incorporating Mercury Bioaccessibility (Model 3) …...……..... 149

Figure 5.4: Summary and Comparison of the Input Data Included in the Models (Differences in the Model Compared to the Previous Model Bolded) ……...….. 149

Supplementary Figure 3.1: Mean Hair Mercury Concentrations and Mean Fish Consumption Frequencies by Time for Those Who Consumed Fish on Average >1 Times per Week …… 92

Supplementary Figure 3.2: Mean Hair Mercury Concentrations and Mean Fish Consumption Frequencies by Time for Those Who Consumed Fish on Average <1 Times per Week …… 93

Supplementary Figure 5.1A: Histograms of the average estimated methylmercury intakes for Models 1-3 for the Dehcho region ……. 172
Supplementary Figure 5.1B: Histograms of the average estimated methylmercury intakes for Models 1-3 for the Sahtú region …… 173
Supplementary Figure 5.2: Histogram of the hair mercury concentrations for the Dehcho and Sahtú regions ..…… 174
Supplementary Figure 5.3: Histogram of the blood mercury concentrations for the Dehcho and Sahtú regions ……. 175
Supplementary Figure 5.4A: Histograms of the average estimated selenium intakes for Models 1-2 for the Dehcho region …… 176
Supplementary Figure 5.4B: Histograms of the average estimated selenium intakes for Models 1-2 for the Sahtú region ……... 177
Supplementary Figure 5.5: Histogram of the blood selenium concentrations for the Dehcho and Sahtú regions ……..… 178
Supplementary Figure 5.6A: Histograms of the average estimated EPA+DHA intakes for Models 1-2 for the Dehcho region …… 179
Supplementary Figure 5.6B: Histograms of the average estimated EPA+DHA intakes for Models 1-2 for the Dehcho region …… 180
Supplementary Figure 5.7: Histogram of the plasma EPA+DHA concentrations for the Dehcho and Sahtú regions ……..… 181
Supplementary Figure 6.1: Flowchart of the inclusion and exclusion criteria for the final analytical samples for the associations between prenatal (first trimester or cord blood Hg concentrations) or postnatal (child blood Hg concentrations) Hg exposure and child IQ, MIREC (2008-2011) and MIREC-CD Plus (2013-2015) Studies ……..……..... 209
Supplementary Figure 6.2: Directed Acyclic Graph (DAG) of potential confounders or effect modifiers of the association between prenatal Hg exposure and child IQ, MIREC (2008-2011) and MIREC-CD Plus (2013-2015) Studies ……..……..... 210
Supplementary Figure 6.3: Directed Acyclic Graph (DAG) of potential confounders or effect modifiers of the association between child Hg exposure and child IQ, MIREC (2008-2011) and MIREC-CD Plus (2013-2015) Studies ……..……..... 210

List of Tables

Table 2.1: Total Mercury Concentrations and Percent Bioaccessibility in Raw Fish Samples …..... 36

Table 2.2: Fatty Acid Concentrations in Raw Fish Samples ……..... 37

Table 2.3: Total Mercury Concentrations and Percent Bioaccessibility in Cooked Fish Samples ... 39

Table 3.1: Demographic Information for Participants Providing a Blood and/or Hair (Proximal 2 cm) Sample from the Dehcho and Sahtú Regions ……..……..…..... 70

Table 3.2: Summary Statistics for Hair (Proximal 2 cm Segment) and Blood Mercury Concentrations in the Dehcho and Sahtú Regions ……..……..... 72

Table 3.3: Summary Statistics for Calculated Hair-Blood Mercury Ratios (from Subset 1) ……. 73

Table 3.4: Summary Statistics for Estimated Blood Mercury Concentrations using Various Ratios (for Subset 1) ……..……...…..... 75

Table 3.5: Frequency of Blood Mercury Estimates Occurring within a Range of Percent Error (from Subset 1) ……..……..... 76

Table 3.6: Total mercury concentrations in hair segments based on Bi-Monthly Time Period ……. 78

Table 3.7: Model Set and AICc Values for Mixed Effects Models (from Subset 3) …….....… 82

Table 4.1: Descriptive statistics for participant characteristics by region …… 105

Table 4.2: Percentage of participants who reported consuming select traditional foods in the past year ……..…….. 106

Table 4.3: Descriptive statistics for participant biomarker concentrations by region ……. 107

Table 4.4: Continuous potential determinants of levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) levels by region ……..……..... 110

Table 4.5: Binary potential determinants of levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) levels for all regions (pooled) ……. 111

Table 4.6: Parameter estimates for the model to explain $\log_2$-tranformed hair mercury concentrations ……..…….. 113

Table 4.7: Parameter estimates for the Tobit model to explain log_2-tranformed blood mercury concentrations …….…….. 113

Table 4.8: Parameter estimates for the model to explain blood selenium concentrations …… 114

Table 4.9: Parameter estimates for the model to explain log_2-transformed plasma EPA+DHA concentrations …….…….. 115

Table 5.1: Exposure Estimates for Methylmercury Intake from the Probabilistic Models …….. 152

Table 5.2A: Summary Statistics and Percent Exceedances for Participants with Model Estimates and Hair Mercury Concentrations …….…….. 153

Table 5.2B: Summary Statistics and Percent Exceedances for Participants with Model Estimates and Blood Mercury Concentrations …….……..... 154

Table 5.3: Exposure Estimates for Selenium Intake ……..……... 158

Table 5.4: Summary Statistics and Percent Exceedances for Participants with Model Estimates and Blood Selenium Concentrations ……..…….. 159

Table 5.5: Exposure Estimates for EPA and DHA Intake ……..…….. 160

Table 5.6: Summary Statistics for Model Estimates of EPA + DHA Intake and Plasma EPA + DHA Concentrations ……..……..... 161

Table 6.1: Participant characteristics stratified by child sex, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……..…… 195

Table 6.2A: Prospective associations between first trimester blood Hg concentrations and child IQ for male children and stratified by prenatal fish, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……..…… 199

Table 6.2B: Prospective associations between first trimester blood Hg concentrations and child IQ for female children and stratified by prenatal fish consumption, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies …… 199

Table 6.3A: Prospective associations between cord blood Hg concentrations and child IQ for male children and stratified by prenatal fish consumption, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……..……... 200

Table 6.3B: Prospective associations between cord blood Hg concentrations and child IQ for female children and stratified by prenatal fish consumption, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies …… 200

Table 6.4: Concurrent associations between child blood Hg concentrations and IQ, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies …….. 201

Table 7.1: Examples of some of the health messages released about fish consumption and Hg at various levels in Canada …….. 240

Supplementary Table 2.1: Bioaccessibility of Total Mercury in Fish, Seafood and Traditional Foods Previously Reported in the Literature ……... 48

Supplementary Table 2.2: Sample Sizes by Lake, Fish Species, and Chemical Analysis ……... 53

Supplementary Table 2.3: List of the nine a priori general linear models to explore variability in percent mercury bioaccessibility among species and lakes and in relation to total mercury concentrations …….. 54

Supplementary Table 2.4: Model Set, AICc values, and log likelihood values for general linear models to explore variability in percent mercury bioaccessibility among species and lakes and in relation to total mercury concentrations…….. 54

Supplementary Table 2.5: Pairwise Comparisons for Ln-Transformed Total Mercury Concentrations by Fish Species (Tukey's test) …….. 55

Supplementary Table 2.6: Pairwise Comparisons for Ln-transformed Total Mercury Concentrations by Lake (Tukey's Test) …….. 56

Supplementary Table 2.7: Pairwise Comparisons for Mercury Bioaccessibility in Raw Fish Muscle by Fish Species (Tukey's Test) …….. 58

Supplementary Table 2.8: Pairwise Comparisons for Mercury Bioaccessibility in Raw Fish Muscle by Lake (Tukey's Test) …….. 59

Supplementary Table 2.9: Total Mercury and Gastric Phase Only Total Mercury Bioaccessibility for Fish from Kakisa Lake (NWT) …….. 60

Supplementary Table 3.1: Hair Mercury Concentrations, Blood Mercury Concentrations, and Hair:Blood Mercury Ratios Stratified by Age Tertile …….. 87

Supplementary Table 3.2: Hair Mercury Concentrations, Blood Mercury Concentrations, and Hair:Blood Mercury Ratios Stratified by Sex ……..……...…… 88

Supplementary Table 3.3: Summary Statistics for Calculated Hair-Blood Hg Ratios (including outliers) ……..……..… 88

Supplementary Table 3.4: Total mercury concentrations in hair segments based on Bi-Monthly Time Periods Comparing by Dehcho and Sahtú Region ……..……...… 89

Supplementary Table 3.5: Pairwise Comparisons of Log Mean Hair Mercury Concentrations ..… 90

Supplementary Table 3.6: Differences in Hair Mercury Concentrations between Time Periods (µg/g) ……..……...……...… 91

Supplementary Table 4.1: Summary statistics for plasma EPA+DHA presented as a % of the total fatty acids ……..……..…… 124

Supplementary Table 4.2: Pair-wise comparisons with the Tukey test for the biomarker concentrations by region ……..……..…… 124

Supplementary Table 4.3A: Binary potential determinants of levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) levels for the Dehcho region ……...… 125

Supplementary Table 4.3B: Binary potential determinants of levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) levels for the Sahtú region ...……127

Supplementary Table 4.3C: Binary potential determinants of levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) levels for the Old Crow region …… 129

Supplementary Table 4.4: Demographic characteristics and traditional food consumption for the subset of participants with complete case data for the models to explain hair mercury concentrations ……..……. 131

Supplementary Table 4.5: Demographic characteristics and traditional food consumption for the subset of participants with complete case data for the models to explain blood mercury, blood selenium, and plasma omega-3 fatty acid concentrations ……..…… 133

Supplementary Table 4.6: AIC values for backwards selection of model to explain log2-tranformed hair mercury concentrations ……..……..…… 135

Supplementary Table 4.7: AIC values for backwards selection of Tobit model to explain log2-tranformed blood mercury concentrations …..…….…… 136

Supplementary Table 4.8: AIC values for backwards selection of model to explain blood selenium concentrations …….……..…….…… 137

Supplementary Table 4.9: AIC values for backwards selection of model to explain log2-tranformed plasma EPA+DHA concentrations …….……..…….…… 138

Supplementary Table 5.1: Summary Statistics for Calculated Intake Rates Used in the Models by Region ……...……..… 182

Supplementary Table 5.2: Summary Statistics for Participant Age, Sex, and Bodyweight Used in the Models by Region …….……...……...… 182

Supplementary Table 5.3: Summary statistics used as input parameters to shape the log-normal distributions for the species-specific fish analyte concentrations used in Model 1 ……..……..… 183

Supplementary Table 5.4: Summary statistics used as input parameters to shape the log-normal distributions for the species and waterbody-specific fish analyte concentrations used in Model 2 .. 184

Supplementary Table 5.5: Summary statistics for total mercury bioaccessibility by fish-species included in Model 3 ……...……...……..… 186

Supplementary Table 6.1A: Frequency of prenatal consumption of fish high in Hg (male children), MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies …… 211

Supplementary Table 6.1B: Frequency of prenatal consumption of fish high in Hg (female children), MIREC (2008-2011) and MIREC-CD Plus (2013-2015) Studies …… 211

Supplementary Table 6.2: Odds ratios and 95% confidence intervals for variables included in the inverse probability weighting (IPW) model to determine the predicted probabilities of participation at follow up (reference = did not participate at follow-up); MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……..…… 212

Supplementary Table 6.3A: Descriptive statistics stratified by prenatal fish consumption for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies …… 213

Supplementary Table 6.3B: Descriptive statistics stratified by prenatal fish consumption for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies …… 215

Supplementary Table 6.4: Comparison of Hg concentrations and prenatal fish consumption in the full MIREC cohort sample versus the analytical sample, MIREC study (2008–2011) …… 217

Supplementary Table 6.5A: Pearson correlations among IQ scales for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies …….…… 218

Supplementary Table 6.5B: Pearson correlations among IQ scales for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies …….…… 218

Supplementary Table 6.6A: IQ measures stratified by covariates for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies …….……... 219

Supplementary Table 6.6B: IQ measures stratified by covariates for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies …….…… 221

Supplementary Table 6.7A: Prenatal blood Hg concentrations (first trimester blood and cord blood Hg concentrations) for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……...…….…… 223

Supplementary Table 6.7B: Prenatal blood Hg concentrations (first trimester blood and cord blood Hg concentrations) for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……...…….…… 224

Supplementary Table 6.8: Child blood Hg concentrations stratified by covariates for male and female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies …….…… 225

Supplementary Table 6.9A: Sensitivity analyses for associations between first trimester blood Hg concentrations and IQ for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……...…….…… 226

Supplementary Table 6.9B: Sensitivity analyses for associations between first trimester blood Hg concentrations and child IQ for sensitivity analyses for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……..… 227

Supplementary Table 6.10A Sensitivity analyses for associations between cord blood Hg concentrations and child IQ for sensitivity analyses for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……..… 228

Supplementary Table 6.10B: Sensitivity analyses for associations between cord blood Hg concentrations and child IQ for sensitivity analyses for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……..… 229

Supplementary Table 6.11: Regression coefficients and 95% CI for associations between the child blood Hg and selenium interaction term and child IQ, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……..……..…230

Supplementary Table 6.12A: Regression coefficients and 95% CI for associations between child blood Hg concentrations and child IQ for sensitivity analyses for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……..……..… 231

Supplementary Table 6.12B: Regression coefficients and 95% CI for associations between child blood Hg concentrations and child IQ for sensitivity analyses for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies ……..… 231

List of Abbreviations

AIC: Akaike Information Criterion

AMAP: Arctic Monitoring and Assessment Program

BE: Biomonitoring Equivalent

BMI: Body Mass Index

CHMS: Canadian Health Measures Survey

CI: Confidence interval

DHA: docosahexaenoic acid

DRI: Dietary Reference Intake

EPA: eicosapentaenoic acid

FFQ: Food Frequency Questionnaire

FNBI: First Nations Biomonitoring Initiative

FSIQ: Full-scale IQ

g: gram

GLC: General language composite score

GM: Geometric mean

GWF: Global Water Futures

Hg: Mercury

IPY-IHS: International Polar Year Inuit Health Survey

IQ: Intelligence quotient

IR: Intake rate

kg: kilogram

LOD: Limit of detection

LOQ: Limit of quantification

MeHg: Methylmercury

mg: milligram

µg: microgram

NA: Not applicable

NCP: Northern Contaminants Program

NHANES: National Health and Nutrition Examination Survey

NSTP: Northern Scientific Training Program

NWT: Northwest Territories

NWF: Northern Water Futures

Omega-3 PUFAs: Omega-3 polyunsaturated fatty acids

PBDEs: Polybrominated diphenyl ethers

PCBs: polychlorinated biphenyls

PIQ: Performance IQ

POP: Persistent organic pollutant

PUFA: Polyunsaturated fatty acid

ppm: parts per million

RF: Reference value

RfD: Reference Dose

Se: Selenium

TDI: Tolerable Daily Intake

TUL: Tolerable Upper Limit

U.S. EPA: United States Environmental Protection Agency

VIQ: Verbal IQ

WHO: World Health Organization

Chapter 1: Introduction

1.1 Benefits of Fish Consumption

Fish and seafood are considered a vital food source for populations around the world and their consumption is increasing globally (FAO, 2022). Fish are an important source of high-quality protein (Tidwell & Allan, 2001) as well as other essential nutrients, such as omega-3 polyunsaturated fatty acids (PUFA), vitamins, and essential elements (such as selenium (Se)) (Chen et al., 2022; Carvalho et al., 2005; Mahaffey, 2004). These nutrients are not only essential for general health, but also include those required for optimal fetal development (Health Canada, 2007) and ultimately children's neurodevelopment (Avella-Garcia & Julvez, 2014).

Due to the relatively high levels of omega-3 PUFA in fish, consumption of fish is recommended for the general population for health benefits including the reduction of cardiovascular disease (Chen et al., 2022; Mahaffey, 2004; Kris-Etherton et al., 2002). Adequate omega-3 PUFA and omega-6 PUFA intakes are important for platelet aggregation, immune function, blood vessel constriction, and proper development and maintenance of visual function (Mahaffey, 2004). In particular, fish and seafood are an important source of the dietary omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Swanson et al., 2012; Mahaffey, 2004). Although EPA and DHA can be found in low concentrations in other animal foods (such as meat and eggs), the consumption of fish and seafood is the most common way to obtain these omega-3 PUFAs (Mahaffey, 2004). Fish livers have long been used as a source of oils (for example cod liver oil), and the consumption of cod liver oil has been associated with increased intake of PUFAs, including EPA and DHA, in mothers and increased levels of these fatty acids in their breast milk (Olafsdottir et al., 2006).

EPA, and in particular DHA, are important during fetal development for cell membrane function and the development of brain and retinal tissues (Swanson et al., 2012). In epidemiological studies, lower biomarker levels of DHA have been associated with increased risk of negative visual and neurodevelopmental outcomes in infants and children (Innis, 2008). EPA and DHA are also considered to have anti-inflammatory properties which may explain their protective effect against cardiovascular disease and potentially against Alzheimer's disease, dementia, and cognitive decline (Swanson et al., 2012; Innis, 2008). Due to the many health

benefits associated with fish consumption (Chen et al., 2022; Mahaffey, 2004; Kris-Etherton et al., 2002; Herold & Kinsella, 1986), Canada's Food Guide recommended that Canadians consume at least 150 grams of fish, or two fish meals, a week (Health Canada, 2009).

Fish and seafood, along with other protein-rich foods such as meat (from mammals and birds), eggs, and cereals, also tend to be relatively high in concentrations of the essential element Se (Ullah et al., 2018). Se is incorporated into a number of selenoproteins (Zhang et al., 2014; Mozaffarian, 2009; Brown & Arthur, 2001), which have many important roles, including in metabolic processes, the immune system, and the endocrine system (Mojadadi, 2021; Ullah et al., 2018). Consequently, several adverse health effects have been associated with Se deficiency, including lower immune function, issues with reproduction/fertility, increased risk of some cancers, and increased mortality (Rayman, 2020). However, at excessive levels of intake, Se is toxic and exposure can pose a health risk (Rayman, 2020; Vinceti et al., 2018). Chronic exposure to elevated levels of Se can lead to the condition selenosis which is associated with hair and nail brittleness, fatigue, and gastrointestinal symptoms (Haye et al., 2014). Therefore, it is important to have adequate Se intake while avoiding excessive exposures.

Locally caught fish in northern Canada are also an important dietary source of nutrients, including Se and omega-3 PUFAs, for northern Indigenous populations. Traditional foods, which include locally caught fish and seafood, have higher nutrient density compared to store-bought foods (Sheehy et al., 2015; Kuhnlein, 1995). It should be noted that the term "traditional foods" is typically used in First Nations and Métis communities, while the term "country foods" is typically used in Inuit communities (Robin et al., 2021). These terms are for any foods (including fish, mammals, birds, and plants) that are locally caught, gathered, hunted and/or harvested by Indigenous populations and that have sociocultural meanings. For consistency, the term traditional foods will be used throughout this thesis. Colonial impacts have influenced a shift away from traditional foods, to increased processed food consumption, which is associated with decreased micronutrient intake in Indigenous populations (AMAP, 2021a; Batal et al., 2021; Sheehy et al., 2015; Kuhnlein, 2003). Decreasing traditional food consumption can negatively affect the diet quality and health of Indigenous populations (AMAP, 2021a; Rudolph et al., 2013; Receveur et al., 1997). Previous research has shown that on days when Indigenous peoples reported eating traditional foods, they had decreased intakes of fat, carbohydrates, and sugar, and increased intakes of protein, vitamin A, vitamin D, vitamin E, vitamin B-6, riboflavin, zinc,

copper, magnesium, manganese, phosphorus, potassium, and Se (Kuhnlein, et al., 2004). Along with nutritional benefits (AMAP, 2021a; Van Oostdam et al., 2005; Kuhnlein, 1995), traditional foods also provide Indigenous peoples with important economic (AMAP, 2021; Lamden et al., 2007; Van Oostdam et al., 2005), social (AMAP, 2021a; Lamden et al., 2007; Van Oostdam et al., 2005), and cultural benefits (AMAP, 2021a; Lamden et al., 2007; Van Oostdam et al., 2005).

1.2 Risks of Fish Consumption – Mercury

Although fish contain many important nutrients, they can also contain environmental contaminants such as mercury (Hg). Hg is an ubiquitous environmental contaminant and is a global public health concern because of its deleterious health effects (Basu et al., 2023; United Nations, 2019; Chen et al., 2018; Kim & Choi, 2012; Choi & Grandjean, 2008; Mergler et al., 2007; Goldman et al., 2001). Although Hg is naturally occurring in the earth's crust, human activities have released large amounts of Hg and Hg emitted from anthropogenic sources can be transmitted long distances through air and ocean currents even to remote areas including the Canadian arctic and sub-arctic (AMAP, 2021a; AMAP, 2021b; Mason et al., 1994). Physical and ecological changes due to climate change, especially in northern regions (such as increased permafrost thaw and increased river runoff), may change the transport and cycling of Hg in the environment (Dastoor et al., 2022; AMAP, 2021b; St. Pierre et al., 2018). Hg can occur as multiple species in the environment, each with different toxicities and effects (Ruggieri et al., 2017). Of the species most commonly observed in the environment, methylmercury (MeHg), an organic form of Hg, is considered the most toxic form and is a potent neurotoxin (Ruggieri et al., 2017). MeHg bioaccumulates and biomagnifies in food webs more readily than other species of Hg (AMAP, 2021b; Chételat & Braune, 2012; Kid et al., 2012; Berti et al., 1998; Watras et al., 1998).

While there are multiple routes of exposure to MeHg for humans, fish consumption is considered a major source, including for the Canadian general population (CHMS, 2019). This can represent a human health risk, particularly for populations that consume significant amounts of fish and/or those containing elevated levels of Hg (Driscoll et al., 2013; AMAP, 2021b). This may include northern Indigenous populations since locally-harvested foods (such as fish) are important components of their diet (AMAP, 2021) and elevated concentrations of Hg have been

measured in some fish species in some waterbodies in northern Canada which has resulted in the release of consumption notices (DHSS, 2012).

Exposure to elevated levels of MeHg is associated with neurodevelopmental impairment as well as harmful neurological, cardiovascular, and reproductive effects (Basu et al., 2023; United Nations, 2019; Chen et al., 2018; Kim & Choi, 2012; Khan & Wang, 2009; Choi & Grandjean, 2008; Mergler et al., 2007; Goldman et al., 2001). Early life stages, especially the developing fetus and infants are particularly susceptible to the harmful neurodevelopmental effects of Hg exposure as their nervous system is undergoing rapid development (Ruggieri et al., 2017 Clifton II, 2007; Goldman et al., 2001). Epidemiological studies have demonstrated that prenatal exposure to Hg can have harmful impacts on children's cognitive function (Jeong et al., 2017; Jacobson et al., 2015; Suzuki et al., 2010; Crump et al., 1998; Grandjean et al., 1997), such as intelligence quotient (IQ) (Jeong et al., 2017; Jacobson et al., 2015). In addition, childhood exposure to Hg has also been negatively associated with neurodevelopmental outcomes (dos Santos-Lima et al., 2020; Myers et al., 2009). However, some studies have not found associations between early life Hg exposure and cognitive function (Golding et al., 2022; 2016; 2017; Hibbeln et al., 2018; Oken et al., 2016; Cao et al., 2010; Davidson et al., 1998); these discrepant findings may be due to the protective role of fish consumption during pregnancy.

1.3 Co-exposures to Mercury and Nutrients

When examining Hg exposures, it is important to consider the co-exposures to nutrients such as Se and omega-3 PUFAs, as they may have common dietary sources, in particular fish and seafood consumption, and they may have interactions. In several epidemiological studies, the consumption of fish has been suggested to be an effect modifier of the associations between Hg exposures and child neurodevelopment (Golding et al., 2017; Jeong et al., 2017; Llop et al., 2017; Jacobson et al., 2015; Jacobson et al., 2008), and this could be due to the nutritional benefits of fish consumption. Se may be protective against the toxic effects of Hg exposure (Ralston & Raymond, 2010; Cuvin-Aralar & Furness, 1991; Pelletier, 1986; Parizek et al., 1969) as Hg and Se interact and can form complexes reducing the biological availability of Hg (Raymond & Ralston, 2004). Several animal studies have shown that Se supplementation can act to decrease the toxicity of Hg in mammals, birds, and fish (Choi et al., 2008; Raymond & Ralston, 2004) and a study in humans, where Se supplementation was implemented for a group

with low Se intake, showed a reduction in hair Hg levels by a third (Alkazemi et al., 2013). It has also been suggested that the cardiovascular benefits of omega-3 PUFA intake from fish consumption may be reduced by the presence of MeHg in fish which may account for inconsistent findings in some studies examining the associations between fish consumption and cardiovascular risk (Mahaffey, 2004).

Previously, levels of Hg, Se, and EPA + DHA, have been found to be correlated in samples from human populations (Caspersen et al., 2019; Nakayama et al., 2019; Turunen et al., 2010; Bates et al., 2007; Dewailly et al., 2001; Granjean et al., 1992). These correlations may be due to common exposure sources for Hg, Se, and EPA+DHA, as fish/seafood consumption has been associated with human levels of Hg (Caspersen et al., 2019; Monastero et al., 2015; Gaxiola-Robles et al., 2014; Gari et al., 2013; Jenssen et al., 2012; Gundacker et al., 2006), Se (Junque et al., 2022), and EPA+DHA (Monastero et al., 2015; Friedman et al., 2006). Additionally, these analytes may have other determinants in common, as previously age, population location/region, and the consumption of alcohol have also been associated with differences in Hg (Caspersen et al., 2019; Jenssen et al., 2012; Bates et al., 2007; Gundacker et al., 2006; Mahaffey et al., 2004), Se (Junque et al., 2022; Bates et al., 2007; Gundacker et al., 2006), and EPA+DHA biomarker levels (Stark et al., 2016; Flock et al., 2013; Friedman et al., 2006). This highlights the need to consider both the nutritional benefits and the contaminant risks of fish consumption, along with other potential confounders (such as age, sex, BMI, location/region, alcohol use, and smoking status), when examining Hg exposures.

1.4 Sub-populations Examined in this Thesis

The two sub-populations that this thesis focused on were 1) Dene/Métis communities in northern Canada, and 2) pregnant women and children in Canada. This thesis focused on northern Indigenous communities, as these communities frequently consume locally harvested freshwater fish (Ratelle et al., 2020a), and elevated levels of Hg have been measured in some freshwater species of fish from some local northern waterbodies (DHSS, 2012). Those who frequently consume fish and/or consume fish with elevated Hg concentrations, could be exposed to elevated concentrations of Hg (Driscoll et al., 2013; AMAP, 2021b). This thesis also focused on pregnant women and children in Canada, as early life stages are particularly sensitive to the

neurotoxic effects of Hg as their nervous systems are undergoing rapid development (Ruggieri et al., 2017; Clifton II, 2007; Goldman et al., 2001).

1.5 Determining Mercury Exposures - Human Biomonitoring of Mercury

Human biomonitoring projects involve the detection and measurement of chemicals and/or their metabolites in collected human biological samples (known as biomarkers of exposure) and this considers all routes and sources of exposure (Angerer et al., 2007; Paustenbach & Galbraith, 2006). The samples collected and analyzed can include blood, hair, urine, breast milk, finger/toe nails, saliva, fat, placenta, and even human breath (Paustenbach & Galbraith, 2006). Generally, for practical purposes, only a few types of samples are used in a study and often multiple chemicals, which can include environmental contaminants and nutrients, are measured (Paustenbach & Galbraith, 2006). Human biomonitoring can be used to establish baseline levels of exposure, determine if exposures are changing over time, identify new and emerging exposures, and to identify populations at risk and/or those with elevated levels of exposure (Angerer et al., 2007). Biomarkers measured in a human biomonitoring study can be compared to those measured in other populations, and they can be compared to biomonitoring equivalents/health-based guidance values. Biomonitoring equivalents are a concentration, or range of concentrations, for a chemical or its metabolite, that are consistent with exposure guidelines, such tolerable daily intakes (TDIs) or reference doses (RfDs) (Hays et al., 2011; Hays et al., 2009). Biomonitoring equivalents can be used as a screening tool in human biomonitoring projects at a population level (Hays et al., 2011; Hays et al., 2009).

Health-based guidance values and TDIs are available for MeHg. A TDI of 0.47 μg/kg/day of MeHg (Health Canada, 2007) has been established for the Canadian general population, and due to the associations between Hg and negative effects on neurodevelopment, a lower TDI of 0.23 μg/kg/day of MeHg has been proposed for children (<18 years of age) and women of childbearing age (WHO, 2003). A health-based guidance value of 8.0 μg/L in whole blood for women of child-bearing age and children, and a value of 20 μg/L whole blood for the general population (Legrand et al., 2010) has been developed. Additionally, a health-based guidance value of 2μg/g hair Hg for women of child-bearing age and children and 5 μg/g for the general population (Delormier et al., 2012), has been proposed.

Guidelines and biomonitoring equivalents have also been established for nutrients. For example, as Se is an essential element, tolerable upper limits (TULs) ranging between 23-400 μg/day for age and sex categories, have been established (National Academies, 2022). A biomonitoring equivalent for total Se in whole blood of 100 μg/L and 400-480 μg/L for an adequate Se intake and to protect against excessive Se intake (i.e., selenosis), respectively, has been proposed (Hays et al., 2014). Although a biomonitoring equivalent is yet to be established for plasma EPA+DHA concentrations, the American Dietetic Association/Dietitians of Canada recommend an intake of 500 mg/day of EPA + DHA (Kris-Etherton & Innis, 2007) and the World Health Organization recommends the equivalent of 200-500 mg/day of EPA + DHA (FAO/WHO, 2003). These guidelines and guidance-values can be used as comparisons when examining population-level exposures in human biomonitoring projects.

As Hg is a global public health concern, Hg has been included as an analyte in biomonitoring studies in numerous countries around the world including the Czech Republic, Germany, Belgium, France, Italy, Austria, Greece, Slovenia, Sweden, Spain, Poland, The United States of America, Canada, Greenland, South Korea, Faeroe Island, Hong Kong, Taiwan, Japan, and Polynesia (Ruggieri et al., 2017). Hg can be measured in a variety of human tissues each with benefits and drawbacks (Ruggieri et al., 2017; Esteban and Castaño, 2009), including blood and hair. Blood Hg concentrations are useful as a biomarker for short-term exposure to Hg, and blood can be used as a long-term measure of Hg in individuals that have a consistent diet with regular fish consumption (Ruggieri et al., 2017). In pregnant individuals, blood Hg concentrations can also provide information about the exposure to the developing fetus, as Hg in the blood crosses the placenta to the developing fetus (Ruggieri et al., 2017; Council, 2000). Cord blood can be used to determine fetal exposure as it is a more proximal measure than maternal blood Hg concentrations, which can be much lower than cord blood Hg concentrations (Ruggieri et al., 2017; Council, 2000). Although blood is a useful matrix for measuring Hg exposure, collecting blood samples is a relatively invasive procedure and samples can be difficult to transport and store, especially from remote regions (Esteban and Castaño, 2009).

Hair samples are useful for determining long-term exposure to Hg, and for observing changes in exposure over time (Ruggieri et al., 2017; Esteban and Castaño, 2009). Most consider the collection of hair to be less invasive, and hair is more easily preserved and shipped than blood. However, there is a risk of outside contamination for hair samples and there are several

assumptions associated with the interpretation of results from hair (Ruggieri et al., 2017; Mahaffey, 2005). Typically, when analyzing hair samples researchers assume that hair grows at a rate of approximately 1 cm per month, but hair growth rates are not uniform and can be affected by a number of factors including age and levels of hormones (LeBeau et al., 2011; Robbins, 2002). The amount of MeHg in hair has been reported to be over 80% of the total Hg in hair, therefore total Hg is often measured in hair and used as a proxy for MeHg (Mahaffey, 2005; Dolbec, et al., 2000).

Studies that measure Hg in both blood and hair samples allow researchers to examine relationships between these matrices as exposure biomarkers (Noisel et al., 2011; Bartell et al., 2000, Phelps et al., 1980). Generally, Hg levels in hair are much higher (often 250–300 times higher), than those observed in blood because the cysteine sites of keratin in hair binds MeHg and accumulates it (Ruggieri et al., 2017; Mahaffey, 2005). The World Health Organization derived a hair to blood Hg concentration ratio of 250:1 (WHO, 1990) and since then several studies have found generally consistent hair to blood Hg ratios across populations (Noisel et al., 2010; Berglund et al., 2005; Johnsson et al., 2005). However, hair to blood mercury concentration ratios can vary for different subpopulations (Singh et al., 2023; Budtz-Jørgensen, 2004) and may vary with different factors such as sex (Liberda et al., 2014), age (Liberda et al., 2014; Budtz-Jørgensen, 2004), blood mercury concentrations (Berglund et al., 2005; Budtz-Jørgensen, 2004), and hair structure (Budtz-Jørgensen, 2004). In a recent study of hair to blood Hg ratios in the Canadian general population, a geometric mean ratio of 293 was calculated, with the ratio for different subpopulations within the Canadian general population ranging between 181 to 382 (Singh et al., 2023). A 250:1 hair to blood Hg ratio has been used to estimate Hg levels in blood from Hg levels in hair, in risk and exposure assessments. It should be noted, however, that hair to blood Hg ratios rely on the assumption of steady state equilibrium, and steady states likely do not occur for populations with seasonal variability in diet, and consequently Hg exposures (Gosselin et al., 2006; Bartell et al., 2000). In some studies, researchers have found that inter-individual variation in hair to blood Hg concentration ratios can be very high (Liberda et al., 2014; Noisel et al., 2011; Berglund et al., 2005; Budtz-Jørgensen et al., 2004; McDowell et al., 2004). For example, in one study conducted in First Nations communities in Northern Quebec, Canada, individual hair to blood Hg ratios ranged from 3 to 2845 (Liberda et al., 2014). Therefore, for populations with seasonal variations in diet, resulting

in seasonal variations in Hg exposures, hair to blood Hg concentration ratios should be examined, as they may deviate from the established 250:1 ratio.

Similar to the United States (with their National Health and Nutrition Examination Survey (NHANES)) and some European countries (such as Germany's German Environmental Survey (GerEs), Beligium's The Flemish Environment and Health Study (FLEHS), and France's The French National Survey on Nutrition and Health (ENNS)), Canada has an established national human biomonitoring program. The Canadian Health Measures Survey (CHMS) is a national survey that gathers information related to the health of Canadians and includes a human biomonitoring component to measure the Canadian general population's exposure to a number of environmental chemicals (CHMS, 2019). However, this survey does not specifically focus on populations that could be disproportionately exposed to or affected by environmental chemicals. For example, due to logistical constraints, the CHMS does not include participants from the Canadian territories (northern Canada) or Indigenous peoples living on reserves (Health Canada, 2019a). To fill these gaps, several focused biomonitoring projects have been implemented in Canada. This includes the Maternal Infant Research on Environmental Chemicals (MIREC) cohort study, which focuses on prenatal and childhood exposures to environmental chemicals, and biomonitoring projects that focus on Indigenous populations (Drysdale et al., 2021; Cordier et al., 2020; Ratelle et al., 2018a; Curren et al., 2015; AFN, 2013; Gosselin et al., 2006; Walker et al., 2006).

1.5.1 The Maternal Infant Research on Environmental Chemicals (MIREC) Cohort Study

The MIREC study is an ongoing Canadian pregnancy cohort study that was designed to address research questions about the exposure of pregnant individuals, infants, and children to environmental chemicals in Canada (Arbuckle et al., 2013). The original cohort study has been expanded to include several follow-up studies to examine the effects of these chemical exposures on: the health of the participating children as they go through various life stages from birth to adolescence, and the health of the participating pregnant individuals as they go from pregnancy to the postnatal period to perimenopause. A cohort profile for the MIREC study has been previously published (Arbuckle et al., 2013). Approximately 2000 pregnant individuals were recruited into the study from 2008-2011 from ten cities (Vancouver, Edmonton, Winnipeg, Toronto, Sudbury, Hamilton, Kingston, Ottawa, Montreal, and Halifax) from across the

provinces in Canada. Biological samples were collected at multiple time-points during the prenatal time period, postnatally, and for a subset of participants, during follow-up studies. The MIREC follow-up studies are included in **Figure 1.1** (MIREC, 2023). This thesis describes results from the MIREC study and the MIREC-CD Plus follow-up study. The MIREC study included the collection of maternal blood samples during each trimester of pregnancy, and cord blood samples at birth. The MIREC-CD Plus follow-up study was conducted when children were 3-4 years of age and included the collection of child blood samples and a neurodevelopmental assessment, including the assessment of child IQ (Ashley-Martin, et al., 2019).

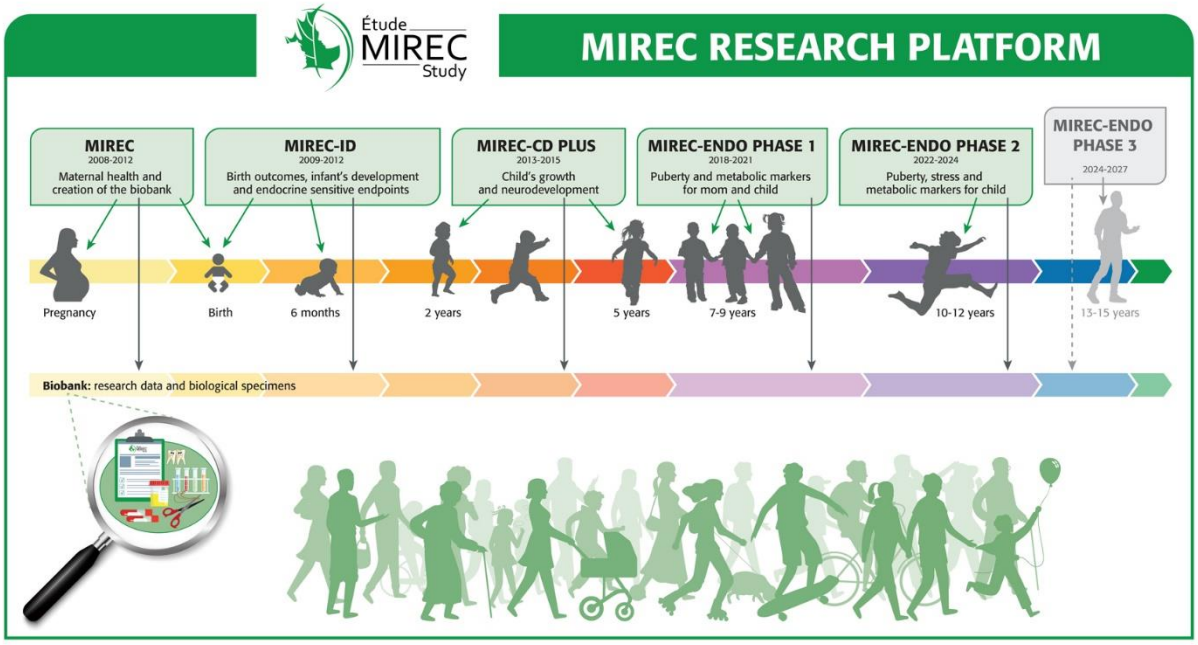


Figure 1.1: Visual summary of the MIREC Research Platform including the MIREC study and the follow-up studies. This figure is from the MIREC Research Platform Website (MIREC, 2023).

Hg concentrations have been measured in first trimester, third trimester, cord, and child blood samples from the MIREC cohort study (Ashley-Martin, et al., 2019; Arbuckle et al., 2016). Median cord blood Hg concentrations were higher than median Hg concentrations in first and third trimester maternal blood (Arbuckle et al., 2016). The vast majority (99%) of participants had blood Hg concentrations below reporting levels (set at 5.1 µg/L total Hg in blood) and blood Hg concentrations were similar or lower than those reported for the Canadian general population through the CHMS (Arbuckle et al., 2016). At follow-up, Hg concentrations were also measured in child blood samples (collected when children were 3-4 years of age) as part of the MIREC-CD

Plus study and were also similar to, or lower than, those measured in the Canadian general population through the CHMS (Ashley-Martin, et al., 2019). Data from the MIREC cohort study has been used to examine the associations between environmental chemical exposures and pregnancy outcomes (Thomas et al., 2015), and other outcomes including child neurodevelopment (Desrochers-Couture et al., 2018). For example, Desrochers-Couture et al., (2018) examined the associations between prenatal and childhood blood lead concentrations and child IQ in the MIREC cohort study. However, associations between prenatal and childhood blood Hg concentrations had not yet been examined. The availability of prenatal and childhood blood Hg concentration and child IQ data, for Canadian children enrolled in the MIREC cohort study, provides a unique opportunity to examine associations between low levels of Hg exposure that are typically seen in the general Canadian population and child IQ.

1.5.2 Human Biomonitoring Projects in Northern Dene/Métis Communities

To address the concerns of northern First Nations communities regarding health risks posed by elevated levels of contaminants in some local traditional foods, including concerns about Hg in locally caught freshwater fish, the Mackenzie River Valley Human Biomonitoring project was designed and implemented in the NWT (Ratelle et al., 2018a; Ratelle et al., 2018b). This project took a community-based approach in its design and implementation. Nine communities, six from the Dehcho region and three from the Sahtú region, took part and the project included the collection of hair, blood, and urine samples, the collection of demographic and biometric information, and the administration of three questionnaires (Ratelle et al., 2018a).

Sampling took place in 2016-2018 and members of the participating communities were eligible to participate in the study if they were over the age of 6 and could provide free and informed consent (for minors they needed to provide consent as well as have the consent of their parent/guardian). Participation rates ranged between 12-40% for the participating communities and represented approximately 9% of the residents of the Dehcho and Sahtú regions (Ratelle et al., 2020b). A total of 537 participants agreed to take part and 917 biological samples (443 hair samples, 276 blood samples, and 198 urine samples) were collected. The biological samples were analyzed for several environmental contaminants and nutrients including Hg (in hair and blood), Se (in blood), and EPA+DHA (in blood plasma). The research team also conducted a similar biomonitoring study, the Old Crow Biomonitoring Project, in the community of Old

Crow, Yukon, in 2019 and 44% of the eligible residents of Old Crow participated (Drysdale et al., 2021).

These projects provided these communities with important baseline data on their exposure to environmental contaminants and their nutrient status. Results on the levels of contaminant and nutrient biomarkers and the other components of the study were returned to communities in the form of community reports, and individual-level biomarker concentrations were returned to each participant that provided a biological sample in the form of personal results letters (Ratelle et al., 2018b; Drysdale et al., 2021). Additionally, a report summarizing the results from the Mackenzie River Valley Biomonitoring project has been published (Ratelle et al., 2019), and several papers have been published on the design (Ratelle et al., 2018a), implementation (Ratelle et al., 2018b), and results of these northern biomonitoring projects (Ratelle et al., 2020a; 2020b; 2020c; 2018b; Drsydale et al., 2021). These projects found that generally levels of environmental contaminants in these communities were similar to those measured in other Canadian biomonitoring projects (Drysdale et al., 2021; Garcia-Barrios et al., 2021; Ratelle et al., 2020b; 2019; 2018b), however, concentrations of some of the metals/elements were elevated in these sub-populations compared to the Canadian general population (Ratelle et al., 2020b). This included Hg concentrations in the NWT regions, but interestingly, Hg concentrations were only elevated relative to the Canadian general population in hair; concentrations of Hg in blood were similar to those measured in blood from the Canadian general population (Ratelle et al., 2020b). Results of the dietary surveys administered have also been published (Ratelle et al., 2023b; 2021; 2020b; Ramirez Prieto et al., 2022). The majority of participants reported consuming several traditional foods over the previous year (Ratelle et al., 2020a), although these foods made up a relatively small percent of their total dietary intake of food (Ramirez Prieto et al., 2022). Additionally, determinants of biomarker levels of some of the measured nutrients and contaminants have been examined (Drysdale, 2023; Ratelle et al., 2018c). For example, the determinants of cadmium biomarkers have been explored and reported for participants in the Mackenzie River Valley NWT biomonitoring project (Ratelle et al., 2018c). However, the determinants of biomarker levels of Hg have not yet been explored. As both hair and blood Hg concentration data is available from these studies, this would provide a unique opportunity to determine if hair to blood Hg concentration ratios for these northern communities are consistent with other populations. Additionally, these studies also present an

opportunity to fill a gap in the literature on the determinants of biomarkers of Hg, Se, and EPA+DHA concentrations in First Nations communities in northern Canada.

1.6 Exposure Assessments

Along with directly measuring exposures to environmental chemicals through biomonitoring, levels of exposures in populations are also commonly estimated according to concentrations of contaminants in environmental matrices (both biotic and abiotic). Dietary exposure assessments are an essential component of risk assessments, and subsequently risk analyses, for food chemicals (FAO/WHO, 2005). Dietary exposure assessments often rely on two main components: data on food analyte concentrations and data on food consumption/intake rates (FAO/WHO, 2005; Tran et al., 2004). They can also include several other variables such as bodyweight, and the bioavailability/bioaccessibility of an analyte in a particular food matrix (Tran et al., 2004). Dietary risk assessments can be performed for environmental contaminants and for nutrients (FAO/WHO, 2008). Often such intake reconstructions take the form of a deterministic model utilizing point estimates (e.g., mean, median, maximum) for the inputs (Solomon, 1996). Alternatively, probabilistic approaches can be used to reconstruct exposure by, instead of relying on point estimates, modeling the distributions of the various exposure determinants like food Hg concentration (Tran et al., 2004; Finley & Paustenbach, 1994). Such probabilistic models, e.g., those based upon Monte Carlo techniques, randomly sample within each of the input variable distributions in order to generate forecasted distributions of exposure. These probabilistic models provide more information than deterministic models that use point estimates and make use of more of the available data (Finley & Paustenbach et al., 1994).

Health Canada has conducted a risk assessment for Hg in fish, which included an exposure assessment and considered the benefits of fish consumption (Health Canada, 2007). The exposure assessment was for the Canadian general population and took into account intake rates of fish commonly consumed by the general Canadian population and levels of MeHg in these fish (fish that are sold commercially in Canada), and deterministic models were used to make conservative estimates of Hg exposures from the consumption of fish for several age and/or sex categories (Health Canada, 2007). However, this exposure assessment was for the Canadian general population and only included the consumption of commercially sold fish. Hg exposure assessments have been conducted for other sub-populations in Canada that consume

locally caught fish and seafood (Juric et al., 2017; Chan & Receveur, 2000). These exposure assessments have found that Hg exposures amongst communities that consume locally caught (not commercially sold and tested) fish and seafood can be variable and can in some cases be elevated (Juric et al., 2017; Chan & Receveur, 2000). In addition to the fish they consume, subpopulations' exposure to Hg may also be impacted by intrinsic factors such as their genetics, microbiome, and traditional practices (such as cooking and preparation methods) (Eagles-Smith et al., 2018). This highlights the importance of conducting site-specific Hg (and nutrient) exposure assessments for populations in Canada that consume locally-harvested (non-commercially tested and sold) fish, including northern Indigenous populations that rely on locally harvested traditional foods including locally harvested fish. These site-specific exposure assessments should include site-specific concentration data (and potentially bioaccessibility/bioavailability and speciation data if available) for analytes (including both contaminants, like Hg, and nutrients, like Se and EPA+DHA) in foods that are commonly consumed at that site/for that sub-population. Additionally, commonly consumed foods and site-specific consumption frequencies should also be obtained for the sub-population(s) included in site-specific dietary exposure assessments.

1.6.1 Food Analyte Concentrations

The availability of analyte concentration data for relevant foods is essential for a dietary exposure assessment. Fish and seafood sold commercially in Canada is regularly tested by the Canadian Food Inspection Agency (CFIA), and regulatory standards (maximum allowable limits) for Hg in fish sold commercially in Canada have been established. For the majority of fish species, the commercial sale guideline is 0.5 ppm (0.5 mg/kg wet weight) of total Hg and for select fish species (fresh/frozen tuna, shark, swordfish, escolar, marlin, and orange roughy) the commercial sale guideline is 1.0 ppm (CFIA, 2018). However, the concentrations of Hg in non-commercially caught fish within Canada can vary. In northern Canada, fish monitoring research, including the measurement of fish Hg concentrations in freshwater fish from waterbodies in the Northwest Territories, has been conducted for over 30 years (NCP, 2017). Although Hg concentrations in the Arctic seemed to have peaked in the mid-2000's and appear to be stabilizing, they are still higher than in pre-industrial times and vary across the Arctic and sub-arctic (NCP, 2017). Elevated Hg concentrations, over 0.5 ppm, have been measured in some

freshwater fish species from some waterbodies in northern Canada, such as in the Northwest Territories Mackenzie River Valley, which has resulted in the release of consumption notices (DHHS, 2016). In the freshwater fish examined, Hg concentrations tend to be highest in piscivorous species that feed at a relatively higher trophic level like Walleye and Northern Pike (Rohonczy et al., 2020; Depew et al., 2013; Evans et al., 2005), while Hg concentrations tend to be lowest in lower trophic level species like Lake Whitefish (Laird et al., 2018; Reyes et al., 2017; Depew et al., 2013; Lockhart et al., 2005).

Although Hg is typically measured in fish samples as total Hg, the majority of Hg in fish is typically in the form of MeHg (Health Canada, 2007). Several studies examining the speciation of Hg in fish have confirmed this (Arroyo-Abad et al., 2016; Kuwabara et al., 2007; Storelli et al., 2005; Cabanero et al., 2005; Gray et al., 2000), however, the percent of total Hg that is MeHg can vary widely (between 30-95%) both between and within fish species (Health Canada, 2007; Yamashita et al., 2005) and between tissue types (Arroyo-Abad et al., 2016; Yamashita et al., 2005). Hg speciation has been determined in fish sold commercially in Canada, and in these fish the majority of Hg was also in the form of MeHg (Forsyth et al., 2004). In exposure assessments, often it is assumed that 100% of the total Hg in fish samples is in the form of MeHg, especially when speciation data is lacking, to provide a conservative estimate.

Concentration data for other analytes, such as Se and omega-3 PUFAs, are also available but are more limited in freshwater fish species in northern Canada compared to Hg concentration data (Macdonald, 2013; 2011; 2009; 2002; Fisheries and Aquatic Resources, 2010; Doetzel, 2007; Rescan Environmental Services, 2005; Stewart et al., 2003; Evans, 1998; Grey et al., 1995). The Hg concentration data that is available (and the more limited Se and omega-3 PUFA concentration data) from several freshwater fish species and waterbodies in northern Canada, would allow for the development of a probabilistic Hg exposure assessment model for the dietary exposure to Hg through the consumption of freshwater fish from northern Canada if consumption rates of these specific fish could be estimated.

1.6.2 Food Consumption/Intake Rates

The use of either 24-hour dietary recalls or food frequency questionnaires (FFQs) to estimate dietary intakes is common. Each dietary survey type has its strengths and limitations. The use of 24-hour dietary recalls is common in various types of studies including national

nutrition surveys (such as the Canadian Community Health Survey (CCHS) in Canada and the National Health and Nutrition Examination Study (NHANES) in the United States) and epidemiological studies (Castell et al., 2015). This method is considered to have relatively high precision and validity for the assessment of energy and nutrient intakes, and only relies on participants' recent memory (within the past 24-hours) (Castell et al., 2015). However, a person's diet can vary from day to day, so a single 24-hour recall cannot estimate that individual's usual intake (Castell et al., 2015). Similarly, FFQs are also commonly used for the collection of data on food consumption. FFQs are considered to have reasonable relative reliability of food estimates and can provide estimates of usual intakes (Rendo-Urteaga et al., 2020; Vézina-Im et al., 2016). However, as responses rely on participant memory over a longer period of time, commonly over the past year, consumption of foods can be subject to recall bias and consumption frequencies may be overestimated or underestimated (Ratelle et al., 2020a; Rodrigo et al., 2015).

In Canada, dietary intake data is available for many foods for the general Canadian population and is commonly used in exposure assessments (Health Canada, 2018). For example, results from the CCHS are commonly used in dietary exposure assessments and includes data relevant for the Canadian general population (Health Canada, 2018). The CCHS includes estimates of daily intakes (including stratified by age and sex categories) of foods commonly consumed by the Canadian general population including grain products, dairy products, meats (including fish and shellfish), meat alternatives, vegetables, fruits, and beverages (Health Canada, 2017). This data was collected through the use of 24-hour dietary recalls (Health Canada, 2017). It should be that the CCHS did not include participants living in the Canadian Territories and/or those living on reserves. However, several other research projects have collected dietary data for sub-populations living in northern Canada.

Several studies have been conducted in Canada that have included the collection of dietary data for northern populations, including northern Indigenous populations (Pakseresht & Sharma, 2010). Previously, FFQ and/or 24-hour dietary recalls surveys have been administered to examine the energy and nutrient intakes of Inuit adults in Nunavut, Canada (Pakseresht & Sharma, 2010a) and Inuvialuit adults in the Northwest Territories, Canada (Kolahdooz et al., 2014; Pakseresht & Sharma, 2010b). FFQs have also been used to determine the frequency of consumption of specific foods, including traditional foods, in northern Indigenous populations

(Kolahdooz et al., 2014; Erber et al., 2010). FFQs and/or 24-hour recall data also exists for First Nations and Dene/Métis populations in the Northwest Territories and Yukon (Kuhnlein et al., 2008; Receveur et al., 1996). More recently, the Mackenzie River Valley Human Biomonitoring project in the Northwest Territories (Ratelle et al., 2018a; Ratelle et al., 2018b) and the Old Crow Biomonitoring Project in the Yukon (Drysdale et al., 2021), along with the collection of biological samples for biomonitoring, included the collection of dietary data through the use of a 24-hour recall and FFQ. The 24-hour recall collected information about participants' consumption of both market (commercial) and traditional foods over the previous 24 hours and energy and nutrient intakes have been estimated (Ratelle et al., 2023b). The FFQ was used to collect information about the frequency of consumption of traditional foods over the past year and information about the preparation method(s) and portion sizes of these foods (Ratelle et al., 2020a). The FFQ was refined for use in these regions through the use of focus groups and the traditional foods included on the questionnaire included locally caught/harvested freshwater fish, land mammals, birds, and plants (Ratelle et al., 2020a). Of the participants that completed the FFQ, the vast majority consumed locally caught fish. This information, on the frequency of consumption of specific fish species and typical portion sizes, could be used to calculate intake rates for these fish species. The co-location of dietary data (from the Mackenzie Valley Human Biomonitoring project), and fish analyte data (from ongoing fish monitoring research) in the Mackenzie River Valley of the Northwest Territories, Canada, could provide a unique opportunity for a site-specific dietary Hg exposure assessment based on the consumption of local freshwater fish species.

1.6.3 Bioaccessibility Data

The inclusion of bioaccessibility or bioavailability data can be used to refine exposure assessments (Lu et al., 2021; Xu et al., 2021). Bioaccessibility is the amount (fraction) of a chemical present in ingested food, water, or soil, that is available for absorption in the gastrointestinal tract after digestion, while bioavailability is the amount of a chemical present in ingested food, water, or soil, that is absorbed and reaches systemic circulation (Peijnenburg & Jager, 2003). Bioaccessibility testing simulates human digestion by using a relatively inexpensive *in vitro* method and allows for conservative estimations of Hg bioavailability from fish and seafood (Costa et al., 2015; Laird and Chan, 2013; Laird et al., 2009). Bioaccessibility is

often used as a conservative estimate for bioavailability in exposure assessments (Bradley et al., 2017). It is important to study Hg bioaccessibility, as although often exposure assessments assume 95-100% of ingested Hg reaches systemic circulation, *in vitro* studies have indicated that Hg bioaccessibility and bioavailability for many species and foods can be far less than 95% (Bradley et al., 2017; Siedlikowski et al., 2016). Assuming 100% Hg bioaccessibility can result in an overestimation of Hg exposures from fish.

Hg bioaccessibility has been reported to vary between fish species (Liao et al., 2020; Alves et al., 2018; Bradley et al., 2017; Yassine, 2017; Cano-Sancho, 2015; Torres-Escribano, 2011, Laird et al., 2009), by the location caught (Kumari & Maiti, 2021; Anacleto et al., 2020; Marmelo et al., 2020), and the type of tissue tested (Yassine, 2017; Laird and Chan, 2013). For example, in one study average total Hg bioaccessibility ranged between 28-61%, with 61% total Hg bioaccessibility for the fish species Hake (*Merlucius australis*) versus 28% for Tuna (*Katsuwonus pelamis*) (Alves et al., 2018). Similarly, when comparing fish tissue types, Laird and Chan, (2013) found that Hg bioaccessibility was 49% for Chinook Salmon (*Oncorhynchus tshawytscha*), and 46% for Sockeye Salmon (*Oncorhynchus nerka*), but only 10% for Salmon eggs.

The preparation or cooking method used has also been shown to affect bioaccessibility of Hg (Liao et al., 2020; Laird & Chan, 2013; Ouédraogo and Amyot, 2011). Although higher Hg concentrations (8 - 93% higher) have been measured in fish tissues after cooking, likely due to moisture loss during the cooking process (Afonso et al., 2018; Afonso et al., 2015a; Torres-Escribano et al., 2011), decreased bioaccessibility of Hg has been reported for cooked compared to raw fish (Liao et al., 2020; Afonso et al., 2015a; 2015b; Ouédraogo and Amyot, 2011). For example, Afonso et al., (2015a) found that Hg bioaccessibility in Tuna (*Thunnus* spp.) was 78%, 48%, and 39% for raw, boiled, and grilled preparations, respectively and Matos et al., (2015) found that Hg bioaccessibility was 94%, 55%, and 52% for raw, steamed, and grilled Blue Shark muscle, respectively. Reduced Hg bioaccessibility in cooked samples could be due to the thermal denaturation of proteins (Anacleto et al., 2020; Costa et al., 2015; Matos et al., 2015; Ouédraogo and Amyot, 2011). The denaturation of proteins at high temperatures has been reported to reduce protein solubility by making it harder for digestive enzymes to access the active sites of the Hg-protein complex and by promoting protein aggregation (Anacleto et al., 2020; Matos et al., 2015; Ouédraogo and Amyot, 2011). Additionally, the conformational changes from protein

denaturation may create additional binding sites, sequestering Hg and rendering it inaccessible (Anacleto et al., 2020; Ouédraogo and Amyot, 2011).

The majority of published data for Hg bioaccessibility in fish samples is for marine species from coastal oceans in areas with a relatively mild yearlong climate (Afonso et al., 2015a; Matos et al., 2015; Calatayud et al., 2012). Little data exist for northern Canada, especially for freshwater fish species. Given the importance of freshwater fish for northern Indigenous communities (Ratelle et al., 2020), this is an important gap in the literature. There is a need to address the lack of bioaccessibility data for commonly consumed freshwater fish from northern latitudes (Yassine, 2017). Additionally, the majority of published bioaccessibility data is for raw samples (Liao et al., 2020; Costa et al., 2015; Ouédraogo & Amyot, 2011), while in northern Canada locally harvested freshwater fish are normally cooked or otherwise prepared (such as smoked or dried) before consumption (Ratelle et al., 2020), so there is an additional need to address the lack of bioaccessibility data for cooked freshwater fish.

1.7 Objectives and Research Questions

The main aim of this thesis was to use targeted biomonitoring to examine Hg exposures, in the context of fish consumption, among sub-populations in Canada. This thesis draws on the results of the Mackenzie River Valley (NWT) Human Biomonitoring project, along with results and samples from on-going fish sampling projects and research in the Northwest Territories, to construct and refine an exposure assessment model to estimate Hg, Se, and EPA and DHA intakes, for the Dehcho and Sahtú regions of the Northwest Territories, Canada, from the consumption of locally harvested northern freshwater fish. Additionally, this thesis sought to understand the dietary and sociodemographic determinants of human biomarker levels of Hg, Se, and omega-3 PUFA, in these northern regions, and how these levels of exposure compared to those in the Canadian general population. Finally, using data from the MIREC cohort study, this thesis sought to determine if there are associations between early life exposures to Hg, at the levels of Hg exposure typically seen in Canada, and child neurodevelopment, and whether the prenatal consumption of fish modifies these associations. In order to achieve these aims, this thesis had five main objectives which were to: 1. measure Hg bioaccessibility in commonly consumed northern freshwater fish; 2. examine and describe Hg biomarker levels in members of northern Dene/Métis communities; 3. identify potential determinants of hair Hg, blood Hg, blood

Se, and plasma EPA+DHA concentrations in members of northern Dene/Métis communities; 4. construct and refine a site-specific exposure assessment model for Hg, Se, and EPA+DHA, from the consumption of local freshwater fish, for the Dehcho and Sahtú regions of the NWT; and 5. determine if there are associations between early life exposures to low levels of Hg and child IQ, and if these associations are modified by prenatal fish consumption. The connections between the various research projects, research chapters/manuscripts, and flow of the results chapters that are included in this thesis are shown in **Figure 1.2**.

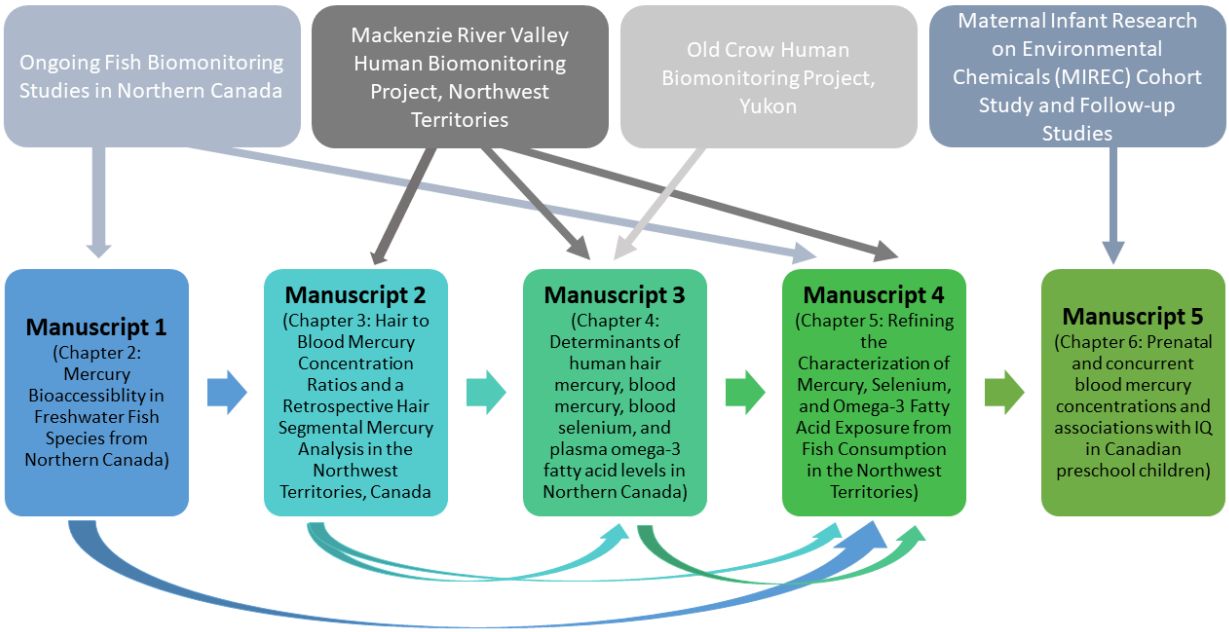


Figure 1.2: Depiction of the connections between the research projects and manuscripts/chapters in this manuscript-based thesis

1.7.1 Objective 1: Measure Hg Bioaccessibility in Commonly Consumed Northern Freshwater Fish

The first objective of this thesis, to address the lack of Hg bioaccessibility data for northern freshwater fish, was to measure the bioaccessibility of Hg in the muscle tissue of several species of freshwater fish that are commonly consumed by Indigenous populations in northern Canada. Whether Hg concentrations, bioaccessible Hg concentrations, and percent Hg bioaccessibility, differed among fish species and waterbodies was explored. Additionally, the livers of fish, in particular burbot livers, are consumed in northern Canada (Ratelle et al., 2019) but to the best of my knowledge, Hg bioaccessibility has never been measured in the livers of

freshwater fish. To address this, Hg bioaccessiblity in burbot livers versus muscle was also determined and compared. Finally, to contribute to the limited literature on the bioaccessibility of Hg in raw versus cooked samples, this thesis examined Hg bioaccessibility in raw versus cooked (pan fried in water) freshwater fish.

1.7.2 Objective 2: Examine and Describe Hg Biomarker Levels in Members of Northern Dene/Métis Communities

The second objective of this thesis, to address community concerns about exposure to Hg from locally caught northern freshwater fish, and to address the gap in the human biomonitoring of Hg in northern Dene/Métis communities in the Northwest Territories, concentrations of Hg in blood and hair samples collected as part of the Mackenzie River Valley Biomonitoring project were examined. The concentrations of these Hg biomarkers in this biomonitoring project were summarized and compared to those measured in the Canadian general population through the national CHMS biomonitoring project. Additionally, region specific hair to blood Hg concentration ratios were calculated to determine if they differed from the expected 250:1 ratio and Hg concentrations between segments of long hair samples were compared to determine if participants' Hg exposure varied seasonally and if this was related to seasonal variation in the frequency of total fish consumption.

1.7.3 Identify Potential Determinants of Hair Hg, Blood Hg, Blood Se, and Plasma EPA+DHA Concentrations in Members of Northern Dene/Métis Communities

The third objective of this thesis was to examine if sociodemographic, anthropometric, and dietary factors were determinants of Hg biomarker concentrations, as well as the concentrations of the essential nutrients Se, and omega-3 PUFA (specifically EPA+DHA). Particular focus was on traditional foods, including specific species of locally caught freshwater fish, that could be an important source or contain elevated levels of Hg, Se, or EPA+DHA. This was to fill the gap in the literature on the determinants of Hg, Se, and EPA+DHA biomarker concentrations in Dene/Métis communities in northern Canada.

1.7.4 Construct and Refine a Site-specific Exposure Assessment Model for Hg, Se, and EPA+DHA, from the Consumption of Local Freshwater Fish, for the Dehcho and Sahtú regions of the Northwest Territories

The fourth objective of this thesis was to use the information generated from the previous objectives to construct and refine a site-specific probabilistic dietary exposure assessment model for Hg, and the nutrients Se and EPA+DHA, from the consumption of local freshwater fish for the Dehcho and Sahtú regions of the Northwest Territories. The exposure assessment models included fish species-specific analyte (Hg, Se, and EPA+DHA) concentration data and species-specific intake rates calculated from the FFQ administered as part of the Mackenzie River Valley Human Biomonitoring project. The models were refined through the inclusion of waterbody-specific fish analyte data and Hg bioaccessiblity data. Exposure estimates were compared to relevant guidelines (such as TDIs, TULs, and adequate daily intakes (ADIs)) and the associations between the exposure estimates and biomarker levels of these analytes were examined.

1.7.5 Objective 5: Determine if there are Associations between Early Life Exposures to Low Levels of Hg and Child IQ, and if these Associations are Modified by Prenatal Fish Consumption

Since early life stages are particularly vulnerable to the neurotoxic effects of Hg, the final objective of this thesis was to use focused biomonitoring to determine if there are associations between early life exposures to Hg and child neurodevelopment at the levels of Hg exposure typically seen in Canada, and whether the prenatal consumption of fish modifies these associations. Associations between prenatal Hg exposures (first trimester and cord blood Hg concentrations) and child IQ (at age 3-4 years) were examined stratified by child sex. These associations were further stratified by the consumption of fish in early pregnancy to determine if prenatal fish consumption modifies the associations. Further, the associations between concurrent exposures (childhood blood Hg concentrations at age 3-4 years) and child IQ were also examined.

Chapter 2: Mercury Bioaccessiblity in Freshwater Fish Species from Northern Canada

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2.1 Abstract

Indigenous communities in northern Canada rely on locally harvested traditional foods, including fish, which provides them with nutritional, cultural, and social benefits. However, Hg exposure from fish consumption can pose a health risk for populations that consume large amounts of fish with elevated Hg concentrations. The bioaccessiblity of Hg in the tissue of northern Canadian freshwater fish is not yet known. To address this, samples from five commonly consumed freshwater fish species (Lake Trout, Northern Pike, Walleye, Lake Whitefish, and Burbot) caught from lakes in the Northwest Territories and Yukon, Canada were examined. Total Hg concentrations, fatty acid composition, and total Hg bioaccessibility differed significantly among fish species and lakes. Mean total Hg bioaccessibility using an *in vitro* gastrointestinal model ranged between 56% and 96% in muscle tissue across fish species and waterbodies examined and was 39% in liver tissue from Burbot. Mean total Hg bioaccessibility was much lower (range: 38% to 42%) for a subset of samples run through only the gastric phase of the digestion model. Total Hg bioaccessibility was significantly lower (on average 40% lower) in a subset of samples that were pan-fried in water. Thus, although cooking increased total Hg concentrations in pan-fried fish samples (likely due to moisture loss), bioaccessible concentrations of total Hg were lower (on average 32% lower). Results from this study contribute to addressing a large knowledge gap in the literature regarding bioaccessibility of total Hg in northern freshwater fish species. To the best of our knowledge, this is the first study to examine Hg bioaccessibility in raw and cooked liver samples from freshwater fish. It also adds to the growing literature indicating that Hg bioaccessibility varies among fish species, locations, and cooking/preparation methods.

2.2 Introduction

Fish are an important dietary source of nutrients, such as omega-3 polyunsaturated fatty acids (PUFA), protein, vitamins, and essential elements (such as selenium) (Chen et al., 2022;

Carvalho et al., 2005; Mahaffey, 2004). The consumption of fish is recommended for the reduction of cardiovascular disease and is associated with numerous health benefits (Chen et al., 2022; Mahaffey, 2004; Kris-Etherton et al., 2002). Fish consumption is considered to be a major source of Hg exposure for the Canadian general population (Health Canada, 2019a), however exposure to elevated levels of Hg, particularly MeHg, is associated with harmful effects, including neurodevelopmental impairment and negative cardiovascular and reproductive effects (Ruggieri et al., 2017; Rice et al., 2014; Alkazemi et al., 2013; Driscoll et al., 2013; Khan and Wang, 2009). Fish consumption can therefore present a human health risk, particularly for populations that consume large amounts of fish, or consume fish with elevated Hg concentrations (AMAP, 2021b). Assessments of Hg exposure are critical to assessing risks and benefits of fish consumption, but exposure assessments are based on dose reconstructions that typically assume that 95-100% of ingested Hg reaches systemic circulation. *In vitro* studies have indicated that Hg bioavailability (and bioaccessibility) for many fish species and other foods can be far less than 95% (Bradley et al., 2017; Siedlikowski et al., 2016). More detailed evaluations of Hg bioavailability and bioaccessibility are thus warranted.

Bioaccessibility, in this context, refers to the proportion of an analyte (in this case mercury) in a matrix (ex. ingested fish or other food) that is available for absorption after digestion and bioavailability refers to the proportion of that analyte from that matrix that is absorbed and enters into circulation in the body. Bioaccessibility testing simulates human digestion by using a relatively inexpensive *in vitro* method and allows for conservative estimations of Hg bioavailability from fish and seafood (Costa et al., 2015; Laird and Chan, 2013; Laird et al., 2009). Often total mercury concentrations and total Hg bioaccessibility are reported and used as a proxy for methylmercury concentrations, as the majority of mercury in fish muscle tends to be in the form of methylmercury (Arroyo-Abad et al., 2016; Kuwabara et al., 2007; Storelli et al., 2005). In the literature, average bioaccessibility of total Hg in fish ranges between 65 and 97% (results from select studies presented in **Supplementary Table 2.1**). Bioaccessibility of total Hg can vary based on a number of factors, including: species (Liao et al., 2020; Alves et al., 2018; Yassine, 2017; Laird et al., 2009), location caught (Kumari & Maiti, 2021; Marmelo et al., 2020; Anacleto et al., 2020;), and the type of tissue tested (Yassine, 2017; Laird and Chan, 2013). For example, Alves et al., (2018) found that total Hg bioaccessibility was on average 61% for Hake (*Merlucius australis*) but only 28% for Tuna (*Katsuwonus pelamis*). Laird and Chan (2013)

found that total Hg bioaccessibility was 49% for Chinook Salmon (*Oncorhynchus tshawytscha*), and 46% for Sockeye Salmon (*Oncorhynchus nerka*), but only 10% for Salmon eggs. Measured total mercury bioaccessiblity can also vary based on the *in vitro* digestion model used (Li & Wang, 2019; Bradely et al., 2017). Method of preparation has also been shown to affect bioaccessibility of total Hg (Liao et al., 2020; Laird and Chan, 2013; Ouédraogo and Amyot, 2011). Previous literature has shown that cooking fish decreases the bioaccessibility of total Hg (Liao et al., 2020; Afonso et al., 2015a; Afonso et al., 2015b; Ouédraogo and Amyot, 2011). For example, Afonso et al., (2015a) found that total Hg bioaccessibility in Tuna (*Thunnus* spp.) was 78%, 48%, and 39% for raw, boiled, and grilled preparations, respectively.

Most published data for bioaccessibility of total Hg in fish samples is for marine species from coastal oceans in areas with a relatively mild yearlong climate (Afonso et al., 2015a; Matos et al., 2015; Calatayud et al., 2012). Little data exist for northern Canada, where locally harvested traditional foods, including freshwater fish, have a critical role in the diet and culture of Indigenous peoples (AMAP, 2009; Lambden et al., 2007; van Oostdam et al., 2005; Kuhnlein, 1995). Given the importance of freshwater fish for these communities (Ratelle et al., 2020), there is a need to address the lack of bioaccessibility data for freshwater fish and in particular those from northern latitudes (Yassine, 2017). Furthermore, many fish harvested from northern Canada are prepared/cooked before consumption (Ratelle et al., 2020), while the majority of published bioaccessibility data is for raw samples (Liao et al., 2020; Costa et al., 2015; Ouédraogo and Amyot, 2011), so there is an additional need to address the lack of bioaccessibility data for cooked freshwater fish.

This study aimed to quantify total Hg bioaccessibility in locally caught freshwater fish species from northern Canada using an *in vitro* gastrointestinal digestion model. The fish species examined included: Lake Trout (*Salvelinus namaycush*), Lake Whitefish (*Coregonus clupeaformis*), Northern Pike (*Esox lucius*), Walleye (*Sander vitreus*), and Burbot (*Lota lota*) which were caught from several waterbodies in the Northwest Territories (NWT) and Yukon, Canada. Effects of cooking (pan frying in water) on total Hg bioaccessibility for a subset of samples was examined. Secondary objectives included: 1) determining if total Hg bioaccessibility differs when using the gastric phase only versus the full gastrointestinal *in vitro* digestion model, 2) exploring if total Hg bioaccessibility varied among fish species and waterbodies and if it was correlated with total Hg concentrations, 3) exploring associations

between total Hg bioaccessibility and fatty acid concentrations, and 4) determining if total Hg bioaccessibility differs between tissue types (muscle versus liver tissue).

2.3 Methods

2.3.1 Fish Samples

Fish were wild-caught and sampled as part of ongoing projects in the NWT and Yukon, Canada. Details regarding sampling and capture of fish from lakes in the NWT are described in Moslemi-Aqdam et al. (2021) and Moslemi-Aqdam et al. (2022). Briefly, samples from five fish species (Lake Whitefish (*Coregonus clupeaformis*), Lake Trout (*Salvelinus namaycush*), Northern Pike (*Esox lucius*), Walleye (*Sander vitreus*), and Burbot (*Lota lota*) that were previously identified as being commonly consumed by northern communities (Ratelle et al., 2020) were collected from several lakes (Mustard Lake, Kakisa Lake, Ekali Lake, Fish Lake, Tathlina Lake, Sanguez Lake, and Dezadeash Lake), which were selected based on the availability of adequate mass for bioaccessibility testing. Sample size for each species, lakes, and chemical analyses are included in **Supplementary Table 2.2**. The majority of samples were dorsal muscle tissue, with the exception of Burbot samples from Dezadeash Lake which included both dorsal muscle and liver tissue. Sampling, handling, and processing of fish followed protocols approved by the University of Waterloo Animal Care Committee (permit # 42081, 40576).

2.3.2 Sample Preparation

2.3.2.1 Sample Preparation for Chemical Analysis

Each fish sample was subsampled for analysis of total Hg concentration and a subsample of at least 6 grams was taken for bioaccessibility testing Depending on the remaining sample mass, an additional subsample was taken for fatty acid analysis (not all samples had enough mass). Subsamples intended for analysis for total Hg concentrations were freeze-dried at −54 °C and 0.014 mBar for 48 h using a LabConco® FreeZone freeze-drier (USA) and homogenized using scissors inside of 20 mL borosilicate scintillation vials. The subsamples for fatty acid analysis were frozen and pulverized under liquid nitrogen in a mortar and pestle and stored at -80 ^{o}C until analysis.

2.3.2.2 Cooking Samples

For fish samples that had sufficient mass, a subsample was also taken for cooking. These subsamples were cooked by pan frying in water. Pan frying in water was selected as this preparation method was identified as being commonly used for cooking Burbot livers and is also a method used for cooking fish muscle in northern First Nations communities (Drysdale, 2019, personal communication). Each subsample was individually fried by placing it in a heated frying pan on a hot plate set at 250 ^{o}C with 20 mL of MilliQ filtered water. The samples were kept in the frying pan until cooked through (approximately 6-7 minutes). They were then cooled and stored in the freezer at -20 ^{o}C until subsampled for analyses of total Hg concentration (see previous section for details about subsample preparation for total Hg analysis) and total Hg bioaccessibility.

2.3.2.2.1 Sample Preparation for Bioaccessibility Testing

Raw and cooked fish samples intended for total Hg bioaccessibility testing were stored at -20 ^{o}C. The day before total Hg bioaccessibility analyses were carried out, a subsample of approximately 6 grams of the frozen tissue was chopped coarsely and split into two gram triplicates before being placed in acid-washed serum bottles to thaw in the refrigerator (4°C) for up to 24 hours.

2.3.3 *In vitro* Gastrointestinal Digestion Model

Thawed triplicate samples were run through a simulated gastrointestinal digestion model that was adapted from Laird and Chan (2013). To begin, 30 mL of simulated gastric juice was added to each sample triplicate (in serum bottles), the pH was adjusted to 2.0 ± 0.2 using hydrochloric acid, and the samples were incubated in a shaker at 37 ^{o}C and 180 rpm for 2 hours. The simulated gastric juice contained pepsin (6 grams/L), and sodium chloride (8.5g/L) dissolved in MilliQ water that had been adjusted to pH 2.0 with hydrochloric acid. After the 2-hour incubation (to simulate gastric digestion), 5 mL of sodium bicarbonate (0.5 M) and 15 mL of simulated intestinal juice were added to the samples in the serum bottles. Simulated intestinal juice contained oxgall bile (6g/L), pancreatin (3g/L), sodium chloride (8.5g/L), and sodium bicarbonate (12.5g/L) dissolved in MilliQ water. The pH was adjusted to 6.5 ± 0.5 and the samples were incubated again in a shaker at 37 ^{o}C and 180 rpm for 3 hours. After the incubation, the samples were filtered through a 0.45 μm Cytiva Whatman Glass Micro Fiber syringe filter

and the solution was stored at -20 ºC until analysis for total Hg concentrations. All glassware used for the bioaccessibility protocol was acid-washed (10% trace-metal grade nitric acid) for 24 hours and rinsed three times with MilliQ water prior to use. Blanks were run through the gastrointestinal model for each batch of approximately 12 samples. Blanks consisted of empty serum bottles that were then run through the full gastrointestinal digestion (addition of simulated gastric and intestinal juices, incubations, filtration etc.). Samples of the MilliQ water used to create the gastric and intestinal juices were also collected during each sample run and analyzed for background total Hg contamination.

A subset of the samples (n=26, from Kakisa Lake, NWT), were run through only the gastric phase of the digestion model. After the first incubation (2 hours with the simulated gastric juice), instead of proceeding to the intestinal phase of the digestion model, these samples were then filtered through a 0.45 um filter and the solution was stored at -20 ºC until analysis for total Hg concentrations.

2.3.4 Chemical Analyses

Freeze-dried sub-samples for total Hg analysis were analyzed for total Hg concentrations using a direct Hg analyzer DMA-80 in accordance with U.S. EPA method 7473 (US EPA, 1998) at the Biotron Analytical Services Laboratory at Western University, London, Ontario, Canada. Quality control-quality assurance (QA/QC) was completed in accordance with the lab's ISO 17025 accreditation. The mean method detection limit (MDL) was 0.01 µg/g of total Hg (dry weight) and the mean method reporting limit (MRL) was 0.02 µg/g of total Hg (dry weight) across the batches of samples analyzed. Total Hg concentrations were converted to wet weight as previously described (Laird et al., 2018). Mean relative percent difference between duplicate samples was <10%, mean recovery of matrix spiked samples was >90%, and mean recovery of certified reference materials (DORM-4, National Research Council of Canada 2012) ranged between 90-110%.

Fatty acid concentrations in a subset of the samples were determined in the lab of Dr. Ken Stark at the University of Waterloo, Waterloo, Ontario, Canada, using high throughput gas chromatography as previously described (Reyes et al., 2017). Briefly, pulverized tissues were extracted with 2:1 chloroform:methanol containing an internal standard (C 22:3n-3 ethyl ester, Nu-Chek Prep Inc., Elysian, MN) and butylated hydroxytoluene as an antioxidant. Fatty acid

methyl esters were created from the total lipid extracts using 14% boron trifluoride in methanol and convectional heat for 1 h at 95°C using a block heater. The fatty acid methyl esters were then analyzed on a Varian 3900 gas chromatograph equipped with a flame ionization detector and a DB-FFAP 15 m × 0.1 mm ID × 0.1 μm film thickness capillary column (J&W Scientific from Agilent Technologies, Mississauga, ON, Canada) with hydrogen as the carrier gas. The samples run through the gastrointestinal digestion model were diluted and analyzed for total Hg concentrations on a Tekran 2600 Hg analyser using cold vapor atomic fluorescence spectroscopy at the Biotron Analytical Services Laboratory. Quality control-quality assurance (QA/QC) was also completed for these analyses in accordance with the lab's ISO 17025 accreditation. The mean MDL was 0.11 ng/L of total Hg and the mean MRL was 0.34 ng/L of total Hg across the batches of samples analyzed. Mean percent difference between duplicate samples was <10%, mean recovery of matrix spiked samples was >90%, and mean recovery of quality control samples was >95%. Additionally, triplicates of Dorm-4 were included in several (n=6) of the batches of gastrointestinal digestions, were analyzed, and the coefficient of variation across the batches was 12%. Total mercury concentrations were also measured in the blank samples and water samples.

2.3.5 Statistical Analyses

Data analysis was conducted using SPSS statistics (IBM, Version 27), and Microsoft Excel® (2019, Version 2104). The arithmetic mean total Hg concentration was calculated from the triplicates for each sample run through the digestion model and these arithmetic means were used for further statistical analyses. Percent total Hg bioaccessibility was calculated by dividing the total Hg concentration in the digested fraction by the total Hg concentration measured in the undigested tissue. The coefficient of variation (CV) between triplicates was also calculated and examined. One sample (including all triplicate subsamples) was excluded from all reported statistics and analyses due to an external contamination or labelling issue. If total Hg was detectable in the water blanks for the batch, bioaccessible total Hg concentrations in this batch were adjusted by subtracting the mean water blank total Hg concentrations (this was the case for one subset of samples, n=10 mean total mercury concentrations in these blanks and water samples= 0.70 ± 0.22 ng/L).

For each lake and species, summary statistics were calculated for total Hg concentrations, bioaccessible total Hg concentrations, percent total Hg bioaccessibility, and fatty acid concentrations (by total fatty acids, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (omega-3 PUFA, and omega-6 fatty acids)). Summary statistics were also calculated using a dataset where samples with triplicate CVs greater than 20% (data not shown) were excluded; results were similar to those estimated using the full dataset so the full dataset was used for all other analyses. For further statistical analyses, concentrations of total Hg and fatty acids were Ln-transformed ($\log_{(\text{base e})}$ transformed). Two-way analyses of variance (ANOVA) were conducted to determine if total Hg concentrations, bioaccessible concentrations of total Hg, and percent total Hg bioaccessibility, differed among lakes and fish species, separately for the raw and cooked samples. Pair-wise comparisons were also determined using the Tukey test to adjust for multiple comparisons. Due to the limited number of lakes that fatty acid data and cooked fish samples were available for, one-way ANOVAs were conducted to determine if fatty acid concentrations differed among fish species and if total Hg bioaccessibility in cooked muscle samples differed significantly among fish species. Paired-samples t-tests were used to determine if total Hg concentrations, the bioaccessible concentration of total Hg, and percent total Hg bioaccessibility, differed by: tissue type (in the burbot samples, muscle versus liver), preparation method (raw versus cooked samples), and digestion type (for a small subset of samples, through the full gastrointestinal digestion model versus through only the gastric phase of the digestion model). Spearman's rank correlations were performed to determine if percent total Hg bioaccessibility was associated with total Hg concentrations and fatty acid concentrations (for total fatty acid concentrations, categories of fatty acids, and for individual fatty acids). For all analyses, alpha was set at 0.05. Additionally, for the raw muscle samples, *a priori* statistical models were constructed and run to explore variability in total Hg bioaccessibility among species and lakes, and in relation to total Hg concentrations (**Supplementary Table 2.3**), and results were consistent with the other statistics conducted (**Supplementary Table 2.4**).

2.4 Results

2.4.1 Bioaccessibility of Mercury in Raw Fish Samples

Results for total Hg concentrations and total Hg bioaccessibility for raw samples by fish species and waterbody are included in **Table 2.1**. Mean total Hg concentrations (wet weight) were highest in Walleye muscle (arithmetic mean=0.62 mg/kg, n=25), followed by: Northern Pike muscle (arithmetic mean=0.51 mg/kg, n=39), Burbot muscle (arithmetic mean=0.29 mg/kg, n=10), Lake Trout muscle (arithmetic mean=0.18 mg/kg, n=7), Lake Whitefish muscle (arithmetic mean=0.11 mg/kg, n=20), and Burbot liver (arithmetic mean=0.08 mg/kg, n=10). For the paired Burbot liver and muscle samples, total Hg concentrations were significantly lower in the liver samples, and this difference was statistically significant for the Ln-transformed data (Paired Samples T-Test, $t=16.67$, $p<0.05$, $df=9$). Total Hg concentrations in the paired muscle and liver samples were correlated (Spearman's Rank Correlations, $rho= 0.78$, $p<0.05$, $df=8$). Patterns of among-lake difference in fish total Hg concentrations varied by fish species, but generally Sanguez Lake and Fish Lake had particularly high fish total Hg concentrations and Mustard Lake, Dezadeash Lake, and Kakisa Lake had some of the lowest fish total Hg concentrations (**Table 2.1**). Ln-transformed total Hg concentrations in fish muscle tissue were significantly different among fish species and lakes (**Figure 2.1**) and the interaction term was not statistically significant, indicating that differences among species were consistent among lakes (and vice versa) (Two-way ANOVA, $F= 17.04$, $p<0.05$, $df=15, 85$). Pairwise comparisons among individual lakes and species are included in **Supplementary Tables 2.5** and **2.6**.

For a subset of the raw fish muscle and liver samples, fatty acid concentrations were also determined (**Table 2.2**). Total fatty acid concentrations were highest on average in Burbot liver, Lake Whitefish muscle, and Lake Trout muscle and lowest in Burbot muscle, Walleye muscle, and Northern Pike muscle (**Table 2.2**). Fatty acid concentrations were only available from two different lakes for the Northern Pike muscle samples, and fatty acid concentrations were similar between these two lakes (Kakisa Lake and Mustard Lake). Across the categories of fatty acids (saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids – including omega-6 and omega-3 PUFA), concentrations were consistently highest in Burbot liver samples followed by Lake Whitefish muscle and Lake Trout muscle samples, and lowest in Walleye muscle, Northern Pike muscle, and Burbot muscle samples (**Table 2.2**). For example, omega-3

fatty acid concentrations were highest on average in Burbot liver, followed by: Lake Whitefish muscle, Lake Trout muscle, Northern Pike muscle, Walleye muscle, and Burbot muscle (**Table 2.2**). Mean Ln-transformed omega-3 fatty acid concentrations were statistically significantly higher in the Burbot liver compared to the paired Burbot muscle samples (Paired Samples T-Test, $t=-18.20$, $p<0.05$, $df=9$). Mean Ln-transformed omega-3 fatty acid concentrations in muscle samples were significantly different among fish species (One-Way ANOVA, $F=35.58$, $p<0.05$, $df=4, 24$).

Total Hg bioaccessibility was determined in the full set of raw samples. Arithmetic mean percent total Hg bioaccessibility in muscle tissue was highest in Northern Pike muscle (86%), followed by: Lake Whitefish muscle and Walleye muscle (78%), Lake Trout muscle (73%), Burbot muscle (56%), and Burbot liver (39%) (**Table 2.1, Figure 2.2**). Similar to total Hg concentrations, the bioaccessible concentrations of total Hg were still highest in Northern Pike and Walleye muscle samples and lowest in Lake Whitefish muscle and Burbot liver samples (**Table 2.1, Figure 2.3**). Total Hg bioaccessibility in muscle tissue differed significantly among fish species and lakes and the interaction term was significant (Two-way ANOVA, $F= 6.77$, $p<0.05$, $df=15, 85$), indicating that differences among lakes varied among species and vice versa. Pairwise comparisons indicated that percent total Hg bioaccessibility was significantly lower in Burbot muscle and significantly higher in Northern Pike muscle compared to the other fish species examined (pair-wise comparisons by species and by lake are included in **Supplementary Tables 2.7** and **2.8)**. Percent total Hg bioaccessibility was not significantly correlated with total Hg concentrations when the data were stratified by species and lake, with the exception of the Burbot liver samples (Spearman's Rank Correlations, $rho=-0.66$, $p<0.05$, $df=8$), and the Lake Whitefish muscle samples from Fish Lake (Spearman's Rank Correlations, $rho=-0.93$, $p<0.05$, $df=5$). The difference in percent total Hg bioaccessibility between paired Burbot muscle and liver samples was not statistically significant (Paired Samples T-Test, $t=2.16$, $p=0.06$, $df=9$) and percent total Hg bioaccessibility was not statistically significantly correlated between the paired Burbot muscle and liver samples (Spearman's Rank Correlations, $rho= -0.6$, $p=0.07$, $df=8$).

For the subset of samples with fatty acid concentration data, correlations between fatty acids and total Hg bioaccessibility were examined. Percent total Hg bioaccessibility was significantly correlated with several individual fatty acids and categories of fatty acids but this differed based on the fish species examined. In Burbot liver samples, percent total Hg

bioaccessibility was significantly positively correlated with the omega-3 fatty acid C 22:6(n-3) i.e., Docosahexaenoic acid (DHA) (Spearman's Rank Correlations, rho=0.69, $p<0.05$, df=8) but was not statistically significantly correlated with total omega-3 PUFA (Spearman's Rank Correlations, rho=0.25, p=0.49, df=8). In Burbot muscle samples, percent total Hg bioaccessibility was not significantly correlated with omega-3 fatty acid concentrations but was correlated with two saturated fatty acids: C 12:0 (Lauric acid) (Spearman's Rank Correlations, rho=0.71, $p<0.05$, df=8) and C 23:0 (Tricosylic acid) (Spearman's Rank Correlations, rho=0.72, $p<0.05$, df=8). In Northern Pike muscle samples, percent total Hg bioaccessibility was not significantly correlated with omega-3 PUFA but was significantly positively correlated with 20:5n-3 + 22:6n-3 (eicosapentaenoic acid (EPA) + DHA) (Spearman's Rank Correlations, rho=0.79, $p<0.05$, df=7), total fatty acids (Spearman's Rank Correlations, rho=0.74, $p<0.05$, df=7), the saturated fatty acid C 18:0 (Stearic acid) (Spearman's Rank Correlations, rho=0.74, $p<0.05$) and the omega-6 fatty acid C 22:4(n-6) (Adrenic acid)(Spearman's Rank Correlation, rho=0.74, $p<0.05$, df=7).

A small subset of samples of Lake Whitefish, Lake Trout, and Walleye from Kakisa Lake (n=26) were run through only the gastric phase of the digestion model (presented in **Supplementary Table 2.9)**. Nine of these samples had also been run through the full digestion model, and for these paired samples, mean percent total Hg bioaccessiblity was much higher (on average 42% higher) in samples run through the full digestion model (gastric and intestinal phases) compared to those run through only the gastric phase of the digestion model and this difference was statistically significant (Paired Samples T-Test, t=10.80, $p<0.05$, df=8).

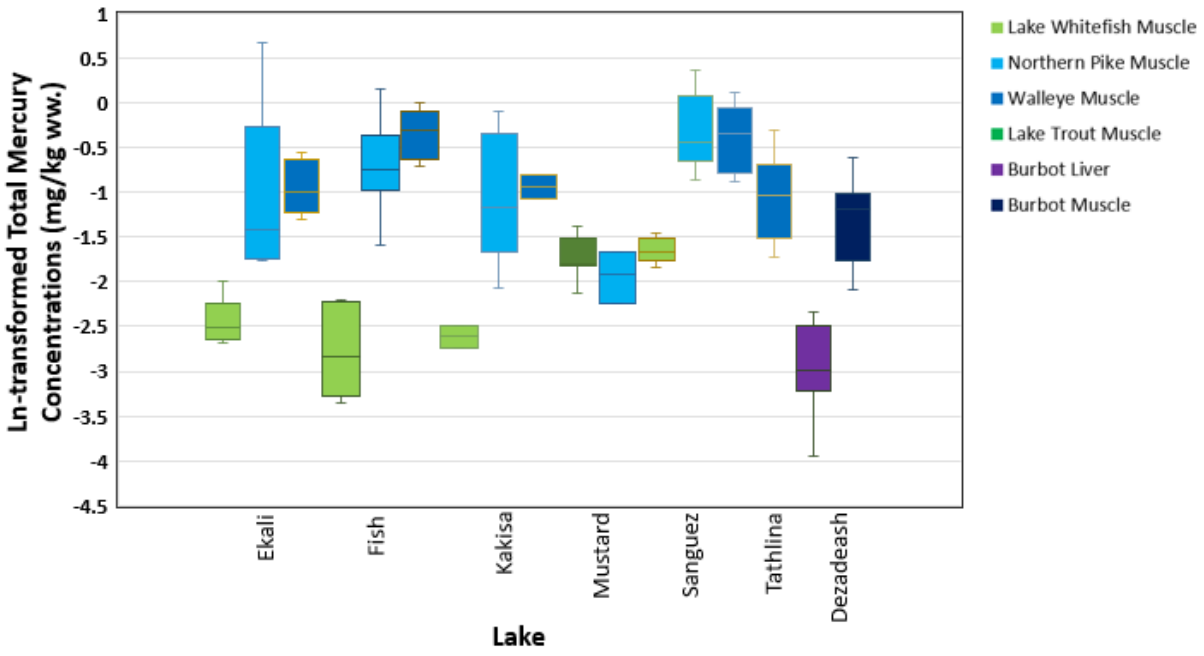


Figure 2.1: Boxplot of Ln-transformed Total Mercury Concentrations by Lake and Species for Raw Fish Muscle and Liver Samples (Plot indicates the median, interquartile range, and min and max values)

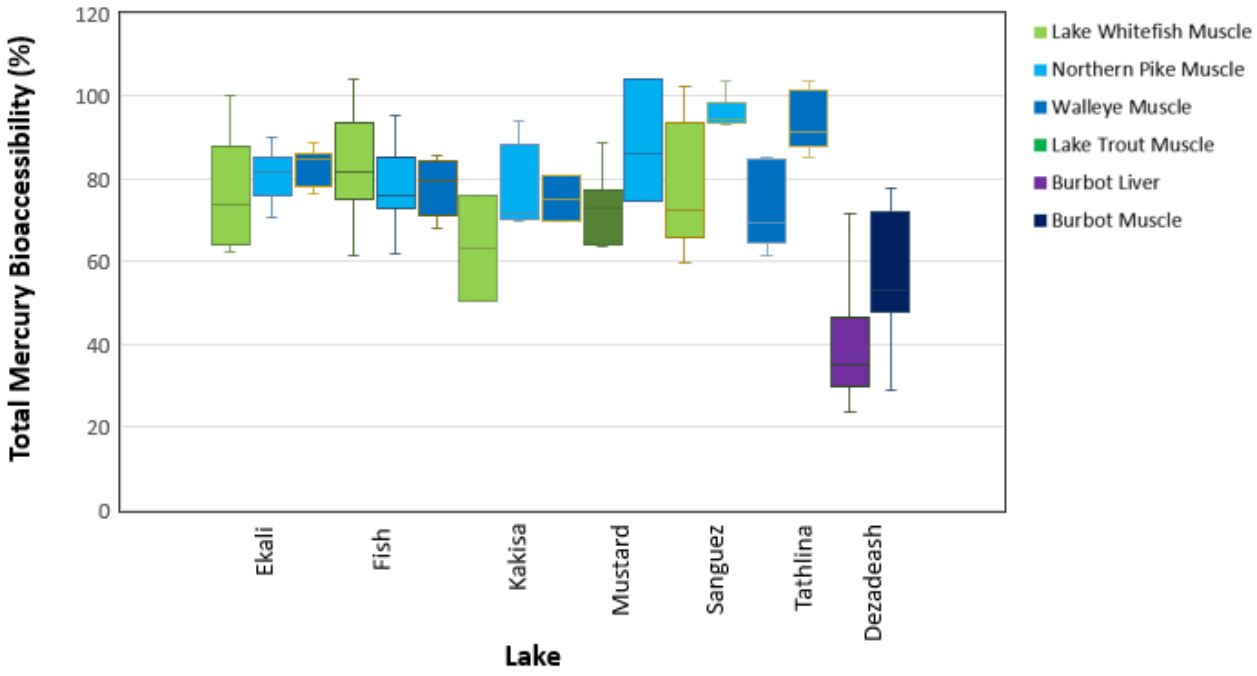


Figure 2.2: Boxplot of Percent Total Mercury Bioaccessibility by Lake and Species for Raw Fish Muscle and Liver Samples (Plot indicates the median, interquartile range, and min and max values)

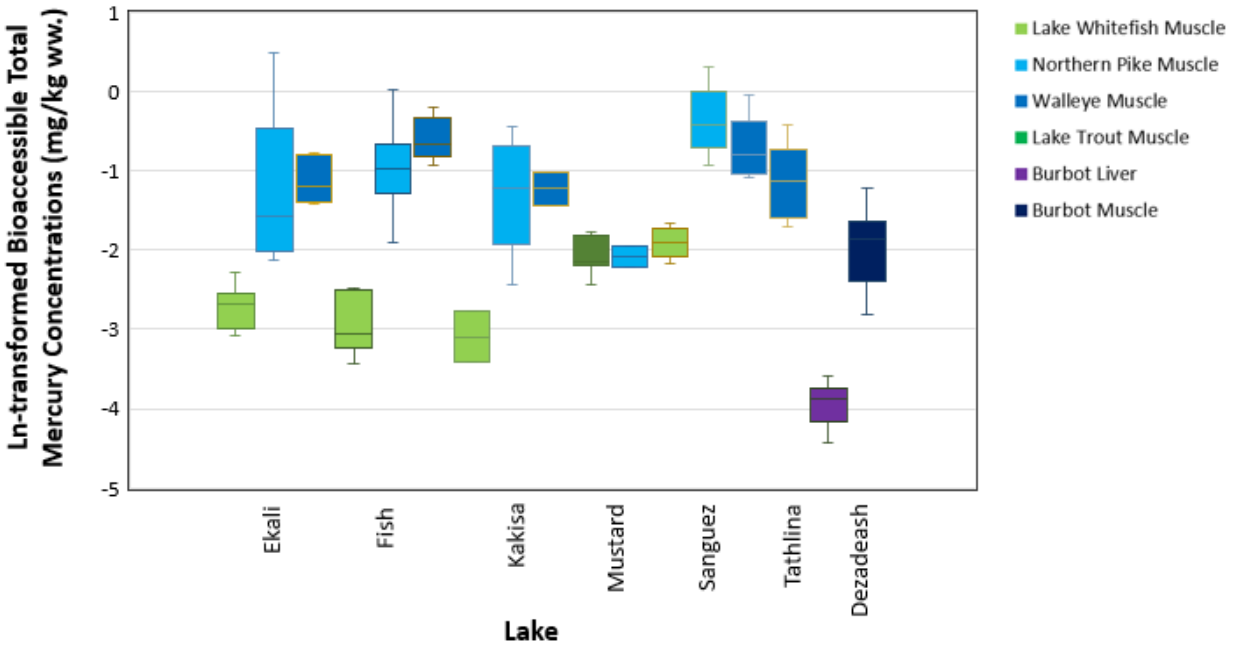


Figure 2.3: Boxplot of Ln-transformed Bioaccessible Total Mercury Concentrations by Lake and Species for Raw Fish Muscle and Liver Samples (Plot indicates the median, interquartile range, and min and max values)

Table 2.1: Total Mercury Concentrations and Percent Bioaccessibility in Raw Fish Samples

Fish Species	Lake[1]	Tissue Type	Sample Size	Total Mercury Concentration (mg/kg ww.)		Bioaccessible Concentration (mg/kg)		% Bioaccessibility	
				Arithmetic Mean	Standard Deviation	Arithmetic Mean	Standard Deviation	Arithmetic Mean	Standard Deviation
Burbot (Loche)	**Dezadeash Lake**	**Muscle**	10	0.29	0.13	0.16	0.08	56	15
		Liver	10	0.06	0.02	0.02	0.01	39	4.7
Lake Trout	**Mustard Lake**	**Muscle**	7	0.18	0.04	0.13	0.03	73	8.5
Lake Whitefish	**Ekali Lake**	**Muscle**	6	0.09	0.03	0.07	0.02	76	14
	Fish Lake	**Muscle**	7	0.07	0.03	0.06	0.02	83	14
	Kakisa Lake	**Muscle**	2	0.07	0.01	0.05	0.02	63	18
	Sanguez Lake	**Muscle**	5	0.19	0.03	0.15	0.03	78	16
Northern Pike	**Ekali Lake**	**Muscle**	6	0.57	0.70	0.47	0.59	81	6.5
	Fish Lake	**Muscle**	8	0.55	0.30	0.43	0.26	78	10
	Kakisa Lake	**Muscle**	5	0.44	0.30	0.33	0.21	78	10
	Mustard Lake	**Muscle**	3	0.15	0.04	0.13	0.02	88	15
	Sanguez Lake	**Muscle**	8	0.78	0.35	0.75	0.33	96	3.7
	Tathlina Lake	**Muscle**	9	0.38	0.19	0.35	0.17	94	6.9
Walleye	**Ekali Lake**	**Muscle**	7	0.40	0.12	0.33	0.09	83	4.7
	Fish Lake	**Muscle**	10	0.74	0.20	0.57	0.15	78	6.6
	Kakisa Lake	**Muscle**	2	0.40	0.08	0.30	0.09	75	7.8
	Sanguez Lake	**Muscle**	6	0.72	0.27	0.53	0.23	73	10

[1]All samples were from lakes in the Northwest Territories with the exception of the Burbot (Loche) samples which were from Yukon

Table 2.2: Fatty Acid Concentrations in Raw Fish Samples

Fish Species	Lake[1]	Tissue Type	Sample Size	Saturated Fatty Acids (mg/g)	Monounsaturated Fatty Acids (mg/g)	Polyunsaturated Fatty Acids (mg/g)				Total Fatty Acids (mg/g)
						Total	Omega-3 PUFA		Omega-6 Fatty Acids	
							Total	20:5n-3 + 22:6n-3		
Burbot (Loche)	**Dezadeash Lake**	**Muscle**	10	2.3 ± 0.81	0.51 ± 0.16	1.6 ± 0.47	0.75 ± 0.25	0.49 ± 0.15	0.88 ± 0.23	4.5 ± 1.4
		Liver	10	39 ± 13	39 ± 14	34 ± 14	19 ± 9.3	6.7 ± 3.1	15 ± 5.4	110 ± 40
Lake Trout	**Mustard Lake**	**Muscle**	7	4.5 ± 3.3	6.0 ± 6.4	7.2 ± 4.9	4.8 ± 3.1	3.0 ± 1.2	2.3 ± 1.8	18 ± 15
Lake Whitefish	**Kakisa Lake**	**Muscle**	2	7.9 ± 1.9	13 ± 3.0	8.7 ± 0.85	6.0 ± 0.10	4.0 ± 0.06	2.7 ± 0.96	29 ± 5.8
Northern Pike	**Kakisa Lake**	**Muscle**	5	1.4 ± 0.37	0.88 ± 0.44	3.0 ± 0.60	2.3 ± 0.43	1.8 ± 0.28	0.75 ± 0.23	5.3 ± 1.4
	Mustard Lake	**Muscle**	3	1.6 ± 0.06	0.83 ± 0.14	3.4 ± 0.35	2.4 ± 0.14	2.1 ± 0.14	1.1 ± 0.33	5.9 ± 0.54
Walleye	**Kakisa Lake**	**Muscle**	2	1.5 ± 0.10	0.94 ± 0.04	2.6 ± 0.01	1.9 ± 0.05	1.5 ± 0.05	0.77 ± 0.04	5.1 ± 0.08

[1]All samples were from lakes in the Northwest Territories with the exception of the Burbot (Loche) samples which were from the Yukon. Values are mean ± stdev.

2.4.2 Cooked Fish Mercury Bioaccessibility (Gastrointestinal Model)

A sub-set of the samples were also cooked (pan-fried in water) and analyzed for total Hg bioaccessibility using the full *in vitro* gastrointestinal digestion model (summary statistics presented in **Table 2.3**). Total Hg concentrations were on average 53% higher in muscle tissue after cooking compared to raw tissue and mean Ln-transformed total Hg concentrations in raw versus cooked muscle samples were statistically significantly different (Paired Samples T-Test, $t=-12.67$, $p<0.05$, $df=34$). Total Hg concentrations in cooked liver tissue samples were on average 19% higher compared to raw liver samples and mean Ln-transformed total Hg concentrations in raw versus cooked Burbot liver samples were statistically significantly different (Paired Samples T-Test, $t=-2.98$, $p<0.05$, $df=5$).

Unlike the total Hg bioaccessibility results for the raw muscle samples, in the cooked fish muscle samples, total Hg bioaccessibility was similar in Northern Pike muscle (41%, n=17), Walleye muscle (38%, n=6), Burbot muscle (36%, n=6), Lake Whitefish muscle (32%, n=4), and Lake Trout muscle (31%, n=2) and mean percent total Hg bioaccessibility in the cooked muscle samples did not differ significantly among fish species (One-Way ANOVA, $F=2.50$, $p=0.06$, $df=4, 30$). However, mean total Hg bioaccessiblity in cooked Burbot liver (11%, n=6). was statistically significantly lower than in the paired cooked muscle samples (Paired Samples T-Test, $t=8.75$, $p<0.05$, $df=5$). Comparing paired cooked and raw muscle samples, total Hg bioaccessibility was on average 42% lower in cooked tissue and this difference was statistically significant (Paired Samples T-Test, $t=-16.20$, $p<0.05$, $df=34$). In Burbot liver samples, total Hg bioaccessibility was on average 29% lower in cooked tissue compared to raw, and this difference was statistically significant (Paired Samples T-Test, $t=-3.42$, $p<0.05$, $df=5$). Similar to in the raw samples, on average, the bioaccessible concentration of total Hg was lowest in cooked Burbot liver and Lake Whitefish muscle samples, and highest in Walleye muscle and Northern Pike muscle samples (**Table 2.3**).

Table 2.3: Total Mercury Concentrations and Percent Bioaccessibility in Cooked Fish Samples

Fish Species	Lake[1]	Tissue Type	Sample Size	Total Mercury Concentration (mg/kg ww.)		Bioaccessible Concentration (mg/kg)		% Bioaccessibility	
				Arithmetic Mean	Standard Deviation	Arithmetic Mean	Standard Deviation	Arithmetic Mean	Standard Deviation
Burbot (Loche)	**Dezadeash Lake**	**Muscle**	6	0.39	0.15	0.14	0.06	36	5.9
		Liver	6	0.07	0.04	0.01	0.01	11	4.3
Lake Trout	**Mustard Lake**	**Muscle**	2	0.25	0.10	0.08	0.05	31	8.0
Lake Whitefish	**Ekali Lake**	**Muscle**	3	0.17	0.08	0.06	0.04	32	8.3
	Sanguez Lake	**Muscle**	1	0.32	NA	0.10	NA	31	NA
Northern Pike	**Fish Lake**	**Muscle**	6	0.85	0.46	0.33	0.15	40	4.6
	Sanguez Lake	**Muscle**	6	0.99	0.21	0.39	0.12	39	6.4
	Tathlina Lake	**Muscle**	5	0.56	0.14	0.27	0.11	46	8.0
Walleye	**Fish Lake**	**Muscle**	6	1.23	0.64	0.44	0.15	38	8.2

[1]All samples were from lakes in the Northwest Territories with the exception of the Burbot (Loche) samples which were from Yukon

2.5 Discussion

2.5.1 Fish Mercury and Fatty Acid Concentrations

Total Hg concentrations in the fish samples included in this study were similar to levels reported for these fish species for other locations in northern Canada (Ponton et al., 2022; Rohonczy et al., 2020; Depew et al., 2013; Evans et al., 2013; Evans et al., 2005; Lockhart et al., 2005). Consistent with previous results and the knowledge that total Hg concentrations tend to be highest in piscivorous species that feed at a relatively higher trophic level (Rohonczy et al., 2020; Depew et al., 2013; Evans et al., 2005), total Hg levels were highest in Walleye and Northern Pike samples and lowest in Lake Whitefish samples (Laird et al., 2018; Reyes et al., 2017; Depew et al., 2013; Lockhart et al., 2005). Also as expected, the highest total Hg concentrations were generally observed in fish from Sanguez Lake and Fish Lake. Previously, elevated levels of total Hg have been measured in some of these species and lakes, resulting in the territorial government releasing fish species-specific consumption notices for Sanguez Lake, Fish Lake, Ekali Lake, and Tathlina Lake in the NWT (DHHS, 2016). The majority of samples included in this study had total Hg concentrations below the Canadian commercial sale guideline of 0.5 ppm (0.5 mg/kg wet weight) (CFIA, 2018). All of the Lake Whitefish muscle samples (from Ekali Lake, Fish Lake, Kakisa Lake, and Sanguez Lake), Lake Trout muscle samples (from Mustard Lake), and Burbot liver samples (from Dezadeash Lake) included in this study were below the commercial sale guideline and only one burbot muscle sample from Dezadeash lake exceeded the guideline. However, several Northern Pike and Walleye samples exceeded 0.5 mg/kg total Hg. This includes the majority of Northern Pike and Walleye samples from Sanguez Lake and Fish Lake; both of these lakes have posted fish consumption notices for Northern Pike and Walleye (DHHS, 2016). Our results regarding current fish total Hg concentrations in these lakes highlights the need for continued monitoring.

In a previous study on freshwater fish in a central European River, Havelková et al., (2008) found that total Hg concentrations were higher in muscle samples versus liver samples when concentrations were relatively low (<0.5 mg/kg Hg concentrations in fish muscle samples). Our results are consistent with this; total Hg concentrations were significantly lower in the paired burbot liver samples compared to the muscle samples, and the total Hg levels in these burbot were relatively low (mean total Hg concentration in muscle = 0.29 mg/kg). Similarly, Goldstein

et al., (1996) measured lower levels of total Hg in the liver versus muscle of carp from a North American river. These results have implications for human populations that consume the livers of fish in addition to the muscle tissue. Future work should examine the concentrations of total Hg in liver versus muscle of Burbot from additional lakes in northern Canada. This is especially important in lakes where elevated levels of Hg have been detected in Burbot, as previous studies have found that in fish exposed to higher levels of Hg (>1 mg/kg Hg concentrations in fish muscle samples), tissue Hg concentrations are higher in liver than in muscle tissue (Havelková et al., 2008).

The fatty acid concentrations of fish samples analyzed in this study were similar to levels previously reported for these and other projects in North America (Neff et al., 2014; Williams et al., 2014). As expected and consistent with previous work (Laird et al., 2018), total fatty acid concentrations, as well as saturated fatty acid, monounsaturated and polyunsaturated (including omega-3 and omega-6) fatty acid concentrations tended to be highest in muscle samples from Lake Trout and Lake Whitefish (Laird et al., 2018), and lowest in muscle samples from Walleye and Northern Pike. The fatty acid content was much higher (almost 25 times higher) in Burbot liver samples compared to Burbot muscle samples. This was expected as the liver is the central organ for fatty acid metabolism. Fish livers have long been used as a source of oils (for example cod liver oil), and the consumption of fish liver oil, in particular cod liver oil, has been associated with increased intake of PUFA, including eicosapentaenoic acid (20:5n-3), docosahexaenoic acid (22:6n-3), and n-3 docosapentaenoic acid (22:6n-3) in mothers and increased levels of these fatty acids in their breast milk (Olafsdottir et al., 2006). Increased dietary intake of omega-3 PUFA from fish and fish oils has been associated with reduced risk of cardiovascular disease and other health benefits (Chen et al., 2022; Kris-Etherton et al., 2002; Mahaffey, 2004; Herold & Kinsella, 1986). Due to these benefits, Canada's Food Guide recommended that Canadians consume at least 150 grams of fish or two fish meals a week (Health Canada, 2009) and the World Health Organization recommends an intake of 20:5n-3 and 22:6n-3 of approximately 200-500 mg per day (Agostoni et al., 2012). Additionally, it should be noted that these freshwater fish can also be an important source of the essential nutrient selenium for local communities. Selenium may be protective against the toxic effects of methylmercury exposure (Ralston & Raymond, 2010; Cuvin-Aralar & Furness, 1991) as mercury and selenium interact and can form complexes reducing the biological availability of mercury (Raymond & Ralston, 2004).

Although not measured in the fish samples used in this analysis, selenium concentrations have previously been measured in samples of these freshwater fish species as part of the larger fish monitoring project in the NWT (Reyes et al., 2017). Mean selenium concentrations in the muscle of Lake Whitefish, Lake Trout, Northern Pike, Walleye, and Burbot (as well as Cisco and Longnose Sucker) from the NWT were similar between fish species and mean selenium concentrations ranged between 0.14 to 0.20 mg/kg (w.w.) (Reyes et al., 2017). The findings discussed in this study suggest that locally caught northern freshwater fishes, especially Lake Whitefish (which on average has some of the highest omega-3 PUFA concentrations but also the lowest total Hg concentrations in the fish muscle samples examined), can be an important dietary source of nutrients such as fatty acids and selenium for local communities.

2.5.2 Total Mercury Bioaccessibility in Raw Fish Tissue

Results for total Hg bioaccessibility in the raw freshwater fish muscle tissue samples run through the full gastrointestinal digestion in this study, with mean total Hg bioaccessibility ranging from 56% to 86%, are similar to the results of previous studies (**Supplementary Table 2.1**) of raw muscle samples of marine fish (Liao et al., 2020; Marmelo et al., 2020; Alves et al., 2018; Costa et al., 2015; Matos et al., 2015; Calatayud et al., 2012; Torres-Escribano et al., 2011). For example, Calatayud et al., (2012) found that total Hg bioaccessibility was on average 68% in the fish species they examined. A few studies have also examined freshwater fish species including Catfish and Carp, and the mean percent bioaccessibility was within the range of percent bioaccessibility reported for the species in this study (Kumari & Maiti, 2021; Liao et al., 2020). Yassine (2017) reported a percent total Hg bioaccessibility of 29% for Lake Trout samples (n=3), which is much lower than the percent total Hg bioaccessibility found for the Lake Trout samples in this study (73%), but both are within the range of total Hg bioaccessibility reported in other studies (Liao et al., 2020; Marmelo et al., 2020; Alves et al., 2018; Costa et al., 2015; Matos et al., 2015; Calatayud et al., 2012; Torres-Escribano et al., 2011). Additionally, some of the differences in total mercury bioaccessibility seen between studies may be due to differences in the *in vitro* gastrointestinal digestion methods used. Previously, total mercury bioaccessibility has been shown to vary considerably between different in vitro bioaccessibility methods (Li & Wang, 2019; Bradley et al., 2017). The lack of a standardized method for determining total mercury bioaccessibility is a challenge when comparing total mercury

bioaccessibility between studies.In the literature, total Hg bioaccessibility is commonly measured using an *in vitro* gastrointestinal digestion model although conditions used (such as the concentrations of enzymes, length of digestions, and filtration/separation methods) can vary (Bradely et al., 2017). Additionslly, some studies have examined total Hg bioaccessibility after only an *in vitro* gastric digestion. Previously, Cabanero et al., (2004) reported that total Hg concentrations varied significantly between samples after gastric digestion versus after gastrointestinal digestion. In this study, the mean percent total Hg bioaccessibility (38-42%) of the samples that were run through the gastric phase only of the digestion model was similar to the total Hg bioaccessibility measured by Maulvault et al., (2011) for samples that also underwent the gastric phase only of a digestion model. Percent total Hg bioaccessibility was lower on average in samples run through the gastric phase only of the digestion model compared to the full gastrointestinal model. This is likely due to the full gastrointestinal digestion model, which included the additional intestinal digestion stage, allowing for more digestion of the tissue.

Moreover, the literature is very limited on total Hg bioaccessibility in different tissues of fish, but similar to the findings of this study, it indicates that total Hg bioaccessibility can differ based on the tissue type examined (Yassine et al., 2017; Laird & Chan, 2013). For example, previously Laird & Chan (2013) found total Hg bioaccessibility to be lower in Salmon eggs than in Salmon muscle tissue and Yassine (2017) reported lower total Hg bioaccessibility in Ringed Seal liver tissue versus muscle tissue. Similarly, in this study total Hg bioaccessibility was significantly different between the two different tissue types examined (paired Burbot muscle and liver samples). Total Hg bioaccessibility was on average 17% lower in the paired Burbot liver samples compared to the muscle samples. It should be noted, that to the best of our knowledge, this is the first study to examine total Hg bioaccessibility in fish liver tissue.

Finally, a number of studies have previously shown that percent total Hg bioaccessibility differs by a number of factors. For example, total Hg bioaccessibility has been reported to vary by fish species (Liao et al., 2020; Alves et al., 2018; Laird et al., 2009). Studies of marine fish that included the same fish species, but from different locations, also report among-location differences in measured bioaccessibility (Kumari & Maiti, 2021; Anacleto et al., 2020; Marmelo et al., 2020), which could be due to differences in the bioaccessibility testing method used or could suggest that total Hg bioaccessibility varies based on where the fish are from. Differences among locations could reflect differences in diet, habitat use, and other ecological factors, and

potentially differences in fatty acid composition. Similarly, in this study, total Hg bioaccessibility varied significantly by fish species and lake and was correlated with total Hg concentrations, and concentrations of several fatty acids (but sample size was very limited for the fatty acid data). Overall, this work confirms that total Hg bioaccessibility varies by fish species and location and fills an important gap in the literature regarding freshwater fish species commonly consumed by Indigenous communities in northern Canada.

2.5.3 Total Mercury Bioaccessibility in Cooked Fish Tissue

Total Hg concentrations increased in fish muscle and liver samples that were cooked by pan frying in water. Higher total Hg concentrations in tissues after cooking (compared to paired raw samples) was likely due to moisture loss that occurred during the cooking process (Afonso et al., 2018; Afonso et al., 2015a; Torres-Escribano et al., 2011). Other authors have reported that total Hg concentration increased between 8 and 93% after cooking, depending on cooking method and fish species (Afonso et al., 2015a; Torres-Escribano et al., 2011). For example, Afonso et al (2015a) found that total Hg concentrations in Gilthead Seabream muscle samples were 1.2 and 1.4 times higher (than uncooked) after boiling and grilling, respectively. Total Hg concentrations in cooked Whitefish liver have been recorded as 1.1-1.5 times higher compared to raw tissue (Perello et al., 2008).

Although cooking increased the concentrations of total Hg in fish tissue, the percent total Hg bioaccessibility was much lower for cooked samples compared to raw samples, resulting in lower bioaccessible total Hg concentrations for the cooked compared to raw samples. Total Hg bioaccessibility was 51% lower and 67% in muscle and liver samples, respectively, in cooked compared to raw fish samples, and this is consistent with previous findings. For example, Matos et al., (2015) measured percent bioaccessibility in raw, steamed, and grilled Blue Shark muscle and found a decrease from 94% to 55% and 52% bioaccessibility, respectively (Matos et al., 2015). Reduced total Hg bioaccessibility in cooked samples could be due to thermal denaturation of proteins (Anacleto et al., 2020; Costa et al., 2015; Matos et al., 2015; Ouédraogo and Amyot, 2011). Previous studies have found that denaturation in response to high temperatures reduces protein solubility by making it harder for digestive enzymes to access the active sites of the Hg-protein complex and by promoting protein aggregation (Anacleto et al., 2020; Matos et al., 2015; Ouédraogo and Amyot, 2011). Additionally, the conformational change may create additional

binding sites, sequestering Hg and rendering it inaccessible (Anacleto et al., 2020; Ouédraogo and Amyot, 2011). The mean percent total Hg bioaccessibility in muscle tissue (ranging between 31% to 46% for the species and lakes examined) in the cooked samples in this study, are similar to those reported in the literature for cooked marine fish samples (Afonso et al., 2018; Alves et al., 2018; Torres-Escribano, et al., 2011). However, to the best of our knowledge this is the first study to measure total Hg bioaccessiblity in cooked fish liver tissue. Therefore, given the much lower percent bioaccessibility, although cooking increased the concentrations of total Hg in fish tissue, the overall bioaccessible concentration was lower for cooked samples compared to raw samples. This means that using the percent bioaccessibility or bioaccessible fraction for raw tissue samples can result in an overestimation of total Hg exposure. However, caution should be taken with the interpretation of these results, as findings from *in vitro* bioaccessibility testing may not translate directly to findings from *in vivo* bioavailability testing. For example, Charette et al., found that bioavailability of methylmercury was not affected by cooking in an *in vivo* porcine bioavailability model in contrast to their findings from *in vitro* bioaccessiblity testing (Charette et al., 2021). Further investigation is needed to help understand the impacts of various cooking and preparation methods, including traditional preparation methods, on total Hg bioaccessibility.

2.5.4 Strengths, Limitations, and Recommendations

This study fills an important gap in the literature regarding the bioaccessibility of total Hg in the tissue of freshwater fish species caught from waterbodies in Northern Canada. A total of 101 samples of raw muscle tissue and 10 samples of raw liver tissue from five commonly consumed fish species in the NWT and the Yukon (Canada) were examined. These samples were from 7 lakes, including those that currently have consumption notices posted due to the previous measurement of elevated levels of total Hg in fish tissue. This provides an important dataset for freshwater fish from northern Canada.

There are several limitations to this study. Due to the sample mass required for each chemical analysis, only a relatively small set of samples could be analyzed for total Hg bioaccessibility and fatty acid composition, and only a small set of samples could be analyzed for both raw and cooked total Hg bioaccessibility. It is recommended that for future collections of fish for bioaccessibility analysis, a larger portion of the fillet be collected whenever possible. It is

also recommended that larger fish samples should be obtained so that they can be used to compare total Hg bioaccessibility in samples exposed to additional preparation/cooking methods. This study was limited to one cooking method, pan frying in water, due to the limited sample masses available. However, many different preparation methods are reported as being used by local communities including smoking, drying, roasting/baking, and in soup (Ratelle et al., 2020). Cooking did have a significant effect on the total Hg bioaccessibility of the samples examined, so future work should examine the effects of other common preparation methods on total Hg bioaccessibility. If larger samples of fish could be obtained, along with additional cooking methods, additional analytes (such as selenium), and analyses (such as mercury speciation), could also be measured in the same fish sample. The lack of mercury speciation in this analysis is a further potential limitation of this study, especially for the liver samples. Mercury is commonly measured as total mercury in fish, and the majority of mercury in fish is typically in the form of methylmercury (Arroyo-Abad et al., 2016; Kuwabara et al., 2007; Storelli et al., 2005). However, the percent of mercury that is in the form of methylmercury can vary between and within fish species (Health Canada, 2007; Yamashita et al., 2005) and between tissue types (Arroyo-Abad et al., 2016; Yamashita et al., 2005). In particular, fish liver, compared to muscle, can contain a higher proportion of inorganic mercury (Arroyo-Abad et al., 2016). We recommend that further studies of mercury bioaccessibility in these freshwater fish are designed to obtain larger samples of fish and include the speciation of mercury in the fish tissue and bioaccessible fraction. Finally, a limitation of bioaccessibility testing is that the use of relatively simple *in vitro* digestion models may not directly translate to bioavailability in humans and that there is not standard accepted and validated method to determine total mercury bioaccessibility in fish and other food stuffs. Further studies are warranted to develop and standardize a method for assessing total mercury bioaccessiblity in fish and other foodstuffs.

A strength of this study is that it examined multiple species of fish from several different waterbodies in northern Canada, including those with consumption notices. However, since findings from this study indicate that total Hg bioaccessibility differed by species and by lake, in the future this testing should be expanded to additional waterbodies and fish species commonly used by local communities.

2.6 Conclusions

This study adds to the literature on total Hg bioaccessibility in fish tissue and addresses a gap in the literature for northern freshwater fish species. Mean total Hg concentrations, fatty acid composition, and total Hg bioaccessibility varied significantly among fish species and lakes, highlighting the importance of gathering site- and species-specific data. Bioaccessibility of total Hg varied among tissues and between cooked and raw samples; bioaccessibility of total Hg was lower in cooked samples. Results also indicate differences in estimates of bioaccessibility that reflect methods (i.e., gastric only vs gastrointestinal models), and which should be considered when comparing results among studies. Future work with increased sample sizes to investigate the speciation of Hg and the effects of other commonly used cooking/preparation methods, and other analytes such as selenium, on total Hg bioaccessibility is warranted.

2.7 Supplementary Tables

Supplementary Table 2.1: Bioaccessibility of Total Mercury in Fish, and Select Traditional Foods Previously Reported in the Literature

Animal	Tissue Type	Preparation Method	Bioaccessibility Method	Bioaccessibility (%)		References
				Average	Standard Deviation	
Marine Fish						
Anchovy	Muscle	Raw	Gastrointestinal	77 - 86		(Calatayud et al., 2012)
Anglerfish	Muscle	Raw	Gastrointestinal	57		(Calatayud et al., 2012)
Blue Shark	Muscle	Raw	Gastrointestinal	94	3	(Matos et al., 2015)
		Steamed	Gastrointestinal	55	5	(Matos et al., 2015)
		Grilled	Gastrointestinal	52	5	(Matos et al., 2015)
Blue Whiting	Muscle	Raw	Gastrointestinal	62 - 68		(Calatayud et al., 2012)
Bonito (Frozen)	Muscle	Raw	Gastrointestinal	17 - 23		(Torres-Escribano et al., 2011)
		Cooked	Gastrointestinal	12 - 17		(Torres-Escribano et al., 2011)
Hake	Muscle	Raw	Gastrointestinal	61 - 81	3	(Alves et al., 2018; Calatayud et al., 2012)
		Steamed	Gastrointestinal	19	2	(Alves et al., 2018)
		Frozen	Gastrointestinal	59 - 92		(Calatayud et al., 2012)
Hake – Small	Muscle	Raw	Gastrointestinal	58 - 89		(Calatayud et al., 2012)
		Frozen	Gastrointestinal	98 - 105		(Calatayud et al., 2012)
Mackerel	Muscle	Raw	Gastrointestinal	34	4	(Alves et al., 2018)
		Steamed	Gastrointestinal	10	0	(Alves et al., 2018)
Meagre	Muscle	Raw	Gastrointestinal	87	2.4	(Afonso et al., 2015a)
		Boiled	Gastrointestinal	91	5.1	(Afonso et al., 2015a)
		Grilled	Gastrointestinal	54	14	(Afonso et al., 2015a)

		Roasted	Gastrointestinal	83	2.6	(Afonso et al., 2015a)
Monkfish	Muscle	Raw	Gastrointestinal	42	2	(Alves et al., 2018)
		Steamed	Gastrointestinal	14	1	(Alves et al., 2018)
Sardine	Muscle	Raw	Gastrointestinal	35 - 50		(Calatayud et al., 2012)
Silver Pomfret	Muscle	Raw	Gastrointestinal	38.3	4.2	(Liao et al., 2020)
Snakehead – Spotted (*Channa punctatus*)	Muscle	Dried	Gastrointestinal	61	12	(Kumari and Maiti, 2021)
Snakehead – Striped (*Channa striata*)	Muscle	Dried	Gastrointestinal	59	7	(Kumari and Maiti, 2021)
Snapper	Muscle	Raw	Gastrointestinal	101.8	7.7	(Liao et al., 2020)
Sole	Muscle	Raw	Gastrointestinal	67		(Calatayud et al., 2012)
		Frozen	Gastrointestinal	105		(Calatayud et al., 2012)
Swamp Barb (*Puntis chola*)	Muscle	Dried	Gastrointestinal	70	16	(Kumari and Maiti, 2021)
Swordfish	Muscle	Raw	Gastrointestinal	42 - 87		(Calatayud et al., 2012; Torres-Escribano et al., 2011)
		Cooked (frying pan)	Gastrointestinal	35 - 49	24	(Jadán-Piedra et al., 2016; Torres-Escribano et al., 2011)
		Frozen	Gastrointestinal	55 - 75		(Calatayud et al., 2012)
Tope Shark	Muscle	Raw	Gastrointestinal	43 - 69		(Torres-Escribano et al., 2011)
		Cooked	Gastrointestinal	34 - 47		(Torres-Escribano et al., 2011)
Tuna	Muscle	Raw	Gastrointestinal	13 - 78	1.9 - 6	(Afonso et al., 2015a; Alves et al., 2018; Marmelo et al., 2020; Torres-Escribano et al., 2011)
		Cooked	Gastrointestinal	6 - 10		(Torres-Escribano et al., 2011)
		Boiled	Gastrointestinal	48	10	(Afonso et al., 2015a)
		Grilled	Gastrointestinal	39	9	(Afonso et al., 2015a)

		Steamed	Gastrointestinal	8	1	(Alves et al., 2018)
		Canned (olive oil)	Gastrointestinal	18	4	(Afonso et al., 2015a)
		Canned (water)	Gastrointestinal	20	5	(Afonso et al., 2015a)
		Stewed	Gastrointestinal	13.5	0.8	(Marmelo et al., 2020)
		Stewed with onion stew	Gastrointestinal	21.8	1.5	(Marmelo et al., 2020)
		Stewed with onion stew and tomato rice	Gastrointestinal	31.2	2.2	(Marmelo et al., 2020)
		Stewed with onion stew, potato, and broccoli	Gastrointestinal	28.4	0.7	(Marmelo et al., 2020)
		Stewed with onion stew and potato	Gastrointestinal	30.7	0.7	(Marmelo et al., 2020)
		Stewed with onion stew and broccoli	Gastrointestinal	30.9	1.3	(Marmelo et al., 2020)
Yellow Croaker	Muscle	Raw	Gastrointestinal	76	3.5	(Liao et al., 2020)
Freshwater Fish						
Bronze Featherback (*Noropterus noropterus*)	Muscle	Dried	Gastrointestinal	65	1.5	(Kumari and Maiti, 2021)
Carp – Common (*Cyprinus carpio*)	Muscle	Dried	Gastrointestinal	62	2	(Kumari and Maiti, 2021)
Carp – Indian (*Catla catla*)	Muscle	Dried	Gastrointestinal	60	17	(Kumari and Maiti, 2021)
Carp – Mrigal (*Cirrhina mrigala*)	Muscle	Dried	Gastrointestinal	71	14	(Kumari and Maiti, 2021)
Carp – Rohu (*Labeo rohita*)	Muscle	Dried	Gastrointestinal	75	12	(Kumari and Maiti, 2021)
Catfish – Walking (*Clarias batrachus*)	Muscle	Dried	Gastrointestinal	61	9	(Kumari and Maiti, 2021)

Catfish – Asian Shovelnose (*Aoricchthys seenghala*)	Muscle	Dried	Gastrointestinal	61	14	(Kumari and Maiti, 2021)
Catfish – Bagrid (*Rita rita*)	Muscle	Dried	Gastrointestinal	63	7	(Kumari and Maiti, 2021)
Eel – Swamp (*Monopterus cuchia*)	Muscle	Dried	Gastrointestinal	73	9	(Kumari and Maiti, 2021)
Lake Trout	Muscle	Raw	Gastrointestinal	28.8		(Yassine, 2017)
Orangefin Labeo (*Labeo calbasu*)	Muscle	Dried	Gastrointestinal	67	13	(Kumari and Maiti, 2021)
Ticto Barb (*Puntius ticto*)	Muscle	Dried	Gastrointestinal	63	21	(Kumari and Maiti, 2021)
Tilapia	Muscle	Raw	Gastrointestinal	69.6	6.9	(Liao et al., 2020)
Anadromous Fish						
Catfish – Striped Dwarf (*Mystus vittatus*)	Muscle	Dried	Gastrointestinal	55	15	(Kumari and Maiti, 2021)
Salmon – Atlantic (Farmed)	Muscle	Raw	Gastrointestinal	89.8	0.1	(Costa et al., 2015)
		Grilled	Gastrointestinal	32.2	9.4	(Costa et al., 2015)
Salmon – Chinook	Muscle	Raw	Gastrointestinal	49	22.1	(Laird and Chan, 2013)
Salmon – Sockeye	Muscle	Raw	Gastrointestinal	46	21.3	(Laird and Chan, 2013)
Salmon - Unspecified	Muscle	Raw	Gastrointestinal	57.3 - 106	8	(Calatayud et al., 2012; Liao et al., 2020)
	Eggs	Raw	Gastrointestinal	10	7.6	(Laird and Chan, 2013)
Seabream	Muscle	Raw	Gastrointestinal	80-89		(Afonso et al., 2018)
		Boiling	Gastrointestinal	52		(Afonso et al., 2018)
		Grilled	Gastrointestinal	39		(Afonso et al., 2018)
		Roasting	Gastrointestinal	38		(Afonso et al., 2018)
Turbot	Muscle	Raw	Gastrointestinal	73.4	2.5	(Liao et al., 2020)
Marine Mammals						
Ringed Seal	Muscle	Raw	Gastrointestinal	69		(Yassine, 2017)

	Liver	Raw	Gastrointestinal	32.3		(Yassine, 2017)
Terrestrial Mammals						
Deer	Muscle	Raw	Gastrointestinal	106	40.7	(Laird and Chan, 2013)
	Liver	Raw	Gastrointestinal	138	5.1	(Laird and Chan, 2013)
Moose	Kidney	Raw	Gastrointestinal	63	27.1	(Laird and Chan, 2013)
Rabbit	Liver	Raw	Gastrointestinal	100	65.8	(Laird and Chan, 2013)

Supplementary Table 2.2: Sample Sizes by Lake, Fish Species, and Chemical Analysis

Fish Species	Lake	Tissue Type	Sample Size					
			Raw Tissue				Cooked Tissue	
			Total Mercury Concentrations	Total Mercury Bioaccessibility (full gastrointestinal digestion model)	Total Mercury Bioaccessibility (gastric phase only of the digestion model	Fatty Acid Concentrations	Total Mercury Concentrations	Total Mercury Bioaccessibility
Burbot (Loche)	**Dezadeash Lake**	**Muscle**	10	10	NA	10	6	6
		Liver	10	10	NA	10	6	6
Lake Trout	**Mustard Lake**	**Muscle**	7	7	NA	7	2	2
Lake Whitefish	**Ekali Lake**	**Muscle**	6	6	NA	NA	3	3
	Fish Lake	**Muscle**	7*	7*	NA	NA	NA	NA
	Kakisa Lake	**Muscle**	2	2	9	2	NA	NA
	Sanguez Lake	**Muscle**	5	5	NA	NA	1	1
Northern Pike	**Ekali Lake**	**Muscle**	6	6	NA	NA	NA	NA
	Fish Lake	**Muscle**	8	8	NA	NA	6	6
	Kakisa Lake	**Muscle**	5	5	9	5	NA	NA
	Mustard Lake	**Muscle**	3	3	NA	3	NA	NA
	Sanguez Lake	**Muscle**	8	8	NA	NA	6	6
	Tathlina Lake	**Muscle**	9	9	NA	NA	5	5
Walleye	**Ekali Lake**	**Muscle**	7	7	NA	NA	NA	NA
	Fish Lake	**Muscle**	10	10	NA	NA	6	6
	Kakisa Lake	**Muscle**	2	2	8	2	NA	NA
	Sanguez Lake	**Muscle**	6	6	NA	NA	NA	NA

NA = No samples from that lake and species were analyzed for that analyte

***** An additional sample was analyzed for this species and lake but was excluded from the summary statistics and statistical analyses in this study

Supplementary Table 2.3: List of the nine *a priori* general linear models to explore variability in percent mercury bioaccessibility among species and lakes and in relation to total mercury concentrations

Model number	Model formula
1	Percent Mercury Bioaccessibility = Species
2	Percent Mercury Bioaccessibility = Lake
3	Percent Mercury Bioaccessibility = Ln-transformed mercury concentrations
4	Percent Mercury Bioaccessibility = Species + Lake
5	Percent Mercury Bioaccessibility = Species + Ln-transformed mercury concentrations
6	Percent Mercury Bioaccessibility = Lake + Ln-transformed mercury concentrations
7	Percent Mercury Bioaccessibility = Species + Lake + Species*Lake
8	Percent Mercury Bioaccessibility = Species + Lake + Ln-transformed mercury concentrations
9	Percent Mercury Bioaccessibility = Species + Lake + Ln-transformed mercury concentrations + Species*Lake

Supplementary Table 2.4: Model Set, AICc values, and log likelihood values for general linear models to explore variability in percent mercury bioaccessibility among species and lakes and in relation to total mercury concentrations

Model Number	Model Formula	AICc	ΔAICc	Log Likelihood
7	Percent Mercury Bioaccessibility = Species + Lake + Species*Lake	785	0	-372
9	Percent Mercury Bioaccessibility = Species + Lake + Ln-transformed mercury concentrations + Species*Lake	785	0.26	-370
1	Percent Mercury Bioaccessibility = Species	787	2.5	-388
8	Percent Mercury Bioaccessibility = Species + Lake + Ln-transformed mercury concentrations	787	2.5	-378
4	Percent Mercury Bioaccessibility = Species + Lake	788	2.8	-381
5	Percent Mercury Bioaccessibility = Species + Ln-transformed mercury concentrations	789	3.8	-387
2	Percent Mercury Bioaccessibility = Lake	790	5.7	-386
6	Percent Mercury Bioaccessibility = Lake + Ln-transformed mercury concentrations	793	8.1	-386
3	Percent Mercury Bioaccessibility = Ln-transformed mercury concentrations	829	44	-411

Based on the AICc criteria, the best model from this set to explain percent mercury bioaccessibility included fish species, lake, and the interaction term between fish species and lake (R-Squared=0.54).

Supplementary Table 2.5: Pairwise Comparisons for Ln-Transformed Total Mercury Concentrations by Fish Species (Tukey's test)

Fish Species A	Fish Species B	Mean Difference in Ln-Transformed Total Mercury Concentrations[a]	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BURB	**LKTR**	0.41	0.23	0.38	-0.22	1.1
	LKWH	1.0*	0.18	0.00	0.52	1.5
	NRPK	-0.43	0.16	0.07	-0.88	0.03
	WALL	-0.77*	0.17	0.00	-1.3	-0.28
LKTR	**BURB**	-0.41	0.23	0.38	-1.1	0.22
	LKWH	0.61*	0.20	0.03	0.05	1.2
	NRPK	-0.84*	0.19	0.00	-1.4	-0.31
	WALL	-1.2*	0.20	0.00	-1.7	-0.63
LKWH	**BURB**	-1.0*	0.18	0.00	-1.5	-0.52
	LKTR	-0.61*	0.20	0.03	-1.2	-0.05
	NRPK	-1.5*	0.13	0.00	-1.8	-1.1
	WALL	-1.8*	0.14	0.00	-2.2	-1.4
NRPK	**BURB**	0.43	0.16	0.07	-0.03	0.89
	LKTR	0.84*	0.19	0.00	0.31	1.4
	LKWH	1.5*	0.13	0.00	1.1	1.8
	WALL	-0.33*	0.12	0.05	-0.66	-0.00
WALL	**BURB**	0.77*	0.17	0.00	0.28	1.3
	LKTR	1.2*	0.20	0.00	0.63	1.7
	LKWH	1.8*	0.14	0.00	1.4	2.2
	NRPK	0.33*	0.12	0.05	0.00	0.66

[a]Ln-transformed mercury concentrations from Fish Species A subtracting Ln-transformed mercury concentrations from Fish Species B

BURB= Burbot, LKTR= Lake Trout, LKWH= Lake Whitefish, NRPK= Northern Pike, WALL= Walleye

Supplementary Table 2.6: Pairwise Comparisons for Ln-transformed Total Mercury Concentrations by Lake (Tukey's Test)

Lake A	Lake B	Mean Difference in Ln-transformed Total Mercury Concentrations[a]	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Dezadeash	**Ekali**	0.11	0.18	1.0	-0.43	0.66
	Fish	-0.20	0.17	0.90	-0.73	0.32
	Kakisa	0.03	0.21	1.0	-0.61	0.67
	Mustard	0.47	0.21	0.27	-0.15	1.1
	Sanguez	-0.64*	0.18	0.01	-1.2	-0.10
	Tathlina	-0.27	0.21	0.87	-0.91	0.37
Ekali	**Dezadeash**	-0.11	0.18	1.0	-0.66	0.43
	Fish	-0.32	0.14	0.28	-0.74	0.11
	Kakisa	-0.08	0.19	1.0	-0.65	0.48
	Mustard	0.36	0.18	0.43	-0.19	0.90
	Sanguez	-0.76*	0.15	0.00	-1.2	-0.30
	Tathlina	-0.38	0.19	0.40	-0.95	0.18
Fish	**Dezadeash**	0.20	0.17	0.90	-0.32	0.73
	Ekali	0.32	0.14	0.28	-0.11	0.74
	Kakisa	0.23	0.18	0.85	-0.31	0.78
	Mustard	0.68*	0.17	0.00	0.15	1.2
	Sanguez	-0.44*	0.14	0.04	-0.86	-0.01
	Tathlina	-0.07	0.18	1.0	-0.61	0.48
Kakisa	**Dezadeash**	-0.03	0.21	1.0	-0.67	0.61
	Ekali	0.08	0.19	1.0	-0.48	0.65
	Fish	-0.23	0.18	0.85	-0.78	0.31
	Mustard	0.44	0.21	0.37	-0.22	1.1
	Sanguez	-0.67*	0.19	0.01	-1.2	-0.11
	Tathlina	-0.30	0.22	0.82	-0.96	0.36
Mustard	**Dezadeash**	-0.47	0.21	0.27	-1.1	0.15
	Ekali	-0.36	0.18	0.43	-0.90	0.19

	Fish	-0.68*	0.17	0.00	-1.2	-0.15
	Kakisa	-0.44	0.21	0.37	-1.1	0.20
	Sanguez	-1.1*	0.18	0.00	-1.7	-0.57
	Tathlina	-0.74*	0.21	0.01	-1.4	-0.10
Sanguez	**Dezadeash**	0.64*	0.18	0.01	0.10	1.2
	Ekali	0.76*	0.15	0.00	0.30	1.2
	Fish	0.44*	0.14	0.04	0.01	0.86
	Kakisa	0.67*	0.19	0.01	0.11	1.2
	Mustard	1.1*	0.18	0.00	0.57	1.7
	Tathlina	0.37	0.19	0.43	-0.19	0.96
Tathlina	**Dezadeash**	0.27	0.21	0.87	-0.37	0.91
	Ekali	0.38	0.19	0.40	-0.18	0.95
	Fish	0.07	0.18	1.00	-0.48	0.61
	Kakisa	0.30	0.22	0.81	-0.36	0.96
	Mustard	0.74*	0.21	0.02	0.10	1.4
	Sanguez	-0.37	0.19	0.43	-0.94	0.19

[a]Ln-transformed mercury concentrations from Lake A subtracting Ln-transformed mercury concentrations from Lake B

Supplementary Table 2.7: Pairwise Comparisons for Mercury Bioaccessibility in Raw Fish Muscle by Fish Species (Tukey's Test)

Fish Species A	Fish Species B	Mean Difference in % Bioaccessibility	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BURB	**LKTR**	-17*	5.2	0.01	-32	-2.9
	LKWH	-22*	4.1	0.00	-33	-11
	NRPK	-31*	3.7	0.00	-41	-20
	WALL	-22*	3.9	0.00	-33	-11
LKTR	**BURB**	17*	5.2	0.01	2.9	32
	LKWH	-4.6	4.6	0.85	-17	8.2
	NRPK	-13*	4.3	0.02	-25	-1.3
	WALL	-4.6	4.5	0.85	-17	7.9
LKWH	**BURB**	22*	4.1	0.00	11	33
	LKTR	4.6	4.6	0.85	-8.2	17
	NRPK	-8.7*	2.9	0.03	-17	-0.66
	WALL	0.06	3.1	1.0	-8.7	8.8
NRPK	**BURB**	31*	3.7	0.00	20	41
	LKTR	13*	4.3	0.02	1.3	25
	LKWH	8.7*	2.9	0.03	0.66	17
	WALL	8.7*	2.7	0.01	1.3	16
WALL	**BURB**	22*	3.9	0.00	11	33
	LKTR	4.6	4.5	0.85	-7.9	17
	LKWH	-0.06	3.1	1.0	-8.8	8.7
	NRPK	-8.7*	2.7	0.01	-16	-1.3

[a]Ln-transformed mercury concentrations from Fish Species A subtracting Ln-transformed mercury concentrations from Fish Species B

BURB= Burbot, LKTR= Lake Trout, LKWH= Lake Whitefish, NRPK= Northern Pike, WALL= Walleye

Supplementary Table 2.8: Pairwise Comparisons for Mercury Bioaccessibility in Raw Fish Muscle by Lake (Tukey's Test)

Lake A	Lake B	Mean Difference in % Bioaccessibility	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Dezadeash	**Ekali**	-24*	4.1	0.00	-37	-12
	Fish	-23*	3.9	0.00	-35	-11
	Kakisa	-18*	4.8	0.01	-32	-3.4
	Mustard	-22*	4.7	0.00	-36	-7.7
	Sanguez	-28*	4.1	0.00	-40	-16
	Tathlina	-38*	4.8	0.00	-52	-23
Ekali	**Dezadeash**	24*	4.1	0.00	12	37
	Fish	0.92	3.2	1.00	-8.7	11
	Kakisa	6.2	4.2	0.76	-6.6	19
	Mustard	2.4	4.1	1.00	-10	15
	Sanguez	-3.9	3.4	0.91	-14	6.4
	Tathlina	-13	4.2	0.03	-26	-0.66
Fish	**Dezadeash**	23*	3.9	0.00	11	35
	Ekali	-0.92	3.2	1.00	-10	8.7
	Kakisa	5.3	4.1	0.85	-7.0	18
	Mustard	1.5	3.9	1.00	-10	13
	Sanguez	-4.8	3.2	0.74	-14	4.8
	Tathlina	-14*	4.1	0.01	-27	-2.1
Kakisa	**Dezadeash**	18*	4.8	0.01	3.4	32
	Ekali	-6.2	4.2	0.76	-19	6.6
	Fish	-5.31	4.1	0.85	-18	7.0
	Mustard	-3.86	4.8	0.98	-18	11
	Sanguez	-10	4.2	0.22	-23	2.7
	Tathlina	-20*	4.9	0.00	-35	-4.8
Mustard	**Dezadeash**	22*	4.7	0.00	7.7	36
	Ekali	-2.4	4.1	1.00	-15	10

	Fish	-1.5	3.9	1.0	-13	10
	Kakisa	3.9	4.8	0.98	-11	18
	Sanguez	-6.2	4.1	0.73	-19	6.1
	Tathlina	-16*	4.8	0.03	-30	-1.3
Sanguez	**Dezadeash**	28*	4.1	0.00	16	40
	Ekali	3.9	3.4	0.91	-6.4	14
	Fish	4.8	3.2	0.74	-4.8	14
	Kakisa	10	4.2	0.22	-2.7	23
	Mustard	6.2	4.1	0.73	-6.1	19
	Tathlina	-9.6	4.2	0.27	-22	3.2
Tathlina	**Dezadeash**	38*	4.8	0.00	23.	52
	Ekali	13*	4.2	0.03	0.66	26
	Fish	14*	4.1	0.01	2.1	27
	Kakisa	20*	4.9	0.00	4.8	35
	Mustard	16*	4.8	0.02	1.3	30
	Sanguez	9.6	4.2	0.27	-3.2	22

[a]Ln-transformed mercury concentrations from Lake A subtracting Ln-transformed mercury concentrations from Lake B

Supplementary Table 2.9: Total Mercury and Gastric Phase Only Total Mercury Bioaccessibility for Fish from Kakisa Lake (NWT)

Fish Species	**Sample Size**	**Total Mercury Concentration (mg/kg ww.)**		**Bioaccessible Concentration (mg/kg)**		**% Bioaccessibility (Gastric Phase Only)**	
		Arithmetic Mean	**Standard Deviation**	**Arithmetic Mean**	**Standard Deviation**	**Arithmetic Mean**	**Standard Deviation**
Lake Whitefish	9	0.04	0.02	0.02	0.01	42	9.4
Northern Pike	9	0.32	0.26	0.12	0.09	38	9.1
Walleye	8	0.28	0.20	0.12	0.10	42	11

Chapter 3: Hair to Blood Mercury Concentration Ratios and a Retrospective Hair Segmental Mercury Analysis in the Northwest Territories, Canada

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3.1 Abstract

Concentrations of total Hg were measured in blood and hair samples collected as part of a human biomonitoring project conducted in First Nations communities of the Mackenzie Valley, Northwest Territories, Canada. Hair (n = 443) and blood (n = 276) samples were obtained from six communities in the Dehcho region and three communities in the Sahtú region of the Mackenzie Valley. The aim of this paper was to calculate hair to blood Hg ratios (for matched samples) and determine if: 1) ratios differed significantly between the two regions; 2) ratios differed from the 250:1 ratio proposed by the WHO; and, 3) point estimates of hair to blood Hg ratios could be used to estimate blood Hg concentrations. In addition, this paper aims to determine if there were seasonal patterns in hair Hg concentrations in these regions and if so, if patterns were related to among-season variability in fish consumption.

The majority of Hg levels in hair and blood were below relevant health-based guidance values. The geometric mean hair (most recent segment) to blood Hg ratio (stratified by region) was 619:1 for the Dehcho region and 1220:1 for the Sahtú region. Mean log-transformed hair to blood Hg ratios were statistically significantly different between the two regions. Hair to blood ratios calculated in this study were far higher (2–5 times higher) than those typically reported in the literature and there was a large amount of inter-individual variation in calculated ratios (range: 114:1 to 4290:1). Using the 250:1 ratio derived by the World Health Organization to estimate blood Hg concentrations from hair Hg concentrations would substantially over-estimate blood Hg concentrations in the studied regions. However, geometric mean site-specific hair to blood Hg ratios can provide estimates of measures of central tendency for blood Hg concentrations from hair Hg concentrations at a population level.

Hg concentrations were determined in segments of long hair samples to examine exposure of participants to Hg over the past year. Hair segments were assigned to six time periods and the highest hair Hg concentrations were generally observed in hair segments that aligned with September/October and November/December, whereas the lowest hair Hg concentrations were aligned with March/April and May/June. Mean log-transformed hair Hg concentrations were statistically significantly different between time periods. Between time periods (e.g., September/October vs. March/April), the geometric mean Hg concentration in hair differed by up to 0.22 μg/g, and the upper margins of Hg exposure (e.g., 95th percentile of hair Hg) varied by up to 0.86 μg/g. Results from self-reported fish consumption frequency questionnaires (subset of participants; n = 170) showed total fish intake peaked in late summer, decreased during the winter, and then increased during the spring. Visual assessment of results indicated that mean hair Hg concentrations followed this same seasonal pattern. Results from mixed effects models, however, indicated that variability in hair Hg concentrations among time periods was not best explained by total fish consumption frequency. Instead, seasonal trends in hair Hg concentrations may be more related to the consumption of specific fish species (rather than total wild-harvested fish in general). Future work should examine whether seasonal changes in the consumption of specific fish species are associated with seasonal changes in hair Hg concentrations.

3.2 Introduction

Hg is a ubiquitous environmental contaminant that can come from natural as well as anthropogenic sources, and it is considered a global public health concern (Ruggieri et al., 2017; World Health Organization, 2007). Hg emitted from anthropogenic sources can be transmitted long distances through air and ocean currents to remote areas, including the Canadian Arctic and subarctic (AMAP, 2021b; Mason et al., 1995). Exposure to elevated levels of Hg, particularly MeHg, can have negative effects on human development and health, and adverse effects include neurodevelopmental impairment and increased risk of cardiovascular disease (Alkazemi et al., 2013; Khan and Wang, 2009). MeHg is a potent neurotoxin, and developing fetuses, infants, and children are particularly susceptible to the harmful effects of Hg exposure because the nervous system is still developing (Ruggieri et al., 2017). Although there are multiple routes of exposure to MeHg, fish consumption is considered a major source for the general population (Clifton, 2007; UNEP, 2002). Fish consumption can thus present a human health risk, particularly for

populations that consume relatively large amounts of fish, and/or if the fish consumed have elevated Hg concentrations (AMAP, 2021b).

Since Hg is a global public health concern, biomonitoring studies that include the measurement of Hg in human biological samples have been completed or are underway in numerous countries (Ruggieri et al., 2017). In Canada, a number of projects have included measurements of Hg in human biological samples, including the national Canadian Health Measures Survey (CHMS). However, the CHMS does not include participants from the Canadian territories or Indigenous peoples living on reserves (Health Canada, 2019a). For Indigenous peoples in Canada, consuming locally harvested traditional foods, including fish, confers important nutritional, economic, and cultural benefits (Laird and Chan, 2013, Receveur et al., 1997). Fish and other traditional foods are important sources of various nutrients and vitamins, including omega-3 PUFA and Se (Chan et al., 2006). However, increased consumption of locally harvested traditional foods may also increase the risk of contaminant exposure (Van Oostdam et al., 1999). Therefore, additional research initiatives in Canada, including human biomonitoring that focus on Indigenous populations, have been implemented (Cordier et al., 2020; Curren et al., 2015; Assembly of First Nations (AFN), 2013; Gosselin et al., 2006; Walker et al., 2006). For example, a human biomonitoring project was implemented to address the concerns of First Nations communities regarding health risks posed by elevated levels of contaminants in local traditional foods of the Mackenzie Valley, Northwest Territories, Canada (Ratelle et al., 2018a, 2018b).

Hg can be measured in a variety of sample types, and previous human biomonitoring projects have measured Hg in hair, blood, urine, foetal/cord blood, and fingernail samples (Ruggieri et al., 2017; Esteban and Castaño, 2009). Blood levels are useful as a biomarker for short-term exposure to Hg, and blood can be used as a long-term measure of Hg in individuals that have a consistent diet with regular fish consumption (Ruggieri et al., 2017). However, collecting blood is a relatively invasive procedure and samples can be difficult to transport and store (Esteban and Castaño, 2009). Hair samples are useful for determining long-term exposure to Hg, and for observing changes in exposure over time (Ruggieri et al., 2017; Esteban and Castaño, 2009). Most consider the collection of hair to be less invasive, and hair is more easily preserved and shipped than blood. There is a risk of outside contamination for hair samples, however (Ruggieri

et al., 2017; Mahaffey, 2005), and there are several assumptions associated with the interpretation of results from hair. Typically, researchers assume that hair grows at a rate of approximately 1 cm per month, but hair growth rates are not uniform and can be affected by a number of factors including age and levels of hormones (LeBeau et al., 2011; Robbins, 2002).

Studies that measure Hg in both blood and hair samples allow researchers to examine relationships between these matrices as exposure biomarkers (Noisel et al., 2011; Bartell et al., 2000, Phelps et al., 1980). For example, Hg levels in hair are generally 250–300 times higher than those observed in blood; a hair to blood Hg ratio of 250:1 was derived by the World Health Organization (WHO, 1990). This 250:1 hair to blood Hg ratio has been used to estimate Hg levels in blood from Hg levels in hair in risk and exposure assessments. Several studies have found consistent hair:blood Hg ratios across populations (Berglund et al., 2005; Johnsson et al., 2005), however, hair to blood Hg ratios rely on the assumption of steady state equilibrium, and steady states likely do not occur for populations with seasonal variability in diet (Gosselin et al., 2006; Bartell et al., 2000). In some studies researchers have found, that inter-individual variation in hair:blood Hg ratios can be very high (Liberda et al., 2014; Noisel et al., 2011; Berglund et al., 2005; Budtz-Jørgensen et al., 2004; McDowell et al., 2004). For example, in one study conducted in First Nations communities in Northern Quebec, Canada, individual hair:blood Hg ratios ranged from 3 to 2845 (Liberda et al., 2014).

In this study, Hg concentration data from hair and blood samples were obtained from two regions (Dehcho and Sahtú) of the Mackenzie Valley of the Northwest Territories, Canada. Our aims were to determine whether hair:blood Hg ratios differed between the two sampled regions, and to compare estimated ratios for each region to the 250:1 ratio proposed by the WHO (WHO, 1990). The implications of using point-estimates (i.e., a mean ratio or the proposed 250:1 ratio) when predicting hair Hg concentrations from blood Hg concentrations are examined and discussed. Further, retrospective Hg concentrations obtained via segmental analysis of hair samples were used to examine the seasonality of Hg exposure, and related to seasonal fish consumption. Secondary objectives included comparing hair to blood Hg concentration ratios from this study to those reported in previous studies and comparing hair and blood Hg concentrations between regions.

3.3 Materials and Methods

3.3.1 Study Design

As previously described (Ratelle et al., 2018a, 2018b), six communities from the Dehcho region and three communities from the Sahtú region of the Mackenzie Valley in the Northwest Territories took part in a human biomonitoring project in 2016–2018. The project was developed in consultation with First Nations communities to address their concerns about environmental contaminants. Community members aged 6 years or older were eligible to participate, and the research team used both active and passive recruitment measures. The informed consent process followed an opt-in framework for each of the project components. For minors (under the age of 18), a parent or guardian also provided written informed consent and consent was also received from the minor for each study component they chose to participate in. These components included collection of biological samples (hair and blood), demographic information, dietary information (through the use of a food frequency questionnaire (FFQ) and a 24-h dietary recall), and information about participants' awareness and perception of contaminant issues. Registered nurses and phlebotomists were hired for the blood sample collection. Results described herein focus on levels of Hg in blood and hair, demographic variables, and fish consumption data collected via the FFQ.

Prior to the publication of results in academic venues, the results were first returned to participating communities (e.g., in the form of community reports describing aggregate results) and to individual participants who provided biological samples (in the form of a confidential results letter specific to that individual). All participants who provided a biological sample were offered confidential results letters, which included levels of contaminants and nutrients that were measured in their samples. Ethics approval was received from all relevant authorities and data was managed according to the approved data management plan (Ratelle et al., 2018a).

3.3.2 Collection and Chemical Analyses of Human Biological Samples

Human biological samples (hair and blood) were collected during clinics that took place in communities in 2016, 2017, and 2018 in the winter months (January, February, March, November, December). It was recommended that participants not eat before giving blood

samples but participants were still accepted if they had eaten prior to blood collection. Samples were collected during the clinic's hours of operation (generally 9:00 a.m. to 5:00 p.m.).

One 6 mL tube (metal-free) containing sodium heparin was collected per participant for whole blood metal analysis, and samples were kept at 4 °C until shipping. Metals, including total Hg, in whole blood samples were measured in the laboratory of Dr. Michèle Bouchard at the Université de Montréal using an inductively coupled plasma mass spectrometer (ICP-MS). All samples were run in triplicate and reference materials were used (INSPQ blood control QM-B-Q1721 with a reference value of 15.9 nmol/L) to assess the quality of the method. Recovery of reference materials was 94.5 ± 2.83 %. The limit of detection for blood Hg ranged between 0.03 and 0.04 μg/L.

Hair samples were collected by local research coordinators and other members of the research team by cutting a full-length lock of hair with sterilized scissors using previously described methods (Ratelle et al., 2018b). The hair was placed in a polyethylene bag and stapled at the proximal end to keep the hair sample together and so that the proximal end could be easily identified later on during chemical analyses. For participants with short hair, trimmings were cut and collected on a sheet of paper which was folded, stapled shut, and placed in a polyethylene bag. These samples were then shipped for analysis.

Total Hg analysis on hair samples was conducted in the laboratory of Dr. Brian Branfireun at the University of Western Ontario (Biotron Analytical Services). For long hair samples, a 2 cm segment was cut from the end of the hair that was most proximal to where the hair was cut from the scalp (referred to in this report as the initial hair segment) to determine the most recent hair Hg concentrations. A minimum weight of 30 mg of hair was analyzed. For short hair samples, the hair was collected and placed into sample boats (also minimum weight of 30 mg). Analyses for total Hg were then conducted with a Milestone Direct Mercury Analyzer (DMA-80; Milestone, Sorisole, Italy) following the procedure outlined in U.S. Environmental Protection Agency (U.S. EPA) method 7473 (U.S. EPA, 1998). A duplicate, certified reference material for human hair (IAEA 086), and blank were analyzed in between batches of 10 hair segments for quality control.

Up to five additional hair segments (each 2 cm in length) were measured for total Hg in each hair sample, depending on the length of hair available. As such, between 1 and 6 hair segments per participant were analyzed. A method reporting limit of 0.24 ng Hg was derived from the 0.08 ng Hg detection limit of the method. The recovery for the reference materials was 100 ± 20 % and duplicates were within 20 % relative percent difference of each other.

3.3.3 Statistical Analyses

Data analysis was conducted in Microsoft Excel® (2019, Version 2104) and IBM SPSS Statistics software (Version 27). Similarly, figures were created using Microsoft Excel® and IBM SPSS Statistics software. For all participants that submitted a sample, summary statistics were calculated for concentrations of Hg in blood (µg/L) and for concentrations of Hg in proximal hair segments (µg/g). Samples with Hg levels below the detection limit were imputed with 50% the limit of detection.

For participants with hair and blood Hg both above the LOD (data subset 1, n=87), hair: blood Hg ratios were calculated. As per previous studies (JECFA, 2004; Liberda et al., 2014; Legrand et al., 2010), summary statistics were calculated for the ratio of paired hair and blood Hg concentration data:

$$Hair{:}\,Blood\ Hg\ Ratio = \frac{Hair\ Hg\ concentration\ (\mu g/g)}{Blood\ Hg\ concentration\ (\mu g/L)} \times 1000$$

The hair:blood Hg ratios were log-transformed to address the assumption of normality for analysis (Cousineau & Chartier, 2010), and after visual inspection, one extreme outlier (a ratio of 34,000:1) was removed. The criterion of values > than three standard deviations from the mean was used to identify additional outliers (Osborne & Overbay, 2004); only one additional outlier was identified using this method. Summary statistics for hair:blood Hg ratios were then calculated from the un-transformed data to maintain interpretability of the ratios. Summary statistics for hair:blood Hg ratios, including statistics stratified by region, were calculated in Excel. A t-test was performed on the log-transformed hair:blood Hg ratios to determine if mean log-transformed hair:blood Hg ratios differed significantly between regions. Further, a multivariable linear regression was also performed with the log transformed hair:blood Hg ratios as the responding variable, and region, participant age, and participant sex as the predictor variables. The utility of the point-estimates (geometric means) from the hair:blood Hg ratios (as

well as the default assumption of 250:1 used in risk assessments) to estimate blood Hg levels based on levels of Hg in hair was also assessed.

Hg concentrations in hair segments (2 cm per segment) were compared between time periods by assigning hair segments to bimonthly time periods. Assignments were based on the assumption that the most recent 2 cm segment of hair represented the prior two months of Hg exposure (Nuttall, 2006). Segments were assigned to bi-monthly time periods (i.e., Jan-Feb; Mar-Apr; May-June; Jul-Aug; Sept-Oct; Nov-Dec) according to sample collection date. To determine the extent to which hair Hg concentrations varied throughout the year, differences in log transformed hair Hg concentrations between bi-monthly periods were then investigated using a repeated measures ANOVA with a Greenhouse-Geisser correction in IBM SPSS Statistics software. This analysis was performed for those participants that had all six hair segments with Hg concentrations above the limit of detection (data subset 2, n=170). The Greenhouse-Geisser correction was used as Sphericity could not be assumed (Mauchly's test of Sphericity, $p<0.05$). Alpha was set at 0.05, and normality of the residuals was assessed (Shapiro-Wilk, $p\geq0.05$). The residuals were normally distributed. Pair-wise comparisons were also determined for the bi-monthly periods using the Bonferroni method to adjust for multiple comparisons.

Seasonal fish consumption (categorical ordinal) data were described by participants using a FFQ. Participants self-reported how many times per week they ate locally caught fish per bi-monthly period. Options in the questionnaire included: less than once per week, 1-2 times per week, 3-5 times a week, and 6-7 times a week. To determine if variability in hair Hg concentrations among time periods corresponded to variability in frequency of fish consumption, mixed models were conducted using SPSS Statistics software. Five *a priori* models were constructed using fish consumption and participant age as fixed effects. Only data from participants with both hair Hg concentration data (for all six segments, above the detection limit) and fish consumption frequency data (data subset 3, n=54), were included in these models. Log-transformed hair Hg concentration was the response variable and fish consumption frequency and participant age were included as potential predictor variables. Hair Hg concentrations for each bi-monthly time period and fish consumption frequency for each bi-monthly time period were treated as repeated measures and time period was included in the model as a random effect. The five models were: model 1 was the base model, model 2 had fish consumption as the fixed

effect, model 3 had participant age as the fixed effect, model 4 had fish consumption and participant age as fixed effects, and model 5 had fish consumption and participant age as fixed effects with an interaction term between fish consumption and participant age. Fish consumption and age were included in the *a priori* models as fish consumption and age have been associated with hair Hg concentrations in previous studies (Yusà et al., 2017; Shao et al., 2013; Dunn et al., 2008; Pesch et al., 2002). Previously, Shao et al., included an interaction term between fish consumption and age in their analysis but found it was not significant (Shao et al., 2013). To determine if this is consistent with this population, an interaction term between fish consumption and age was included in Model 5 in this paper. Model selection was based on AIC_C values.

3.4 Results and Discussion

3.4.1 Mercury Concentrations in Blood Samples and Initial Hair Segments

A total of 515 participants from the Dehcho (n=267) and Sahtú (n=248) regions provided a hair and/or blood sample as part of the biomonitoring project in the Mackenzie Valley of the Northwest Territories. Demographic information for the participants that provided each type of sample by region is included in **Table 3.1**. The age and sex of participants was similar between the regions and overall, 49.7% of participants were male, 50.3% of participants were female, and the mean age was 41 (±20) years old. Summary statistics for hair and blood Hg concentrations by region are included in **Table 3.2** and this data is presented stratified by age in **Supplementary Table 3.1** and stratified by sex in **Supplementary Table 3.2**.

In both regions, for those who submitted a hair and/or blood sample, approximately half the participants were male and half were female and participant age was similar between the regions. However, the demographic data differed for participants in subset 1 of the data (i.e., provided both hair and blood samples that had Hg concentrations above the detection limit for both samples). For example, as shown in **Table 3.1** participants subset 1 were more likely to be male and were, on average, approximately 10 years older than was seen for participants who provided at least one sample (includes participants with Hg concentrations below the detection limit). Both blood and hair Hg have been reported to be positively associated with age (Dong et al., 2015; Liberda et al., 2014; Airey, 1983). Therefore, the older age of participants in subset 1 is due to the exclusion of participants with blood Hg data below the limit of detection.

Table 3.1: Demographic Information for Participants Providing a Blood and/or Hair (Proximal 2 cm) Sample from the Dehcho and Sahtú Regions

Sample	Region	Sample Size	Participant Sex		Participant Age[a] (Years)			
			% Male	% Female	Median	Arithmetic Mean	Standard Deviation	Range
All Participants Who Provided a Sample[b]								
Hair	**Dehcho**	231	52%	48%	42	40	19	6-79
	Sahtú	212	49%	51%	46	43	22	6-88
	Total	443	50%	50%	44	42	21	6-88
Blood	**Dehcho**	136	48%	52%	44	43	18	10-79
	Sahtú	140	49%	51%	44	44	20	8-88
	Total	276	49%	51%	44	43	19	8-88
Subset 1[c]								
Hair and Blood	**Dehcho**	48	56%	44%	55	54	16	15-79
	Sahtú	39	59%	41%	62	59	16	25-88
	Total	87	57%	43%	56	56	16	15-88

[a]A small number of participants preferred not to give their age. Of those who provided a hair sample, 3% of participants chose not to provide their age (Dehcho 3%, Sahtú 3%). Of those who provided a blood sample, 4% chose not to provide their age. Of those who provided both blood and hair samples, 2% chose not to provide age.
[b]Demographic data for all participants that submitted a hair sample and/or blood sample
[c]Demographic data for those who submitted both a hair and blood sample that had Hg concentrations above the detection limit in both the proximal 2 cm of the hair sample and the blood sample

In both regions, for those who submitted a hair and/or blood sample, approximately half the participants were male and half were female and participant age was similar between the regions. However, the demographic data differed for participants in subset 1 of the data (i.e., provided both hair and blood samples that had Hg concentrations above the detection limit for both samples). For example, as shown in **Table 3.1** participants subset 1 were more likely to be male and were, on average, approximately 10 years older than was seen for participants who provided at least one sample (includes participants with Hg concentrations below the detection limit). Both blood and hair Hg have been reported to be positively associated with age (Dong et al., 2015; Liberda et al., 2014; Airey, 1983). Therefore, the older age of participants in subset 1 is due to the exclusion of participants with blood Hg data below the limit of detection.

The vast majority of hair and blood Hg levels in participants from communities in both regions were below relevant health-based guidance values: 5 µg/g Hg in hair (2 µg/g hair Hg for children and women of childbearing age) (Delormier, 2012), and 20 µg/L in blood (8 µg/L blood Hg for children and women of childbearing age) (Legrand et al., 2010). In both regions, over 50% of blood Hg levels were below the detection limit. Blood and hair Hg levels appeared to be low relative to other northern Indigenous populations (Cordier et al., 2020; Curren et al., 2015; Walker et al., 2006). As well, geometric mean blood Hg levels were below those previously measured in the Canadian general population through the Canadian Health Measures Survey (CHMS) (Health Canada, 2019a) who had geometric mean blood Hg levels of 0.69 µg/L, 0.79 µg/L, and 0.64 µg/L in cycle 2, cycle 3, and cycle 5 of the survey, respectively. Geometric mean and arithmetic mean blood Hg levels in this study were also below those measured in First Nations through the First Nations Biomonitoring Initiative (FNBI) (AFN, 2013), who had a geometric mean blood Hg concentration of 0.95 µg/L and an arithmetic mean blood Hg concentration of 2.47 µg/L. However, geometric mean hair Hg concentrations in this population appeared to be approximately 2.5 times higher than those levels measured in hair samples from the Canadian general population (Health Canada, 2019a) that had a geometric mean hair Hg concentration of 0.19 µg/g.

Hair Hg concentrations appeared on average 1.5-fold higher in the Sahtú region than in the Dehcho region. As well, the 95th percentile for hair Hg concentrations was approximately 1.4 times higher in the Sahtú region than in the Dehcho region. In contrast, blood Hg levels were on average 1.9-fold higher in the Dehcho region than in the Sahtú region and the 95th percentile for blood Hg concentrations in the Dehcho region was approximately 1.8 times higher than the Sahtú region. When only data in subset 1 were examined, mean hair Hg concentrations still appeared to be 1.5-fold higher in the Sahtú region than in the Dehcho region, but the 90th percentile was only approximately 1.1-fold higher in the Sahtú region and the 95th percentiles were similar. Blood Hg concentrations appeared to be only 1.1-fold higher on average in the Dehcho region compared to the Sahtú region when subset 1 data were examined. However, the 95th percentile of the subset 1 data was approximately 1.5-fold higher in the Dehcho region compared to the Sahtú region.

Table 3.2: Summary Statistics for Hair (Proximal 2 cm Segment) and Blood Mercury Concentrations in the Dehcho and Sahtú Regions

Sample		Sample Size	Geometric Mean	Arithmetic Mean	Standard Deviation	Median	75th Percentile	90th Percentile	95th Percentile	% Below Limit of Detection
					All Samples[a]					
Hair (Proximal 2 cm Segment) Mercury (µg/g)	**Dehcho Region**	231	0.39	0.75	1.02	0.44	0.87	1.71	2.30	0.43%
	Sahtú Region	212	0.59	1.06	1.04	0.65	1.62	2.54	3.13	1.42%
	Total	443	0.47	0.90	1.04	0.50	1.16	2.19	2.78	0.90%
Blood Mercury (µg/L)	**Dehcho Region**	136	0.13	1.32	3.57	0.02	1.21	3.15	5.63	56%
	Sahtú Region	140	0.07	0.68	1.97	0.02	0.59	1.74	2.99	67%
	Total	276	0.09	1.00	2.89	0.02	0.80	2.52	4.68	61%
					Subset 1[b]					
Hair (Proximal 2 cm Segment) Mercury (µg/g)	**Dehcho Region**	48	0.90	1.38	1.55	0.97	1.66	2.84	4.17	
	Sahtú Region	39	1.82	2.09	1.16	1.95	2.53	3.19	4.11	
	Total	87	1.24	1.70	1.43	1.27	2.18	3.14	4.42	
Blood Mercury (µg/L)	**Dehcho Region**	48	1.46	3.03	5.04	1.42	3.08	6.56	9.18	
	Sahtú Region	39	1.30	2.27	3.23	1.11	2.37	5.11	5.99	
	Total	87	1.39	2.69	4.32	1.32	2.65	5.62	8.36	

[a]Samples less than LOD was imputed at 0.5*LOD during the calculation of these summary statistics.
[b]Data for participants where both hair Hg (from the proximal 2 cm segment) and blood Hg data was available and above the detection limit, this data was used to calculate hair:blood Hg ratios

3.4.2 Hair Mercury to Blood Mercury Ratios to Estimate Past Blood Mercury Concentrations from Retrospective Hair Mercury Analysis

When site-specific data are unavailable, researchers often assume a hair:blood Hg ratio of 250:1, which was derived by the WHO to convert between hair and blood Hg levels (WHO, 1990). However, as noted above, the inter-individual variation in hair:blood Hg ratios within populations can be very high and differences in the hair:blood Hg ratio have been observed among populations (Liberda et al., 2014; Berglund et. al., 2005; Budtz-Jørgensen et al., 2004; McDowell et al., 2004). As expected, initial hair segment Hg concentrations were significantly positively associated with blood Hg concentrations in this population (Spearman's $\rho=0.55$, $p<0.05$), and this remained true when data were stratified by region (Dehcho: Spearman's $\rho=0.68$, $p<0.01$; Sahtú: Spearman's $\rho=0.55$, $p<0.01$). To further investigate the relationship between these biomarkers in the Northwest Territories, hair to blood Hg ratios were calculated. Only the data from participants with both hair and blood Hg concentration data available and above the detection limit (outliers removed as described in the methods, see **Supplementary Table 3.3** for summary statistics with the outliers included) were used to calculate these ratios (subset 1, Total: n=85, Dehcho: n=48, Sahtú: n=37). Summary statistics for the calculated hair Hg: blood Hg ratios are presented in **Table 3.3**.

Table 3.3: Summary Statistics for Calculated Hair-Blood Mercury Ratios (from Subset 1)

	Sample Size	Geometric Mean	Arithmetic Mean	Standard Deviation	Percentile					
					10th	25th	50th	75th	90th	95th
Dehcho Region	48	619	833	729	244	370	577	980	1970	2360
Sahtú Region[a]	37	1220	1440	845	509	915	1210	1850	2390	2860
Total	85	830	1100	834	312	462	873	1440	2290	2790

[a]The two hair:blood Hg ratios identified as outliers are not included in these summary statistics

Mean calculated hair:blood Hg ratios were much higher in this study than the 250:1 ratio determined by the WHO (WHO, 1990) and those typically seen in the literature (Johnsson et. al., 2005; Berglund et. al., 2005). However, the blood samples collected in this study were collected throughout the day and participants were allowed to give blood samples even if they had eaten recently. This could have affected the calculated hair:blood Hg ratios, as blood Hg

concentrations are influenced by the timing of exposure/food intake (Gosselin et al., 2006). There was also a large amount of inter-individual variation in the hair:blood Hg ratios calculated in this study; hair:blood Hg ratios ranged from 114:1 to 4290:1. In a study of First Nations communities in Northern Quebec, Canada, hair:blood Hg ratios ranged from 3:1 to 2845:1 (Liberda et al., 2014).

Mean hair:blood Hg ratios varied significantly between the two regions included in this study ($t=-3.5$, $df=2, 85$, $p<0.01$, $n=87$ (data were log-transformed)). Region was a significant predictor of log transformed hair to blood Hg ratios (multiple linear regression, $F=6.67$, $p<0.05$, $df=3,79$) while participant age and sex were not significant predictors ($p>0.05$). Liberda et al., (2014) also reported that mean hair/blood Hg ratios differed between participating First Nations communities. The factors governing such regional and/or community differences in hair:blood ratios are currently unknown.

3.4.3 Comparison of Hair: Blood Mercury Ratios for Estimating Blood Mercury Concentrations

As the calculated hair:blood Hg ratios varied by region and were higher than the 250:1 ratio determined by the WHO (WHO, 1990), geometric mean ratios for each region and the 250:1 WHO ratio were compared to determine how well these ratios estimated blood Hg concentrations based on the initial hair Hg concentrations. Summary statistics on the estimated blood Hg concentrations compared to the measured blood Hg concentrations are included in **Table 4.4**. The percent error between the estimated and measured blood Hg concentrations was calculated and the frequency of estimates occurring within a range of percent error is included in **Table 3.5**.

Table 3.4: Summary Statistics for Estimated Blood Mercury Concentrations using Various Ratios (for Subset 1)

Region		Geometric Mean	Arithmetic Mean	Percentile					
				10th	25th	50th	75th	90th	95th
Dehcho Region (n=48)	**Measured Blood Mercury (µg/L)**	1.46	3.03	0.33	0.62	1.42	3.01	6.55	9.18
	Estimated Blood Mercury (µg/L) by Regional Geometric Mean Ratio	1.46	2.22	0.44	0.79	1.56	2.68	4.59	6.74
	Estimated Blood Mercury (µg/L) by WHO 250:1 Ratio	3.61	5.51	1.09	1.96	3.86	6.64	11.4	16.7
Sahtú Region (n=39)	**Measured Blood Mercury (µg/L)**	1.30	2.27	0.45	0.67	1.11	2.37	5.11	5.99
	Estimated Blood Mercury (µg/L) by Regional Geometric Mean Ratio	1.50	1.72	0.82	1.01	1.60	2.09	2.63	3.38
	Estimated Blood Mercury (µg/L) by WHO 250:1 Ratio	7.28	8.37	3.98	4.94	7.80	10.1	12.8	16.4

Table 3.5: Frequency of Blood Mercury Estimates Occurring within a Range of Percent Error (from Subset 1)

Region	Method of Estimating Blood Mercury from Hair Mercury	Range of Percent Errors[a]								
		Percent of Blood Mercury Estimates in this Range (%)								
		Underestimated Blood Mercury			Within ±10% of Blood Mercury		Overestimated Blood Mercury			
		-50 to -100%	-25 to -50%	-10 to -25%	0 to -10%	0 to 10%	10 to 25%	25 to 50%	50 to 100%	> 100%
Dehcho Region	**Geometric Mean from Calculated Ratios**	19%	21%	8%	6%	4%	2%	10%	10%	29%
	WHO Ratio (250:1)	2%	0%	4%	6%	2%	4%	8%	17%	56%
Sahtú Region	**Geometric Mean from Calculated Ratios**	18%	5%	10%	15%	5%	5%	5%	21%	15%
	WHO Ratio (250:1)	0%	0%	0%	0%	0%	0%	5%	5%	90%

[a] Percent error was calculated as: Percent error = (estimated blood Hg – measured blood Hg)/measured blood Hg x 100%

Using the WHO 250:1 hair:blood Hg ratio to estimate blood Hg concentrations from hair Hg concentrations consistently resulted in overestimates. Using the 250:1 ratio, 56% of the estimated blood Hg levels for the Dehcho region and 90% of the estimated blood Hg levels for the Sahtú region had a percent error over 100% when compared to the measured blood Hg levels. Unsurprisingly, geometric mean ratios calculated at the regional level better estimated blood Hg levels from the hair Hg levels, especially for the measures of central tendency, compared to using the 250:1 ratio. Using regional geometric mean ratios, however, tended to result in over-estimates of blood Hg concentrations at the lower end of the distribution, and under-estimates of blood Hg concentrations at the upper end of the distribution. Both the regional geometric mean ratios and the WHO 250:1 ratio had high percent errors associated with estimates (**Table 3.5**). Using the geometric mean ratios calculated at the regional level, only 10% of the estimated blood Hg levels for the Dehcho region and 20% of the estimated blood Hg levels for the Sahtú region were within 10% of the measured blood Hg concentrations, and thus considered accurate. Using the WHO 250:1 ratio, only 8% of the estimated blood Hg levels for the Dehcho region and 0% of the estimated blood Hg levels for the Sahtú region were considered accurate. Previously, when Liberda et al (2014) used the WHO 250:1 ratio to estimate blood Hg concentrations from hair Hg concentrations, they found that at the individual level, percent error ranged from -98.7% to 1040%. In this study, the percent error ranged from -54.4% to 1210% in the Dehcho region, and from 30.5% to 13500% for the Sahtú region when using the 250:1 WHO ratio, and from -81.6% to 430% in the Dehcho region and from -73.2% to 2710% for the Sahtú region when using the geometric mean of the calculated ratios. Site-specific hair to blood Hg ratios (region specific geometric mean ratios) can provide estimates of measures of central tendencies for blood Hg concentrations at a population level, however, this approach still appears unable to estimate blood Hg concentrations at the upper and lower ends of the distribution.

3.4.4 Retrospective Hair Mercury Analysis

For long hair samples, Hg concentrations were determined not only in the initial hair segment, but also in additional 2 cm segments until a maximum of 6 segments were measured for each participant. This approach was undertaken to lend insight into participants' exposure to Hg over the past year. As previously described, 2 cm segments of hair were assigned to approximate bi-monthly time periods. For example, hair collected in November was assigned to

represent Hg exposure in September-October whereas hair collected in December was assigned to November-December. Summary statistics for hair Hg concentrations by approximate bi-monthly time period pooled by region for all available hair segment data and for subset 2 is shown in **Table 3.6** (summary statistics for the data stratified by region are shown in **Supplementary Table 3.4**).

Table 3.6: Total mercury concentrations in hair segments based on Bi-Monthly Time Period

	Hair Mercury Concentrations by Time Period (µg/g)				
	Sample Size	**Arithmetic Mean**	**Geometric Mean**	**Median**	**95th Percentile**
			All Samples[x]		
JAN/FEB	279	0.88	0.46	0.42	2.77
MAR/APR	183	0.58	0.33	0.31	2.11
MAY/JUN	198	0.70	0.39	0.35	2.46
JUL/AUG	215	0.85	0.48	0.46	2.87
SEP/OCT	255	0.93	0.53	0.53	3.22
NOV/DEC	350	0.97	0.51	0.53	3.15
			Subset 2 [y]		
JAN/FEB [d, e, f]	170	0.61	0.35	0.33	2.29
MAR/APR [c, d, e, f]		0.60	0.34	0.31	2.15
MAY/JUN [b, d, e, f]		0.67	0.39	0.34	2.26
JUL/AUG [a, b, c, e]		0.82	0.50	0.46	2.81
SEP/OCT [a, b, c, d]		0.91	0.56	0.55	3.01
NOV/DEC [a, b, c]		0.85	0.52	0.46	2.88

[x] This includes those participants with Hg concentrations above the detection limit and method reporting limit for any hair segment

[y] This includes those participants with Hg concentrations above the detection limit and method reporting limit for all 6 hair segments

The time periods that each time period is significantly different from are indicated as follows: [a]=Jan/Feb, [b]=Mar/Apr, [c]=May/Jun, [d]=Jul/Aug, [e]=Sep/Oct, and [f]=Nov/Dec

The highest hair Hg concentrations were generally observed in segments assumed to represent exposure in September/October and November/December. In contrast, hair Hg levels were lowest in segments assumed to represent exposure in March/April and May/June. Since

participants had hair that varied in length, sample size varied among time periods. The sample size for each time period ranged between 183 (Mar-Apr) and 350 (Nov-Dec). Of the 443 participants that provided hair samples, 170 participants had hair Hg concentrations available and above the detection limit for all six hair segments (subset 2). These participants in subset 2 followed the same temporal patterns as those described above. Due to higher frequency of longer hair for female participants, 94% of the 170 participants who had hair Hg concentrations for all six hair segments were female.

To determine if mean Hg concentrations differed significantly between time periods, a repeated measures ANOVA was conducted on subset 2 (n=170, participants with hair Hg concentrations above the detection limit for all six hair segments). As patterns between hair segments were similar between regions, hair Hg data were pooled for Dehcho and Sahtú participants. Log hair Hg concentrations varied significantly among the bi-monthly periods (Repeated measures ANOVA with a Greenhouse-Geisser correction, $F=51.43$, $df=2.78, 469.05$, $p<0.05$, $n=170$). Results from post-hoc tests using the Bonferroni correction revealed that the mean log hair Hg concentrations were significantly different for 11 of 15 pair-wise comparisons (**Supplementary Tables 3.5** and **3.6**). Between time periods (e.g., September/October vs. March/April), the geometric mean of hair Hg varied by up to 0.22 µg/g, and 95th percentile of hair Hg varied by up to 0.86 µg/g. The geometric mean of the intra-individual range for Hg in hair was 0.40 µg/g. Of the 170 participants in subset 2, 39% of participants had an intra-individual range > 0.5 µg/g, 18% had an intra-individual range > 1.0 µg/g, and 2% had an intra-individual range > 2.5 µg/g.

Seasonal variability in hair Hg concentrations have been observed in previous studies in populations around the world (Vega et al., 2018; Dong et al., 2015; Bélanger et al., 2008; Dolbec et al., 2001; Akagi et al., 1995). For example, in Indigenous populations from the Brazilian Amazon, Hg concentrations increased during the dry season, from December to April, and decreased during the rainy season, from June to September (Vega et al., 2018). The authors suggested that these seasonal variations in hair Hg concentrations were due to variability in frequency of fish consumption (increased frequency of fish consumption during the dry season), as well as variation in the type of fish consumed (increased consumption of piscivorous versus herbivorous fish) (Vega et al, 2018; Dolbec et al., 2001). In a study of consumers of freshwater

fish from the Grand Lake watershed (Oklahoma, USA), Hg concentrations in hair were lowest in winter and highest in summer (Dong et al., 2015). Again, in this study it was suggested that seasonal variation in hair Hg concentrations was due to differences in total fish consumption, and differences in the consumption of certain fish species (Dong et al., 2015).

3.4.5 Fish Consumption Frequencies as a Predictor of Seasonal Variation in Hair Mercury Concentrations

Of the 537 participants of the biomonitoring study, 171 completed a survey documenting seasonality of fish consumption. There was a seasonal trend of decreasing frequency in fish consumption over the winter and increasing frequency in the spring, with a peak in late summer (**Figure 3.2**). The highest self-reported fish consumption frequency was in July/August, and the lowest was in November/December. In July/August, 9% more participants reported consuming fish at least 3 times a week compared to in November/December. In November/December, 11% more participants reported consuming fish less than once a week compared to in July/August.

To relate seasonal patterns in fish consumption frequencies with seasonal patterns in hair Hg concentrations, arithmetic mean hair Hg concentrations and fish consumption frequencies were overlaid (**Figure 3.1**). Mean hair Hg concentrations and mean fish consumption frequencies seemed to follow similar patterns; both increased during summer/fall. Increases in hair Hg concentrations appeared to lag behind increases in fish consumption frequency, however, as the highest frequency of fish consumption was in July/August while peak mean hair Hg concentration was in September/October. When the data was stratified by those who consumed fish more than one times per week on average over the year versus those who consumed fish less than times per week on average over the year, the trend in hair Hg concentrations was similar between these groups although it was less pronounced in the low consumers and the low consumers had lower average hair Hg concentrations (**Supplementary Figures 3.1 and 3.2**).

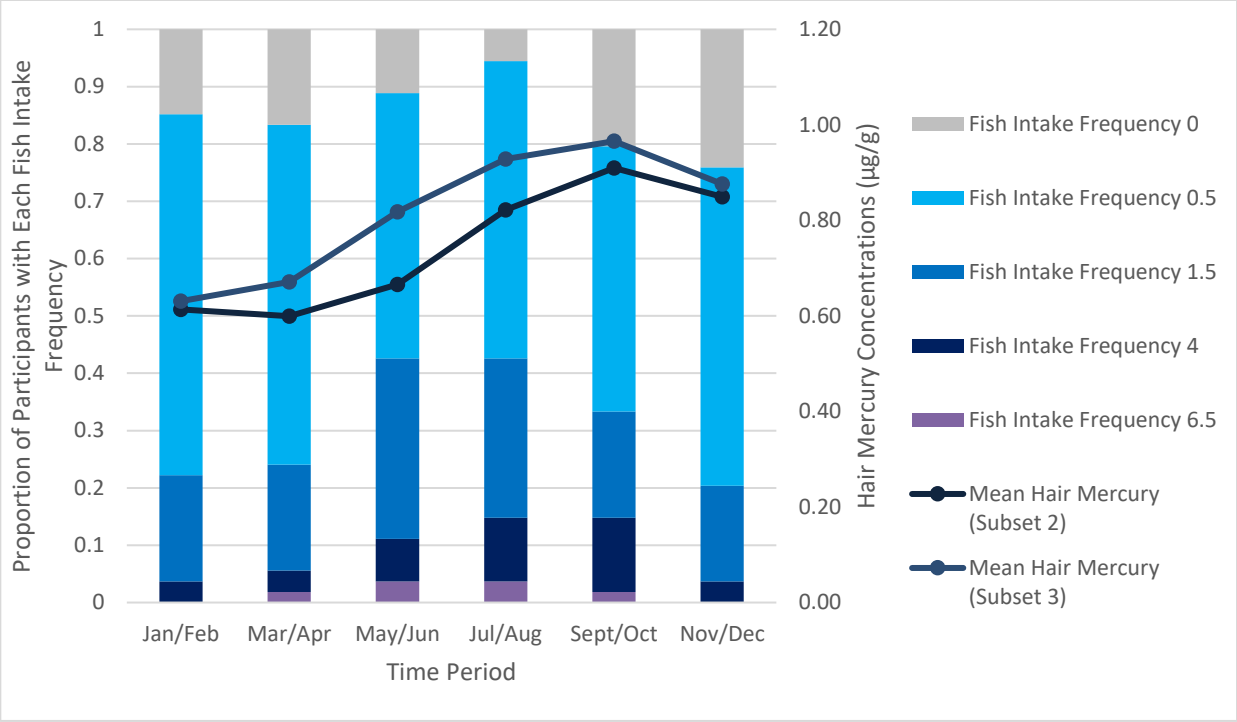


Figure 3.1: Mean Hair Mercury Concentrations (Subset 2 versus Subset 3) and Mean Fish Consumption Frequencies by Time

The peak in mean hair Hg concentrations could lag behind the peak in fish consumption frequency for a number of reasons, including the time required for increased fish consumption to result in sustained increased blood Hg levels and a corresponding increase in monthly average hair Hg concentration (Gosselin et al., 2006). There could also be an issue with the initial alignment of hair segments to bi-monthly time periods, as hair samples from communities were collected on different dates (but had to be assigned to the six time periods for comparability). Additionally, the alignment of 2 cm hair segments to a bi-monthly time period relies on the assumption that hair grows at a rate of 1 cm per month. Hair growth rate has been reported to range between 0.65 cm to 2.2 cm a month (LeBeau et al., 2011). This alignment also assumes that the first hair segment was cut directly adjacent to the scalp. However, during hair sample collection, it is not possible to cut the hair directly at the scalp without risk of causing an abrasion. It is likely that for most samples, the hair was cut several millimeters from the scalp. LeBeau et al., (2011) found that mean length of hair remaining at the scalp after sample collection was 0.8 cm (LeBeau et al., 2011). If hair samples were collected at an average distance of 0.8 cm from the scalp, the hair may represent exposure from 3 weeks prior to what was

assumed. Altogether, these factors may have resulted in hair samples being assigned to a more recent time period than assumed, which could partially explain the lag between reported increases in fish consumption, and observed increases in Hg levels in hair.

It seems likely that fish consumption frequency is associated with hair Hg concentrations in this population, since both followed similar seasonal trends but with a lag in hair Hg concentrations. To determine if fish consumption frequencies were a significant predictor of seasonal variation in hair Hg concentrations, mixed models were constructed. Overall, there were 54 participants with complete data (subset 3) which included: hair Hg concentrations above the detection limit for six hair segments representing approximately a year of Hg exposure, complete answers to the food frequency questionnaire questions about fish consumption over the past year, and participant age. Based on exploratory data analysis, *a priori* models were constructed using fish consumption and age as fixed effects. Due to the small number of males with hair Hg data for all six hair segments, participant sex could not be included as a factor in the models. In the models, time period was aligned so that the fish consumption frequency data from the previous bi-monthly time period was aligned with the hair Hg concentration data of the next bi-monthly time period (for example January/February fish consumption frequency data was paired with March/April hair Hg concentration data), because as previously discussed, mean hair Hg concentrations seemed to lag behind the peak of fish consumption frequency by a two-month time period. Model selection was accomplished using AICc due to the small sample size in these models (**Table 3.7)**. Based on the AICc criteria, the best model from this set was the model with participant age as predictor variable and time period as a random effect (**Model 3**).

Table 3.7: Model Set and AICc Values for Mixed Effects Models (from Subset 3)

Model Number	Model Formula	AICc	ΔAICc
1	lnHairHg =	393.408	25.811
2	lnHairHg = fish consumption (past 2 months)	401.735	34.138
3	lnHairHg = participant age	367.597	0
4	lnHairHg= fish consumption (past 2 months) + participant age	375.248	7.651
5	lnHairHg= fish consumption (past 2 months) + participant age + fish consumption * participant age	379.859	12.262

As expected, age was a predictor variable in the best model (**Model 3**) for hair Hg concentration (**Table 3.7**). Age has previously been associated with hair Hg concentrations (and/or blood Hg concentrations) (Dong et al., 2015; Liberda et al., 2014; Airey, 1983). However, fish consumption frequency was not included as a predictor variable in the model with the lowest AICc value. This could be due to a number of factors, including the limitations of this study/model (discussed below). These seasonal differences in hair Hg concentrations may also be explained by seasonal differences in the frequency of consumption of specific species of fish or seasonal differences in the source of fish (the waterbodies accessed), rather than total fish consumption frequencies. The most commonly consumed fish species that participants reported eating in the communities sampled was Lake Whitefish (87% of participants reported consuming Whitefish in the past year) which is low in Hg (Ratelle et al., 2020). The next most consumed fish species in the communities were Lake Trout (consumed by 62% of participants), Northern Pike (consumed by 44% of participants), and Walleye (consumed by 34% of participants) (Ratelle et al., 2020). Lake Trout, Northern Pike, and Walleye are generally considered to have higher Hg levels compared to Lake Whitefish (Laird et al., 2018; Reyes et al., 2017). Seasonal differences in the consumption of these higher Hg fish could relate to seasonal differences observed in human hair Hg concentrations.

3.4.6 Limitations

3.4.6.1 Limitations in the Hair:Blood Mercury Ratio Analysis

Analyses of hair:blood Hg ratios in this study were limited by the proportion of participants with blood Hg concentrations below the limit of detection. In both regions, over 50% of blood Hg levels were below the detection limit. This meant that the population included in the hair:blood Hg ratio analysis tended to be older, so these ratios may not be generalizable to the general population in these regions. As well, this research documented the challenges of relying on the 250:1 hair to blood Hg ratio (or even the geometric mean ratios at the regional level) to estimate blood Hg concentrations at an individual level. Even when differences in ratios by region were taken into account, a number of additional factors could affect hair:blood Hg ratios. Previous studies have shown that hair to blood Hg ratios may differ between sexes (Liberda et al., 2014), and vary with age (Liberda et al., 2014; Budtz-Jørgensen, 2004), blood Hg

concentrations (Berglund et al., 2005; Budtz-Jørgensen, 2004), and hair structure (Budtz-Jørgensen, 2004). In this study, region was a significant predictor (multiple linear regression, $F=6.67$, $p<0.05$, $df=3,79$), while age and sex were not significant predictors ($p>0.05$), of log-transformed hair:blood Hg ratios. Hair growth rates were not examined in this study, but previous studies have examined factors affecting inter-individual differences in hair growth rates. Differences in human hair growth rates have been associated with: age (Loussouam et al., 2016; Saitoh et al., 1970), sex/gender (Loussouam et al., 2016; Loussouam et al., 2005), hair position/location (Loussouam et al., 2016; Saitoh et al., 1970), hair type (Loussouam et al., 2016), race/ethnicity (Loussouam et al., 2016; Loussouam et al., 2005), geographical location (Loussouam et al., 2016), and season (Loussouam et al., 2016; Liu et al., 2014; Randall et al., 1991). To our knowledge, such factors in hair growth rates have not been studied in these northern populations. Future studies could examine whether differences in hair growth rates and associated factors could explain the regional differences in hair:blood Hg concentration ratios seen in this study. Additionally, in this study total Hg was measured in hair and blood. In future work, determining the speciation of Hg in blood and hair samples could provide more insight into drivers of inter-individual and inter-regional variation in hair to blood Hg ratios.

An additional factor that could affect hair:blood Hg ratios measured in this study is whether the population Hg exposures are in a steady state. Hair to blood Hg ratios rely on the assumption of steady state equilibrium. Although hair to blood Hg ratios approach a constant value when steady state is reached, true steady states may not occur for populations with seasonal variability in diet (Gosselin et al., 2006; Bartell et al., 2000). Given the seasonal changes in hair Hg levels and total fish consumption frequency seen in this study, this population is likely not in steady state. Violations to the assumption of steady state may explain the large differences in hair to blood Hg ratios reported in the literature (Tsuchiya, 2012) and in this study. Differences in dietary and other factors should be explored in future studies, to understand the determinants of regional differences in hair to blood Hg concentration ratios in this study.

Although the use of site-specific (region specific geometric mean ratios) hair to blood Hg ratios appears unable to estimate blood Hg concentrations at the upper and lower ends of the distribution, they can provide estimates of measures of central tendencies for blood Hg concentrations at a population level. This is a strength of this approach compared to the use of

the WHO derived 250:1 ratio, with which blood Hg concentrations were consistently overestimated in this population.

3.4.6.2 Limitations in the Temporal Hair Mercury Analysis

For the segmental hair Hg analysis, a limitation of this study was the small number of male participants with hair Hg data for all six hair segments. Female participants were more likely to have long hair, so 94% of the 170 participants with hair Hg data for all six hair segments were female. In order to determine if the results of this study are generalizable to males, future studies could include the longitudinal collection of short hair samples. This way, seasonal trends in hair Hg concentrations could be compared between males and females.

For the analysis of seasonal trends in hair Hg with the mixed effects models, total fish consumption frequency was not included in the final model. Notably, upon visual inspection, total fish consumption frequency and hair Hg concentrations seemed to follow similar seasonal trends. Total fish consumption frequency may not have been included in the final mixed effects model due to the sample size or the use of FFQ data in the model. The model had a low sample size (n=54) because it only included those with both hair Hg data for all six hair segments and fish consumption frequency data. As previously discussed, participants could opt into providing a hair sample and completing the FFQ, so only a sub-set of participants completed both these components of the biomonitoring project. The analysis would be strengthened by a higher sample size, and as previously discussed, the inclusion of species- and season-specific fish consumption frequency. Further, FFQ data can be subject to significant measurement error. Because responses rely on participant memory over the past year, consumption of foods can be overestimated or underestimated (Ratelle et al., 2020; Rodrigo et al., 2015). However, this FFQ was refined for these regions (Ratelle et al., 2020) and FFQs are commonly used and considered to have reasonable relative reliability of food estimates (Rendo-Urteaga et al., 2020; Vézina-Im et al., 2016). Additionally, seasonal differences in species-specific consumption frequencies or seasonal differences in the consumption of fish from different waterbodies, could likely better explain the seasonal trends in hair Hg concentrations rather than total fish consumption frequencies. Future studies should explore whether seasonal differences in species-specific fish consumption or lake-specific fish consumption relate to seasonal Hg exposure.

3.5 Conclusions

Although Hg levels are sometimes elevated in certain fish species and waterbodies in these regions, based on the collection and analysis of both blood and hair samples, it appears that risks to human health due to Hg exposure in these regions is relatively low. Disproportionately impacted populations, however, such as women of childbearing age and children, should continue to follow local fish consumption guidance. There appeared to be regional differences in Hg exposure, with higher blood Hg concentrations in the Dehcho region compared to the Sahtú region. However, when hair Hg concentrations were examined, the Sahtú region had higher levels than the Dehcho region. This suggested that there were regional differences in hair to blood Hg ratios. Calculated hair to blood Hg ratios were highly variable, and the mean ratio differed significantly by region. Use of a generalized, default hair to blood Hg ratio of 250:1 did not allow for an accurate predication of blood Hg concentrations based on hair Hg concentrations. Site-specific hair to blood Hg ratios provided better estimates of measures of central tendencies for blood Hg concentrations at a population level, but individual-level risk still could not be accurately predicted based on a hair sample.

Analysis of multiple hair segments revealed seasonal trends in inferred Hg exposure. Highest Hg concentrations were measured in hair estimated to have accreted during fall, and lowest Hg concentrations were measured in hair estimated to have accreted during spring/early summer. In future studies in these regions, hair samples collected in late fall (when hair Hg concentrations are highest) would result in the most conservative risk assessments. Qualitatively similar seasonal trends were observed for total fish consumption frequency and Hg concentrations in hair. Future work should examine whether seasonal changes in the consumption of specific fish species or harvest locations are associated with seasonal changes in hair Hg concentrations (rather than total wild-harvested fish in general). To get a representative sample of the population, future work could also involve the longitudinal collection of short hair samples so that more men could also be included the analysis of seasonal trends.

3.6 Supplementary Tables

Supplementary Table 3.1: Hair Mercury Concentrations, Blood Mercury Concentrations, and Hair:Blood Mercury Ratios Stratified by Age Tertile

	Age Tertile	Sample Size	Geometric Mean	Arithmetic Mean	Standard Deviation	Percentile				
						10th	25th	50th	75th	90th
Hair Mercury Concentrations	Tertile 1 (15-48)	29	0.82	1.17	0.94	0.24	0.39	0.80	1.71	2.59
	Tertile 2 (49 – 67)	27	1.16	1.38	0.82	0.56	0.97	1.12	1.81	2.18
	Tertile 3 (67 – 88)	27	1.92	2.51	1.96	0.83	1.16	2.03	3.13	5.21
Blood Mercury Concentrations	Tertile 1 (15-48)	29	0.93	1.43	1.42	0.24	0.39	0.80	1.71	2.59
	Tertile 2 (49 – 67)	27	1.48	2.25	2.25	0.46	0.65	1.32	3.11	4.42
	Tertile 3 (67 – 88)	27	2.30	4.71	6.90	0.54	1.06	2.16	5.52	11.7
Hair:Blood Mercury Concentration Ratios	Tertile 1 (15-48)	29	879	1060	600	473	545	984	1390	1862
	Tertile 2 (49 – 67)	27	782	1080	894	318	420	655	1480	2520
	Tertile 3 (67 – 88)	27	834	1150	988	309	392	950	1370	2280

Supplementary Table 3.2: Hair Mercury Concentrations, Blood Mercury Concentrations, and Hair:Blood Mercury Ratios Stratified by Sex

	Sex	Sample Size	Geometric Mean	Arithmetic Mean	Standard Deviation	Percentile				
						10th	25th	50th	75th	90th
Hair Mercury Concentrations	Female	35	1.19	1.54	1.13	0.42	0.64	1.20	2.07	2.92
	Male	50	1.23	1.76	1.61	0.28	0.83	1.24	2.16	3.24
Blood Mercury Concentrations	Female	35	1.27	2.09	2.91	0.43	0.70	1.00	2.41	4.16
	Male	50	1.62	3.21	5.11	0.37	0.76	1.76	2.89	7.61
Hair:Blood Mercury Concentration Ratios	Female	35	930	1190	784	366	496	997	1740	2290
	Male	50	761	1034	870	280	435	793	1250	2085

Supplementary Table 3.3: Summary Statistics for Calculated Hair-Blood Hg Ratios (including outliers)

	Sample Size	Geometric Mean	Arithmetic Mean	Standard Deviation	Percentile					
					10th	25th	50th	75th	90th	95th
Dehcho Region	48	619	833	729	244	370	577	980	1970	2360
Sahtú Region	39	1402	2530	5490	512	924	1250	1980	2860	4980
Total	87	893	1600	3790	317	465	915	1560	2350	2980

Supplementary Table 3.4: Total mercury concentrations in hair segments based on Bi-Monthly Time Periods Comparing by Dehcho and Sahtú Region

Time Period	Hair Mercury Concentrations by Time Period (µg/g)									
	Sample Size		Arithmetic Mean		Geometric Mean		Median		95th Percentile	
	Dehcho	Sahtú	Dehcho	Sahtú	Dehcho	Sahtú	Dehcho	Sahtú	Dehcho	Sahtú
All Samples										
JAN/FEB	112	167	0.59	1.07	0.31	0.60	0.30	0.63	2.07	3.40
MAR/APR	107	76	0.50	0.69	0.29	0.40	0.27	0.36	1.36	2.40
MAY/JUN	115	83	0.60	0.83	0.35	0.45	0.31	0.45	2.18	2.59
JUL/AUG	125	90	0.71	1.04	0.42	0.58	0.40	0.57	2.21	3.46
SEP/OCT	157	98	0.70	1.30	0.42	0.76	0.44	0.79	2.15	4.12
NOV/DEC	187	163	0.66	1.32	0.36	0.76	0.40	0.76	2.10	3.91
Subset 2[a]										
JAN/FEB	n = 96	n = 74	0.47	0.80	0.28	0.46	0.27	0.44	1.57	2.53
MAR/APR			0.53	0.69	0.31	0.39	0.27	0.35	1.54	2.42
MAY/JUN			0.62	0.73	0.37	0.42	0.30	0.41	2.17	2.30
JUL/AUG			0.76	0.90	0.47	0.54	0.44	0.55	2.36	2.81
SEP/OCT			0.72	1.16	0.47	0.71	0.46	0.75	2.16	3.68
NOV/DEC			0.59	1.19	0.39	0.74	0.37	0.74	2.04	3.50

[a] This includes those participants with mercury concentrations above the detection limit for all 6 hair segments

Supplementary Table 3.5: Pairwise Comparisons of Log Mean Hair Mercury Concentrations

Bi-Monthly Time Period vs. Bi-Monthly Time Period		Mean Difference	Std. Error	Sig.[b]
January-February	**March-April**	.018	.032	1.000
	May-June	-.116	.041	.075
	July-August	-.364*	.050	.000
	September-October	-.481*	.051	.000
	November-December	-.396*	.045	.000
March-April	**January-February**	-.018	.032	1.000
	May-June	-.134*	.024	.000
	July-August	-.382*	.042	.000
	September-October	-.500*	.050	.000
	November-December	-.414*	.051	.000
May-June	**January-February**	.116	.041	.075
	March-April	.134*	.024	.000
	July-August	-.248*	.028	.000
	September-October	-.365*	.044	.000
	November-December	-.280*	.052	.000
July-August	**January-February**	.364*	.050	.000
	March-April	.382*	.042	.000
	May-June	.248*	.028	.000
	September-October	-.117*	.032	.006
	November-December	-.031	.050	1.000
September-October	**January-February**	.481*	.051	.000
	March-April	.500*	.050	.000
	May-June	.365*	.044	.000
	July-August	.117*	.032	.006
	November-December	.086	.034	.177
November-December	**January-February**	.396*	.045	.000
	March-April	.414*	.051	.000
	May-June	.280*	.052	.000
	July-August	.031	.050	1.000
	September-October	-.086	.034	.177

* Result is statistically significant at $p<0.05$

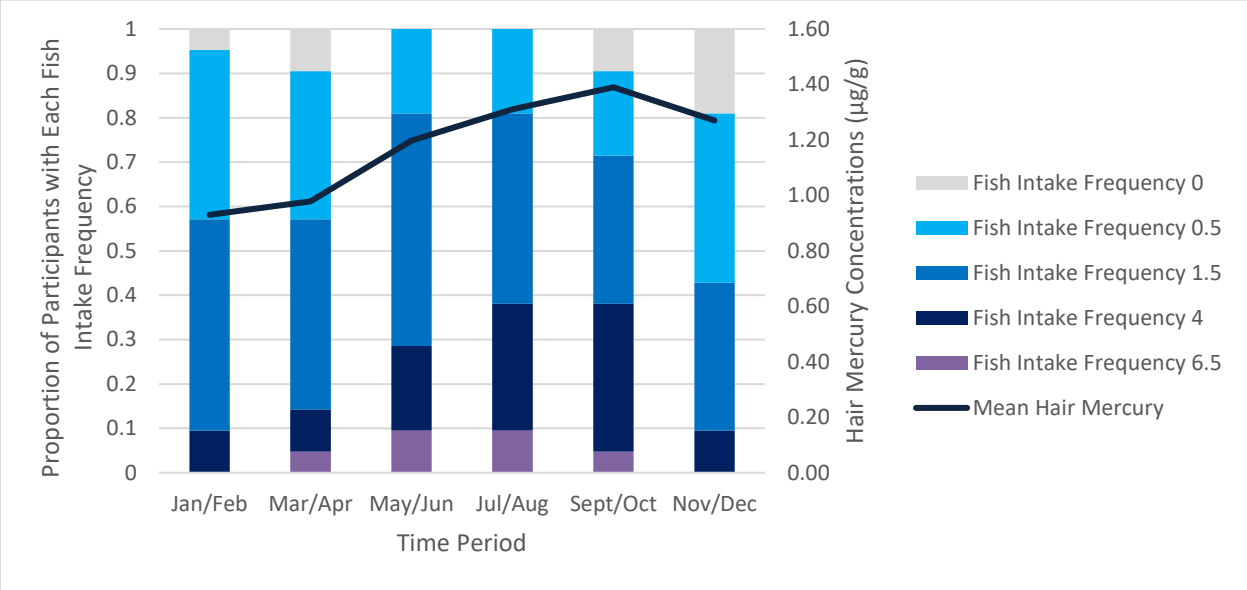
Supplementary Table 3.6: Differences in Hair Mercury Concentrations between Time Periods (µg/g)

Bi-Monthly Time Period vs. Bi-Monthly Time Period		Difference in Arithmetic Means	Difference in Geometric Means	Difference in 95th Percentiles
January-February	**March-April**	0.01	0.01	0.14
	May-June	-0.05	-0.04	0.03
	July-August*	-0.21	-0.15	-0.52
	September-October*	-0.30	-0.21	-0.72
	November-December*	-0.24	-0.17	-0.59
March-April	**January-February**	-0.01	-0.01	-0.14
	May-June*	-0.07	-0.05	-0.11
	July-August*	-0.22	-0.16	-0.66
	September-October*	-0.31	-0.22	-0.86
	November-December*	-0.25	-0.17	-0.73
May-June	**January-February**	0.05	0.04	-0.03
	March-April*	0.07	0.05	0.11
	July-August*	-0.16	-0.11	-0.55
	September-October*	-0.24	-0.17	-0.75
	November-December*	-0.18	-0.13	-0.62
July-August	**January-February***	0.21	0.15	0.52
	March-April*	0.22	0.16	0.66
	May-June*	0.16	0.11	0.55
	September-October*	-0.09	-0.06	-0.20
	November-December	-0.03	-0.02	-0.07
September-October	**January-February***	0.30	0.21	0.72
	March-April*	0.31	0.22	0.86
	May-June*	0.24	0.17	0.75
	July-August*	0.09	0.06	0.20
	November-December	0.06	0.05	0.13
November-December	**January-February***	0.24	0.17	0.59
	March-April*	0.25	0.17	0.73
	May-June*	0.18	0.13	0.07
	July-August	0.03	0.02	0.07
	September-October	-0.06	-0.05	-0.13

* Mean log transformed hair mercury concentrations are statistically significantly different at $p<0.05$ between these time periods based on post hoc tests using the Bonferroni correction.

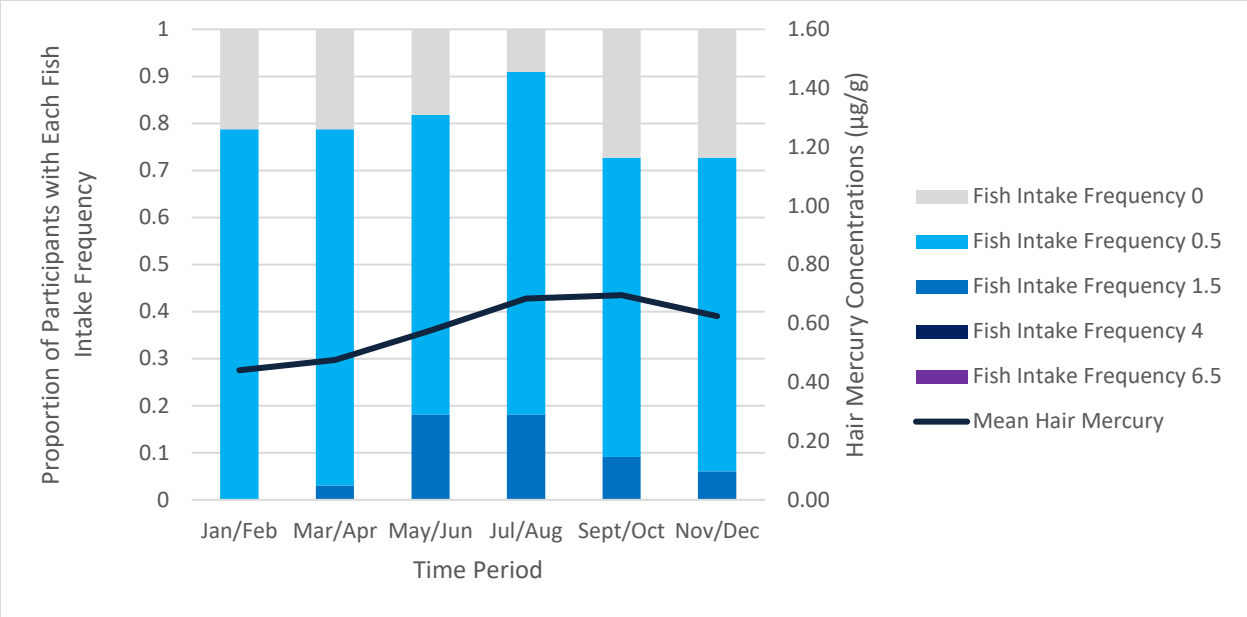
This includes those participants with mercury concentrations above the detection limit for all 6 hair segments, n=170

3.7 Supplementary Figures



Supplementary Figure 3.1: Mean Hair Mercury Concentrations and Mean Fish Consumption Frequencies by Time for Those Who Consumed Fish on Average >1 Times per Week

Classification in those who consumed fish more than once a week was based on the average across all six time periods. Therefore, some people included in this analysis may have reported consuming fish zero times per week for one or more of the time periods (but overall their average across all six time periods was >1 times per week).



Supplementary Figure 3.2: Mean Hair Mercury Concentrations and Mean Fish Consumption Frequencies by Time for Those Who Consumed Fish on Average <1 Times per Week

Chapter 4: Determinants of human hair mercury, blood mercury, blood selenium, and plasma omega-3 fatty acid levels in Northern Canada

Packull-McCormick, S., Ratelle, M., Skinner, K., Drysdale, M., Borghese, M., Bouchard, M., Stark, K., Gamberg, M., & Laird, B. (2023b). Determinants of Human Hair Mercury, Blood Mercury, Blood Selenium, and Plasma Omega-3 Fatty Acid Levels in Northern Canada. *Manuscript in Preparation*.

4.1 Abstract

To address concerns about exposure to environmental contaminants in Northern Canada, human biomonitoring projects were conducted and measured both environmental contaminants and nutrients in human biological samples. This included the measurement of hair and blood total Hg concentrations, as well as blood Se and omega-3 polyunsaturated fatty acids in blood plasma (specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)). The primary aim of this study was to identify demographic and dietary characteristics that could explain variability in the concentrations of these biomarkers within and between the Northern Canadian regions sampled and to determine if these biomarkers were correlated. Hair total Hg, blood total Hg, blood Se, and plasma EPA+DHA concentrations were measured in samples collected from biomonitoring projects conducted in the Dehcho and the Sahtú regions of the Northwest Territories, and Old Crow in the Yukon, Canada. Participants provided demographic data and completed a food frequency questionnaire for the locally harvested traditional foods consumed over the past year. Concentrations of hair total Hg, blood total Hg, blood Se, and plasma EPA+DHA differed by several demographic and dietary factors. Final models explaining variability in hair and blood total Hg, and plasma EPA+DHA concentrations, included participant age, the consumption of at least one species of fish, and the consumption of waterfowl (those that are omnivorous or piscivorous). The final model explaining variability in blood Se concentrations included region, and the consumption of several species of fish, burbot livers, and caribou livers and kidneys. Hair and blood total Hg concentrations were moderately/strongly correlated with plasma EPA+DHA concentrations across all three regions (r_s = 0.36 to 65) while correlations between Se concentrations and the other biomarkers were less consistent (r_s = 0.04 to 0.28). These findings provide important information about which

demographic and dietary factors contribute to variability in hair and blood total Hg, blood Se, and plasma EPA+DHA concentrations in these northern regions. These findings highlight the need to consider the nutritional benefits of traditional foods while seeking to understand and reduce the risks of contaminant exposure.

4.2 Introduction

Hg is a global pollutant that can be transmitted long distances through air and ocean currents even to remote areas, including northern Canada (AMAP, 2021). Exposure to elevated levels of Hg, particularly MeHg, is associated with adverse neurological and cardiovascular health effects (Ruggieri et al., 2017; Rice et al., 2014; Alkazemi et al., 2013; Driscoll et al., 2013; Khan & Wang, 2009). Fish consumption is considered a major route of exposure to MeHg, particularly for populations that consume significant amounts of fish containing high levels of MeHg (AMAP, 2021; Driscoll et al., 2013). This may include northern populations since locally-harvested foods (such as fish) are important components of their diet (AMAP, 2021) and elevated concentrations of total Hg have been measured in some fish species in some waterbodies in northern Canada which has resulted in the release of consumption notices (DHSS, 2012). However, fish and other traditional foods are important sources of nutrients and vitamins including omega-3 polyunsaturated fatty acids (PUFA) (including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) and Se (Chan et al., 2006; Van Oostdam et al., 1999). Se is an essential trace element that has been shown to protect, at least in part, against some of Hg's adverse health effects (Zhang et al., 2014; Mozaffarian, 2009; Brown & Arthur, 2001) as Hg and Se interact and can form complexes reducing the biological availability of Hg (Raymond & Ralston, 2004). Due to the relatively high levels of omega-3 PUFA in fish, consumption of fish is recommended for the reduction of cardiovascular disease and other health benefits (Chen et al., 2022; Mahaffey, 2004; Kris-Etherton et al., 2002). However, increased fish consumption and increased levels of biomarkers of omega-3 PUFA have been associated with increased levels of biomarkers of Hg exposure (Turunen et al., 2010; Dewailly et al., 2000). When examining exposure to Hg, it is important to also consider the co-exposure to beneficial nutrients from fish consumption.

To address concerns about environmental contaminants in traditional foods in northern Canada, human biomonitoring projects were completed in the Dehcho and Sahtú regions of the Northwest Territories (Ratelle et al., 2020; Ratelle et al., 2018), and Old Crow in the Yukon (Drysdale et al., 2021). These studies included the measurement of total Hg, Se, and EPA+DHA in human biological samples, and collection of demographic and dietary data. Hair total Hg concentrations and blood Se concentrations differed according to the northern regions examined (Ratelle et al., 2020). In other studies, levels of total Hg, Se, and EPA+DHA in humans have been associated with various demographic and dietary factors (Caspersen et al., 2019; Gaxiola-Robles et al., 2014; Gari et al., 2013; Jenssen et al., 2012). Several of these dietary and demographic determinants of total Hg, Se, and/or EPA+DHA biomarker levels can overlap and these biomarkers can be correlated with each other (Caspersen et al., 2019; Nakayama et al., 2019; Turunen et al., 2010; Laird et al., 2013; Bates et al., 2007; Dewailly et al., 2001; Grandjean et al., 1992). For example, fish/seafood consumption (including the consumption of specific types or species of fish) has been associated with biomarkers of total Hg (Caspersen et al., 2019; Monastero et al., 2015; Gaxiola-Robles et al., 2014; Gari et al., 2013; Jenssen et al., 2012; Gundacker et al., 2006), Se (Junque et al., 2022), and EPA+DHA (Monastero et al., 2015; Friedman et al., 2006). Age, location/region, and the consumption of alcohol have also been associated with differences in total Hg (Caspersen et al., 2019; Jenssen et al., 2012; Bates et al., 2007; Gundacker et al., 2006; Mahaffey et al., 2004), Se (Junque et al., 2022; Bates et al., 2007; Gundacker et al., 2006), and EPA+DHA biomarker levels (Stark et al., 2016; Flock et al., 2013; Friedman et al., 2006). Total Hg and EPA+DHA biomarkers have also been reported to differ based on sex (Flock et al., 2013; Gari et al., 2013; Jenssen et al., 2012; Friedman et al., 2006), and race/ethnicity (Friedman et al., 2006; Mahaffey et al., 2004), and total Hg and Se biomarker levels have been reported to differ based on tobacco use/exposure to smoke (Caspersen et al., 2019; Gaxiola-Robles et al., 2014; Gari et al., 2013), body mass index (BMI) (Caspersen et al., 2019; Gaxiola-Robles et al., 2014), and income (Caspersen et al., 2019). However, it is unclear which biometric, demographic, and/or dietary factors are associated with total Hg, Se, and EPA+DHA biomarkers in First Nations communities from northern Canada, and whether this varies between northern regions.

The aim of this study was to identify demographic and dietary characteristics that could explain variability in concentrations of total Hg (in hair and blood), Se (in blood), and EPA + DHA (in plasma) in First Nations communities, between and within regions in northern Canada. Additionally, we sought to determine if these biomarkers were correlated with each other in the context of northern Canadian populations, and if there were common dietary and/or demographic determinants for these biomarkers.

4.3 Methods

4.3.1 Study design

Biomarker, demographic, and dietary data were collected as part of a biomonitoring study conducted in the Dehcho and Sahtú regions of the Northwest Territories, Canada, in 2016-2018 (Ratelle et al., 2018) and a biomonitoring study conducted in the community of Old Crow of the Yukon, Canada, in 2019 (Drysdale et al., 2021). These studies have been previously described (Drysdale et al., 2021; Ratelle et al., 2020b; Ratelle et al., 2018b). In brief, six communities from the Dehcho region of the Northwest Territories, three communities from the Sahtú region of the Northwest Territories, and one community, Old Crow, from the Yukon, took part in the studies. These projects were developed in consultation with participating First Nations communities to address their concerns about exposure to environmental contaminants and with the goal of better understanding the risks and benefits of traditional food consumption.

Members of the communities were eligible to participate if they were over the age of 6 for the Northwest Territories biomonitoring project and over the age of 4 for the Yukon project. Participants were included if they could provide free and informed consent (for minors they needed to provide consent as well as have the consent of their parent/guardian). In the Northwest Territories biomonitoring project, participation rates ranged between 12-40% for the participating communities and represented approximately 9% of the residents of the Dehcho and Sahtú regions (Ratelle et al., 2020) and 44% of the eligible residents of Old Crow participated in the Yukon biomonitoring project (Drysdale et al., 2021). The project included several components that participants could opt into including the collection of biological samples (including hair and blood), the provision of basic demographic/biometric data (including age, sex, height, and weight), and the completion of a food frequency questionnaire (FFQ) adapted to

focus on locally consumed traditional foods (Ratelle et al., 2020). Each participating community member received a confidential plain language letter with their contaminant exposure levels and each community received a plain language report with the aggregate results before results from these studies were shared with the public and published in academic journals. Ethics approval was received from all relevant authorities and the data was managed according to the approved data management plans (Drysdale et al., 2021; Ratelle et al., 2018).

4.3.2 Collection and chemical analysis of human biological samples

Collection and analysis of human biological samples from these studies has previously been described (Drysdale et al., 2021; Ratelle et al., 2018). Methods described herein are specific to the collection and analysis of hair total Hg, blood total Hg, blood Se, and plasma fatty acid concentrations. One 6 mL tube (containing heparin) was collected per participant for whole blood metal analysis (including total Hg and Se). The whole blood metals analysis was conducted at the Université de Montréal using an inductively coupled plasma mass spectrometer (ICP-MS). Reference materials were used (INSPQ blood control QM-B-Q1721 with a reference value of 15.9 nmol/L) and recovery of the reference materials was 94.5 ± 2.83% for total Hg and 103.4 ± 5.7% for Se (n = 5 replicate analyses). All samples were run in triplicate in the ICP-MS. The limit of detection for blood total Hg ranged between 0.03 and 0.04 μg/L and the limit of detection for blood Se was 3.97 μg/L.

For plasma analysis, two plastic 6 mL vacutainer tubes (containing K_2EDTA) of whole blood were collected and centrifuged at 3000 rpm for 15 minutes in a portable centrifuge and then separated and transferred into 1.2 mL polypropylene vials. One 1.2 mL vial was pre-loaded with dibutylhydroxytoluene and this vial with plasma was sent to the University of Waterloo for fatty acid analysis. Plasma fatty acid concentrations, including EPA+DHA concentrations, were determined using high throughput gas chromatography as previously described (Metherel et al., 2012). Lipids were extracted from the plasma with 2:1 chloroform:methanol containing an internal standard (C 22:3n-3 ethyl ester, Nu-Chek Prep Inc., Elysian, MN). Boron trifluoride (14% boron trifluoride in methanol) and convectional heat for 1 h at 95°C using a block heater, was used to create fatty acid methyl esters from the total lipid extracts. The fatty acid methyl esters were then analyzed on Varian 3900 gas chromatograph equipped with a flame ionization

detector and a DB-FFAP 15 m × 0.1 mm ID × 0.1 μm film thickness capillary column (J&W Scientific from Agilent Technologies, Mississauga, Canada) with hydrogen as the carrier gas.

Hair samples were collected by cutting a full-length lock of hair with sterilized scissors for long hair (stapled at the proximal end to maintain the orientation) or for short hair by taking trimmings (collected on paper that was then folded and stapled shut) and placing in a polyethylene bag for shipping, as previously described (Packull-McCormick et al., 2022; Ratelle et al., 2018). These samples were analyzed for total Hg concentrations at the University of Western Ontario (Biotron Analytical Services). The most proximal (to the scalp) 2 cm segment for the long hair samples, and a portion of the trimmings for the short hair, was collected and weighed and a minimum weight of 30 mg of hair was analyzed for total Hg concentrations with a Milestone Direct Mercury Analyzer (DMA-80; Milestone, Sorisole, Italy) following the procedure outlined in U.S. Environmental Protection Agency (U.S. EPA) method 7473 (U.S. EPA, 1998). A duplicate, certified reference material for human hair (IAEA 086), and blank were analyzed in between batches of 10 hair segments for quality control. The analysis of additional segments of the long hair samples has been previously described (Packull-McCormick, 2022).

4.3.3 Collection of dietary, demographic, and biometric data

The development and implementation of the FFQ has previously been described (Ratelle et al., 2020) and was based on previous FFQs (Chan, 2012; Receveur et al., 1996). The questionnaire asked participants about which traditional foods they had consumed over the past year along with the frequency of consumption, preparation methods they used, parts of foods consumed, and portion sizes. The foods included specific species of fish, wild birds, game/land animals, and plants/berries. The survey was administered on iPads using QuickTapSurvey (by Formstack). Generally, participants completed the survey on their own, but members of the research team were available to assist participants with the completion of the survey if needed. Additionally, demographic and biometric data was collected by the research team. Participants self-reported their age (in years, continuous), their sex (male or female), and whether they had smoked (binary, yes or no) or consumed alcohol (binary, yes or no) in the previous 24-hours. A member of the research team measured the participants' height (in cm, continuous) using a standard tape measure affixed to a nearby wall, and the participants' weight (in kg, continuous)

on a medical scale (Health o meter® 753KL Legal for Trade Medical Scale, McCook, IL, USA). For each participant that had height and weight measured, their body mass index (BMI) was calculated by dividing their weight (in kg) by their height (in m) squared.

4.3.4 Statistical analyses

Data analysis was conducted in Microsoft Excel (2019, Version 2104) and SAS Studio (Release 3.81 Enterprise Edition). Summary statistics such as arithmetic mean, geometric mean, or percent of participants were calculated for the demographic and/or dietary variables that were selected for inclusion in the analyses in this study. Demographic/biometric variables were selected for inclusion in analyses based on the identification of these variables being associated with human biomarker concentrations of total Hg, Se, or EPA+DHA (Caspersen et al., 2019; Gaxiola-Robles et al., 2014; Gari et al., 2013; Jenssen et al., 2012). These included participants age (in years, continuous), sex (male or female), BMI (continuous), smoking status (binary, yes or no), and alcohol consumption (binary, yes or no). Smoking in the previous 24 hours and alcohol consumption in the previous 24 hours, were used as a proxy for smoking status and alcohol consumption, respectively.

Similarly, dietary factors were selected from the FFQ based on factors that have previously been reported to be associated with human biomarker concentrations of total Hg, Se, or EPA+DHA. Previously, the consumption of fish/seafood has been associated with hair/blood total Hg concentrations (Caspersen et al., 2019; Gaxiola-Robles et al., 2014; Jenssen et al., 2012; Gundacker et al., 2006), blood/plasma omega-3 PUFA concentrations (Monastero et al., 2015; Friedman et al., 2006), and occasionally blood/urine Se concentrations (Junque et al., 2022). However, in these regions, the consumption of fish (any species) was very high (93–99% of participants who completed the FFQ reported eating fish) so the consumption of specific fish species was instead examined. The specific fish species included: whitefish (*Coregonus clupeaformis*), inconnu (*Stenodus leucichthys*, also known as coney), lake trout (*Salvelinus namaycush*), northern pike (*Esox lucius*, also known as Jackfish), burbot (*Lota lota*, also known as loche), grayling (*Thymallus arcticus*, also known as bluefish), sucker (including longnose sucker (*Catostomus catostomus*) also known as red sucker, and white sucker (*Catostomus commersonii*)), cisco (*Coregonus artedii*, also known as herring), walleye (*Sander vitreus*, also known as pickerel), chinook salmon (*Oncorhynchus tshawytscha*, also known as king salmon),

chum salmon (*Oncorhynchus keta*, a also known as dog salmon), and coho salmon (*Oncorhynchys kisutch*). The consumption of one seafood/marine mammal, muktuk (a traditional food consisting of whale skin and blubber), was also examined. However, there were some differences in the fish species/foods included in the version of the FFQ administered among NWT communities versus Old Crow due to regional differences in the traditional foods consumed. Cisco and walleye were only included on the version of the FFQ administered in the NWT whereas chinook salmon, chum salmon, and coho salmon, were the fish species only included on the Yukon version. Additionally, muktuk was only on the FFQ for the Yukon, but it should be noted that Old Crow is located in-land (not on the coast) so the muktuk consumed in Old Crow is harvested elsewhere.

Along with the consumption of specific fish species, the consumption of several other traditional foods was also examined in this analysis including the consumption of: fish eggs (all species), burbot livers, moose (*Alces americanus*) kidneys, moose livers, caribou (*Rangifer tarandus*) kidneys, caribou livers, waterfowl (those that are omnivorous or piscivorous), and wild caught bird eggs (all species). Wild caught birds (gamebirds and waterfowl) that were included in either or both versions of the FFQ were: spruce grouse (*Canachites canadensis*, also known as spruce hen), sharp-tailed grouse (*Tympanuchus phasianellus*, prairie chicken), ptarmigan (*Lagopus muta*)), black duck (*Anas rubripes*), mallard (*Anas platyrhynchos*), fish duck (hooded merganser (*Lophodytes cucullatus*), common merganser (*Mergus merganser*), and arctic loon (*Gavia arctica*) also known as black-throated loon), long-tailed duck (*Clangula hyemalis*, also known as oldsquaw), American wigeon (*Mareca americana*, also known as whistling duck), canvasback (*Aythya valisineria*), Canada goose (*Branta canadensis*), snow goose (*Anser caerulescens*, also known as Wavies), pintail (*Anas acuta*), swan (*Cygnus columbianus*), white-winged scooter (*Melanitta deglandi*), and greater white-fronted goose (*Anser albifrons*, also known as speckle-belly goose). The following birds were considered to be waterfowl with either an omnivorous or piscivorous diet: black duck, mallard, fish duck/merganser, oldsquaw/long-tailed duck, canvasback, pintail, and white-winged scoter. Although the FFQ included several other traditional foods, the subset of the foods from the FFQ examined in this analysis were selected based on their potential to be associated with at least one of the biomarkers of interest (hair total Hg, blood total Hg, blood Se, or plasma EPA+DHA concentrations). For example, the

consumption of fish eggs was included in this analysis due to the relatively high levels of Se and omega-3 PUFA that have been measured in fish eggs (Lemire et al., 2015). The consumption of burbot livers was included in this analysis as burbot livers can contain much higher levels of omega-3 PUFA, including EPA+DHA, compared to burbot muscle tissue (Packull-McCormick et al., 2023). As well, the consumption of moose and caribou livers and moose and caribou kidneys were examined as land mammal organs have been reported to sometimes contain elevated levels of environmental contaminants including total Hg (Chan et al., 2021). Finally, the consumption of waterfowl and wild bird eggs were examined, as aquatic birds can contain higher total Hg concentrations relative to land animals (Golzadeh et al., 2020) and the seasonal consumption of marine bird eggs has been suggested as a possible seasonal source of Hg exposure for some northern populations (Lemire et al., 2015).

The consumption of each fish species, fish part, or other traditional food were treated as a binary variable (yes or no during the past year for the consumption of that food). Some foods were not included in the FFQ administered in particular regions based on local input during the questionnaire design about which foods were either unavailable or not eaten in that region. Additionally, each category of the FFQ included open-field options whereby participants could note whether they had eaten other traditional foods not included in the FFQ. Therefore, in analyses that pooled data across regions, the foods that were not included within the FFQ for that region, were coded as not consumed for those participants from that region.

Summary statistics for the biomarker concentrations, including the geometric mean and 95% confidence intervals (CIs), arithmetic mean, standard deviation, median, 75^{th} percentile, and 95^{th} percentile, were calculated for all participants and separately by region. Samples with concentrations below the LOD were imputed at half the LOD for the analyses. Spearman's rank correlations were used to determine the strength of correlations between biomarker concentrations. For subsequent analyses, the hair total Hg, blood total Hg, and plasma EPA+DHA concentrations were $\log_2$-transformed. To determine if biomarker concentrations differed by region, one-way Analysis of Variance (ANOVA)s were conducted. Post-hoc pair-wise comparisons were conducted using the Tukey test to adjust for multiple comparisons. To determine if the biomarker levels varied by the demographic/dietary variables, Pearson correlations were calculated for the continuous explanatory variables (age and BMI) and

geometric means (and 95% CIs) were calculated and compared for the binary variables (consumption of specific fish species and other select traditional foods, consumption of alcohol in the previous 24 hours, and smoking in the previous 24 hours). It should be noted that the geometric means were not calculated and examined for the dietary variables for traditional foods that were consumed by less than 5% or more than 90% of participants.

Finally, all of the potential determinants examined in the bivariate analyses were included in models and backwards selection based on Akaike Information Criterion (AIC) values was used to determine which variables best explained the variation in biomarker concentrations. For hair total Hg concentrations (log_2-transformed), blood Se concentrations, and plasma EPA+DHA concentrations (log_2-tranformed), general linear models were used. However, due to the relatively high percentage of blood total Hg concentrations below the limit of detection in the NWT, Tobit models (censored regression models) were used to explain the variation in log_2-transformed blood total Hg concentrations. For these Tobit models, blood total Hg concentrations below the limit of detection were treated as left-censored data and the final model was selected through backwards selection based on AIC values.

4.4 Results

4.4.1 Participant Characteristics

A total of 610 participants (279 from the Dehcho region, 254 from the Sahtú region, and 77 from Old Crow) provided a hair sample, blood sample, and/or completed the FFQ. The arithmetic mean age of participants was 41 and approximately half the participants were female (51.1%). Descriptive statistics for the participants by region are reported in **Table 4.1**. Participant characteristics were similar across the regions. For those participants that completed the FFQ, the percent that consumed select traditional foods are reported in **Table 4.2**. For all three regions**,** the vast majority of participants that completed the FFQ reported consuming fish and land mammals over the past year. However, the percent reporting consuming individual fish species, land mammal organs (liver or kidney), waterfowl, and wild bird eggs varied between the regions. For the Dehcho region, the most consumed fish species were whitefish, northern pike, and lake trout while in the Sahtú region they were whitefish, lake trout, and grayling. In Old

Crow, the most commonly consumed fish species were chinook salmon, whitefish, and chum salmon.

4.4.2 Biomarker Concentrations

Summary statistics for hair total Hg concentrations, blood total Hg concentrations, blood total Se concentrations, and plasma EPA+DHA concentrations, by region, are presented in **Table 4.3** (EPA+DHA levels in plasma are also presented as a % of the total fatty acids in **Supplementary Table 4.1**). Log_2-tranformed total Hg concentrations in hair (One-way ANOVA, F=6.5, df=2,513 p=<0.01), log_2-tranformed total Hg concentrations in blood (One-way ANOVA, F=25.5, df=2, 329, p<0.01), and blood Se concentrations (One-way ANOVA, F=32.2, df=2, 329, p<0.01) differed by region while log_2-transfomed plasma EPA+DHA concentrations were not different by region (One-way ANOVA, F=0.41, df=2, 326, p=0.66). Pair-wise comparisons for hair and blood total Hg concentrations and blood Se concentrations by region are presented in **Supplementary Table 4.2**. Geometric mean hair total Hg concentrations were higher in the Sahtú region than in the Dehcho region and Old Crow. However, geometric mean blood total Hg concentrations were higher in Old Crow compared to the Dehcho and Sahtú region. Mean blood Se concentrations were different between all three regions and were highest in the Dehcho region and lowest in the Sahtú region.

Several of the biomarkers were also correlated with each other. In the full sample, hair total Hg concentrations were correlated with blood total Hg (r_s=0.51, n=272), and plasma EPA+DHA concentrations (r_s=0.48, n=270), but not blood Se concentrations (r_s=0.05, n=272). However, blood total Hg concentrations were correlated with plasma EPA+DHA (r_s=0.45, n=328), and weakly correlated with blood Se concentrations (r_s=0.18, n=332), Plasma EPA+DHA concentrations were also weakly correlated with blood Se (r_s=0.15, n=328). The correlations pooled and stratified by region are presented in **Figure 4.1**. When stratified by region the results were similar with consistent correlations between hair total Hg, blood total Hg, and plasma EPA+DHA concentrations and weak or no correlation between Se concentrations and the other biomarkers.

Table 4.1: Descriptive statistics for participant characteristics by region

Characteristic	Characteristic Level	Dehcho		Sahtú		Old Crow		Total	
		N	Arithmetic mean ± SD or n (%)	n	Arithmetic mean ± SD or n (%)	n	Arithmetic mean ± SD or n (%)	n	Arithmetic mean ± SD or n (%)
Age (years)		266	39 ± 19	243	43 ± 21	77	44 ± 16	586	41 ± 20
BMI		235	27.8 ± 7.0	207	25.8 ± 5.5	71	28.5 ±6.3	513	27.1 ± 6.4
Sex	**Female**	143	51%	126	50%	43	56%	312	51%
	Male	136	49%	128	50%	34	44%	298	49%
Smoking (in the previous 24 hours)	**Yes**	90	32%	122	49%	35	56%	247	42%
	No	187	68%	129	51%	27	44%	343	58%
Alcohol consumption (in the previous 24 hours)	**Yes**	27	10%	41	16%	6	10%	74	13%
	No	242	90%	210	84%	57	90%	509	87%

[a]Sample sizes differ across variables based on the study components that participants completed. A total of 610 participants (279 from the Dehcho region, 254 from the Sahtú region, and 77 from Old Crow) provided a hair sample, blood sample, and/or completed the FFQ.

Table 4.2: Percentage of participants who reported consuming select traditional foods in the past year

Food Category	Traditional Food	Percent Consuming (%)			
		Dehcho	Sahtú	Old Crow	Total
Fish	**Total fish (Any Species)**	96	93	99	96
	Chinook salmon	NA	NA	90	21
	Chum salmon	NA	NA	52	12
	Cisco	3	14	NA	5
	Coho salmon	NA	NA	39	9
	Grayling	12	42	27	23
	Inconnu	28	30	21	27
	Lake trout	54	75	15	51
	Burbot	13	24	28	20
	Northern pike	60	16	10	37
	Sucker	26	6	3	15
	Walleye	49	5	NA	26
	Whitefish	92	76	85	86
	Fish eggs (total)	31	35	45	35
	Burbot Livers	6	12	14	10
Marine Mammals	**Muktuk**	NA	NA	24	5
Land Mammals	**Total land mammals (any species)**	94	98	100	96
	Moose liver	26	17	15	21
	Moose kidney	31	23	34	30
	Caribou liver	14	14	30	18
	Caribou kidney	12	24	54	25
Birds	**Total gamebirds and waterfowl (any species)**	70	70	49	61
	Waterfowl (omnivorous or piscivorous)	58	45	41	51
	Total wild bird eggs (any species)	6	2	6	5

Sample size (those that completed the FFQ) was 156 for the Dehcho region, 83 for the Sahtú region, 71 for Old Crow, and 310 when the data was pooled across the communities

NA = Not applicable because that food/food item was not included in the FFQ given to that region

Table 4.3: Descriptive statistics for participant biomarker concentrations by region

Biomarker	Region	n[a]	%>LOD	Geometric mean (95% CI)	Arithmetic mean (SD)	Median	75th Percentile	95th Percentile
Total mercury in hair (µg/g)	Total	516	99%	0.43 (0.38-0.49)	0.83 (0.99)	0.46	1.00	2.57
	Dehcho	231	~100%	0.39 (0.31-0.47)	0.75 (1.02)	0.44	0.87	2.30
	Sahtú	212	99%	0.59 (0.46-0.69)	1.06 (1.04)	0.65	1.62	3.13
	Old Crow	73	100%	0.30 (0.25-0.36)	0.40 (0.33)	0.30	0.53	1.00
Total mercury in blood (µg/L)	Total	332	48%	0.12 (0.09-0.15)	1.06 (2.69)	<LOD[b]	1.12	4.59
	Dehcho	136	44%	0.13 (0.07-0.17)	1.32 (3.57)	<LOD[b]	1.21	5.63
	Sahtú	140	33%	0.07 (0.04-0.09)	0.68 (1.97)	<LOD[b]	0.59	2.98
	Old Crow	56	93%	0.71 (0.48-1.05)	1.38 (1.35)	1.11	2.01	3.75
Total selenium in blood (µg/L)	Total	332	100%	169.54 (166.27-172.88)	172.24 (30.47)	169.75	188.58	224.95
	Dehcho	136	100%	183.22 (178.32-188.26)	185.59 (30.45)	183.40	203.65	232.39
	Sahtú	140	100%	156.24 (151.73-160.90)	158.44 (24.60)	159.79	173.60	193.96
	Old Crow	56	100%	172.23 (165.30-179.46)	174.34 (29.19)	168.18	186.63	218.51
EPA+DHA in plasma (mg/L)	Total	329	100%	72.78 (69.49-76.22)	79.86 (37.46)	71.65	94.49	149.52
	Dehcho	136	100%	71.34 (66.76-76.23)	76.95 (31.03)	71.33	89.79	143.22
	Sahtú	137	100%	72.99 (67.16-79.33)	82.79 (46.21)	71.61	98.85	173.30
	Old Crow	56	100%	75.83 (69.58-82.65)	79.75 (26.10)	78.90	93.10	119.69

[a]Sample sizes differ across the biomarkers based on the study components that participants completed
[b,]The LOD for blood mercury concentrations was between 0.03 and 0.04 µg/L

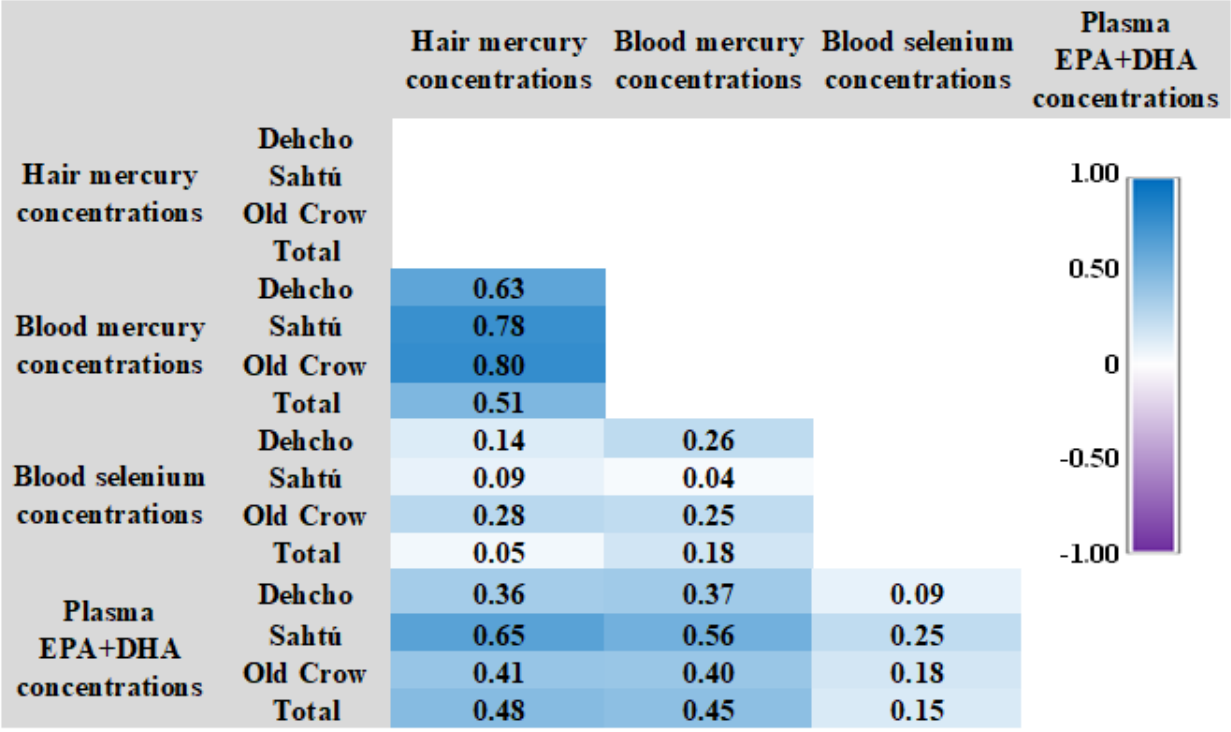


Figure 4.1: Heatmap of the Spearman's rank correlation coefficients for the correlations between biomarker concentrations

4.4.3 Bivariate associations between levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) and demographic and dietary factors

Participant characteristics and their consumption of several traditional foods were examined as potential determinants of exposure in bivariate analyses. The correlations between the biomarker concentrations and age and BMI are shown in **Table 4.4** pooled and by region. Biomarker concentrations seemed to increase with increasing participant age, and positive correlations were observed between hair total Hg (Pearson correlation coefficients between 0.39-0.61), blood total Hg (Pearson correlation coefficients between 0.32-0.50), and plasma EPA+DHA concentrations (Pearson correlation coefficients between 0.27-0.56) and age, but blood Se concentrations (Pearson correlation coefficients between 0.10-0.19) were only weakly correlated with age (**Table 4.4**). Hair total Hg, blood Se, and plasma EPA+DHA concentrations seemed to increase with increasing BMI and these biomarker concentrations were moderately or weakly positively correlated with BMI (Pearson correlation coefficients between 0.12-0.29) but these associations were not consistent when stratified by region. The geometric mean and 95% CIs of the biomarker concentrations by level of the binary variables are presented in **Table 4.5**

(and presented stratified by region in **Supplementary Tables 4.3A-4.3C)**. Geometric mean biomarker concentrations did not seem to differ by sex (**Table 4.5**). Since the percent of participants consuming fish and land mammals was high (more than 90% for all three regions) these were not included in the bivariate analyses. Instead, the consumption of specific fish species and land mammal organs (moose and caribou livers or kidneys) were examined. Geometric mean biomarker concentrations differed in consumers versus non-consumers for several of the traditional foods (**Table 4.5**). In particular, geometric mean hair total Hg concentrations were higher (between 1.7-2.6 times) in consumers of inconnu, lake trout, burbot, burbot livers, fish eggs, moose kidneys, and waterfowl. Geometric mean blood total Hg concentrations were higher (between 2.4-8.9 times) in consumers of whitefish, grayling, chinook salmon, chum salmon, coho salmon, fish eggs, moose kidneys, caribou kidneys, and waterfowl. However, it should be noted that three of the fish species, with the largest difference in geometric mean blood total Hg concentrations between consumers versus non-consumers (6.0-8.9 times), were only included in the FFQ for Old Crow (Yukon), and this region overall had higher geometric mean blood total Hg concentrations than the Dehcho and Sahtú regions (as these regions only had 44-48% of blood total Hg concentrations >LOD). When examining the data stratified by region (**Supplementary Tables 4.3A- 4.3C**), geometric mean blood total Hg concentrations were only 1.1 to 4.2 times higher in consumers of chinook, chum, and coho salmon from Old Crow. Geometric mean blood Se concentrations were 1.1 times higher in consumers of whitefish and walleye but were 1.1 times lower in consumers of grayling. Geometric mean plasma EPA+DHA concentrations were higher (between 1.2-1.3 times) in consumers of whitefish, lake trout, and waterfowl, and were 1.2 times lower in those who had smoked in the previous 24 hours. The biomarkers examined appeared to be fairly similar by sex, although geometric mean blood total Hg concentrations were higher in males compared to females and geometric mean plasma EPA+DHA concentrations were higher in females compared to males, but the confidence intervals for these geometric means overlapped. The trends in these findings were similar when stratified by region (**Supplementary Tables 4.3A-4.3C**).

Table 4.4: Continuous potential determinants of levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) levels by region

Predictor	Region	Hair Mercury[b] (µg/g)			Blood Mercury[b] (µg/L)			Blood Selenium (µg/L)			Plasma EPA+DHA[b] (mg/L)		
		n[a]	Pearson Correlation Coefficient	p-value	n[a]	Pearson Correlation Coefficient	p-value	n[a]	Pearson Correlation Coefficient	p-value	n[a]	Pearson Correlation Coefficient	p-value
Age	**Dehcho**	223	0.45	<0.01	130	0.50	<0.01	130	0.19	0.03	130	0.56	<0.01
	Sahtú	205	0.61	<0.01	135	0.52	<0.01	135	0.19	0.03	132	0.54	<0.01
	Old Crow	73	0.39	<0.01	56	0.32	0.02	56	0.10	0.47	56	0.27	0.04
	Total	501	0.51	<0.01	321	0.44	<0.01	321	0.15	0.01	318	0.51	<0.01
BMI	**Dehcho**	201	0.08	0.28	120	-0.04	0.71	120	-0.04	0.69	120	0.32	<0.01
	Sahtú	174	0.27	<0.01	114	0.13	0.16	114	0.16	0.10	113	0.34	<0.01
	Old Crow	59	0.13	0.34	52	0.19	0.17	52	0.14	0.32	52	0.19	0.18
	Total	434	0.12	0.02	286	0.10	0.11	286	0.14	0.02	285	0.29	<0.01

[a]Sample sizes differ across variables based on the study components that participants completed
[b]Log_2-transformed biomarker concentrations were used to calculate the correlation coefficients and p-values

Table 4.5: Binary potential determinants of levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) levels for all regions (pooled)

Predictor	Predictor Level	Hair Mercury		Blood Mercury		Blood Selenium		EPA+DHA	
		n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)
Sex	**Male**	255	0.44 (0.36-0.53)	161	0.16 (0.11-0.24)	161	169.87 (164.81-175.09)	158	70.22 (65.65-75.11)
	Female	261	0.43 (0.37-0.51)	171	0.09 (0.07-0.13)	171	169.24 (165.02-173.57)	171	75.22 (70.57-80.17)
Smoking in the previous 24 hours	**Yes**	205	0.44 (0.37-0.54)	146	0.10 (0.07-0.14)	146	168.63 (163.67-173.75)	145	65.91 (61.61-70.51)
	No	294	0.44 (0.36-0.52)	174	0.13 (0.09-0.18)	174	170.52 (165.89-175.28)	172	78.93 (74.05-84.12)
Alcohol use in the previous 24 hours	**Yes**	65	0.60 (0.44-0.83)	42	0.11 (0.06-0.24)	42	165.73 (156.67-175.31)	41	77.29 (67.57-88.42)
	No	427	0.42 (0.36-0.48)	274	0.11 (0.09-0.15)	274	169.92 (166.31-173.61)	272	71.55 (67.97-75.32)
Consumed whitefish	**Yes**	231	0.40 (0.32-0.48)	167	0.18 (0.12-0.25)	167	172.6 (168.46-176.84)	167	77.04 (72.65-81.71)
	No	38	0.22 (0.11-0.45)	24	0.05 (<LOD-0.11)	24	150.46 (137.43-164.72)	24	61.46 (49.87-75.75)
Consumed inconnu	**Yes**	69	0.53 (0.41-0.67)	48	0.22 (0.11-0.43)	48	168.28 (161.05-175.82)	48	73.38 (65.57-82.12)
	No	200	0.32 (0.25-0.41)	143	0.13 (0.09-0.19)	143	170.11 (165.14-175.23)	143	75.40 (70.43-80.72)
Consumed cisco[b]	**Yes**	13	0.44 (0.04-5.37)	8	0.34 (LOD-3.18)	8	150.87 (127.14-179.02)	8	80.97 (53.2-123.24)
	No	256	0.36 (0.30-0.43)	183	0.15 (0.11-0.20)	183	170.52 (166.35-174.79)	183	74.63 (70.38-79.14)
Consumed lake trout	**Yes**	137	0.55 (0.44-0.69)	106	0.15 (0.09-0.23)	106	167.22 (161.66-172.96)	105	80.17 (73.87-87.01)
	No	132	0.24 (0.17-0.32)	85	0.16 (0.10-0.25)	85	172.73 (166.59-179.10)	86	68.91 (63.67-74.57)
Consumed burbot	**Yes**	53	0.60 (0.45-0.80)	41	0.39 (0.20-0.79)	41	174.18 (165.59-183.22)	41	78.90 (70.40-88.43)
	No	216	0.32 (0.25-0.41)	150	0.12 (0.08-0.17)	150	168.43 (163.73-173.26)	150	73.83 (69.02-78.96)
Consumed northern pike	**Yes**	97	0.41 (0.29-0.58)	62	0.14 (0.08-0.25)	62	172.72 (166.87-178.77)	62	74.30 (66.57-82.92)
	No	172	0.34 (0.27-0.44)	129	0.16 (0.11-0.23)	129	168.19 (162.79-173.77)	129	75.17 (70.19-80.51)
Consumed grayling	**Yes**	59	0.52 (0.33-0.81)	45	0.32 (0.17-0.62)	45	158.39 (151.82-165.25)	45	82.23 (72.38-93.43)
	No	210	0.33 (0.26-0.41)	146	0.12 (0.08-0.17)	146	173.27 (168.36-178.34)	146	72.76 (68.19-77.63)
Consumed walleye[b]	**Yes**	74	0.39 (0.25-0.61)	46	0.10 (0.05-0.20)	46	179.25 (171.96-186.85)	46	82.90 (73.53-93.47)
	No	128	0.35 (0.28-0.44)	94	0.17 (0.12-0.25)	94	166.71 (161.90-171.66)	94	72.51 (67.88-77.46)
Consumed sucker	**Yes**	41	0.47 (0.25-0.9)	27	0.25 (0.10-0.63)	27	178.61 (167.65-190.28)	27	85.90 (73.85-99.92)
	No	228	0.35 (0.28-0.43)	164	0.14 (0.10-0.20)	164	168.22 (163.78-172.77)	164	73.21 (68.77-77.94)
Consumed chinook salmon[b]	**Yes**	60	0.30 (0.25-0.38)	46	0.80 (0.53-1.22)	46	174.07 (165.77-182.78)	46	77.80 (70.79-85.51)
	No	210	0.38 (0.30-0.49)	145	0.09 (0.06-0.13)	145	168.27 (163.52-173.16)	145	73.99 (68.94-79.39)
	Yes	36	0.29 (0.21-0.39)	27	0.74 (0.40-1.35)	27	174.31 (162.07-187.47)	27	75.69 (68.27-83.92)

Consumed chum salmon[b]	**No**	234	0.38 (0.30-0.47)	164	0.12 (0.08-0.16)	264	168.89 (164.51-173.39)	164	74.75 (70.01-79.82)
Consumed coho salmon[b]	**Yes**	27	0.28 (0.20-0.39)	22	0.72 (0.37-1.39)	22	173.35 (161.75-185.79)	22	80.98 (71.50-91.73)
	No	243	0.38 (0.30-0.47)	169	0.12 (0.09-0.17)	169	169.17 (164.74-173.72)	169	74.13 (69.56-78.99)
Consumed muktuk[b]	**Yes**	16	0.26 (0.15-0.43)	11	0.44 (0.11-1.70)	11	167.51 (154.40-181.72)	11	67.10 (51.97-86.64)
	No	254	0.37 (0.30-0.46)	180	0.14 (0.10-0.20)	180	169.78 (165.46-174.21)	180	75.39 (71.01-80.04)
Consumed fish eggs	**Yes**	93	0.59 (0.49-0.72)	70	0.28 (0.16-0.48)	70	172.75 (166.74-178.98)	69	81.22 (74.54-88.50)
	No	176	0.28 (0.21-0.37)	121	0.11 (0.07-0.16)	121	167.84 (162.34-173.52)	122	71.45 (66.20-77.12)
Consumed burbot liver	**Yes**	86	0.85 (0.59-1.23)	54	0.52 (0.16-1.75)	54	179.84 (165.77-195.10)	54	86.45 (68.84-108.56)
	No	183	0.33 (0.27-0.41)	137	0.13 (0.10-0.18)	137	168.62 (164.31-173.04)	137	73.78 (69.50-78.32)
Consumed moose liver	**Yes**	56	0.43 (0.26-0.70)	41	0.16 (0.08-0.35)	41	173.47 (166.63-180.60)	41	82.58 (71.73-95.07)
	No	213	0.35 (0.28-0.43)	150	0.15 (0.10-0.21)	150	168.62 (163.72-173.66)	150	72.91 (68.45-77.66)
Consumed moose kidney	**Yes**	81	0.57 (0.39-0.82)	67	0.28 (0.16-0.49)	66	174.47 (167.16-182.10)	66	81.90 (74.30-90.28)
	No	188	0.30 (0.24-0.38)	124	0.11 (0.07-0.16)	124	167.10 (162.14-172.21)	125	71.43 (66.50-76.72)
Consumed Caribou Liver	**Yes**	48	0.48 (0.35-0.66)	32	0.28 (0.12-0.64)	32	169.83 (161.97-178.08)	32	80.24 (68.96-93.36)
	No	221	0.34 (0.27-0.43)	159	0.13 (0.09-0.19)	159	169.61 (164.90-174.45)	159	73.85 (69.34-78.66)
Consumed caribou kidney	**Yes**	66	0.48 (0.38-0.61)	48	0.43 (0.23-0.80)	48	175.69 (167.79-183.96)	48	83.55 (74.57-93.61)
	No	203	0.33 (0.26-0.43)	143	0.11 (0.07-0.15)	143	167.67 (162.86-172.61)	143	72.19 (67.51-77.18)
Consumed waterfowl	**Yes**	140	0.50 (0.40-0.63)	96	0.24 (0.15-0.38)	96	172.59 (165.99-179.45)	95	81.51 (75.31-88.23)
	No	129	0.26 (0.19-0.36)	95	0.10 (0.06-0.14)	95	166.72 (161.76-171.84)	96	68.86 (63.42-74.77)
Consumed wild bird eggs	**Yes**	16	0.63 (0.38-1.03)	13	0.31 (0.06-1.52)	13	183.34 (165.40-203.22)	13	75.30 (61.69-91.92)
	No	253	0.35 (0.29-0.43)	178	0.14 (0.10-0.20)	178	168.69 (164.46-173.02)	178	74.86 (70.44-79.55)

[a]Sample sizes differ across variables based on the study components that participants completed

[b]For the region(s)/community(s) where these foods were not included in the Food Frequency Questionnaire (as these were not commonly consumed traditional foods for those regions(s)/community(s), the participants were considered to not be consumers of these foods for the pooled statistics and analyses. See methods for more details.

4.4.4 Explanatory models for levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma)

The potential determinants were also included in multivariable models for those participants that had completed the FFQ and had complete data for the other variables of interest (demographic characteristics, percent consuming the traditional foods of interests, and biomarker concentrations for the complete cases are included by region in **Supplementary Tables 4.4-4.5**) and backwards selection based on AIC values was used to identify the final model for each biomarker. The AIC values for the backwards model selection are included in **Supplementary Tables 4.6-4.9**. The final model explaining log_2-transformed hair total Hg concentrations included age, and the consumption of lake trout and waterfowl as explanatory variables (adjusted R^2 =0.26, n=218, df=3, 221) and the regression coefficients with p-values and confidence intervals are included in **Table 4.6**. Due to the high rate of blood total Hg concentrations below the detection limit in the Dehcho (56%) and Sahtú region (67%), a Tobit model was used for the model examining blood total Hg concentrations. The final model explaining log_2-transformed blood total Hg concentrations included age, region, BMI, and the consumption of grayling, and waterfowl as explanatory variables (adjusted R^2 not available for this type of model, n=162, df=8, 154) and the regression coefficients with p-values are included in **Table 4.7**.

Table 4.6: Parameter estimates for the model to explain log_2-tranformed hair mercury concentrations

Parameter	Estimate	P-value	95% Confidence Limits
Intercept	-4.29	<0.01	-4.99, -3.59
Age	0.04	<0.01	0.03, 0.06
Consuming lake trout	0.63	0.02	0.09, 1.16
Consuming waterfowl	1.13	<0.01	0.58, 1.67

Table 4.7: Parameter estimates for the Tobit model to explain log_2-tranformed blood mercury concentrations

Parameter	Estimate	Approximate P-value
Intercept	-6.24	<0.01
Age	0.11	<0.01
Region: Dehcho vs Sahtú	1.68	0.06
Region: Old Crow vs Sahtú	5.95	<0.01
BMI	-0.09	0.10
Consuming grayling	2.15	<0.01
Consuming waterfowl	2.00	<0.01
_Sigma	3.73	<0.01

Sigma is the estimate of the error variance

For the blood Se concentrations, the final model explaining blood Se concentrations included region, BMI, sex, and the consumption of whitefish, northern pike, grayling, chinook salmon, burbot livers, caribou livers, and caribou kidneys as explanatory variables (AIC= 1212.18, adjusted R^2 =0.32, n=162, df =11, 150) and the regression coefficients with p-values and confidence intervals are included in **Table 4.8**.

Table 4.8: Parameter estimates for the model to explain blood selenium concentrations

Parameter	Estimate	P-value	95% Confidence Limits
Intercept	164.04	<0.01	143.09, 184.98
Region: Dehcho vs Sahtú	32.83	<0.01	21.94, 43.71
Region: Old Crow vs Sahtú	-4.55	0.71	-28.35, 19.24
BMI	-0.73	0.02	-1.35, -0.12
Sex: Male vs Female	5.50	0.17	-2.44, 13.44
Consuming whitefish	11.15	0.09	-1.83, 24.14
Consuming northern pike	-9.05	0.06	-18.33, 0.24
Consuming grayling	-12.28	0.02	-22.21, -2.35
Consuming chinook salmon	28.92	0.02	4.93, 52.92
Consuming burbot liver	11.79	0.09	-1.99, 25.57
Caribou liver	-18.48	0.01	-31.41, -5.56
Caribou kidney	20.09	<0.01	7.74, 32.43

In the pooled analysis, the final model explaining $\log_2$-transformed plasma EPA+DHA concentrations included age, sex, smoking (within the past 24 hours), alcohol (use within the past 24 hours), and the consumption of whitefish, inconnu, lake trout, northern pike, walleye, coho salmon, and waterfowl, as explanatory variables (AIC= -62.26, adjusted R^2 =0.33, n=162, df =11, 150) and the regression coefficients with p-values and confidence intervals are included in **Table 4.9**.

Table 4.9: Parameter estimates for the model to explain log_2-transformed plasma EPA+DHA concentrations

Parameter	Estimate	P-Value	95% Confidence Limits
Intercept	5.45	<0.01	5.13, 5.76
Age	0.01	<0.01	0.01, 0.02
Sex: Male vs Female	-0.16	0.05	-0.32, -0.00
Smoking	-0.31	<0.01	-0.47, -0.15
Alcohol Use	0.49	<0.01	0.25, 0.73
Consuming Whitefish	0.22	0.09	-0.03, 0.46
Consuming Inconnu	-0.30	<0.01	-0.48, -0.11
Consuming Lake Trout	0.19	0.02	0.03, 0.34
Consuming Northern Pike	-0.13	0.16	-0.31, -0.05
Consuming Walleye	0.15	0.15	-0.05, 0.34
Consuming Coho Salmon	0.32	0.01	0.07, 0.58
Consuming Waterfowl	0.19	0.03	0.02, 0.36

4.5 Discussion

4.5.1 Determinants of Hair and Blood Mercury Concentrations

Several dietary and demographic/biometric factors were associated with differences in mean hair and blood total Hg concentrations and after backwards selection, several of these factors were included as potential explanatory variables in the final models to explain the variation in hair and blood total Hg concentrations. Age was strongly positively associated with both hair and blood total Hg concentrations in all three regions and was included in the final regression models. The other demographic and biometric factors examined (BMI, sex, and smoking with the previous 24 hours) were not consistently associated with differences in hair and blood total Hg concentrations, but BMI was included in the Tobit regression model to explain blood total Hg concentrations. The positive association between age and hair and blood total Hg concentrations could be due in part to the increased intakes of fish in older age groups (Ratelle et al., 2020). Additionally, age has been consistently associated with total Hg concentrations in hair and blood across multiple studies (Caspersen et al., 2019; Gundacker et al., 2006; Mahaffey et al., 2004; Bates et al., 2007) while BMI, sex, and smoking status have been less consistently associated with hair/blood total Hg concentrations (Gaxiola-Robles et al., 2014; Gari et al., 2013; Jenssen et al., 2012; Gundacker et al., 2006).

The consumption of several fish species/parts and traditional foods was associated with differences in hair and blood total Hg concentrations and were included in the final regression

models. However, the specific species that were significantly associated with differences in hair or blood total Hg concentrations differed between the regions examined and region was an explanatory variable in the final model to explain blood total Hg concentrations. These differences are likely due to the variations in diet between the regions. For example, whitefish was the most commonly consumed fish in the Dehcho and Sahtú region (consumed by 92% and 76% or participants from each region, respectively) followed by northern pike (consumed by 60% of participants) in the Dehcho region while the second most consumed fish was lake trout for the Sahtú region (consumed by 75% of participants). In Old Crow, chinook salmon was the most commonly consumed fish (by 90% of participants), followed by whitefish (85%). Of note, the fish species whose consumption was associated with higher hair or blood total Hg concentrations and/or were included in the regression models, were species that tend to be higher in total Hg concentrations (Reyes et al., 2017; Laird et al., 2018). For example, the consumption of lake trout was included in the final regression model for hair total Hg concentrations. Lake trout is a predatory fish species that has been reported to have elevated total Hg concentrations (>0.5 mg/kg total Hg concentrations wet weight) in some lakes in Northern Canada (DHHS, 2016; Laird et al., 2018; Reyes et al., 2017). Additionally, it should be noted that lake trout is the second most consumed fish species in Sahtú region (with 75% of participants from the Sahtú region consuming lake trout versus 54% and 15% consuming lake trout in the Dehcho and Old Crow, respectively), which is also the region with the highest hair total Hg concentrations. Interestingly, the consumption of waterfowl was also associated with higher hair and blood total Hg concentrations. Previously the bioaccumulation of total Hg in aquatic/marine birds has been observed in the Canadian arctic (Provencher et al., 2014). However, the identification of waterfowl as a potential dietary source of total Hg exposure, fills a knowledge gap on the sources of total Hg exposure (other than fish) for northern populations that are not in coastal regions (with regular access to seafood).

It should also be noted, as has previously been described (Drysdale et al., 2021; Ratelle et al., 2020b; Ratelle et al., 2018b), the vast majority of participants had hair and blood total Hg concentrations below relevant health-based guidance values and similar to levels in the Canadian General population. However, hair total Hg concentrations, especially from the Sahtú region, were higher on average (geometric mean) than the levels measured in the Canadian general

population (Health Canada, 2019a). Although the consumption of several foods were associated with higher total Hg levels in these populations, total Hg exposure appears to be relatively low in these participating communities.

4.5.2 Determinants of Blood Selenium Concentrations

Very few demographic and dietary factors were associated with differences in blood Se concentrations, however, after backwards selection, several were included in the final model to explain blood Se concentrations. The only demographic/biometric factor associated with blood Se concentrations was age, but when stratified by region, this association was only seen in the Dehcho and Sahtú region and it was not included in the final model. This could be due to the low number of younger participants from Old Crow. Although the mean age was similar between the regions, the percent of children (<18 years of age) participating in the biomonitoring project was much lower in Old Crow (3%) compared to the Dehcho (20%) and Sahtú (15%) regions. Previous studies have found that age is associated with Se concentrations (Bates et al., 2007; Gundacker et al., 2006). Additionally, in the final model to explain blood Se concentrations, BMI and sex were included as potential explanatory variables. In the final model, the association with BMI was negative which is similar to the observations of Caspersen et al. (2019) who also found a negative association between BMI and blood Se concentrations in a Norwegian pregnancy cohort study. However, previously Gundacker et al., (2006) found that blood Se concentrations did not differ by sex.

The consumption of several traditional foods was included in the final model including the consumption of several specific fish species. Although the consumption of several of these fish species/traditional foods were positively associated with blood Se concentrations (whitefish, chinook salmon, burbot livers, caribou kidney), others were negatively associated with blood Se concentrations (northern pike, grayling, caribou liver). While a study of fish from the Dehcho region of the NWT (Reyes et al., 2017) found that Se concentrations were similar between the fish species examined, the mean Se concentrations were slightly higher for whitefish (whose consumption in this study is positively associated with blood Se concentrations) compared to northern pike (whose consumption in this study is negatively associated with blood Se concentrations). Additionally, northern pike from this region were found to have significantly higher total Hg concentrations compared to lake whitefish, and total Hg and total Se

concentrations in the muscle tissue of these fish species were found to be negatively correlated (Reyes et al., 2017). The consumption of burbot livers was included in the final model and positively associated with blood Se concentrations, which was expected, as previously a study of several traditional foods from Alberta, Canada, found that of the foods tested, Se concentrations were highest in fish liver samples (Golzadeh et al., 2020). The consumption of caribou kidneys (positively associated) and caribou livers (negatively associated) were also included in the final model. If levels of Se were higher in the kidneys compared to livers of the caribou that participants from these regions ate, then this could explain the positive effect estimate for the consumption of caribou kidneys and negative effect estimate for the consumption of caribou livers that was observed. Previously a study of two caribou populations in Greenland found that Se concentrations were higher in caribou kidneys compared to caribou livers, both of which were much higher than levels in caribou muscle tissue (Gamberg et al., 2016). Overall, the findings of this analysis, that the consumption of several traditional foods could help explain variability in blood Se concentrations in these regions, were similar to findings of other studies that also found significant associations between the consumption of specific foods and Se biomarker concentrations (Junque et al., 2022; Monastero et al., 2015). For example, in a study of children in northern Spain, urinary Se concentrations were positively associated with the consumption of lean fish (Junque et al., 2022). Differences in the Se concentrations between these traditional foods, could explain why some foods were positively and some were negatively associated with blood Se concentrations in this study.

It should be noted that geometric mean blood Se concentrations from all three regions were similar or below the geometric mean blood Se concentrations measured in the Canadian general population through the CHMS (Health Canada, 2019a) and in First Nations through the FNBI (AFN, 2013). Hays et al., (2014) suggested a biomonitoring equivalent for whole blood of 100 μg/L total Se for an adequate Se intake and a biomonitoring equivalent of 400-480 μg/L total Se in whole blood to protect against excessive Se intake (ie. selenosis). Based on these biomonitoring equivalents, the participating regions appear to have an adequate Se intake without excessive exposure (95^{th} percentile total Se concentrations in blood were well below the upper biomonitoring equivalent of 480 μg/L). Similarly, as previously described (Ratelle et al., 2022), intakes of Se estimated from a 24-hour recall dietary questionnaire that was administered

as part of the Biomonitoring project in the Dehcho and Sahtú regions, were adequate (above dietary reference intakes) for the majority of participants in the Dehcho and Sahtú regions. These estimated Se intakes (based on the 24-hour recall) were higher in the Sahtú region compared to the Dehcho region (Ratelle et al., 2022), however, blood Se concentrations were higher on average in the Dehcho region compared to the Sahtú region. This difference could be due to the short time-frame (24 hours) that the estimated Se intakes were based on. Meanwhile blood Se concentrations would reflect both recent and long-term exposure to Se, as Se has both a rapid phase (half-life 1-3 days) and slow phase (half-life of 30-110 days) of elimination (Alexander, 2015).

4.5.3 Determinants of EPA+DHA Concentrations

As previously described (Drysdale et al., 2021; Ratelle et al., 2018), levels of plasma EPA+DHA in these regions are similar to levels observed in other Canadian studies (Stark et al., 2016). As expected, several demographic/biometric and dietary factors were associated with differences in plasma EPA+DHA concentrations. Age was positively associated with higher average plasma EPA+DHA for all three regions and this association has been previously seen in several studies (Flock et al., 2013; Friedman et al., 2006). However, the association with other demographic/biometric factors was less consistent, as BMI was only associated with increased average plasma EPA+DHA in the Dehcho and Sahtú regions, while sex was only associated with differences in plasma EPA+DHA in Old Crow. The higher plasma EPA+DHA concentrations seen in the women versus men in Old Crow in this study, and included in the final model, agrees with the study by Flock et al., (2013), who also found higher concentrations of EPA+DHA in the women in their study compared to the men. Smoking in the past 24-hours was associated with decreased plasma EPA+DHA concentrations levels in the Dehcho region and was also included in the final regression model. This agrees with the literature as smoking has been associated with differences in fatty acid concentrations, including reduced serum concentrations of omega-3 PUFA (Simon et al., 1996).

In terms of dietary factors, the consumption of several species of fish and the consumption of waterfowl were associated with differences in plasma EPA+DHA concentrations and were included in the final regression model. The consumption of the fish species: whitefish, lake trout, walleye, and coho salmon, were associated with increased plasma EPA+DHA

concentrations in the final model. The inclusion of these fish species in the final model agrees with the findings of a study of fish from the NWT, which found that total omega-3 PUFA concentrations were highest in lake trout, followed by longnose sucker, lake whitefish, cisco, walleye, northern pike, and lastly burbot (Reyes et al., 2017). Meanwhile, the consumption of inconnu and northern Pike was included in the final model but was associated with a decrease in plasma EPA+DHA concentrations. In the NWT, significantly lower omega-3 PUFA concentrations have been reported in northern pike muscle samples relative to lake trout and lake whitefish (Reyes et al., 2017) and in a study of several fish species from Siberian Arctic Lakes, inconnu was found to have significantly lower fat content compared to lake whitefish (Suchchik et al., 2020). As such, the differences in fat/omega-3 PUFA content of these different fish species could explain the direction of their associations in the final model.

4.5.4 Associations between Biomarkers

As expected, several of the biomarkers were correlated with each other. Hair and blood total Hg concentrations were significantly correlated with plasma EPA+DHA concentrations in all three regions. This is consistent with other studies that have found correlations between hair or blood total Hg concentrations and plasma EPA+DHA (Turunen et al., 2010; Dewailly et al., 2001). These correlations are likely due to the common dietary sources of total Hg and EPA+DHA in these populations. Supporting this, the consumption of at least one species of fish was included in each of the models for hair and blood total Hg concentrations and plasma EPA+DHA concentrations and the consumption of lake trout appeared in both the model for hair total Hg concentrations and the model for plasma EPA+DHA concentrations. The consumption of waterfowl was also included in the final models for hair total Hg, blood total Hg, and plasma EPA+DHA concentrations.

The correlations between Se and the other biomarkers were less consistent. Se was only correlated with hair total Hg concentrations for Old Crow, blood total Hg concentrations for the Dehcho region, and plasma EPA+DHA concentrations for the Sahtú region. Several studies have found associations between blood total Hg and Se concentrations (Caspersen et al., 2019; Nakayama et al., 2019; Bates et al., 2007; Granjean et al., 1992). However, correlations may not have been seen across all three of the regions/communities in this study due to differences in diet by region. Additionally, the dietary sources/dietary patterns in these regions that were associated

with higher Se levels may be different than the dietary patterns associated with higher total Hg and EPA+DHA concentrations. Supporting this, several additional dietary factors were included in the model for blood Se concentrations (such as the consumption of caribou kidneys and burbot livers) that were not included in the final models for total Hg or EPA+DHA concentrations.

4.5.5 Limitations and Future Directions

This study provides important information about potential determinants of total Hg, Se, and EPA+DHA acid concentrations for First Nations communities in northern Canada. However, the remote location of the study sites meant that the sample size for this study was relatively small but this did represent approximately 9% of the residents of the Dehcho and Sahtú regions and 44% of the residents of Old Crow. By pooling the analysis across three regions, the associations between several dietary and demographic factors and biomarker concentrations could be examined. Additionally, a challenge for this study was the high percentage of blood samples with total Hg concentrations below the limit of detection for participants from the NWT. A Tobit regression model (to account for left censoring of the data) was used to address this issue. Additionally, these analyses rely on data from a FFQ where participants had to recall their consumption of specific foods from past year. This could result in under or over-estimates of consumption frequencies, so the dietary data used in these analyses were converted to binary variables (consumed or not consumed) rather than using consumption frequencies. As well, FFQs are considered to have reasonable relative reliability of food estimates (Rendo-Urteaga et al., 2020; Vézina-Im et al., 2016) and are frequently used. However, this is a relatively crude categorization and may have contributed non-differential measurement error which could have biased the associations towards the null (Fosgate, 2006). Similarly, the measures used to determine alcohol consumption/use and smoking status were also relatively crude. Whether someone had used alcohol in the past 24 hours or smoked in the past 24 hours were used as a proxy for recent alcohol consumption, and smoking status, respectively. Although a short recall period can reduce issues related to memory when asking about alcohol use, this will not provide accurate information about a participant's typical alcohol consumption, and may not capture infrequent/irregular consumption of alcohol (Gmel & Rehm, 2004; Dawson, 2003). There are currently many methods (such as screening tools, questionnaires, diagnostic tools, and/or guidelines) used in the literature for assessing and categorizing alcohol intake (Tevik et al.,

2021). As alcohol use was included in the final model to predict plasma EPA+DHA concentrations, future studies examining determinants of EPA+DHA biomarker levels should consider collecting information about participants' alcohol use using a measure with a longer recall period. Additionally, although a self-reported measure was used for smoking status, and self-reported measures may underestimate smoking in some populations (Gorber et al., 2009), in previous analyses in a subset of this population, urinary cotinine levels agreed well with self-reported smoking (Ratelle et al., 2018c). Even with these relatively crude estimates of traditional food consumption, alcohol use, and smoking status, the final models explained 26-36% of the variability in hair total Hg, blood Se, and plasma EPA+DHA concentrations. There were also several variables potentially related to the biomarker levels that could not be examined as this data were not available/collected in some or all of the regions. This includes data on whether participants had any Hg containing dental amalgams, and their occupation history. Additionally, it should be noted that the studies were conducted over several years (2016-2018 for the NWT project and 2019 for the Yukon project), however, the sampling was always conducted in the winter season (November-March). It is possible that there were some temporal differences.

Future work could include follow-up studies in these regions to determine if exposures to environmental contaminants and nutrients, and the diets of these populations, are changing over time. Climate change has the potential to impact access to traditional foods including fish in northern Canada (Proverbs, et al., 2020) and levels of environmental contaminants, such as Hg, in those foods (Rohonczy et al., 2020). This study provides important information and begins to fill the gap on potential dietary sources of total Hg, Se, and EPA+DHA concentrations for northern First Nations communities. Although fish consumption is a well-known potential source of Hg, this research highlights the import nutrients that fish are also a source of. Interestingly the consumption of waterfowl was identified as a potential determinant of total Hg and EPA+DHA biomarker levels. Ongoing traditional food sampling projects in northern Canada could include the sampling and analysis of waterfowl tissue for environmental contaminants and nutrients. Future work should continue to monitor Hg concentrations in traditional foods especially in the face of climate change, and the dataset on nutrients in traditional foods should also continue to be expanded.

4.6 Conclusions

Several demographic and dietary factors, including the consumption of specific species of fish and other traditional foods, were identified as potentially explaining the variability in total Hg, Se, and EPA+DHA biomarker levels for these northern First Nations communities. This analysis helps to fill the gap in the literature for the site-specific demographic and dietary determinants of total Hg, Se, and EPA+DHA biomarker concentrations of northern Indigenous populations. These results can be used to inform future and ongoing projects that aim to understand nutrient and contaminant levels in northern traditional foods. For example, over 40% of participants reported consuming waterfowl and the consumption of waterfowl was associated with increased hair and blood total Hg concentrations, and plasma EPA+DHA concentrations in this study. Future research, along with the ongoing monitoring of total Hg concentrations in local fish, could include the collection and analysis of locally caught waterfowl for total Hg and EPA+DHA concentrations, to better inform conversations about maximizing the nutritional benefits of traditional food consumption while minimizing the risks of contaminant exposure. This work also helps to address the concerns of northern First Nations communities from these regions regarding the elevated concentrations of total Hg that were detected in some species of fish from some lakes in these regions. Although levels of total Hg are elevated in some fish in the region, results from these biomonitoring studies indicate that Hg exposures have generally remained low for the majority of participants. Additionally, although the consumption of several traditional foods was associated with increased total Hg biomarker levels, the consumption of these foods was also associated with increased concentrations of the important nutrients Se and EPA+DHA. These findings highlight the need to consider the nutritional benefits of traditional foods when designing consumption notices and messages around traditional food consumption and environmental contaminants. Collectively, this research can be used to support ongoing efforts in these regions to promote the consumption of traditional foods while seeking to understand and reduce the risks of contaminant exposure.

4.7 Supplemental Tables

Supplementary Table 4.1: Summary statistics for plasma EPA+DHA presented as a % of the total fatty acids

Region	n[a]	Geometric mean (95% CI)	Arithmetic mean (SD)	Median	75th Percentile	95th Percentile
Total	329	1.92 (1.85-1.99)	2.04 (0.77)	1.87	2.38	3.54
Dehcho	136	1.84 (1.74-1.94)	1.94 (0.70)	1.78	2.10	3.41
Sahtú	137	2.02 (1.89-2.15)	2.17 (0.87)	2.00	2.57	3.73
Old Crow	56	1.87 (1.73-2.02)	1.95 (0.59)	1.86	2.43	2.85

Supplementary Table 4.2: Pair-wise comparisons with the Tukey test for the biomarker concentrations by region

Dependent Variable	(I) Region	(J) Region	Mean Difference (I-J)	P-Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Log2 hair mercury concentrations	**Dehcho**	**Old Crow**	0.37	0.41	-0.31	1.04
		Sahtú	-0.56	0.02	-1.03	-0.08
	Old Crow	**Dehcho**	-0.37	0.40	-1.04	0.31
		Sahtú	-0.92	<0.01	-1.61	-0.24
	Sahtú	**Dehcho**	0.56	0.02	0.08	1.03
		Old Crow	0.92	<0.01	0.24	1.61
Log2 blood mercury concentrations	**Dehcho**	**Old Crow**	-2.67	<0.01	-3.82	-1.51
		Sahtú	0.81	0.08	-0.07	1.69
	Old Crow	**Dehcho**	2.67	<0.01	1.51	3.82
		Sahtú	3.48	<0.01	2.33	4.63
	Sahtú	**Dehcho**	-0.81	0.08	-1.69	0.07
		Old Crow	-3.48	<0.01	-4.63	-2.33
Blood selenium concentrations	**Dehcho**	**Old Crow**	11.25	0.03	0.82	21.68
		Sahtú	27.15	<0.01	19.24	35.06
	Old Crow	**Dehcho**	-11.25	0.03	-21.68	-0.82
		Sahtú	15.90	<0.01	5.52	26.29
	Sahtú	**Dehcho**	-27.15	<0.01	-35.06	-19.24
		Old Crow	-15.90	<0.01	-26.29	-5.52

Supplementary Table 4.3A: Binary potential determinants of levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) levels for the Dehcho region

Predictor	Predictor Level	Hair Mercury		Blood Mercury		Blood Selenium		EPA+DHA	
		n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)
Sex	**Male**	119	0.41 (0.30-0.55)	65	0.15 (0.08-0.29)	65	186.63 (180.41-193.07)	65	69.63 (62.82-77.19)
	Female	112	0.36 (0.27-0.49)	71	0.08 (0.05-0.14)	71	180.16 (172.75-187.89)	71	72.94 (66.84-79.59)
Smoking in the previous 24 hours	**Yes**	76	0.34 (0.24-0.50)	52	0.09 (0.05-0.16)	52	185.01 (177.4-192.94)	53	59.93 (54.09-66.41)
	No	154	0.40 (0.31-0.52)	83	0.13 (0.08-0.22)	83	182.11 (175.6-188.86)	82	80.51 (74.48-87.03)
Alcohol use in the previous 24 hours	**Yes**	24	0.53 (0.36-0.80)	18	0.08 (<LOD -0.26)	18	187.64 (176.94-198.99)	18	71.73 (59.02-87.19)
	No	199	0.36 (0.29-0.46)	112	0.11 (0.07-0.18)	112	182.13 (176.77-187.66)	112	70.72 (65.66-76.17)
Consumed whitefish	**Yes**	126	0.32 (0.23-0.45)	78	0.09 (0.06-0.16)	78	181.92 (176.12-187.92)	78	75.16 (68.78-82.12)
	No	10	0.21 (0.04-1.10)	6	0.06 (<LOD -0.50)	6	168.87 (138.52-205.88)	6	52.17 (31.70-85.86)
Consumed inconnu	**Yes**	39	0.46 (0.34-0.63)	21	0.10 (<LOD -0.30)	21	182.69 (173.61-192.26)	21	75.20 (65.59-86.22)
	No	97	0.27 (0.17-0.41)	63	0.09 (0.05-0.15)	63	180.38 (173.35-187.71)	63	72.58 (64.98-81.06)
Consumed lake trout	**Yes**	77	0.48 (0.38-0.61)	49	0.13 (0.07-0.25)	49	183.11 (176.01-190.49)	48	79.53 (70.60-89.58)
	No	59	0.18 (0.09-0.35)	35	0.06 (<LOD -0.11)	35	178.00 (168.44-188.09)	36	65.59 (57.64-74.63)
Consumed burbot	**Yes**	19	0.6 (0.37-0.99)	13	0.17 (<LOD -0.83)	13	186.83 (172.99-201.78)	13	81.34 (64.57-102.46)
	No	117	0.28 (0.20-0.41)	71	0.08 (0.05-0.13)	71	179.90 (173.60-186.44)	71	71.83 (65.16-79.17)
Consumed northern pike	**Yes**	82	0.39 (0.27-0.58)	47	0.14 (0.07-0.29)	47	177.57 (170.62-184.81)	47	75.66 (66.31-86.32)
	No	54	0.22 (0.13-0.39)	37	0.05 (<LOD -0.09)	37	185.35 (175.77-195.46)	37	70.24 (62.54-78.89)
Consumed grayling	**Yes**	14	0.53 (0.31-0.88)	6	0.27 (<LOD -3.82)	6	178.15 (163.03-194.68)	6	65.45 (40.18-106.63)
	No	122	0.3 (0.21-0.42)	78	0.08 (0.05-0.14)	78	181.18 (175.10-187.47)	78	73.86 (67.42-80.91)
Consumed walleye	**Yes**	71	0.37 (0.23-0.57)	42	0.09 (<LOD -0.18)	42	181.65 (174.03-189.60)	42	81.70 (72.15-92.51)
	No	65	0.26 (0.16-0.42)	42	0.09 (0.05-0.18)	42	180.27 (171.60-189.38)	42	65.63 (58.12-74.11)
Consumed sucker	**Yes**	35	0.43 (0.20-0.91)	20	0.20 (0.07-0.60)	20	186.68 (174.24-200.00)	20	84.24 (70.92-100.07)
	No	101	0.28 (0.20-0.40)	64	0.07 (<LOD -0.12)	64	179.21 (172.74-185.92)	64	70.08 (63.24-77.67)
Consumed fish eggs	**Yes**	41	0.68 (0.52-0.90)	26	0.17 (0.06-0.44)	26	179.89 (171.26-188.95)	25	89.11 (76.38-103.96)
	No	95	0.22 (0.14-0.34)	58	0.07 (<LOD -0.12)	58	181.47 (174.01-189.24)	59	67.05 (60.52-74.30)
	Yes	11	0.85 (0.37-1.95)	7	0.21 (<LOD -17.11)	7	183.24 (159.09-211.05)	7	102.50 (59.97-175.19)

Consumed burbot liver	**No**	125	0.29 (0.21-0.41)	77	0.09 (0.05-0.14)	77	180.82 (174.85-186.99)	77	71.68 (65.55-78.38)
Consumed moose liver	**Yes**	35	0.39 (0.18-0.83)	24	0.13 (0.05-0.37)	24	177.87 (167.92-188.41)	24	75.10 (62.04-90.91)
	No	101	0.29 (0.20-0.41)	60	0.08 (<LOD -0.13)	60	182.21 (175.19-189.51)	60	72.49 (65.52-80.19)
Consumed moose kidney	**Yes**	44	0.54 (0.29-1.01)	34	0.18 (0.08-0.42)	34	180.95 (171.62-190.79)	33	76.91 (66.03-89.58)
	No	92	0.24 (0.17-0.35)	50	0.06 (<LOD -0.10)	50	180.96 (173.62-188.62)	51	70.93 (63.51-79.21)
Consumed caribou liver	**Yes**	19	0.72 (0.43-1.23)	11	0.33 (0.05-1.97)	11	184.11 (166.71-203.33)	11	82.33 (63.39-106.93)
	No	117	0.27 (0.19-0.39)	73	0.07 (0.05-0.12)	73	180.49 (174.36-186.84)	73	71.94 (65.39-79.15)
Consumed caribou kidney	**Yes**	15	0.7 (0.39-1.25)	10	0.57 (0.09-3.72)	10	189.16 (172.65-207.26)	10	78.12 (54.58-111.81)
	No	121	0.28 (0.20-0.40)	74	0.07 (<LOD -0.11)	74	179.88 (173.75-186.22)	74	72.59 (66.22-79.56)
Consumed waterfowl	**Yes**	84	0.44 (0.31-0.62)	51	0.15 (0.08-0.29)	51	182.78 (175.38-190.50)	50	78.95 (70.97-87.83)
	No	52	0.18 (0.10-0.34)	33	LOD (<LOD -0.08)	33	178.17 (169.02-187.83)	34	65.55 (56.37-76.22)
Consumed wild bird eggs	**Yes**	10	0.57 (0.29-1.12)	7	0.07 (<LOD -0.73)	7	183.12 (173.89-192.84)	7	78.62 (61.57-100.39)
	No	126	0.3 (0.21-0.42)	77	0.09 (0.06-0.15)	77	180.76 (174.59-187.16)	77	72.75 (66.16-80.00)

[a]Sample sizes differ across variables based on the study components that participants completed

Note: Those foods not included in the FFQ for this region or with a sample size of less than 5 for one of the predictor levels are not included in this table.

Supplementary Table 4.3B: Binary potential determinants of levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) levels for the Sahtú region

Predictor	Predictor Level	Hair Mercury		Blood Mercury		Blood Selenium		EPA+DHA	
		n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)
Sex	**Male**	104	0.52 (0.37-0.73)	69	0.10 (0.05-0.17)	69	154.99 (147.64-162.71)	66	72.40 (64.36-81.45)
	Female	108	0.62 (0.49-0.77)	71	LOD (<LOD -0.07)	71	157.48 (152.11-163.03)	71	73.55 (65.20-82.97)
Smoking in the previous 24 hours	**Yes**	97	0.65 (0.51-0.83)	71	0.06 (<LOD -0.10)	71	155.88 (149.95-162.05)	69	68.93 (61.53-77.22)
	No	113	0.51 (0.37-0.71)	69	0.07 (<LOD -0.12)	69	156.62 (149.7-163.86)	68	77.36 (68.39-87.52)
Alcohol use in the previous 24 hours	**Yes**	36	0.75 (0.46-1.23)	21	0.11 (<LOD -0.33)	21	149.36 (138.34-161.26)	20	81.23 (65.13-101.30)
	No	173	0.54 (0.43-0.68)	119	0.06 (<LOD -0.09)	119	157.49 (152.53-162.62)	117	71.67 (65.45-78.49)
Consumed whitefish	**Yes**	48	0.84 (0.64-1.11)	44	0.11 (0.06-0.22)	44	154.61 (148.57-160.9)	44	80.89 (70.86-92.33)
	No	18	0.28 (0.08-1.04)	12	<LOD (<LOD -0.08)	12	139.49 (119.58-162.71)	12	64.70 (45.27-92.47)
Consumed inconnu	**Yes**	16	1.18 (0.77-1.8)	15	0.18 (0.05-0.71)	15	147.18 (136.91-158.22)	15	69.09 (50.87-93.83)
	No	50	0.51 (0.30-0.85)	41	0.06 (<LOD -0.12)	41	152.75 (144.52-161.45)	41	80.27 (69.97-92.08)
Consumed cisco[c]	**Yes**	9	1.80 (1.08-3.01)	7	0.52 (0.05-5.63)	7	144.48 (122.64-170.21)	7	88.35 (57.05-136.81)
	No	57	0.53 (0.34-0.83)	49	0.06 (<LOD -0.11)	49	152.23 (145.25-159.54)	49	75.62 (66.08-86.55)
Consumed lake trout	**Yes**	50	0.78 (0.47-1.28)	46	0.11 (0.05-0.22)	46	151.82 (144.03-160.04)	46	81.71 (71.02-94)
	No	16	0.31 (0.18-0.54)	10	<LOD (<LOD -0.05)	10	148.58 (139.87-157.83)	10	59.06 (45.53-76.62)
Consumed burbot	**Yes**	15	1.19 (0.78-1.80)	12	0.18 (<LOD-0.74)	12	154.89 (144.09-166.51)	12	79.09 (61.96-100.94)
	No	51	0.52 (0.31-0.86)	44	0.07 (<LOD -0.13)	44	150.26 (142.42-158.53)	44	76.58 (65.92-88.96)
Consumed northern pike	**Yes**	9	1.22 (0.59-2.55)	10	0.07 (<LOD -0.41)	10	155.25 (144.49-166.81)	10	71.23 (52.04-97.5)
	No	57	0.56 (0.36-0.88)	46	0.08 (<LOD-0.16)	46	150.38 (142.74-158.43)	46	78.45 (68.07-90.41)
Consumed grayling	**Yes**	27	0.80 (0.32-2.01)	24	0.18 (0.07-0.51)	24	147.79 (139.72-156.32)	24	87.97 (72.73-106.42)
	No	39	0.52 (0.38-0.71)	32	LOD (<LOD -0.09)	32	153.88 (143.97-164.47)	32	69.85 (59.08-82.57)
	Yes	3	2.00 (0.64-6.19)	4	0.50 (<LOD -24.33)	4	155.90 (137.49-176.78)	4	96.67 (44.3-210.94)

Consumed walleye	**No**	63	0.59 (0.39-0.90)	52	0.07 (<LOD -0.13)	52	150.89 (143.94-158.17)	52	75.78 (66.53-86.31)
Consumed sucker	**Yes**	4	NA	5	0.27 (<LOD -6.91)	5	152.33 (131.51-176.44)	5	100.88 (54.26-187.55)
	No	62	NA	51	0.07 (<LOD -0.13)	51	151.13 (144.11-158.49)	51	75.1 (65.94-85.53)
Consumed fish eggs	**Yes**	21	1.05 (0.77-1.41)	19	0.10 (<LOD -0.32)	19	157.78 (151.33-164.5)	19	76.09 (62.96-91.95)
	No	45	0.49 (0.28-0.87)	37	0.07 (<LOD -0.15)	37	147.99 (138.9-157.66)	37	77.64 (65.53-91.98)
Consumed burbot liver	**Yes**	9	1.74 (1.22-2.5)	6	0.38 (<LOD -5.30)	6	160.32 (143.9-178.62)	6	91.28 (54.19-153.78)
	No	57	0.53 (0.34-0.84)	50	0.07 (<LOD -0.12)	50	150.18 (143.12-157.6)	50	75.56 (66.18-86.27)
Consumed moose liver	**Yes**	10	0.83 (0.45-1.51)	9	0.05 (<LOD -0.23)	9	165.48 (153.25-178.68)	9	92.07 (62.23-136.24)
	No	56	0.59 (0.37-0.95)	47	0.09 (0.05-0.18)	47	148.66 (141.45-156.23)	47	74.53 (65.14-85.27)
Consumed moose kidney	**Yes**	14	1.20 (0.75-1.92)	15	0.10 (<LOD -0.39)	15	157.24 (144.61-170.97)	15	88.02 (68.79-112.63)
	No	52	0.52 (0.32-0.86)	41	0.08 (<LOD -0.15)	41	149.10 (141.38-157.24)	41	73.46 (63.27-85.29)
Consumed caribou liver	**Yes**	8	0.90 (0.52-1.55)	7	0.06 (<LOD -0.52)	7	157.78 (144.79-171.92)	7	87.29 (47.26-161.21)
	No	58	0.59 (0.38-0.94)	49	0.09 (<LOD -0.16)	49	150.33 (143.08-157.94)	49	75.75 (66.75-85.98)
Consumed caribou kidney	**Yes**	14	1.19 (0.84-1.68)	12	0.06 (<LOD -0.25)	12	157.73 (149.08-166.89)	12	94.89 (71.47-125.98)
	No	52	0.52 (0.32-0.86)	44	0.09 (<LOD -0.17)	44	149.51 (141.61-157.86)	44	72.86 (63.29-83.89)
Consumed waterfowl	**Yes**	29	0.95 (0.66-1.36)	25	0.19 (0.07-0.53)	25	147.42 (135.60-160.27)	25	90.07 (74.06-109.54)
	No	37	0.45 (0.23-0.87)	31	LOD (<LOD-0.08)	31	154.39 (147.44-161.66)	31	68.02 (58.05-79.71)

[a]Sample sizes differ across variables based on the study components that participants completed

Note: Those foods not included in the FFQ for this region or with a sample size of less than 5 for one of the predictor levels are not included in this table. If there is a sample size less than 5 for one of the predictor levels for less than two of the biomarkers this predictor is still included in the table but no stratified geometric means are presented and are instead denoted by NA.

Supplementary Table 4.3C: Binary potential determinants of levels of mercury (hair and blood), selenium (blood), and EPA+DHA (plasma) levels for the Old Crow region

Predictor	Predictor Level	Hair Mercury		Blood Mercury		Blood Selenium		EPA+DHA	
		n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)	n[a]	Geometric mean (95%CI)
Sex	**Male**	32	0.32 (0.23-0.45)	27	0.77 (0.41-1.45)	27	171.18 (159.02-184.27)	27	66.50 (58.51-75.57)
	Female	41	0.28 (0.23-0.35)	29	0.66 (0.39-1.12)	29	173.22 (165.61-181.18)	29	85.70 (77.29-95.04)
Smoking in the previous 24 hours	**Yes**	32	0.26 (0.19-0.35)	23	0.71 (0.35-1.43)	23	174.34 (159.89-190.09)	23	71.72 (65.05-79.07)
	No	27	0.35 (0.26-0.47)	22	0.70 (0.35-1.39)	22	173.70 (164.64-183.27)	22	77.94 (65.85-92.26)
Alcohol use in the previous 24 hours	**Yes**	5	0.22 (0.10-0.48)	3	0.99 (0.17-5.83)	3	162.95 (123.32-215.30)	3	86.89 (38.18-197.72)
	No	55	0.30 (0.24-0.37)	43	0.67 (0.41-1.09)	43	174.97 (166.29-184.11)	43	73.43 (66.76-80.76)
Consumed whitefish	**Yes**	57	0.33 (0.26-0.40)	45	0.83 (0.53-1.31)	45	175.46 (167.33-183.98)	45	76.69 (69.76-84.29)
	No	10	0.16 (0.12-0.22)	6	0.18 (0.10-0.33)	6	155.95 (136.87-177.69)	6	65.34 (42.73-99.94)
Consumed inconnu	**Yes**	14	0.30 (0.17-0.53)	12	1.14 (0.66-1.96)	12	172.29 (159.39-186.23)	12	75.80 (63.24-90.85)
	No	53	0.29 (0.24-0.36)	39	0.60 (0.35-1.02)	39	173.27 (164.05-183.02)	39	75.09 (67.23-83.87)
Consumed lake trout	**Yes**	10	0.28 (0.13-0.59)	11	0.93 (0.33-2.62)	11	167.13 (158.04-176.74)	11	76.68 (64.65-90.96)
	No	57	0.30 (0.24-0.36)	40	0.64 (0.40-1.04)	40	174.70 (165.32-184.61)	40	74.87 (67.03-83.62)
Consumed burbot	**Yes**	19	0.35 (0.23-0.56)	16	1.40 (0.83-2.34)	16	179.69 (163.92-196.98)	16	76.84 (63.81-92.53)
	No	48	0.27 (0.22-0.33)	35	0.51 (0.29-0.88)	35	170.08 (161.52-179.10)	35	74.54 (66.78-83.20)
Consumed northern pike	**Yes**	6	0.14 (0.05-0.37)	5	0.35 (0.03-3.98)	5	164.72 (150.79-179.93)	5	68.15 (47.76-97.24)
	No	61	0.32 (0.26-0.38)	46	0.75 (0.49-1.16)	46	173.97 (165.69-182.67)	46	76.07 (68.95-83.93)
Consumed grayling	**Yes**	18	0.27 (0.20-0.37)	15	0.84 (0.41-1.73)	15	168.83 (158.74-179.55)	15	80.86 (66.43-98.43)
	No	49	0.30 (0.24-0.39)	36	0.64 (0.38-1.10)	36	174.82 (164.82-185.44)	36	73.03 (65.68-81.21)
Consumed chinook salmon	**Yes**	60	0.30 (0.25-0.38)	46	0.80 (0.53-1.22)	46	174.07 (165.77-182.78)	46	77.80 (70.79-85.51)
	No	7	0.21 (0.14-0.33)	5	0.19 (<LOD-1.80)	5	163.88 (151.98-176.71)	5	55.41 (40.85-75.18)
Consumed chum salmon	**Yes**	36	0.29 (0.21-0.39)	27	0.74 (0.40-1.35)	27	174.31 (162.07-187.47)	27	75.69 (68.27-83.92)
	No	31	0.30 (0.23-0.38)	24	0.65 (0.35-1.23)	24	171.62 (162.73-181.00)	24	74.77 (63.34-88.25)
Consumed coho salmon[c]	**Yes**	27	0.28 (0.20-0.39)	22	0.72 (0.37-1.39)	22	173.35 (161.75-185.79)	22	80.98 (71.50-91.73)
	No	40	0.30 (0.23-0.39)	29	0.68 (0.38-1.22)	29	172.80 (162.48-183.78)	29	71.18 (62.28-81.35)
Consumed muktuk	**Yes**	16	0.26 (0.15-0.43)	11	0.44 (0.11-1.70)	11	167.51 (154.40-181.72)	11	67.10 (51.97-86.64)
	No	51	0.30 (0.25-0.37)	40	0.79 (0.52-1.21)	40	174.59 (165.52-184.16)	40	77.67 (70.40-85.69)

Consumed fish eggs	**Yes**	31	0.33 (0.24-0.46)	25	1.03 (0.62-1.72)	25	177.15 (164.18-191.15)	25	77.51 (68.46-87.76)
	No	36	0.26 (0.21-0.33)	26	0.48 (0.24-0.93)	26	169.17 (160.69-178.11)	26	73.15 (63.45-84.33)
Consumed burbot liver	**Yes**	58	0.45 (0.26-0.77)	44	1.33 (0.48-3.66)	44	195.81 (164.06-233.71)	44	73.06 (48.11-110.96)
	No	9	0.27 (0.22-0.33)	7	0.63 (0.39-1.01)	7	169.67 (162.35-177.32)	7	75.61 (68.85-83.04)
Consumed moose liver	**Yes**	11	0.32 (0.15-0.66)	8	1.29 (0.61-2.74)	8	169.71 (153.17-188.03)	8	97.14 (75.41-125.13)
	No	56	0.29 (0.24-0.35)	43	0.62 (0.38-1.01)	43	173.67 (165.08-182.69)	43	71.77 (65.22-78.97)
Consumed moose kidney	**Yes**	23	0.39 (0.26-0.58)	18	1.43 (0.92-2.23)	18	177.59 (160.09-197.00)	18	86.55 (75.65-99.03)
	No	44	0.25 (0.20-0.31)	33	0.47 (0.26-0.84)	33	170.61 (163.29-178.25)	33	69.73 (61.98-78.44)
Consumed caribou liver	**Yes**	21	0.26 (0.17-0.39)	14	0.52 (0.18-1.50)	14	165.37 (155.54-175.83)	14	75.39 (63.23-89.89)
	No	46	0.31 (0.25-0.39)	37	0.78 (0.49-1.23)	37	176.03 (166.24-186.40)	37	75.20 (67.19-84.17)
Consumed caribou kidney	**Yes**	37	0.29 (0.23-0.38)	26	0.93 (0.54-1.60)	26	179.48 (167.43-192.39)	26	80.84 (71.18-91.83)
	No	30	0.29 (0.21-0.40)	25	0.51 (0.26-1.01)	25	166.59 (157.56-176.14)	25	69.85 (61.04-79.93)
Consumed waterfowl	**Yes**	27	0.39 (0.29-0.51)	20	1.08 (0.67-1.74)	20	181.57 (166.94-197.48)	20	77.94 (68.09-89.21)
	No	40	0.24 (0.19-0.32)	31	0.53 (0.28-0.98)	31	167.75 (159.56-176.37)	31	73.57 (64.69-83.68)

[a]Sample sizes differ across variables based on the study components that participants completed

Note: Those foods not included in the FFQ for this region or with a sample size of less than 5 for one of the predictor levels are not included in this table.

Supplementary Table 4.4: Demographic characteristics and traditional food consumption for the subset of participants with complete case data for the models to explain hair mercury concentrations

Characteristic	Characteristic Level	Dehcho	Sahtú	Old Crow	Total
		Arithmetic Mean ± SD or n (%)	Arithmetic Mean ± SD or n (%)	Arithmetic Mean ± SD or n (%)	Arithmetic Mean ± SD or n (%)
Age (years)		40 ± 20	47 ± 19	43 ± 16	42 ± 19
Sex	**Female**	46%	53%	52%	49%
	Male	54%	47%	48%	51%
BMI		28.72 ± 7.50	26.13 ± 5.58	27.45 ± 5.21	27.81 ± 6.73
Smoking (in the previous 24 hours)	**Yes**	32%	39%	52%	37%
	No	68%	61%	48%	63%
Alcohol consumption (in the previous 24 hours)	**Yes**	10%	16%	8%	11%
	No	90%	84%	92%	89%
Consumed whitefish	**Yes**	92%	74%	95%	88%
	No	8%	26%	5%	12%
Consumed inconnu	**Yes**	29%	26%	25%	28%
	No	71%	74%	75%	72%
Consumed cisco	**Yes**	NA	16%	NA	6%
	No	NA	84%	NA	94%
Consumed lake trout	**Yes**	56%	79%	25%	56%
	No	44%	21%	75%	44%
Consumed burbot	**Yes**	15%	25%	33%	21%
	No	85%	75%	67%	79%
Consumed northern pike	**Yes**	61%	16%	13%	40%
	No	39%	84%	87%	60%
Consumed grayling	**Yes**	10%	41%	28%	21%
	No	90%	59%	72%	79%
Consumed walleye	**Yes**	51%	5%	NA	30%
	No	49%	95%	NA	70%
Consumed sucker	**Yes**	25%	7%	NA	16%
	No	75%	93%	NA	84%
Consumed chinook salmon	**Yes**	NA	NA	87%	16%
	No	NA	NA	13%	84%
Consumed chum salmon	**Yes**	NA	NA	58%	11%
	No	NA	NA	42%	89%
Consumed coho salmon	**Yes**	NA	NA	45%	8%
	No	NA	NA	55%	92%
Consumed muktuk	**Yes**	NA	NA	25%	5%
	No	NA	NA	75%	95%
Consumed fish eggs	**Yes**	30%	37%	50%	35%

	No	70%	63%	50%	65%
Consumed burbot liver	**Yes**	53%	16%	13%	11%
	No	47%	84%	87%	89%
Consumed moose livers	**Yes**	26%	17%	17%	22%
	No	74%	83%	83%	78%
Consumed moose kidneys	**Yes**	30%	21%	37%	29%
	No	70%	79%	63%	71%
Consumed caribou livers	**Yes**	13%	14%	33%	17%
	No	87%	86%	67%	83%
Consumed caribou kidneys	**Yes**	11%	22%	54%	23%
	No	89%	78%	46%	77%
Consumed waterfowl	**Yes**	61%	47%	43%	54%
	No	39%	53%	57%	46%
Consumed wild bird eggs	**Yes**	8%	NA	8%	6%
	No	92%	NA	92%	94%

For these complete cases, the sample size was 121 for the Dehcho region, 57 for the Sahtú region, and 40 for Old Crow. Mean total hair Hg concentrations for the complete case data were 0.65 ± 0.91 µg/g for the Dehcho region, 1.09 ± 0.93 µg/g for the Sahtú region, and 0.43 ± 0.35 µg/g for Old Crow.

Supplementary Table 4.5: Demographic characteristics and traditional food consumption for the subset of participants with complete case data for the models to explain blood mercury, blood selenium, and plasma omega-3 fatty acid concentrations

Characteristic	Characteristic Level	Dehcho	Sahtú	Old Crow	Total
		Arithmetic Mean ± SD or n (%)	Arithmetic Mean ± SD or n (%)	Arithmetic Mean ± SD or n (%)	Arithmetic Mean ± SD or n (%)
Age (years)		45 ± 17	45 ± 19	43 ± 16	44 ± 17
Sex	**Female**	53%	55%	54%	54%
	Male	47%	45%	46%	46%
BMI		29.30 ± 7.47	25.59 ± 5.62	27.49 ± 5.15	27.72 ± 6.57
Smoking (in the previous 24 hours)	**Yes**	39%	47%	49%	44%
	No	61%	53%	51%	56%
Alcohol consumption (in the previous 24 hours)	**Yes**	14%	18%	7%	14%
	No	86%	82%	93%	86%
Consumed whitefish	**Yes**	93%	76%	95%	88%
	No	7%	24%	5%	12%
Consumed inconnu	**Yes**	26%	29%	27%	27%
	No	74%	71%	73%	73%
Consumed cisco	**Yes**	NA	12%	NA	4%
	No	NA	88%	NA	96%
Consumed lake trout	**Yes**	57%	82%	27%	57%
	No	43%	18%	73%	43%
Consumed burbot	**Yes**	18%	18%	34%	22%
	No	82%	82%	66%	78%
Consumed northern pike	**Yes**	56%	18%	12%	33%
	No	44%	82%	88%	67%
Consumed grayling	**Yes**	8%	41%	29%	23%
	No	92%	59%	71%	77%
Consumed walleye	**Yes**	50%	6%	NA	24%
	No	50%	94%	NA	76%
Consumed sucker	**Yes**	24%	8%	NA	14%
	No	76%	92%	NA	86%
Consumed chinook salmon	**Yes**	NA	NA	88%	22%
	No	NA	NA	12%	78%
Consumed chum salmon	**Yes**	NA	NA	56%	14%
	No	NA	NA	44%	86%
Consumed coho salmon	**Yes**	NA	NA	44%	11%
	No	NA	NA	56%	89%
Consumed muktuk	**Yes**	NA	NA	24%	6%
	No	NA	NA	76%	94%

Consumed fish eggs	**Yes**	33%	37%	49%	38%
	No	67%	63%	51%	62%
Consumed burbot liver	**Yes**	53%	12%	12%	11%
	No	47%	88%	88%	89%
Consumed moose livers	**Yes**	30%	16%	17%	23%
	No	70%	84%	83%	77%
Consumed moose kidneys	**Yes**	40%	22%	37%	34%
	No	60%	78%	63%	66%
Consumed caribou livers	**Yes**	14%	12%	29%	17%
	No	86%	88%	71%	83%
Consumed caribou kidneys	**Yes**	12%	18%	49%	23%
	No	88%	82%	51%	77%
Consumed waterfowl	**Yes**	63%	45%	41%	52%
	No	38%	55%	59%	48%
Consumed wild bird eggs	**Yes**	10%	NA	7%	52%
	No	90%	NA	93%	48%

For these complete cases, the sample size was 72 for the Dehcho region, 49 for the Sahtú region, and 41 for Old Crow. Mean total blood Hg concentrations for the complete case data were 0.94 ± 2.46 µg/L for the Dehcho region, 0.76 ± 1.62 µg/L for the Sahtú region, and 1.51 ± 1.44 µg/L for Old Crow.

Supplementary Table 4.6: AIC values for backwards selection of model to explain log_2-tranformed hair mercury concentrations

Step	Variable Removed	AIC	ΔAIC
0	**Base Model**	568.4	31.39
1	**Consuming Caribou Kidneys**	566.4	29.40
2	**Consuming Burbot**	564.4	27.40
3	**Sex**	562.5	25.49
4	**Consuming Chinook Salmon**	560.7	23.66
5	**Consuming Coho Salmon**	558.8	21.78
6	**Consuming Caribou Livers**	557.0	20.03
7	**Consuming Burbot Livers**	555.3	18.30
8	**Consuming Gamebird Eggs**	553.6	16.63
9	**Consuming Northern Pike**	552.0	14.95
10	**Consuming Walleye**	550.3	13.26
11	**Consuming Inconnu**	548.7	11.73
12	**BMI**	547.3	10.28
13	**Consuming Sucker**	545.8	8.79
14	**Consuming Chum Salmon**	544.5	7.49
15	**Consuming Fish Eggs**	543.1	6.05
16	**Smoking**	542.0	5.01
17	**Consuming Muktuk**	541.1	4.05
18	**Consuming Grayling**	540.7	3.71
19	**Alcohol Use**	540.2	3.16
20	**Consuming Whitefish**	539.7	2.72
21	**Consuming Moose Livers**	539.3	2.24
22	**Consuming Moose Kidneys**	538.0	1.01
23	**Region**	538.0	0.96
24	**Consuming Cisco**	537.0*	0.00*

* Optimal AIC value reached, the final model to explain Log_2 hair mercury concentrations had the variables: age, consuming lake trout, and consuming waterfowl.

Supplementary Table 4.7: AIC values for backwards selection of Tobit model to explain log_2-tranformed blood mercury concentrations

Step	Variable Removed	AIC	ΔAIC
0	**Base Model**	597.18	27.52
1	**Consuming Burbot Liver**	595.20	25.54
2	**Consuming Moose Kidneys**	593.22	23.56
3	**Consuming Chum Salmon**	591.27	21.61
4	**Consuming Caribou Livers**	589.36	19.70
5	**Consuming Caribou Kidneys**	587.40	17.74
6	**Consuming Cisco**	585.74	16.08
7	**Consuming Sucker**	584.14	14.48
8	**Consuming Muktuk**	582.69	13.03
9	**Consuming Fish Eggs**	581.40	11.74
10	**Smoking**	580.27	10.61
11	**Consuming Chinook Salmon**	579.28	9.62
12	**Consuming Coho Salmon**	578.11	8.45
13	**Consuming Northern Pike**	577.01	7.35
14	**Consuming Walleye**	576.06	6.40
15	**Consuming Lake Trout**	574.89	5.23
16	**Sex**	573.67	4.01
17	**Consuming Gamebird Eggs**	572.88	3.22
18	**Consuming Burbot**	571.98	2.32
19	**Consuming Inconnu**	570.87	1.21
20	**Consuming Whitefish**	570.26	0.60
21	**Alcohol Use**	569.82	0.16
22	**Consuming Moose Liver**	569.66*	0.00*

* Optimal AIC value reached, the final model to explain Log_2 blood mercury concentrations had the variables: age, region, BMI, consuming grayling, and consuming waterfowl.

Supplementary Table 4.8: AIC values for backwards selection of model to explain blood selenium concentrations

Step	Variable Removed	AIC	ΔAIC
0	**Base Model**	1239.05	26.87
1	**Consuming Lake Trout**	1237.05	24.87
2	**Consuming Sucker**	1235.05	22.87
3	**Consuming Coho Salmon**	1233.06	20.88
4	**Consuming Gamebird Eggs**	1231.10	18.92
5	**Consuming Moose Kidneys**	1229.13	16.95
6	**Consuming Waterfowl**	1227.21	15.03
7	**Consuming Walleye**	1225.31	13.13
8	**Alcohol Use**	1223.42	11.24
9	**Consuming Muktuk**	1221.61	9.43
10	**Consuming Chum Salmon**	1219.87	7.69
11	**Consuming Cisco**	1218.28	6.10
12	**Consuming Burbot**	1216.86	4.68
13	**Age**	1215.51	3.33
14	**Smoking**	1214.36	2.18
15	**Consuming Fish Eggs**	1213.23	1.05
16	**Consuming Moose Liver**	1212.29	0.11
17	**Consuming Inconnu**	1212.18*	0.00*

* Optimal AIC value reached, the final model to explain blood selenium concentrations had the variables: BMI, sex, region, consuming whitefish, consuming northern pike, consuming grayling, consuming chinook salmon, consuming burbot livers, consuming caribou livers, and consuming caribou kidneys.

Supplementary Table 4.9: AIC values for backwards selection of model to explain log_2-tranformed plasma EPA+DHA concentrations

Step	Variable Removed	AIC	ΔAIC
0	**Base Model**	-37.73	24.53
1	**Region**	-40.19	22.07
2	**Consuming Caribou Liver**	-42.18	20.08
3	**Consuming Fish Eggs**	-44.16	18.10
4	**Consuming Grayling**	-46.11	16.14
5	**Consuming Burbot Liver**	-48.00	14.26
6	**BMI**	-49.88	12.38
7	**Consuming Chinook Salmon**	-51.61	10.65
8	**Consuming Burbot**	-53.29	8.97
9	**Consuming Muktuk**	-54.93	7.33
10	**Consuming Cisco**	-56.59	5.66
11	**Consuming Chum Salmon**	-57.66	4.60
12	**Consuming Gamebird Eggs**	-58.41	3.84
13	**Consuming Caribou Kidney**	-59.35	2.91
14	**Consuming Moose Kidney**	-60.32	1.93
15	**Consuming Sucker**	-61.51	0.75
16	**Consuming Moose Liver**	-62.26*	0.00*

* Optimal AIC value reached, the final model to explain log_2 plasma EPA+DHA concentrations had the variables: age, sex, consuming whitefish, consuming inconnu, consuming lake trout, consuming northern pike, consuming walleye, consuming coho salmon, consuming waterfowl, smoking, and alcohol use.

Chapter 5: Refining the Characterization of Mercury, Selenium, and Omega-3 Fatty Acid Exposure from Fish Consumption in the Northwest Territories

5.1 Abstract

Consumption of locally harvested freshwater fish provides northern Indigenous communities with important nutrients such as Se and omega-3 PUFA. However, elevated concentrations of total Hg have been measured in some freshwater species of fish from some waterbodies in northern Canada. In this study, co-located human biomonitoring and fish monitoring projects provides a unique opportunity to conduct a site-specific exposure assessment for MeHg, Se, and omega-3 PUFA from local fish consumption. We constructed a probabilistic exposure assessment model for MeHg, Se, and omega-3 PUFA (specifically Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)) for the Dehcho and Sahtú regions of the Northwest Territories, Canada, using local and species-specific fish analyte data, and compared generated exposure estimates with biomarker-based measures of internal dose. We then refined the model by: 1) including lake-specific fish analyte data; and, 2) including total Hg bioaccessibility data (for the MeHg exposure assessment only) and assessed whether these refinements improved the association between exposure estimates and biomarker concentrations for total Hg, Se, and EPA+DHA.

The inclusion of waterbody-specific MeHg concentrations and fish species-specific Hg bioaccessibility data in the model resulted in lower estimated MeHg intakes. On average estimated MeHg intake was 39% and 23% lower in the model with waterbody-specific fish analyte MeHg data, and 52% and 38% lower in the model with Hg bioaccessibility data, when compared to the base model for the Dehcho and Sahtú regions respectively. Correlations between MeHg exposure estimates and total Hg biomarkers were similar across models. Estimates from the refined model appeared more consistent with hair and blood mercury concentrations (the vast majority of total Hg biomarker levels were below health-based guidance values). Estimates from the refined model were lower and fewer estimates exceeded tolerable daily intakes. Model estimates and total Hg biomarker levels indicated that although Hg concentrations are elevated in certain fish species in some waterbodies in the two northern regions studied, Hg exposures in people living in these regions are relatively low. Model estimates for Se and EPA+DHA

indicated that the consumption of locally harvested fish is an important contributor to dietary intakes of these nutrients for local populations of people. However, similar to other Canadian populations, the majority of estimated EPA+DHA intakes fell below recommendations. In order to refine these assessments, additional fish analyte data (especially Se and EPA+DHA concentrations, and bioaccessibility data) should be generated for the waterbodies that local communities regularly harvest fish from. Although the consumption of locally caught freshwater fish is a source of Hg exposure, Hg concentrations in local people have stayed relatively low and locally caught fish are an important source of essential nutrients.

5.2 Introduction

Locally harvested traditional foods (or country foods)[1] provide Indigenous peoples with important nutritional (AMAP, 2021a; Van Oostdam et al., 2005; Kuhnlein, 1995), economic (AMAP, 2021; Lamden et al., 2007; Van Oostdam et al., 2005), social (AMAP, 2021a; Lamden et al., 2007; Van Oostdam et al., 2005), and cultural benefits (AMAP, 2021a; Lamden et al., 2007; Van Oostdam et al., 2005). Traditional foods have higher nutrient density compared to store-bought foods and colonial impacts have influenced a shift away from traditional foods to increased processed food consumption is associated with decreased micronutrient intake (AMAP, 2021a; Batal et al., 2021; Sheehy et al., 2015; Kuhnlein, 2003). Decreasing traditional food consumption can negatively affect the diet quality and health of Indigenous populations (AMAP, 2021a; Rudolph et al., 2013; Receveur et al., 1997). Fish and other traditional foods are important sources of many nutrients and vitamins including omega-3 PUFA and Se (AMAP, 2021a; Batal et al., 2021; Chan et al., 2006; Van Oostdam et al., 1999). In the Northwest Territories, Canada, monitoring projects have measured fish Hg concentrations in waterbodies for over 30 years (AMAP, 2021b; NCP, 2003).

Hg is a ubiquitous environmental contaminant, and global public health concern (AMAP, 2021b; Ruggieri et al., 2017). Hg emitted from anthropogenic sources can be transmitted long

[1] The term “traditional foods” is typically used in First Nations and Métis communities, while the term “country foods” is typically used in Inuit communities. These terms are for any foods (including fish, mammals, birds, and plants) that are locally caught, gathered, hunted and/or harvested by Indigenous populations and that have sociocultural meanings. As the study included in this manuscript was conducted with First Nations communities in northern Canada, the term traditional foods will be used throughout this manuscript.

distances through air and ocean currents even to remote areas including the Canadian arctic and sub-arctic (AMAP, 2021b). Physical and ecological changes due to climate change, especially in northern regions (such as increased permafrost thaw and increased river runoff), may change the transport and cycling of Hg in the environment (Dastoor et al., 2022; AMAP, 2021b; St. Pierre et al., 2018). Although there are multiple routes of exposure to Hg for humans, fish consumption is considered a major source for the Canadian general population (CHMS, 2019).

Exposure to elevated levels of MeHg can lead to neurodevelopmental impairment as well as harmful cardiovascular and reproductive effects (Rice et al., 2014; Driscoll et al., 2013; Khan & Wang, 2009). Early life stages, especially the developing foetus and infants, are particularly susceptible to the harmful effects of MeHg exposure as their nervous system is developing and sensitive to the neurotoxic effects (Ruggieri et al., 2017; Driscoll et al., 2013). This can represent a human health risk, particularly for populations that consume significant amounts of fish containing elevated levels of Hg (Driscoll et al., 2013; AMAP, 2021b).

In the Dehcho and Sahtú regions of the Mackenzie Valley of the Northwest Territories, elevated concentrations of total Hg were measured in some fish species from some lakes. As a result, food consumption notices were released advising people to limit their consumption of some species of fish (predatory species such as Northern Pike (*Esox lucius*), Lake Trout (*Salvelinus namaycush)*, and Walleye (*Sander vitreus*) from specific lakes (DHSS, 2012). However, if concerns about environmental contaminants contribute to a broad-scale decline in the consumption of many types of traditional foods, they can have the potential to do more harm than good (Zhang et al., 2014; Laird et al., 2013; Berti et al., 1998) Therefore, a community-based human biomonitoring project, that assessed contaminant and nutrient levels, was developed and implemented in First Nations communities in the Dehcho and Sahtú regions to address their concerns about contaminant exposures (Ratelle et al., 2018a; Ratelle et al., 2018b). Additionally, recent efforts have focused on developing communication materials that are based upon not only contaminant risks but also nutritional benefits for traditional foods (AMAP, 2021a).

Hg exposure assessments based upon intake reconstructions rely on food Hg concentrations as well as several other variables (e.g., bodyweight, bioavailability, consumption rates) (Tran et al., 2004). Often such intake reconstructions take the form of a deterministic model utilizing

point estimates (e.g., mean, median, maximum) for the inputs (Solomon, 1996). Probabilistic approaches can alternatively be used to reconstruct exposure by modeling distributions of various exposure determinants, such as food Hg concentrations (Tran et al., 2004; Finley & Paustenbach, 1994). Such probabilistic models, for example, those based upon Monte Carlo techniques, randomly sample within each of the input variable distributions to generate forecasted distributions of exposure. These probabilistic models provide more information than deterministic models (that use point estimates) and make better use of available data (Finley & Paustenbach et al., 1994).

Inclusion of bioaccessibility data may also be useful in Hg exposure assessments. Bioaccessibility is the amount (fraction) of a chemical present in ingested food, water, or soil, that is available for absorption in the gastrointestinal tract after digestion, while bioavailbility is the amount of a chemical present in ingested food, water, or soil, that is absorbed and reaches systemic circulation (Peijnenburg & Jager, 2003). Exposure assessments often assume that 95-100% of ingested Hg reaches systemic circulation, but *in vitro* studies have indicated that Hg bioaccessibility and bioavailability for many species and foods can be far less than 95% (Bradley et al., 2017; Siedlikowski et al., 2016). Bioaccessibility is often used as a conservative estimate for bioavailability in exposure assessments (Bradley et al., 2017). While it is known that total Hg bioaccessibility varies between and within fish species (Bradley et al., 2017; Cano-Sancho, 2015; Torres-Escribano, 2011), bioaccessibility data for North American fish samples, especially northern freshwater fish, is limited. It is also unclear if inclusion of bioaccessibility data improves exposure assessments. For example, Siedlikowski et al. (2016) found that the inclusion of bioaccessibility data did not significantly alter the parameters of their exposure assessment models.

In the Dehcho and Sahtú regions of the Northwest Territories, Canada, the co-location of a human biomonitoring project and on-going fish monitoring research provides a unique opportunity to conduct a site-specific exposure assessment model and to explore whether the inclusion of total Hg bioaccessibility data improves exposure estimates. Further, conducting such analyses in these regions, where elevated total Hg concentrations have been measured in some fish populations, will provide important information to local communities who rely on the consumption of freshwater fish. Because fish consumption is an important dietary source of

nutrients including Se and omega-3 PUFA (such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)), exposure to these nutrients should also be considered in a balanced approach to evaluating risk.

The aims of this study were to: 1) construct a probabilistic exposure assessment model for MeHg, Se, and the omega-3 PUFA EPA and DHA in the Dehcho and Sahtú regions, with species-specific fish analyte data from the Northwest Territories; 2) compare base models of exposure with models that were refined to consider lake-specific fish analyte data and which included mercury bioaccessibility data (mercury exposure assessment only); and, 3) compare generated exposure estimates with biomarker-based measures of internal dose. We hypothesized that inclusion of lake-specific fish analyte data, and inclusion of bioaccessibility data, would improve associations between modeled exposure estimates and measured biomarker concentrations for total Hg, Se, and EPA+DHA in local human populations.

5.3 Methods

As previously described (Ratelle et al., 2018a; Ratelle et al., 2018b), a human biomonitoring project was conducted in the Mackenzie River Valley of the Northwest Territories, Canada, from 2016-2018 to investigate and address concerns of local Indigenous communities about exposure to environmental contaminants. Six communities from the Dehcho region and three communities from the Sahtú region took part in the biomonitoring project. Passive and active recruitment methods were used and anyone over the age of 6 years old (with parent/guardian consent) could participate in the study. Informed consent was obtained, and ethics approval was received from relevant authorities, including: the University of Waterloo Research Ethics Committee, the Stanton Territorial Health Authority for Human Research, and the Aurora Research Institute. Data were managed according to the approved data management plan.

The biomonitoring project included several components that participants could opt-into including: provision of basic demographic information and anthropometric measures (including age, sex, and bodyweight), provision of biological samples (including hair, blood, and/or urine), participation in dietary questionnaires (a food frequency questionnaire (FFQ) and/or 24-hour dietary recall), and participation in a Health Messages Survey about risk perception. The FFQ included questions about the consumption of traditional foods, including specific species of fish,

over the past year. The results of the FFQ have been previously described (Ratelle et al., 2020a); a total of 237 participants (154 from the Dehcho region and 83 from the Sahtú region) completed the FFQ representing approximately 8% of the population of participating communities. Previously, the fish species most commonly consumed by participants were identified as Lake Whitefish (*Coregonus clupeaformis)*, 87% of participants reporting consuming), Lake Trout (*Salvelinus namaycush* , 62% of participants reporting consuming), Northern Pike (*Esox lucius*, also known as Jackfish 44% of participants reporting consuming), and Walleye (*Sander vitreus*, also known as pickerel, 34% of participants reporting consuming) (Ratelle et al., 2020a).

For participants who had completed the FFQ, probabilistic models were constructed to estimate participants' MeHg, Se, and EPA + DHA intake from consumption of the four most commonly consumed locally harvested fish species. These models were constructed using Oracle Crystal Ball (Release 11.1.2.4.600) software and incorporated: 1) dietary, demographic, and anthropometric information of participants from the Mackenzie Valley Biomonitoring project, and 2) fish tissue analyte data (Hg, Se, and EPA + DHA concentrations) from ongoing fish sampling projects in the region and the literature.

5.3.1 Dietary, Demographic, and Anthropometric Data Included in the Models

The FFQ included questions about participants' frequency of consumption of these four most commonly consumed fish (Lake Whitefish, Lake Trout, Northern Pike, and Walleye) over the past year, and about portion sizes. Using the frequency of consumption for each fish species (in times consumed per week, on average, over the past year), and the number of servings participants reported typically consuming in a fish meal, estimated intake rates in grams/week were calculated for each participant for each of the four most commonly consumed fish species. This calculation (**Figure 5.1**) was conducted for those participants who had completed the FFQ and had provided their age and sex. Portion size was determined through the FFQ as previously described (Ratelle et al., 2020) with the use of six pictures of different portion sizes of fish (pan fried sole) representing 0.5, 1. 1.5, 2, 2.5, and 3 servings of fish (based on serving sizes from Canada's Food Guide) and one serving of fish was assumed to be 75 grams (Government of Canada, 2017). For participants for which portion size data were missing (~27%), the arithmetic mean serving size for that age group (<18, 18-40, >40-60, and 60+ years of age) and sex from the other communities from that region (Dehcho versus Sahtú) was substituted into the

calculation so the intake rate for those participants could still be calculated. This resulted in a total of 148 participants from the Dehcho region and 80 from the Sahtú region having species-specific estimated fish intake rates that were included in the models. A maximum total fish intake rate of 870 grams/week (which is approximately the consumption of seven fish meals per week at the mean serving size reported for these regions) was set. For those participants that exceeded this maximum value (<8%), species-specific intake rates were adjusted so that total fish intake rate was equal to 870 grams/week; this was done to reduce overestimates of fish consumption.

$$Fish\ Intake\ Rate = Fish\ Consumption\ Frequency\ \left(\frac{Times}{Week}\right) \times Number\ of\ Servings\ \times 75 \frac{grams}{Serving}$$

Figure 5.1: Formula for Calculating Estimated Fish Species-Specific Intake Rates

Similarly, for participants with missing bodyweight data, arithmetic mean bodyweight (kg) for that age and sex group from that region was substituted for the missing values. Summary statistics for species-specific intake rates calculated for participants and used in the models are presented in **Supplementary Table 5.1**, by region. Summary statistics for the age, sex, and bodyweight of the participants used in the models are presented in **Supplementary Table 5.2**, by region.

5.3.2 Fish Analyte Data Included in the Models

Fish total Hg, Se, and EPA+DHA concentration data for each of: Lake Whitefish, Lake Trout, Northern Pike, and Walleye, from lakes and rivers in the Northwest Territories were gathered from peer-reviewed publications and regional reports (Macdonald, 2013; 2011; 2009; 2002; Fisheries and Aquatic Resources, 2010; Doetzel, 2007; Rescan Environmental Services, 2005; Stewart et al., 2003; Evans, 1998; Grey et al., 1995), and raw data shared by northern partners and researchers (including from ongoing fish sampling programs and from the research projects of Dr. Heidi Swanson and Dr. Marlene Evans). Summary statistics for the fish species-specific concentrations of total Hg, total Se, and EPA + DHA used in the models is included in **Supplementary Table 5.3**. Additionally, participants could indicate through the FFQ where they had gotten fish from in the past year. Local waterbodies that 25% or more of the participants in a community reported getting fish from over the past year included: Great Slave Lake, the

Mackenzie River, Kakisa Lake, Willow Lake, Jean Marie River, Ekali Lake, Kelly Lake, Tathlina Lake, Trout Lake, Great Bear Lake, and Colville Lake. Where data were available, summary statistics for species-specific, and waterbody-specific fish analyte concentrations were calculated and are presented in **Supplementary Table 5.4.** When individual-level fish analyte data were not available for a specific waterbody, aggregate data were extracted from the literature.

Finally, a small dataset for the bioaccessibility of total Hg in fish samples from the Northwest Territories was available and included in the models. As previously described, bioaccessibility of total Hg was measured in the muscle of freshwater fish species from northern Canada, and included samples of Lake Whitefish, Lake Trout, Northern Pike, and Walleye collected from waterbodies in the Northwest Territories (Dehcho region) (Packull-McCormick, 2023a). Summary statistics for bioaccessibility of total Hg in Lake Whitefish, Lake Trout, Northern Pike, and Walleye are included in **Supplementary Table 5.5**.

5.3.3 Probabilistic Models

Three probabilistic exposure assessment models were constructed using Oracle Crystal Ball software. The input variables for Model 1 included species-specific fish analyte data, species-specific fish intake rates of participants, and the body weight of participants. These inputs were used to estimate MeHg, total Se and EPA + DHA, intake from the consumption of Lake Whitefish, Lake Trout, Northern Pike, and Walleye. Although the fish Hg data used in these models was total Hg, due to the relatively limited availability of MeHg data for these fish species from specific waterbodies in the Northwest Territories, in the models the conservative assumption was made that 100% of the total Hg was in the form of MeHg as typically the majority of total Hg in fish muscle is in the form of MeHg (Storelli et al., 2005; Arroyo-Abad et al., 2016; Cabañero et al., 2016). Individual-level fish intake rates were used so that this could be linked to other variables (such as individual-level bodyweight, age, and sex data as well as biomarker data if available). Summary statistics for individual-level intake rates in this model were similar to those generated in previous models that used log-normal probability distributions (Laird., 2017) or custom distributions for four age categories (<18, 18-39, 40-59, and 60+ years of age) (Packull-McCormick et al., 2020b) for fish intake rates. As previously described (Laird et al., 2017), log-normal probability distributions were constructed for species-specific fish tissue

analyte concentrations (see **Supplementary Table 5.3**). Visual inspection of the data and Oracle Crystal Ball distribution fitting confirmed that a log-normal distribution most closely approximated the shape of the distributions for species-specific fish analyte concentrations. Input variables included in Model 1 were used to estimate: MeHg intake, total Se intake, and EPA+DHA intake, from each of the four fish species consumed and summed across the four species (equations for these estimates shown in **Figure 5.2**). For each participant, a total of 50 trials (for a total of 11,400 trials) were run.

$$Individual\ Estimated\ Methylmercury\ Intake\ \left(\frac{\frac{ug}{kg}}{day}\right) = \frac{Intake\ Rate\left(\frac{g}{week}\right) \times Fish\ Mercury\ Concentration\ \left(\frac{ug}{g}\right)}{Bodyweight\ (kg) \times 7\frac{days}{week}}$$

$$Individual\ Estimated\ Total\ Selenium\ Intake\ \left(\frac{ug}{day}\right) = \frac{Intake\ Rate\left(\frac{g}{week}\right) \times Fish\ Selenium\ Concentration\ \left(\frac{ug}{g}\right)}{7\frac{days}{week}}$$

$$Individual\ Estimated\ EPA\&DHA\ Intake\ \left(\frac{mg}{day}\right) = \frac{Intake\ Rate\left(\frac{g}{week}\right) \times Fish\ EPA\&DHA\ Concentrations\ \left(\frac{mg}{g}\right)}{7\frac{days}{week}}$$

Figure 5.2: Equations to Estimate Fish Species-specific Participant Methylmercury, Total Selenium, and EPA+DHA Intake

The second model included the same input data as Model 1 but used both waterbody-specific and species-specific fish analyte concentrations (compared to species-specific only in Model 1). To incorporate waterbody-specific data in the model, log-normal probability distributions were created for each fish species and analyte of interest in each of the most commonly reported waterbodies. When individual-level fish analyte data were not available for a specific fish species and waterbody, aggregate data from the literature for that species and waterbody were used to shape the probabilistic distribution (**Supplementary Table 5.4**). If individual and aggregate level data were not available for a specific fish species and waterbody, then the species-specific fish analyte distributions from Model 1 were used. A weighted average of the fish species-specific analyte concentration selected from each waterbody during each trial was calculated and this weighted average analyte concentration was used as the analyte concentration in the model equations (**Figure 5.2**). The weights for each waterbody, used in the weighted average calculation, were determined at the community level based on the percent of participants reporting consuming fish from each of the eleven selected waterbodies (community-

level data not shown to maintain confidentiality). A total of 50 trials was also run for each participant for Model 2.

Finally, Model 3 included the same input variables and distributions as Model 2, but also included total Hg bioaccessibility data. A normal probability distribution was constructed for the total Hg bioaccessibility data for each fish species. This bioaccessibility data were incorporated into the equation to estimate MeHg intake as shown in **Figure 5.3**. Model 3 was only used to estimate MeHg intake (from each fish species and summed across the fish species) as unfortunately, no bioaccessibility data for Se or EPA+DHA was available for these fish species.

$$Individual\ Estimated\ Methylmercury\ Intake\left(\frac{\frac{ug}{kg}}{day}\right) = \frac{Intake\ Rate\left(\frac{g}{week}\right) \times \left(Fish\ Mercury\ Concentration\left(\frac{ug}{g}\right) \times Mercury\ Bioaccessibility\right)}{Bodyweight\ (kg) \times 7\frac{days}{week}}$$

Figure 5.3: Equation to Estimate Fish Species-specific Participant Methylmercury Intake Incorporating Mercury Bioaccessibility (Model 3)

A summary and comparison of the input data included in the three models in included as **Figure 5.4**. Additionally, for each model, sensitivity analyses were conducted in Crystal Ball to identify the main drivers of variability in estimated intakes of MeHg, total Se, and EPA+DHA from consumption of the four fish species of interest.

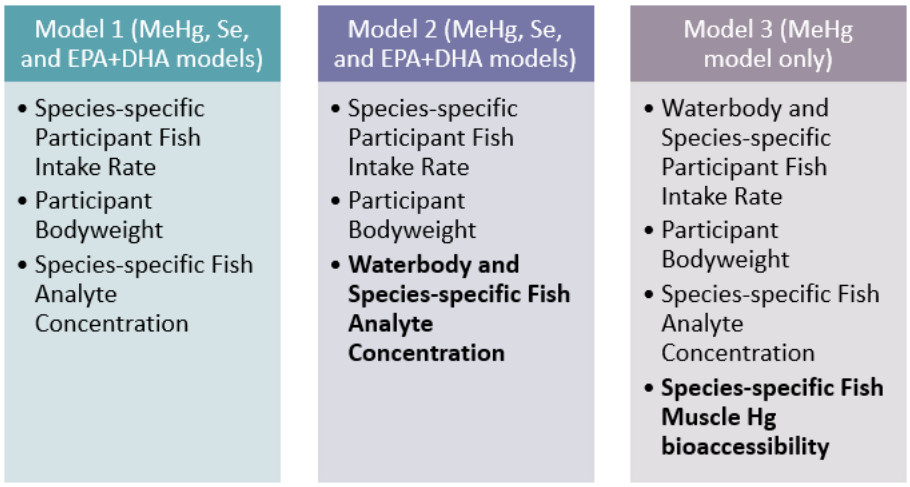


Figure 5.4: Summary and Comparison of the Input Data Included in the Models (Differences in the Model Compared to the Previous Model Bolded)

5.3.4 Statistical Analyses

After running 50 trials per participant (total of 11,400 trials), for each of the three models, summary statistics were calculated for intakes of each analyte from each fish species. For each of the three models, intakes (stratified by region) were then summed across the fish species. Mean intake was calculated for each participant (across the 50 trials), and the mean estimated total intake for each analyte for each participant was compared to relevant tolerable daily intakes (TDI) and/or recommended dietary intakes (RDIs) to determine percent of estimates exceeding TDIs and/or below RDIs. Biomarker data (human hair total Hg, blood total Hg, blood total Se, and plasma EPA+DHA concentrations) were also available for some of the participants. Collection and analysis of these biological samples has been previously described (Ratelle et al., 2020b; 2018a; 2018b; Packull-McCormick et al., 2023b; 2022). For those participants included in the models that also had biomarker concentration data available, Spearman's rank correlations were calculated between the model estimates and the relevant biomarker concentrations for each model. For blood total Hg concentrations, a significant proportion of participants (>50%) had blood total Hg concentrations below limit of detection (LOD) and for these participants, a value of half the LOD was substituted. Biomarker concentrations were also compared to relevant health-based guidance values and/or biomonitoring equivalents, and the percent of participants exceeding health-based guidance values and/or meeting recommended intakes was calculated. Strengths of correlations between model estimates and biomarker concentrations were compared. The percent of participants with estimates exceeding TDIs/RDIs from each model were also compared to the percent of participants with biomarker levels exceeding health-based guidance values/biomonitoring equivalents, when available.

5.4 Results

5.4.1 Exposure Estimates for Mercury Intake

Summary statistics were calculated for estimated MeHg intakes generated from all three models (**Table 5.1**). For participants from the Dehcho region, consumption of Walleye (35-40%) followed by Northern Pike (27-30%) contributed the most to average estimated MeHg intake across all three models. However, in the Sahtú region, the consumption of Lake Trout (63-71%) followed by Lake Whitefish (20-25%) contributed the most to the average estimated MeHg

intake across all three models. Average estimated MeHg intakes were compared to tolerable daily intakes (TDIs) for MeHg. A TDI of 0.23 μg/kg/day of MeHg (WHO, 2003) was used for children (<18 years of age) and women of childbearing age (adult women aged 18-<40 years of age) and a TDI of 0.47 μg/kg/day of MeHg (Health Canada, 2007) was used for adult men (18+ years of age) and women 40 years of age or older. The majority of participants in both regions had an estimated MeHg intake below the relevant TDI for their age and sex group. The percentage of participants with an average estimated MeHg intake above the relevant TDI was highest in Model 1 (Dehcho region: 17%, Sahtú region: 10%), followed by Model 2 (Dehcho region: 6%, Sahtú region: 6%), and was lowest in Model 3 (Dehcho region: 4%, Sahtú region: 1%). Histograms of the average estimated MeHg intakes are compared for the three models by region in **Supplementary Figures 5.1A** and **5.1B**.

Sensitivity analyses were conducted and the four variables that contributed the most to uncertainty in estimated MeHg intakes were consistently the intake rates of the four fish species. For example, in Model 1, the intake rates of Walleye, Northern Pike, Lake Whitefish, and Lake Trout contributed 26.2%, 26.2%, 25.5%, and 15.8% respectively, to the variance in estimates for MeHg intakes. In Model 2 and 3, intake rates of the four fish species still contributed the most to the variance in the estimated MeHg intakes (~55%). Weights assigned to waterbodies (to determine MeHg concentrations) also contributed to the variance in estimated MeHg intakes (~44%).

For the subset of participants with both fish intake data (i.e., who completed the FFQ) and total Hg biomarker data, summary statistics were calculated (presented **Tables 5.2A** and **5.2B**) and Spearman rank correlations were run to examine associations between estimated MeHg intake from each of the models and measured total Hg biomarker levels. For the Dehcho region, measured hair total Hg concentrations were statistically significantly correlated with estimated MeHg intake for all three models ($n = 129$; Model 1: Spearman's rho = 0.23, $p<0.05$; Model 2: Spearman's rho = 0.21, $p<0.05$; Model 3: Spearman's rho = 0.21, $p<0.05$). The same was true for the Sahtú region; measured hair total Hg concentrations were statistically significantly correlated with estimated MeHg intake for all three models ($n = 65$; Model 1: Spearman's rho = 0.53, $p<0.05$; Model 2: Spearman's rho = 0.49, $p<0.05$; Model 3: Spearman's rho = 0.49, $p<0.05$). Notably, measured blood total Hg concentrations in the Dehcho region

were not significantly correlated with estimated MeHg intake from any of the models (n = 80; Model 1: Spearman's rho = 0.07, p = 0.52; Model 2: Spearman's rho = 0.17, p = 0.13; Model 3: Spearman's rho = 0.18, p = 0.11). Similarly, measured blood total Hg concentrations from the Sahtú region were not statistically significantly correlated with the estimated MeHg intake (n = 55; Model 1: Spearman's rho = 0.26, p = 0.05) Model 2: Spearman's rho = 0.23, p = 0.08; Model 3: Spearman's rho = 0.24, p = 0.08), and the strength of the correlations were similar across all three models.

Hair and blood total Hg concentrations were compared to the following health-based guidance values: 8.0 µg/L in whole blood for women of child-bearing age and children (Legrand et al., 2010), 20 µg/L whole blood for the general population (Legrand et al., 2010), 2µg/g hair Hg for women of child-bearing age and children (Delormier et al., 2012), and 5 µg/g for the general population (Delormier et al., 2012). Less than 1% of hair total Hg concentrations (**Table 5.2A**) and 0% of blood total Hg concentrations (**Table 5.2B**) in these participant subsets exceeded relevant health-based guidance values. Histograms of hair and blood total Hg concentrations by region are presented in **Supplementary Figures 5.2** and **5.3**.

Table 5.1: Exposure Estimates for Methylmercury Intake from the Probabilistic Models

Region	Fish Species	Model[a]	Estimated Methylmercury Intake (µg/kg of bodyweight/day)					
			Geometric Mean	Arithmetic Mean	Standard Deviation	Minimum[b]	Maximum	95th Percentile
Dehcho	**Lake Whitefish**	**Model 1**	0.04	0.04	0.07	0.00	1.68	0.14
		Model 2	0.02	0.02	0.02	0.00	0.33	0.06
		Model 3	0.01	0.01	0.02	0.00	0.26	0.05
	Lake Trout	**Model 1**	0.04	0.04	0.08	0.00	1.54	0.17
		Model 2	0.03	0.03	0.05	0.00	0.90	0.13
		Model 3	0.02	0.02	0.04	0.00	0.72	0.10
	Northern Pike	**Model 1**	0.07	0.07	0.12	0.00	1.79	0.28
		Model 2	0.04	0.04	0.05	0.00	0.59	0.15
		Model 3	0.03	0.03	0.05	0.00	0.49	0.13
	Walleye	**Model 1**	0.07	0.08	0.16	0.00	2.27	0.37
		Model 2	0.05	0.06	0.14	0.00	2.63	0.24
		Model 3	0.04	0.04	0.11	0.00	1.87	0.18
	Total Intake	**Model 1**	0.22	0.23	0.24	0.00	2.39	0.70
		Model 2	0.13	0.14	0.18	0.00	2.69	0.43
		Model 3	0.11	0.11	0.14	0.00	1.91	0.33
Sahtú	**Lake Whitefish**	**Model 1**	0.03	0.03	0.07	0.00	1.23	0.10
		Model 2	0.02	0.02	0.04	0.00	0.49	0.07
		Model 3	0.02	0.02	0.03	0.00	0.46	0.06
	Lake Trout	**Model 1**	0.08	0.10	0.20	0.00	2.58	0.41
		Model 2	0.07	0.07	0.11	0.00	1.07	0.28
		Model 3	0.05	0.05	0.08	0.00	0.81	0.22
	Northern Pike	**Model 1**	0.01	0.01	0.03	0.00	0.41	0.07
		Model 2	0.01	0.01	0.02	0.00	0.11	0.04
		Model 3	0.01	0.01	0.01	0.00	0.10	0.04
	Walleye	**Model 1**	<0.01	<0.01	0.01	0.00	0.28	0.00
		Model 2	<0.01	<0.01	0.01	0.00	0.12	0.00
		Model 3	<0.01	<0.01	0.01	0.00	0.09	0.00
	Total Intake	**Model 1**	0.12	0.13	0.22	0.00	2.74	0.50
		Model 2	0.10	0.10	0.12	0.00	1.14	0.35
		Model 3	0.07	0.08	0.09	0.00	0.86	0.26

[a]Model 1: Included species-specific fish Hg concentration data, Model 2: Included species and waterbody-specific fish Hg concentration data, Model 3: Included species and waterbody-specific fish Hg concentration data and species-specific fish Hg bioaccessibility data

[b]As some participants did not report consuming any of the four fish species included in the model, their estimated intake was 0 across the models and therefore minimum estimated intake was 0

Table 5.2A: Summary Statistics and Percent Exceedances for Participants with Model Estimates and Hair Mercury Concentrations

Region	Summary Statistics	Hair Mercury Concentrations (µg/g)	Estimated Methylmercury Intake (µg/kg of bodyweight/day)		
			Model 1[c]	Model 2[c]	Model 3[c]
Dehcho n=129	**Geometric Mean**	0.37	0.22	0.14	0.11
	Arithmetic Mean	0.65	0.24	0.15	0.12
	Standard Deviation	0.82	0.20	0.16	0.13
	95th Percentile	2.21	0.58	0.40	0.32
	% Above Guidance Value or TDI[a,b]	0.78%[a]	17.83%[b]	7.75%[b]	4.65%[b]
Sahtú n=65	**Geometric Mean**	0.68	0.11	0.09	0.07
	Arithmetic Mean	1.09	0.12	0.09	0.07
	Standard Deviation	0.90	0.13	0.10	0.07
	95th Percentile	2.58	0.38	0.29	0.22
	% Above Guidance Value or TDI[a,b]	0%[a]	4.62%[b]	3.08%[b]	1.54%[b]

[a]For hair total Hg concentrations a health-based guidance value of 2µg/g hair Hg for women of childbearing age and children and 5 µg/g for the general population (Delormier et al., 2012) was used

[b] A MeHg tolerable daily intake (TDI) of 0.23 µg/kg/day (WHO, 2003) was used for children (males and females <18 years of age) and women of childbearing age (those 18-<40 years of age) and a TDI of 0.47 µg/kg/day (Health Canada, 2007) was used for adult men (18+ years of age) and women 40 years of age or older

[c]Model 1: Included species-specific fish Hg concentration data, Model 2: Included species and waterbody-specific fish Hg concentration data, Model 3: Included species and waterbody-specific fish Hg concentration data and species-specific fish Hg bioaccessibility data

Table 5.2B: Summary Statistics and Percent Exceedances for Participants with Model Estimates and Blood Mercury Concentrations

Region	Summary Statistics	Blood Mercury Concentrations (µg/L)	Estimated Methylmercury Intake (µg/kg of bodyweight/day)		
			Model 1[c]	Model 2[c]	Model 3[c]
Dehcho n=80	**Geometric Mean**	0.09	0.22	0.14	0.11
	Arithmetic Mean	0.96	0.23	0.15	0.12
	Standard Deviation	2.38	0.19	0.16	0.12
	95th Percentile	4.77	0.56	0.46	0.36
	% Above Guidance Value or TDI[a,b]	0%[a]	17.50%[b]	7.50%[b]	5.00%[b]
Sahtú n=55	**Geometric Mean**	0.08	0.13	0.10	0.07
	Arithmetic Mean	0.81	0.14	0.10	0.08
	Standard Deviation	1.64	0.16	0.11	0.08
	95th Percentile	4.80	0.45	0.32	0.24
	% Above Guidance Value or TDI[a,b]	0%[a]	9.09%[b]	5.45%[b]	1.82%[b]

[a]For blood total Hg concentrations a health-based guidance value of 8.0 µg/L in whole blood for women of childbearing age and children and 20 µg/L whole blood for the general population (Legrand et al., 2010), was used.

[b]A MeHg tolerable daily intake (TDI) of 0.23 µg/kg/day (WHO, 2003) was used for children (males and females <18 years of age) and women of childbearing age (those 18-<40 years of age) and a TDI of 0.47 µg/kg/day (Health Canada, 2007) was used for adult men (18+ years of age) and women 40 years of age or older

[c]Model 1: Included species-specific fish Hg concentration data, Model 2: Included species and waterbody-specific fish Hg concentration data, Model 3: Included species and waterbody-specific fish Hg concentration data and species-specific fish Hg bioaccessibility data

5.4.2 Exposure Estimates for Selenium Intake

Summary statistics were calculated for estimated Se intake for models 1 and 2 and are presented by region (**Table 5.3**). For participants from the Dehcho region, on average the consumption of Northern Pike (32%) followed by Walleye (35%) contributed the most to the average estimated total Se intake in Model 1, but for Model 2 Lake Whitefish (41%) followed by Walleye (27%) contributed the most to average estimated Se intake. In the Sahtú region, consumption of Lake Trout (51-70%) followed by Lake Whitefish (20-46%) contributed most to average estimated total Se intake for both models. Sensitivity analyses were conducted, and in Model 1 intake rates of Walleye, Northern Pike, Lake Whitefish, and Lake Trout contributed 27.5%, 27.5%, 26.3%, and 15.9% respectively, to the variance in estimates for Se intakes. In Model 2, collectively, the weights assigned to waterbodies contributed most to the variance in estimated Se intakes (~57%), followed by intake rates of the four fish species (~41%).

Average estimated total intakes of Se were compared to DRIs and Tolerable Upper Levels (TULs) for Se (National Academies, 2022). The DRI for children aged 4-8 is 23 μg/day, for children aged 9-13 is 35 μg/day, for children aged 14-18 is 45 μg/day, and for adults 18+ is 45 μg/day of Se (National Academies, 2022). The TUL for children aged 4-8 is 90 μg/day, children aged 9-13 is 280 μg/day, for children aged 14-18 is 400 μg/day, and for adults 18+ is 400 μg/day (National Academies, 2022). On average, mean estimated Se intakes from Model 1 were 33% (± 30%) of the relevant DRI (39% ± 32% for the Dehcho region and 21% ± 22% for the Sahtú region) and for Model 2 were 36% (± 45%) of the relevant DRI (43% ± 52% for the Dehcho region and 23% ± 25% for the Sahtú region). Only one mean estimated Se intake from Model 2 exceeded the relevant TUL (in this case the estimate exceeded the TUL as all Se analyte data came from a lake with high Se concentrations (based on weighting) and the lowest TUL was used due to age of the participant, however, estimates from Model 1 for this individual were well below the TUL). Histograms of the average estimated Se intakes are compared for the two models by region in **Supplementary Figures 5.4A** and **5.4B**.

For the subset of participants with both fish intake data (i.e., who completed the FFQ) and blood Se concentrations available, summary statistics were calculated (**Table 5.4)**. Results of Spearman rank correlations indicated that blood Se concentrations were not statistically significantly correlated with the estimated Se intake for either Model 1 of Model 2 in either of

the regions (Dehcho Model 1: Spearman's rho = -0.02, p = 0.87, n = 80; Dehcho Model 2: Spearman's rho = -0.09, p = 0.44, n = 80; Sahtú Model 1: Spearman's rho = 0.17, p = 0.21, n = 55; Sahtú Model 2: Spearman's rho = 0.20, p = 0.14, n = 55). Blood Se concentrations were also compared to a biomonitoring equivalent for total Se in whole blood of 100 µg/L and 400-480 µg/L for an adequate Se intake and to protect against excessive Se intake (i.e., selenosis), respectively (Hays et al., 2014). These biomonitoring equivalents were based on the previously established DRIs and TULs for Se. When comparing to biomonitoring equivalents, 99% of participants (98% from the Dehcho region and 100% from the Sahtú region) had adequate blood Se concentrations and 0% of participants had excessive blood Se concentrations (above 400 µg/L) (**Table 5.4**). Histograms of blood Se concentrations by region are presented in **Supplementary Figure 5.5**.

5.4.3 Exposure Estimates for EPA+DHA Intake

Summary statistics were calculated for estimated EPA + DHA intakes from 50 trials for each participant for models 1 and 2 by region (**Table 5.5**). For participants from the Dehcho region, on average the consumption of Lake Whitefish (48-49%) contributed most to average estimated EPA + DHA total intake for both models. In the Sahtú region, consumption of Lake Trout (57-60%) followed by Lake Whitefish (40%) contributed most to average estimated EPA + DHA total intake for both models. The American Dietetic Association/Dietitians of Canada recommend an intake of 500 mg/day of EPA + DHA (Kris-Etherton & Innis, 2007) and the World Health Organization recommends the equivalent of 200-500 mg/day of EPA + DHA (FAO/WHO, 2003). Only 14% of the mean estimated EPA+ DHA intakes (15% in the Dehcho region and 11% in the Sahtú region) exceeded 200 mg/day and no mean estimated EPA+ DHA intakes exceeded 500 mg/day. Histograms of the average estimated EPA+DHA intakes are compared for the two models by region in **Supplementary Figures 5.6A** and **5.6B**.

Sensitivity analyses indicated that intake rates of the four fish species contributed most to uncertainty in estimated EPA+DHA intakes. In Model 1, intake rates of Lake Whitefish, Lake Trout, Northern Pike, and Walleye, contributed 44.1%, 20.0%, 17.1%, and 12.4% respectively, to the variance in the estimates for EPA + DHA intakes. In Model 2, the intake rates of the four fish species still contributed the most to the variance in estimated EPA+DHA intakes (~58%)

and weights assigned to waterbodies contributed 39% to the variance in estimated EPA + DHA intakes.

For participants with exposure estimates and plasma EPA + DHA concentrations available, results of Spearman rank correlations between estimated EPA+ DHA intake and plasma concentrations were not significant for the models for the Dehcho region (n = 80; Model 1: Spearman's rho = 0.10, p = 0.35; Model 2: Spearman's rho = 0.11, p = 0.35). Sahtú plasma EPA + DHA concentrations were statistically significantly correlated with the estimated EPA + DHA intake from Model 1 (Spearman's rho = 0.28, $p<0.05$, n = 55) but not from Model 2 (Spearman's rho = 0.25, p = 0.07, n = 55). Summary statistics for plasma EPA + DHA concentrations and estimated EPA + DHA intake for this subset is presented in **Table 5.6**. Histograms of plasma EPA+DHA concentrations by region are presented in **Supplementary Figure 5.7**.

Table 5.3: Exposure Estimates for Selenium Intake

Region	Fish Species	Model[a]	Selenium Intake (µg/day)					
			Geometric Mean	Arithmetic Mean	Standard Deviation	Minimum[b]	Maximum	95th Percentile
Dehcho	**Lake Whitefish**	**Model 1**	2.11	2.83	4.69	0.00	96.10	9.89
		Model 2	4.86	7.50	8.83	0.00	72.04	24.51
	Lake Trout	**Model 1**	1.58	2.92	5.98	0.00	127.29	12.44
		Model 2	1.31	2.34	3.63	0.00	46.95	9.29
	Northern Pike	**Model 1**	2.62	5.50	9.32	0.00	140.48	21.79
		Model 2	1.83	3.58	7.565	0.00	188.41	15.58
	Walleye	**Model 1**	2.14	5.93	11.24	0.00	153.93	27.14
		Model 2	1.64	5.03	11.65	0.00	110.89	24.59
	Total Intake	**Model 1**	11.43	17.17	17.90	0.00	176.68	53.19
		Model 2	12.03	18.46	20.41	0.00	207.09	55.51
Sahtú	**Lake Whitefish**	**Model 1**	1.21	1.85	3.74	0.00	47.88	7.18
		Model 2	2.51	4.72	6.46	0.00	53.46	19.38
	Lake Trout	**Model 1**	3.32	6.38	12.04	0.00	138.03	27.25
		Model 2	2.75	5.25	7.17	0.00	43.29	21.57
	Northern Pike	**Model 1**	0.25	0.66	2.18	0.00	33.56	4.53
		Model 2	0.15	0.34	1.96	0.00	78.87	1.65
	Walleye	**Model 1**	0.06	0.18	1.12	0.00	21.14	0.00
		Model 2	0.04	0.05	0.50	0.00	7.69	0.00
	Total Intake	**Model 1**	5.39	9.07	13.17	0.00	146.22	33.40
		Model 2	6.04	10.40	11.41	0.00	96.75	36.54

[a]Model 1: Included species-specific fish selenium concentration data, Model 2: Included species and waterbody-specific fish selenium concentration data

[b]As some participants did not report consuming any of the four fish species included in the model, their estimated intake was 0 across the models and therefore minimum estimated intake was 0

Table 5.4: Summary Statistics and Percent Exceedances for Participants with Model Estimates and Blood Selenium Concentrations

Region	Summary Statistics	Blood Selenium Concentrations (µg/L)	Estimated Total Selenium Intake (µg/ /day)	
			Model 1[c]	Model 2[d]
Dehcho n=80	**Geometric Mean**	180.38	11.92	12.94
	Arithmetic Mean	182.31	18.11	20.38
	Standard Deviation	26.71	15.36	21.69
	95th Percentile	229.52	46.26	76.90
	% Above TUL/BE[a,b]	0%	0%	0%
Sahtú n=55	**Geometric Mean**	151.29	5.63	6.28
	Arithmetic Mean	153.13	8.88	10.07
	Standard Deviation	21.78	8.91	10.55
	95th Percentile	181.94	29.19	33.33
	% Above TUL/BE[a,b]	0%	0%	0%

[a] The TULs used for children aged 4-8 is 90 µg/day, children aged 9-13 is 280 µg/day, for children aged 14-18 is 400 µg/day, and for adults 18+ is 400 µg/day (National Academies, 2022)

[b]The biomonitoring equivalent (BE) for excessive blood selenium concentrations used was 400 µg/L (Hays et al., 2014)

[c]Model 1: Included species-specific fish selenium concentration data

[d]Model 2: Included species and waterbody-specific fish selenium concentration data

Table 5.5: Exposure Estimates for EPA and DHA Intake

Region	Fish Species	Model[a]	EPA and DHA Intake (mg/day)					
			Geometric Mean	Arithmetic Mean	Standard Deviation	Minimum[b]	Maximum	95th Percentile
Dehcho	**Lake Whitefish**	**Model 1**	28.14	47.60	55.77	0.00	620.62	153.36
		Model 2	29.24	49.24	50.24	0.00	369.08	152.45
	Lake Trout	**Model 1**	5.49	19.06	29.04	0.00	317.97	76.04
		Model 2	5.00	17.19	26.18	0.00	359.72	67.46
	Northern Pike	**Model 1**	6.00	17.76	26.37	0.00	303.41	68.28
		Model 2	5.94	18.25	24.75	0.00	410.00	64.78
	Walleye	**Model 1**	3.88	15.63	30.57	0.00	593.73	70.79
		Model 2	3.92	16.36	27.56	0.00	275.36	74.89
	Total Intake	**Model 1**	68.74	100.05	84.07	0.00	635.79	266.79
		Model 2	69.00	101.05	77.95	0.00	560.36	245.21
Sahtú	**Lake Whitefish**	**Model 1**	11.49	30.40	43.77	0.00	468.30	114.16
		Model 2	11.07	29.15	41.00	0.00	447.80	110.43
	Lake Trout	**Model 1**	15.37	43.29	61.86	0.00	449.03	177.76
		Model 2	14.65	41.63	58.97	0.00	520.44	169.73
	Northern Pike	**Model 1**	0.46	2.07	6.04	0.00	66.48	15.37
		Model 2	0.47	2.06	5.98	0.00	93.09	14.42
	Walleye	**Model 1**	0.11	0.54	3.28	0.00	50.73	0.00
		Model 2	0.11	0.52	3.09	0.00	54.84	0.00
	Total Intake	**Model 1**	37.50	76.31	83.50	0.00	522.42	257.58
		Model 2	35.60	70.75	76.56	0.00	534.11	239.25

[a]Model 1: Included species-specific fish EPA + DHA concentration data, Model 2: Included species and waterbody-specific fish EPA + DHA concentration data

[b]As some participants did not report consuming any of the four fish species included in the model, their estimated intake was 0 across the models and therefore minimum estimated intake was 0

Table 5.6: Summary Statistics for Model Estimates of EPA + DHA Intake and Plasma EPA + DHA Concentrations

Region	Summary Statistics	Plasma EPA +DHA Concentrations (mg/L)	Relative Percent Plasma EPA + DHA (% weight EPA+DHA in total fatty acids)	Estimated EPA + DHA Intake (mg/day)	
				Model 1[b]	Model 2[c]
Dehcho n=80	**Geometric Mean**	73.19	1.84	70.93	71.42
	Arithmetic Mean	79.64	1.96	100.92	101.82
	Standard Deviation	34.08	0.73	70.20	69.84
	95th Percentile	148.72	3.58	232.50	229.89
	% Above AI[a]			10.00%	11.25%
Sahtú n=55	**Geometric Mean**	77.73	2.11	39.65	37.49
	Arithmetic Mean	87.11	2.28	71.88	66.59
	Standard Deviation	47.60	0.95	68.41	69.47
	95th Percentile	158.35	4.07	212.72	218.35
	% Above AI[a]			5.45%	7.27%

[a]An adequate intake (AI) of 200 mg/day of EPA + DHA, from the World Health Organization recommendation of 200-500 mg/day of EPA + DHA (FAO/WHO, 2003), was used for comparison for this table

[b]Model 1: Included species-specific fish EPA + DHA concentration data

[c]Model 2: Included species and waterbody-specific fish EPA + DHA concentration data

5.5 Discussion

5.5.1 Estimated Intakes in the Dehcho and Sahtú Regions

5.5.1.1 Estimated Mercury Intakes

The majority of estimated MeHg intakes across all three models were below relevant tolerable daily intakes for MeHg (Health Canada, 2007; WHO, 2003). This is similar to other Hg estimates generated from this dataset, including models that examined the impact of harvest location on Hg exposures (Packull-McCormick et al., 2020b), and models that included additional fish species (Laird, 2017). Results are aligned with previous observations that the vast majority of blood and hair Hg concentrations measured in participants from this project were below relevant health-based guidance values (Packull-McCormick et al., 2022; Ratelle et al., 2020b). This is even with some participants getting some of their fish from waterbodies with consumption advisories. Of the eleven waterbodies participants reported getting their fish from, there are currently consumption notices in place for Ekali Lake and Tathlina Lake (for the consumption of Northern Pike and Walleye), Kelly Lake (for the consumption of Lake Trout), and Trout Lake (for the consumption of Lake Trout and Walleye). Previously, biomarker concentrations from this dataset were compared to results from a national biomonitoring study, the Canadian Health Measures Study (CHMS), and blood total Hg concentrations in this population were similar to those measured in the Canadian general population (Health Canada, 2010; 2017; 2019). Hair total Hg concentrations appeared to be higher than levels measured in cycle 5 of the CHMS (Health Canada, 2019a). As previously reported, however, hair to blood total Hg ratios in this population were much higher (geometric mean ratios of 619:1 for the Dehcho region and 1220:1 for the Sahtú region) than the 250:1 ratio typically seen in other populations (WHO, 1990).

Collectively, these Hg exposure estimates and biomarker results indicate that although fish total Hg concentrations are sometimes elevated in some fish species from some waterbodies in these regions (DHSS, 2016), human exposure to Hg is still relatively low for studied communities in the Dehcho and Sahtú regions. It is important that MeHg intakes stay low, especially for women of childbearing age and children as early life stages are particularly vulnerable to the neurotoxic effects of MeHg because of their developing nervous system

(Ruggieri et al., 2017; Clifton II, 2007; Goldman et al., 2001). Several epidemiological studies have demonstrated that prenatal and childhood Hg exposures have been associated with negative impacts on children's cognitive function (dos Santos-Lima et al., 2020; Jeong et al., 2017; Jacobson et al., 2015; Suzuki et al., 2010; Myers et al., 2009; Crump et al., 1998; Grandjean et al., 1997), while several other studies have not found associations (Golding et al., 2022; 2017; 2016; Hibbeln et al., 2018; Oken et al., 2016; Cao et al., 2010; Davidson et al., 1998). Differences in findings between these studies may be explained by the nutritional benefits of fish and seafood consumption during pregnancy, as many of the nutrients in fish, including omega-3 PUFA and Se, are essential for optimal fetal development (Health Canada, 2007) and neurodevelopment (Avella-Garcia & Julvez, 2014). The majority of studies conducted have been for populations with higher levels of exposure than seen in the Dehcho and Sahtú regions and the Canadian general population. It is unclear if there would be negative associations between early life Hg exposures and child neurodevelopment at the low levels of Hg exposure observed in the Dehcho and Sahtú regions and the Canadian general population, and whether fish consumption would modify these associations.

Estimated MeHg intakes and the major contributors to these model estimates differed between the Dehcho and Sahtú regions. Further, on average, estimated total intakes of MeHg were higher for participants from the Dehcho region compared to the Sahtú region. This is likely due to differences between regions in the frequency of the consumption of the four fish species included in the models. Although Lake Whitefish (87%), Lake Trout (62%), Northern Pike (44%), and Walleye (34%) were overall the most commonly consumed fish (Ratelle et al., 2020a), the percent of participants consuming these fish varied by region. In both regions Lake Whitefish (92% Dehcho, 76% Sahtú), and Lake Trout (54% and 75%) were commonly consumed, but Northern Pike (60% Dehcho, 16% Sahtú) and Walleye (49% Dehcho, and 5% Sahtú) had low consumption in the Sahtú region. Previously, the highest total Hg concentrations in northern Canadian freshwater fish have been measured in predatory/piscivorous fish such as Northern Pike, Walleye, Lake Trout, and Burbot (*Lota lota*, also known as Loche or Ling Cod), while species such as Lake Whitefish, Sucker (*Catostomus* spp.), and Cisco (*Coregonus artedii*, also known as herring), tend to have much lower total Hg concentrations relative to piscivorous freshwater fish species (Laird et al., 2018; Dewpew et al., 2013; Lockhart et al., 2005). The

higher consumption of Northern Pike and Walleye in the Dehcho region, and the higher total Hg concentrations found in these fish (a species-specific arithmetic mean concentration of 0.47 and 0.57 µg/g of Hg for Northern Pike and Walleye respectively, were used in Model 1) compared to others (for example, a species specific arithmetic mean concentration of 0.12 µg/g of Hg was used in Model 1 for Lake Whitefish), explain why on average the estimated total intake of MeHg intakes in the Dehcho region, even though Lake Whitefish was more commonly consumed. Similarly, the increased consumption of Lake Trout (with a species-specific arithmetic mean Hg concentration of 0.37 µg/g used in Model 1) in the Sahtú region, and the decreased consumption of other fish typically high in Hg, e.g., Northern Pike and Walleye, explain why Lake Trout contributed the most on average to estimated MeHg intakes in the Sahtú region.

For the subset of participants with both exposure estimates from the models and biomarker data, estimated MeHg intakes across all models were much more strongly correlated with hair total Hg concentrations (Spearman's correlation coefficients of 0.5) than with blood total Hg concentrations (Spearman's correlation coefficients of 0.2). This could be due to the relative lack of sensitivity for measuring low levels of blood total Hg (over 50% for both regions were below the LOD) while the vast majority of hair total Hg concentrations (>98%) were above the detection limit. Additionally, the correlations were likely strongest between MeHg exposure estimates and hair total Hg concentrations as this biomarker represents a more long-term exposure to Hg (Ruggieri et al., 2017; Esteban & Castano, 2009). The hair samples analyzed in this study were the 2cm most proximal to the scalp representing the past two months of total Hg exposure (assuming that hair grows at a rate of approximately 1 cm per month; Packull-McCormick et al., 2022). The models relied on fish consumption frequency data from an FFQ that asked about typical fish consumption over the past year, also representing a long-term exposure. Blood total Hg concentrations tend to represent more recent exposure to Hg, although they can be used as a long-term measure of Hg exposure when exposure is consistent (Ruggieri et al., 2017). As has been previously described (Packull-McCormick et al., 2022), there are seasonal differences in total fish consumption and hair total Hg concentrations in this population, so blood total Hg levels likely fluctuate considerably over the course of a year. It is also possible that the fish species consumed vary over the course the year. The correlation between exposure estimates and blood total Hg concentrations was likely weaker, therefore, because blood total Hg

concentrations reflect a more short-term than the exposure estimates. Hair total Hg concentrations may be more appropriate for comparisons to exposure estimates for long-term dietary intakes.

When comparing among the three models for estimated MeHg intake (Model 1: fish species-specific Hg concentration data, Model 2: fish species and waterbody-specific Hg concentration data, Model 3: fish species and waterbody-specific Hg concentration data and fish species-specific Hg bioaccessibility data), the strength of the correlations between the exposure estimates and biomarkers were similar. MeHg exposure estimates did differ between the models, however; they decreased on average from Model 1 to Model 2 and from Model 2 to Model 3. The lowest estimated geometric mean MeHg intake (total and for each fish species) was for Model 3, where the model was adjusted with waterbody-specific Hg concentrations and Hg bioaccessibility, and the geometric mean MeHg total intake from Model 3 was approximately 52% and 54% of the geometric mean MeHg intake from Model 1 for the Dehcho region and Sahtú region, respectively. This is consistent with the findings of Siedlikowski et al. (2016), who found that the inclusion of bioaccessiblity data, and even the inclusion of bioavailability data, did not improve the fit of their MeHg uptake models (regression models) to either predict blood Hg concentrations or hair Hg concentrations. However, they did find a decrease in their estimated MeHg intakes when including bioaccessibility and/or bioavailability data.

For the subset of participants with both exposure estimates and biomarker data, percent exceedances (of health-based guidance values for biomarker levels) from measured data were most consistent with the results from Model 3 (with the lowest percent of exposure estimates exceeding TDIs). Therefore, although the strength of the correlations between exposure estimates and biomarker concentrations did not differ, the inclusion of waterbody-specific fish Hg concentrations and bioaccessibility data resulted in exposure estimates that were more representative of biomarker levels. This suggests that the inclusion of Hg analyte data that is fish species- and waterbody-specific, as well as the inclusion of Hg bioaccessibility data, may help prevent overestimates of Hg exposure in Hg exposure assessments as both adjustments assist in refining the intake estimates.

5.5.1.2 Estimated Selenium Intake

On average, estimated Se intakes from the consumption of the four fish species were 39% and 20% of the age and sex specific DRIs (National Academies, 2022) for Se for the Dehcho region and Sahtú region, respectively. This indicates that consumption of these four fish species is an important dietary source of Se for these populations, as was expected because fish and seafood tend to be foods with relatively high Se concentrations (Ullah et al., 2018). As Se is an essential trace element, and selenoproteins have many important roles, including in metabolic processes, the immune system, and the endocrine system (Mojadadi, 2021; Ullah et al., 2018), it is important to have an adequate intake. Along with fish and seafood, other protein-rich foods such as meat (from mammals and birds), eggs, and cereals tend to have high Se concentrations (Ullah et al., 2018). Although the estimated Se intakes from fish consumption in this study are generally below the DRIs for Se, fish is not the only potential source of Se for this population. As has been previously reported (Ratelle et al., 2020a), participants from the Dehcho region and Sahtú region reported frequently consuming other traditional foods, such as land mammals (muscle and organs) and gamebirds/waterfowl that could also be a source of Se. Along with the four fish species included in the models, overall, the most consumed traditional foods in these regions included foods such as: Moose (consumed by 94% of participants), Canada Goose (57%), Rabbit (57%), and Woodland Caribou (56%) (Ratelle et al., 2020a), which would also contribute to dietary Se intakes. These populations also consume market foods which may be a source of Se in their diet. For example, average estimated Se intakes for these populations calculated from a 24-hour recall dietary survey and including both traditional foods and market foods were much higher (more than 10 times higher) than the estimates from the models (only based on the consumption of the four most commonly consumed fish) (Ratelle et al., 2022). Overall, the estimates suggest that the consumption of fish is an important contributor to these populations' Se intake, although the consumption of other foods containing Se remains important for meeting DRIs.

For the subset of participants with both estimated Se intakes and blood Se concentrations, correlations between estimated Se intake and blood Se concentrations in either model were not significant for the Dehcho region and the Sahtú region. The absence of significant correlations is likely due to the models not taking into account all of the main dietary sources of Se in these

populations, as previously discussed. Model 1 and Model 2 generated very similar Se exposure estimates. While inclusion of lake-specific fish analyte data did not seem to change exposure estimates, this could also be due to a lack of waterbody-specific Se concentrations for several of fish species-waterbody combinations. For example, waterbody-specific Se concentration data were not available for at least one fish species for ten out of the eleven waterbodies examined, and of these, lake-specific Se concentration data was not available for any of the four fish species for two of the eleven waterbodies. Additionally, Se bioaccessibility data for these fish species from these waterbodies was not available so the models could not be further refined.

5.5.1.3 Estimated EPA+DHA Intake

Although DRIs for total omega-3 fatty acid intake have not yet been established, the WHO has recommended an intake of 200-500 mg of EPA+DHA per day (FAO/WHO, 2003) and the American Dietetic Association/Dietitians of Canada recommend an intake of 500 mg/day of EPA+DHA (Kris-Etherton & Innis, 2007). Mean estimates of EPA+DHA intake for participants from both regions and from both Model 1 and Model 2 were below 500 mg/day and the majority of mean estimates were also below 200 mg/day. On average, the estimated EPA+DHA intake from the consumption of the four fish species accounted for less than 20% of the recommended intake of EPA+DHA if using the recommendation of 500 mg/day (Kris-Etherton & Innis, 2007). This suggests that participants from these regions may not be getting enough EPA+DHA from fish consumption to reduce the risk of cardiovascular disease. However, this is also an issue in southern Canadian populations (Lacombe et al., 2018; Denomme et al., 2005), and the American general population (Thompson et al., 2019; Zhang et al., 2018), where the majority of the population also have estimated dietary intakes of EPA+DHA that are below recommendations.

The estimated EPA+DHA intakes from these models only considered the consumption of fish, specifically fish muscle, while omega-3 PUFA can also be obtained from other dietary sources and through supplementation (Tur et al., 2012). When considering both traditional (including both fish and other traditional foods) and market foods, however, previous estimates based on a 24-hour recall for a subset of these participants were also below recommended intakes. In fact, mean estimated EPA+DHA intake from this analysis for the Sahtú region (37 or 34 mg/day) was very similar to the estimated EPA+DHA intake using the 24-hour recall (31 mg/day) (Ratelle et al., 2022a). However, the estimated EPA+DHA intake for the Dehcho region

from both Model 1 and Model 2 (68 and 69 mg/day) was approximately two times higher than the estimated intake for the Dehcho region from the 24-hour recall (34 mg/day) (Ratelle et al., 2022a). Differences between the estimates from the 24-hour recall versus the models which were based on the FFQ could be due to seasonal differences in fish consumption. The FFQ asked about fish consumption over the past year, while the 24-hour recall only captured the past 24 hours of food consumption, and as sampling was conducted over the winter months, the fish consumption reflected in the 24-hour recall questionnaire may be an underestimate; total fish consumption for these participants was reported to be the lowest in the winter months (Packull-McCormick et al., 2022). Additionally, in a previous analysis of the data for the Dehcho region only (Laird, 2017), average estimated EPA + DHA dose was higher; however, that model included consumption of three additional fish species.

For the subset of participants with both estimated EPA+DHA intake, and plasma EPA+DHA concentration data available, estimated EPA+DHA intakes were not significantly correlated with participant plasma EPA+DHA concentrations from the Dehcho region but were correlated in the Sahtú region (Spearman's rho of 0.28 to 0.25). Similar to blood total Hg concentrations, these weak-to-moderate correlations could be due to plasma omega-3 fatty acid concentrations reflecting more recent intakes while the model estimates reflected average long-term EPA + DHA intake. Previously, short half-lives (<5 days) have been reported for EPA and DHA in human plasma after supplementation (Zuijdgeest-van Leeuwen, 1999). In these populations, where seasonal variation in total fish intake has been recorded (Packull-McCormick, 2022), plasma DHA+EPA concentrations may not provide an accurate estimate of long-term EPA+DHA intake. Additionally, correlations and model estimates were similar between Model 1 and Model 2. This is likely due to the limited availability of waterbody-specific omega-3 fatty acid concentrations for the waterbodies where participants most commonly reported as a source of their fish.

5.5.1.4 Comparison to Other Regions

Estimated MeHg and EPA + DHA intakes calculated for the Dehcho and Sahtú regions in this analysis were much lower than estimated intakes reported for Inuit participants of the International Polar Year Inuit Health Survey (IPY-IHS) (Hu et al., 2017a). Unlike estimates for the Dehcho and Sahtú region, the average estimated MeHg intakes for the IPY-IHS participants

exceeded MeHg TDIs (Hu et al., 2017a). However, although Hg intakes were much higher in the IPY-IHS, intakes of EPA and DHA were also much higher. Similarly, blood total Hg (geometric mean of 7.0 μg/L) and blood Se levels (geometric mean of 319.5 μg/L) in the IPY-IHS (Hu et al., 2017b) were much higher compared to biomarker concentrations measured in the Dehcho and Sahtú regions. These higher levels are due to higher consumption of marine mammals and marine fish in Inuit populations, which can have elevated levels of Hg and omega-3 PUFA (Hu et al., 2017a). For example, consumption of ringed seal liver accounted for 25% of the estimated Hg intake in the IPY-IHS population (Hu et al., 2017a). Relative to marine mammals, Hg concentrations are lower in the freshwater fish species consumed in the Dehcho and Sahtú regions.

The estimated EPA + DHA concentrations for the Dehcho and Sahtú regions are also lower than estimates that were previously calculated for the First Nations community of Old Crow in the Yukon (Drysdale, 2022). However, the model to estimate EPA + DHA intakes for Old Crow included other dietary sources of EPA+DHA (other than just fish muscle). For example, along with the consumption of fish muscle (Chinook Salmon, Coho Salmon, Chum Salmon, Whitefish) the model for Old Crow also included consumption of Loche/Burbot liver, and Muktuk (a marine mammal food consisting of whale blubber and skin). Although estimated intakes were higher in Old Crow, plasma EPA + DHA concentrations were similar in the Dehcho and Sahtú region compared to Old Crow (Packull-McCormick et al., 2023b).

5.5.2 Limitations and Future Directions

The main limitation of this study is the uncertainty around the input data included in the models. These models rely on fish intake estimates based on data collected through an FFQ, which can be subject to measurement error as participants must recall their consumption of food over the past year and may under or over-estimate their consumption of foods (Ratelle et al., 2020a; Rodrigo et al., 2015). However, FFQs are commonly used and considered to have reasonable relative reliability of food estimates (Rendo-Urteaga et al., 2020; Vézina-Im et al., 2016). To address potential over-estimation of fish consumption, a maximum total fish consumption equivalent to seven fish meals a week was set in these models. As well, the FFQ used in this study was refined for the specific regions of study (Ratelle et al., 2020a). Intake rates of the four fish species and the waterbody weights were important sources of uncertainty in the

models. Future versions of the FFQ could include additional questions to gather more information about the consumption frequency of the most commonly consumed fish species (such as questions about seasonal differences in the intake rates of these fish) and the waterbodies fish are consumed from (such as a question about what proportion of their fish do participants get from each of the commonly accessed waterbodies).

An additional limitation was the availability of waterbody-specific fish Se and omega-3 fatty acid data for the lakes participants commonly reported getting their fish from, limiting the ability to refine the models to estimate Se and omega-3 fatty acid intakes. When possible, aggregate data from the literature was used to fill these gaps but as more information becomes available in the future, these models could be revisited. The lack of bioaccessibility data for these northern freshwater fish species was also a limitation. No Se or omega-3 fatty acid bioaccessibility data was available for fish from these regions. However, fish-species specific Hg bioaccessibility data from a small subset of fish caught in the region was obtained for the four fish species included in the model. Fitting probability distributions for the analyte data and using these in the models also introduced some uncertainty into the estimates.

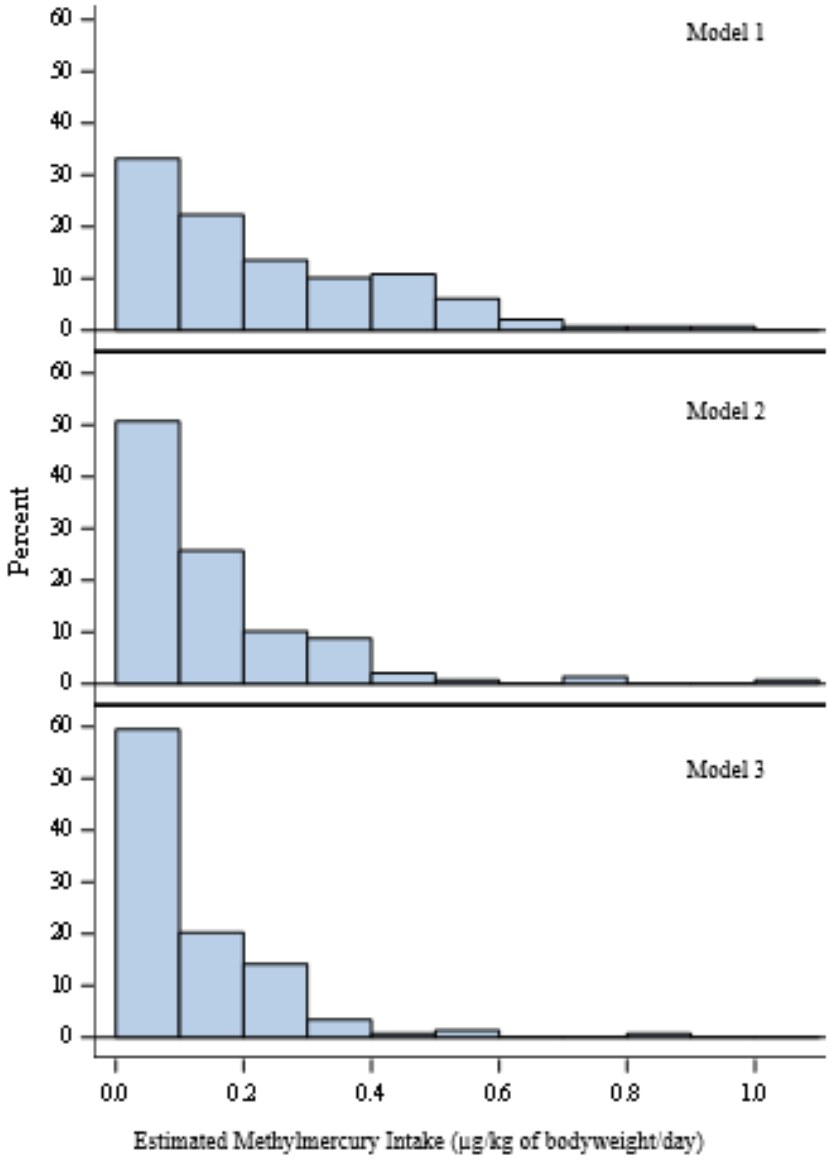
Future research could focus on obtaining additional analyte data for these four fish species from the top eleven waterbodies local communities report getting their fish from (Great Slave Lake, the Mackenzie River, Kakisa Lake, Willow Lake, Jean Marie River, Kelly Lake, Ekali Lake, Tathlina Lake, Trout Lake, Great Bear Lake, and Colville Lake). In particular, there was a gap in waterbody-specific Se and EPA+DHA concentrations. These models could be re-examined when additional waterbody-specific Se and EPA+DHA concentration data becomes available. Additionally, at that time, based on the availability of analyte data from other traditional foods, other foods that could be important sources of Se and/or EPA+DHA could be added to the models and those estimates could be compared to the biomarker concentrations to determine if this improves the correlations. Finally, along with including other traditional foods and additional Se and EPA+DHA data, future work could include other analytes to the models to create a model that considers multiple contaminant exposures and multiple nutrient intakes. For example, recent work has been undertaken in the Sahtú region, to measure several nutrition biomarkers and deficiencies in vitamin D and folate have been identified in this population (Ratelle et al., 2023). Future versions of the models could examine estimated intakes of several

other nutrients and vitamins from traditional food consumption and could be combined with other models that have been developed for other contaminants.

5.6 Conclusions

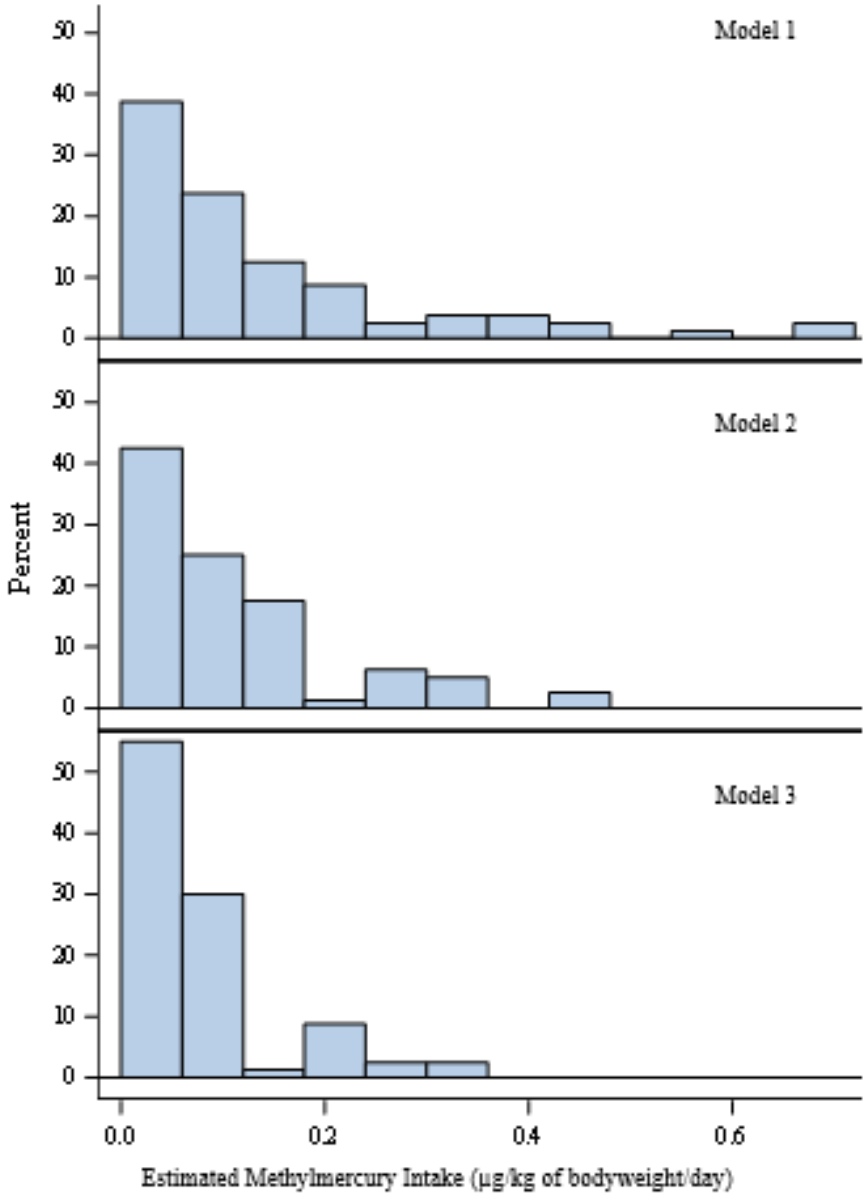
Although the consumption of locally caught freshwater fish species can be a source of MeHg exposure, it is also a source of the important dietary nutrients Se, and omega-3 PUFA (including EPA and DHA). The inclusion of fish species and waterbody-specific Hg concentrations and fish species-specific Hg bioaccessibility data in the model resulted in lower estimated MeHg intakes, which appeared more consistent with hair and blood total Hg concentrations measured in a subset of participants. The majority of estimated MeHg intakes were below TDIs and the vast majority of hair and blood total Hg concentrations were below health-based guidance values, indicating that although total Hg concentrations are elevated in certain fish species in some waterbodies in these regions, MeHg exposures in these regions are relatively low. Model estimates also indicate that consumption of locally harvested fish (Lake Whitefish, Lake Trout, Northern Pike, and Walleye) can be an important contributor to dietary intakes of Se and EPA+DHA in these regions. However, similar to other Canadian populations, the majority of estimated EPA+DHA intakes fall below recommendations. To refine these assessments, additional fish analyte data (especially Se and EPA+DHA concentrations, and bioaccessibility data) should be generated for the waterbodies from which local communities regularly harvest fish. This work highlights just some of the many benefits of local fish consumption for northern communities, and while monitoring of total Hg concentrations in local fish should continue, the consumption of fish, especially those considered low in total Hg (such as Lake Whitefish), should be encouraged.

5.7 Supplementary Figures



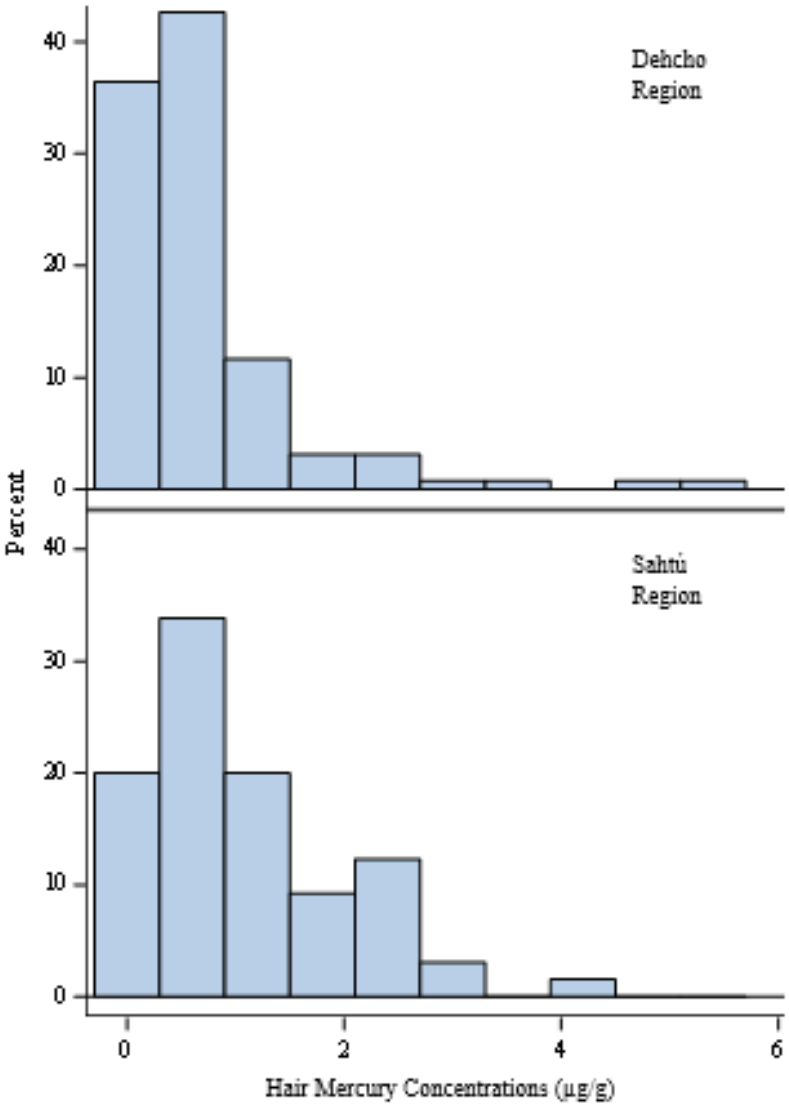
Supplementary Figure 5.1A: Histograms of the average estimated methylmercury intakes for Models 1-3 for the Dehcho region

Model 1: Included species-specific fish mercury concentration data, Model 2: Included species and waterbody-specific fish mercury concentration data, Model 3: Included species and waterbody-specific fish mercury concentration data and species-specific fish mercury bioaccessibility data

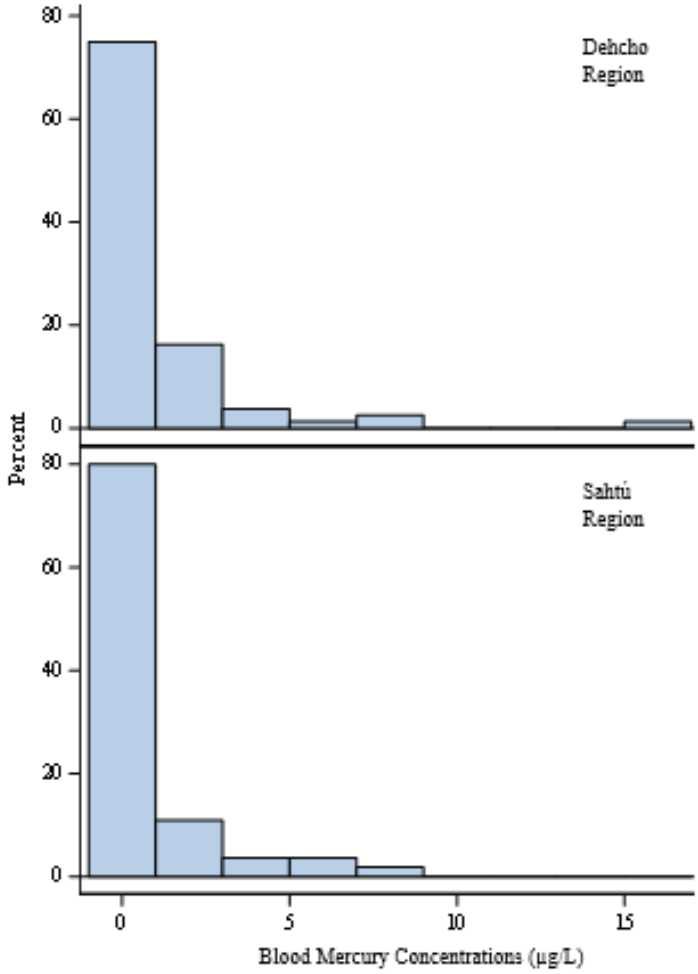


Supplementary Figure 5.1B: Histograms of the average estimated methylmercury intakes for Models 1-3 for the Sahtú region

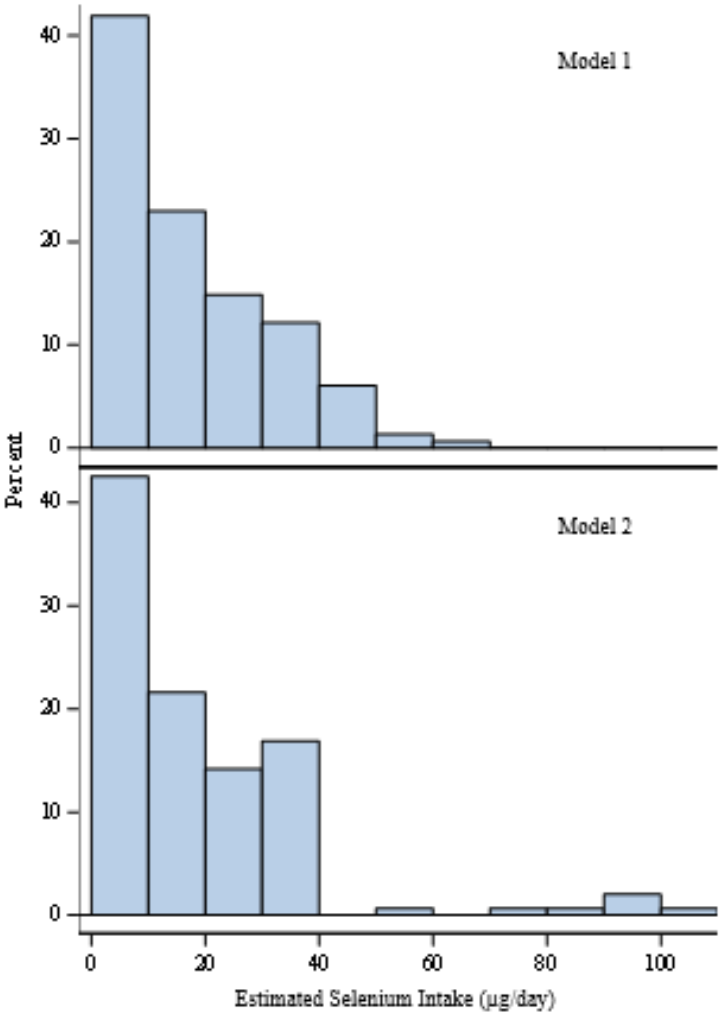
Model 1: Included species-specific fish mercury concentration data, Model 2: Included species and waterbody-specific fish mercury concentration data, Model 3: Included species and waterbody-specific fish mercury concentration data and species-specific fish mercury bioaccessibility data



Supplementary Figure 5.2: Histogram of the hair mercury concentrations for the Dehcho and Sahtú regions

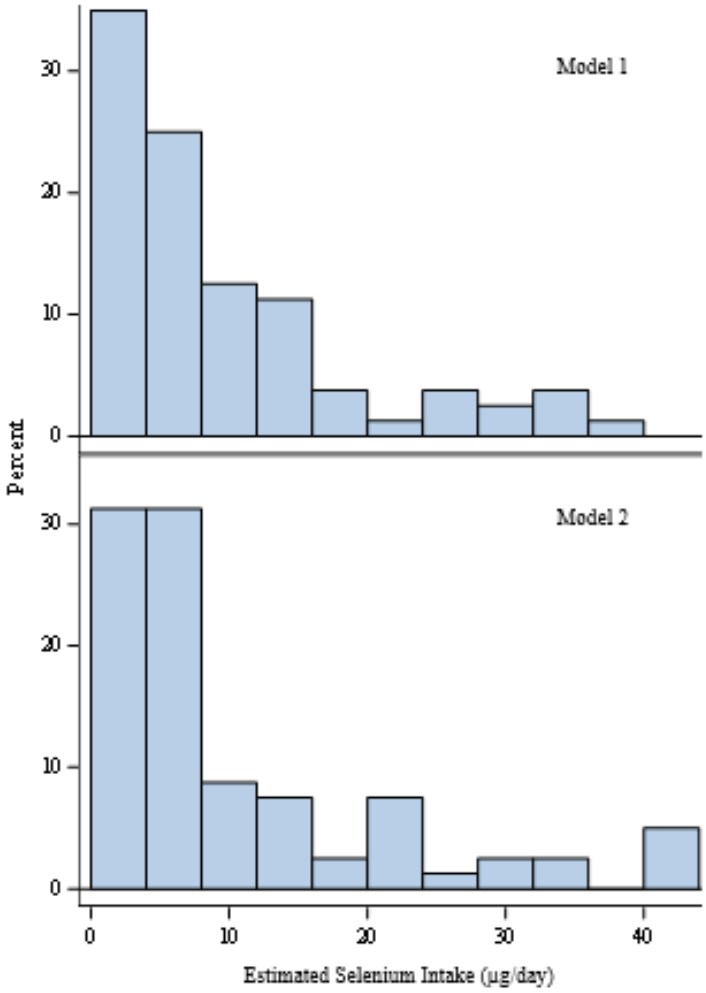


Supplementary Figure 5.3: Histogram of the blood mercury concentrations for the Dehcho and Sahtú regions



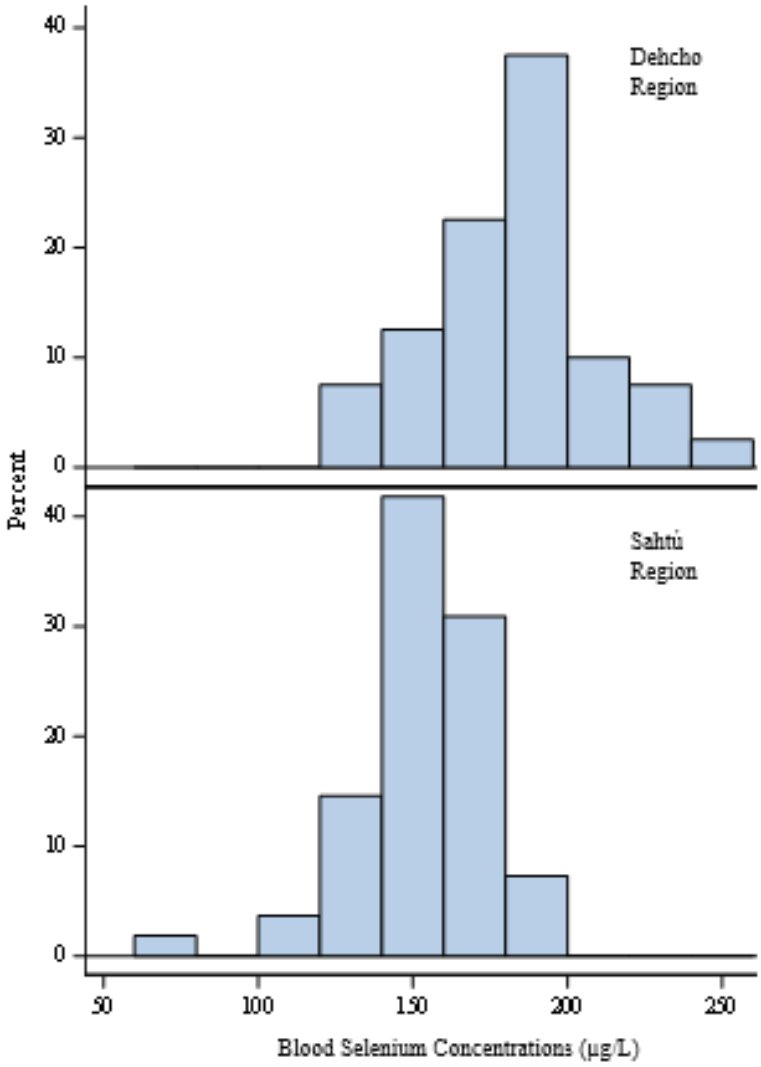
Supplementary Figure 5.4A: Histograms of the average estimated selenium intakes for Models 1-2 for the Dehcho region

Model 1: Included species-specific fish selenium concentration data, Model 2: Included species and waterbody-specific fish selenium concentration data

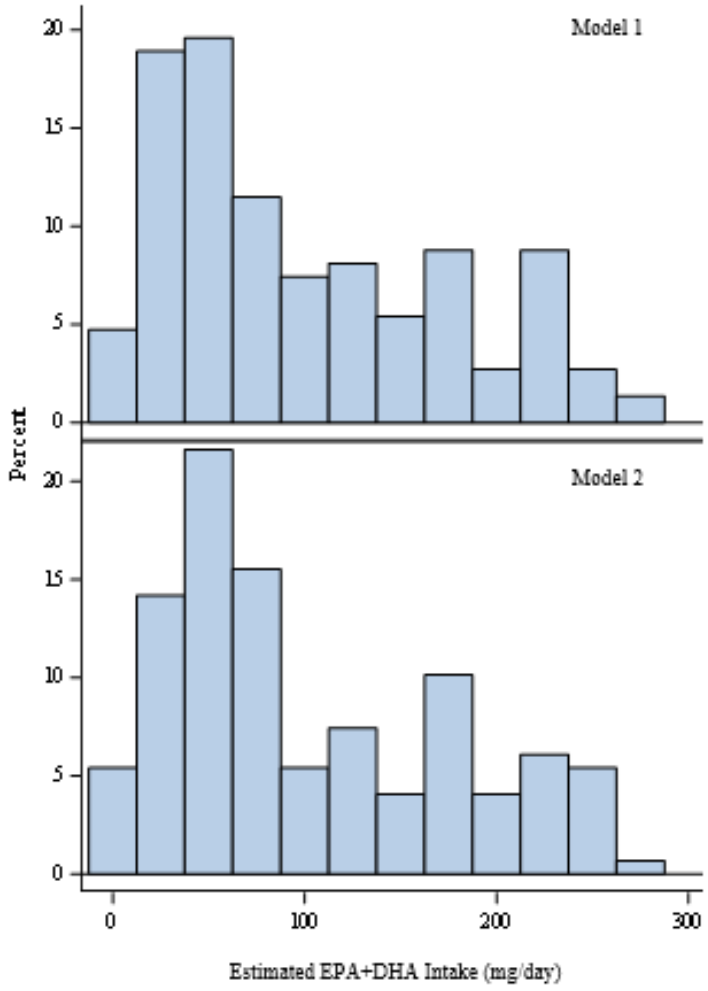


Supplementary Figure 5.4B: Histograms of the average estimated selenium intakes for Models 1-2 for the Sahtú region

Model 1: Included species-specific fish selenium concentration data, Model 2: Included species and waterbody-specific fish selenium concentration data

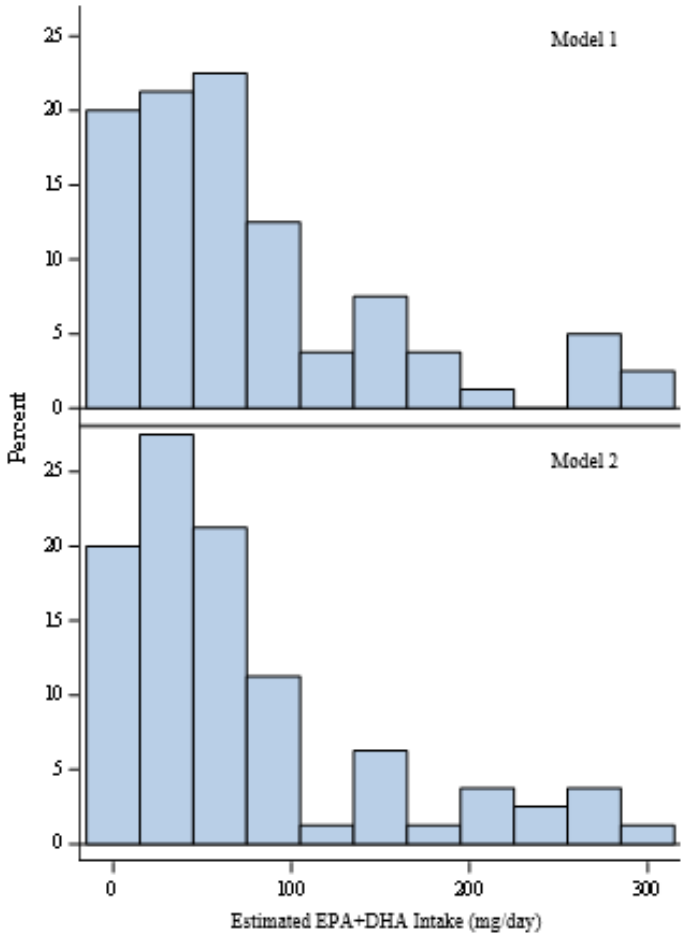


Supplementary Figure 5.5: Histogram of the blood selenium concentrations for the Dehcho and Sahtú regions



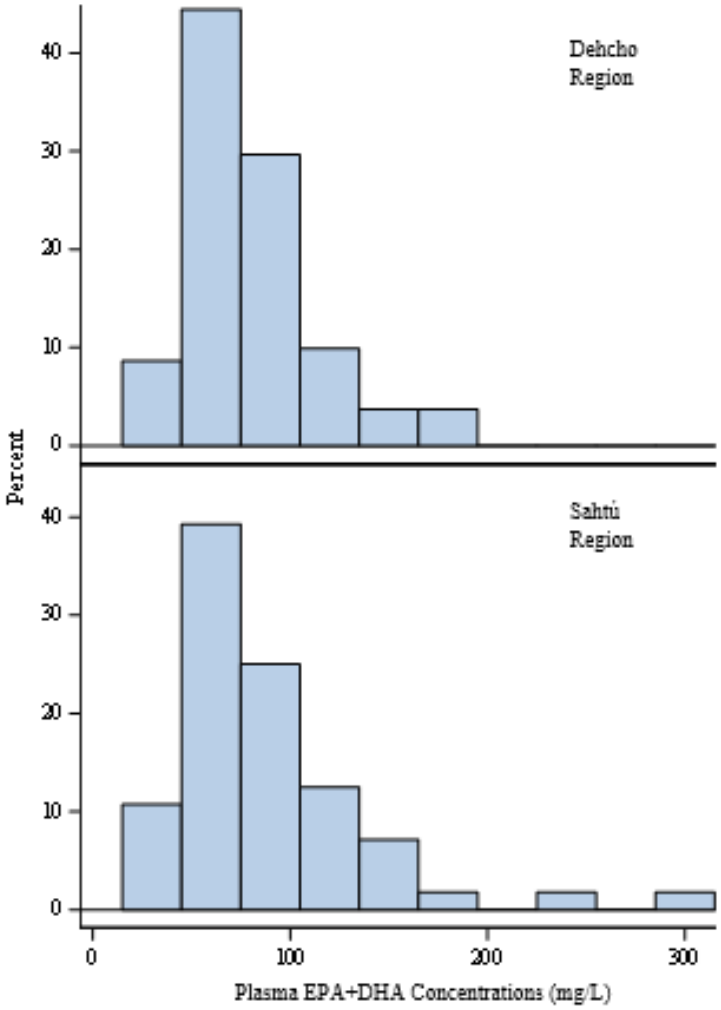
Supplementary Figure 5.6A: Histograms of the average estimated EPA+DHA intakes for Models 1-2 for the Dehcho region

Model 1: Included species-specific fish EPA+DHA concentration data, Model 2: Included species and waterbody-specific fish EPA+DHA concentration data



Supplementary Figure 5.6B: Histograms of the average estimated EPA+DHA intakes for Models 1-2 for the Dehcho region

Model 1: Included species-specific fish EPA+DHA concentration data, Model 2: Included species and waterbody-specific fish EPA+DHA concentration data



Supplementary Figure 5.7: Histogram of the plasma EPA+DHA concentrations for the Dehcho and Sahtú regions

5.8 Supplementary Tables

Supplementary Table 5.1: Summary Statistics for Calculated Intake Rates Used in the Models by Region

Region	Fish Species	Intake Rate (grams/week)				
		Geometric Mean	Arithmetic Mean	Standard Deviation	Minimum	Maximum
Dehcho n= 148	**Lake Whitefish**	89	165	158	0	809
	Lake Trout	11	56	76	0	435
	Northern Pike	16	83	100	0	497
	Walleye	9	72	109	0	600
Sahtú n=80	**Lake Whitefish**	30	106	134	0	750
	Lake Trout	35	125	157	0	632
	Northern Pike	1	10	25	0	113
	Walleye	0	2	12	0	75

In order to calculate fish intake rates, the reported consumption frequency for that fish species was multiplied by the number of servings a participant reported consuming in a typical meal of fish and a serving size was assumed to be 75 grams. Fish consumption frequencies were either: never, <1 times per week, 1-2 times per week, 3-5 times per week, and 6+ times per week and these were coded as 0 times/week, 0.5 times/week, 1.5 times/week, 4 times/week, or 6.5 times/week.

For this subset of participants (included in the models), from the Dehcho region: 93%, 55%, 60%, and 49% of participants consumed Lake Whitefish, Lake Trout, Northern Pike, and Walleye, respectively, and from the Sahtú region 74%, 76%, 15%, and 4% of participants consumed Lake Whitefish, Lake Trout, Northern Pike, and Walleye, respectively.

Supplementary Table 5.2: Summary Statistics for Participant Age, Sex, and Bodyweight Used in the Models by Region

Participant Characteristic	Statistic	Region	
		Dehcho	Sahtú
Sex	**Male (%)**	49%	48%
	Female (%)	51%	52%
Age (Years)	**Geometric Mean**	34	39
	Arithmetic Mean	40	45
	Standard Deviation	20	20
	Minimum	6	7
	Maximum	79	79
Bodyweight (kg)	**Geometric Mean**	74.8	68.3
	Arithmetic Mean	77.7	70.4
	Standard Deviation	20.1	17.5
	Minimum	19.0	30.7
	Maximum	152.9	134.4

Supplementary Table 5.3: Summary statistics used as input parameters to shape the log-normal distributions for the species-specific fish analyte concentrations used in Model 1

Analyte	Fish Species	Summary Statistics				
		N	Arithmetic Mean	Standard Deviation	Minimum	Maximum
Mercury (µg/g)	**Lake Whitefish**	2104	0.12	0.11	0.01	2.50
	Lake Trout	2600	0.37	0.41	0.02	6.71
	Northern Pike	1635	0.47	0.36	0.03	3.12
	Walleye	1170	0.57	0.38	0.04	2.31
Selenium (µg/g)	**Lake Whitefish**	791	0.25	0.15	0.04	1.53
	Lake Trout	1120	0.26	0.11	0.00	1.20
	Northern Pike	352	0.27	0.70	0.02	13.00
	Walleye	232	0.28	0.25	0.09	1.42
EPA+DHA (mg/g)	**Lake Whitefish**	107	2.07	1.11	0.41	5.75
	Lake Trout	58	2.45	1.15	0.22	6.06
	Northern Pike	108	1.53	0.95	0.29	7.07
	Walleye	83	1.53	1.18	0.25	8.07

These summary statistics were used as input parameters to shape the log-normal distributions for the species-specific fish analyte concentrations for Model 1 Summary statistics were calculated from individual level fish analyte data from fish caught in the Northwest Territories. This data was obtained from literature and raw data from northern partners and researchers. The literature included peer-reviewed publications and regional reports (Macdonald, 2013; 2011; 2009; 2002; Fisheries and Aquatic Resources, 2010; Doetzel, 2007; Rescan Environmental Services, 2005; Stewart et al., 2003; Evans, 1998; Grey et al., 1995) and included unpublished data from the research of Dr. Heidi Swanson and Dr. Marlene Evans.

Supplementary Table 5.4: Summary statistics used as input parameters to shape the log-normal distributions for the species and waterbody-specific fish analyte concentrations used in Model 2

Waterbody	Fish Species	Mercury (µg/g)[b]				Selenium (µg/g)				EPA + DHA (mg/g)			
		Arithmetic Mean	Standard Deviation	Minimum	Maximum	Arithmetic Mean	Standard Deviation	Minimum	Maximum	Arithmetic Mean	Standard Deviation	Minimum	Maximum
Great Slave Lake	**Lake Whitefish**	0.01[a]	0.01[a]	0.01[a]	0.10[a]	NA	NA	NA	NA	NA	NA	NA	NA
	Lake Trout	0.17	0.07	0.07	0.41	NA	NA	NA	NA	NA	NA	NA	NA
	Northern Pike	0.22[a]	0.12[a]	0.01[a]	0.66[a]	NA	NA	NA	NA	NA	NA	NA	NA
	Walleye	0.17[a]	0.07[a]	0.15[a]	0.19[a]	NA	NA	NA	NA	NA	NA	NA	NA
Mackenzie River	**Lake Whitefish**	0.10	0.03	0.06	0.18	NA	NA	NA	NA	NA	NA	NA	NA
	Lake Trout	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Northern Pike	0.29	0.12	0.13	0.56	NA	NA	NA	NA	NA	NA	NA	NA
	Walleye	0.36[a]	NA	NA	NA	0.25[a]	NA	NA	NA	NA	NA	NA	NA
Kakisa Lake	**Lake Whitefish**	0.06	0.04	0.02	0.19	0.38	0.04	0.29	0.45	3.21	0.89	2.05	4.76
	Lake Trout	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Northern Pike	0.35	0.20	0.03	1.06	0.32	0.03	0.27	0.37	1.87	0.23	1.38	2.15
	Walleye	0.28	0.15	0.10	1.13	0.41	0.02	0.36	0.44	1.50	0.58	0.01	1.92
Willow Lake	**Lake Whitefish**	0.09	0.06	0.01	0.31	0.15	0.02	0.13	0.17	0.87	0.24	0.59	1.42
	Lake Trout	NA	NA	NA	NA	NA	NA	NA	NA	1.08	0.16	0.90	1.20
	Northern Pike	0.35	0.20	0.03	1.06	0.32	0.03	0.27	0.37	0.52	0.09	0.36	0.63
	Walleye	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Jean Marie River	**Lake Whitefish**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Lake Trout	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Northern Pike	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Walleye	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kelly Lake	**Lake Whitefish**	0.17	0.07	0.03	0.52	NA	NA	NA	NA	NA	NA	NA	NA

	Lake Trout	0.68	0.69	0.22	5.56	0.28	0.13	0.09	0.65	NA	NA	NA	NA
	Northern Pike	0.55	0.19	0.31	0.86	0.22	0.03	0.17	0.26	NA	NA	NA	NA
	Walleye	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Tathlina Lake	**Lake Whitefish**	0.09	0.05	0.04	0.44	0.30	0.04	0.24	0.38	2.19	1.11	0.01	3.16
	Lake Trout	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Northern Pike	0.50	0.29	0.08	1.15	0.30	0.06	0.14	0.40	1.45	0.26	1.04	1.98
	Walleye	0.54	0.23	0.13	1.01	0.45	0.05	0.37	0.57	1.46	0.59	0.00	2.25
Trout Lake	**Lake Whitefish**	0.04	0.01	0.03	0.06	1.19	0.22	0.86	1.53	2.40	0.49	1.82	3.49
	Lake Trout	0.44	0.15	0.17	0.88	0.47	0.32	0.16	1.20	0.90	0.70	0.17	2.33
	Northern Pike	0.15	0.09	0.07	0.29	0.82	0.15	0.69	1.14	2.05	0.40	1.66	2.93
	Walleye	0.71	0.48	0.04	2.00	1.09	0.14	0.97	1.31	1.96	0.32	1.24	2.38
Great Bear Lake	**Lake Whitefish**	0.11	0.07	0.03	0.27	0.40	0.06	0.24	0.50	NA	NA	NA	NA
	Lake Trout	0.24	0.18	0.05	1.12	0.32	0.08	0.04	0.53	NA	NA	NA	NA
	Northern Pike	0.36	0.12	0.22	0.64	NA	NA	NA	NA	NA	NA	NA	NA
	Walleye	0.59	0.13	0.50	0.74	NA	NA	NA	NA	NA	NA	NA	NA
Colville Lake	**Lake Whitefish**	0.04	0.04	0.01	0.20	0.08	0.02	0.04	0.13	NA	NA	NA	NA
	Lake Trout	0.21	0.10	0.02	0.58	0.08	0.02	0.00	0.12	NA	NA	NA	NA
	Northern Pike	0.24	0.04	0.21	0.30	0.07	0.02	0.05	0.11	NA	NA	NA	NA
	Walleye	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ekali Lake	**Lake Whitefish**	0.10	0.04	0.03	0.23	0.20	0.20	0.06	0.66	2.79	1.02	1.87	5.44
	Lake Trout	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Northern Pike	0.44	0.30	0.07	1.97	0.45	0.27	0.08	0.83	1.76	1.09	1.20	5.92
	Walleye	0.36	0.17	0.10	0.99	0.48	0.39	0.14	1.12	2.31	1.82	1.32	8.07

[a]Individual level data was not available for this fish species and waterbody so aggregate level data (summary statistics) were used.

[b]These species and waterbody-specific fish mercury concentration distributions were also used in Model 3

NA = Not available. Individual and aggregate level data was not available for this fish species and waterbody. For Model 2 (and if applicable Model 3) the fish species-specific data from Supplementary Table 3 was used

Summary statistics were calculated from individual level fish analyte data from fish caught in the Northwest Territories. This data was obtained from literature and raw data from northern partners and researchers. The literature included peer-reviewed publications and regional reports (Macdonald, 2013; 2011; 2009; 2002; Fisheries and Aquatic Resources, 2010; Doetzel, 2007; Rescan Environmental Services, 2005; Stewart et al., 2003; Evans, 1998; Grey et al., 1995) and included unpublished data from the research of Dr. Heidi Swanson and Dr. Marlene Evans.

Supplementary Table 5.5: Summary statistics for total mercury bioaccessibility by fish-species included in Model-3

Fish Species	Total Mercury Bioaccessibility			
	Arithmetic Mean	**Standard Deviation**	**Minimum**	**Maximum**
Lake Whitefish	78%	15%	51%	104%[a]
Lake Trout	73%	9%	64%	89%
Northern Pike	86%	11%	62%	104%[a]
Walleye	78%	8%	62%	89%

[a]For these fish-species the experimental maximum value exceeded 100% but in reality this cannot exceed 100% so for the models a maximum of 100% was imputed

Chapter 6: Prenatal and concurrent blood mercury concentrations and associations with IQ in Canadian preschool children

Packull-McCormick, S., Ashley-Martin, J., Singh, K., Fisher, M., Arbuckle, T. E., Lanphear, B., Laird, B. D., Muckle, G., Booij, L., Asztalos, E., Walker, Mark., Bouchard, M. F., Saint-Amour, D., Boivin, M., & Borghese, M. (2023). Prenatal and concurrent blood mercury concentrations and associations with IQ in Canadian preschool children. *Environmental Research*.

6.1 Abstract

Background: Prenatal and childhood mercury (Hg) exposures have been associated with negative impacts on child neurodevelopment. It is unclear if associations persist at the low Hg exposures typical in Western countries.

Objective: To examine associations between prenatal/childhood blood Hg concentrations and child IQ in Canadian male and female children while considering the potential modifying role of prenatal fish consumption.

Methods: We analyzed data from the Maternal-Infant Research on Environmental Chemicals study. Hg was measured in first trimester (n = 527), cord (n = 430), and child (at 3–4 years of age, n=355) blood and examined sex-stratified associations between blood Hg and children's Full Scale IQ (FSIQ), Verbal IQ (VIQ), Performance IQ (PIQ), and General Language Composite (GLC) scores (assessed with WPPSI-III). Prenatal Hg analyses were further stratified by prenatal fish consumption (low: 0–2, moderate: 3–7, or high: ≥ 8 times/month).

Results: Higher cord blood Hg concentrations were associated with lower PIQ (β=-3.27; 95%CI: -6.44, -0.09) in male children with the lowest prenatal fish consumption. Progressively stronger positive associations were observed with PIQ in male children for moderate (β=1.08; 95%CI: -0.10, 2.26) and high (β=3.07; 95%CI: 1.95, 4.19) prenatal fish consumption. Cord blood Hg concentrations were positively associated with female children's FSIQ (β = 1.29; 95% CI: 0.77, 1.81) and PIQ (β = 2.01; 95% CI: 1.19, 2.83); however, when stratified only in the highest fish consumption subgroup. Among female children, higher child blood Hg concentrations were associated with an approximately 1-point increase in FSIQ, VIQ, and GLC.

Conclusions: Prenatal exposure to low levels of Hg was associated with lower PIQ scores in male children with low prenatal fish intake. Positive associations between cord and child blood Hg

concentrations and IQ were primarily observed in female children and may be due to beneficial effects of prenatal fish intake.

6.2 Introduction

Hg, an ubiquitous environmental contaminant, is a global public health concern because of its deleterious health effects, such as those on the nervous system and impairments in cognitive development (Basu et al., 2023; United Nations, 2019; Chen et al., 2018; Kim & Choi, 2012; Choi & Grandjean, 2008; Mergler et al., 2007; Goldman et al., 2001). The developing fetus is particularly susceptible to the neurotoxic effects of Hg because their nervous system is undergoing rapid development (Ruggieri et al., 2017; Clifton II, 2007; Goldman et al., 2001). Epidemiological studies have demonstrated that prenatal exposure to Hg can have harmful impacts on children's cognitive function (Jeong et al., 2017; Jacobson et al., 2015; Suzuki et al., 2010; Crump et al., 1998; Grandjean et al., 1997), such as intelligence quotient (IQ) (Jeong et al., 2017; Jacobson et al., 2015). In addition, childhood exposure to Hg has also been negatively associated with some neurodevelopmental outcomes (dos Santos-Lima et al., 2020; Myers et al., 2009), including Verbal IQ (VIQ) (dos Santos-Lima et al., 2020).

Most studies of Hg and cognitive function have been conducted in populations with higher levels of exposure than is typically experienced by pregnant women and children in the general Canadian population, such as that observed in the Canadian Health Measures Survey (CHMS) (Health Canada, 2011, 2019a). Moreover, few studies have measured Hg at multiple time points to directly compare the impacts of prenatal and childhood exposures on cognitive function (Hsi et al., 2014; Davidson, et al., 2010; Plusquellec et al., 2010). Several epidemiological studies have not found associations between early life Hg exposure and cognitive function (Golding et al., 2022; 2017; 2016; Hibbeln et al., 2018; Oken et al., 2016; Cao et al., 2010; Davidson et al., 1998); these discrepant findings may be due to the protective role of fish and shellfish consumption during pregnancy.

While some fish species can be a common source of Hg, especially for the more toxic form MeHg, fish are also an important source of omega-3 PUFA, protein, vitamin D, and other minerals essential for optimal fetal development (Health Canada, 2007) and ultimately children's neurodevelopment (Avella-Garcia & Julvez, 2014). Several studies have found that fish consumption during pregnancy can modify the association between prenatal Hg exposure and neurodevelopmental outcomes (Golding et al., 2017; Jeong et al., 2017; Jacobson et al., 2015), showing that the beneficial effects of nutrients in fish and seafood sources may offset the adverse effects of exposure to low levels of Hg.

However, co-exposures to other contaminants (e.g., lead, polybrominated diphenyl ethers (PBDEs), or polychlorinated biphenyls (PCBs)), nutrients (e.g., Se), as well as sociodemographic factors may confound or modify associations between prenatal and childhood Hg exposures and child neurodevelopment (Azar et al., 2021; Castriotta et al., 2020; Kim et al., 2020; Tratnik et al., 2017a; Orenstein et al., 2014). Se may be protective against the toxic effects of Hg exposure (Ralston &

Raymond, 2010; Cuvin-Aralar & Furness, 1991; Pelletier, 1986; Parizek et al., 1969) as Hg and Se interact and can form complexes reducing the biological availability of Hg (Raymond & Ralston, 2004).

Finally, these associations between prenatal Hg and child neurodevelopment may differ according to child sex (Wang et al., 2019; Llop et al., 2012; Sagiv et al., 2012). Sex-specific associations between prenatal exposures to other environmental contaminants and child IQ have been observed in previous analyses from our Maternal-Infant Research on Environmental Contaminants (MIREC) cohort study (Azar et al., 2021; Nkinsa et al., 2020; Green et al., 2019; Desrochers-Couture et al., 2018). Generally, negative associations were more commonly seen for the male children in these studies.

Further studies are needed to examine the sex-specific associations between the low levels of Hg exposure during pregnancy and early childhood that are typical in Western countries (Basu et al., 2018; Wong & Lye, 2008) and child cognitive function, with consideration of the potential modifying role of fish consumption during pregnancy.

The goal of the present study was to examine the sex-specific associations between Hg exposure during two critical developmental windows (prenatal and early childhood) and early childhood IQ, as well as to determine whether fish consumption during pregnancy modifies these associations. Additionally, we explored the potential for an interaction between child blood Hg and child blood Se concentrations. Finally, we adjusted for co-exposure to lead, PBDEs, or PCBs to determine if co-exposure to these chemical contaminants affect the associations observed between early life Hg exposures and child IQ.

6.3 Methods

6.3.1 Study Design and Population

We analyzed data from the MIREC pregnancy cohort (Arbuckle et al., 2013) and the MIREC-Child Development (CD) Plus follow-up study (Fisher et al., 2023). In the MIREC study, participants were recruited in their first trimester of pregnancy between 2008 and 2011 from obstetric and prenatal clinics from ten cities in Canada. Participants were eligible if they were able to communicate in English or French, > 18 years of age, < 14 weeks gestation, and planning to deliver at a local hospital. A subset of participants were followed from six of the initial 10 MIREC sites in 2013–2015 when the children were 2–5 years old (Fisher, 2023). In the MIREC-CD Plus follow-up study, the birthing parent completed a questionnaire to capture covariate information while children provided a blood sample and participated in neurodevelopment tests administered by trained examiners. Both studies were approved by the Research Ethics Boards of Health Canada/Public Health Agency of Canada, Sainte-Justine University Hospital, as well as the ethic boards for all MIREC-affiliated study sites. Informed consent was obtained from all participants, as well as child assent. Of the participants recruited for the MIREC study (n = 2001), this

analysis was restricted to the subset of participants that were included in the MIREC-CD Plus follow-up study and had complete neurodevelopment data (n = 527) (**Supplementary Figure 6.1**).

6.3.2 Hg and Other Analyte Concentrations

Total Hg concentrations were measured in whole blood samples collected during the first trimester (6–13 weeks), from the umbilical cord at birth (Arbuckle et al., 2016; 2013), and from children collected concurrently with the IQ assessment (Fisher, 2023). Hg can readily cross the blood-brain barrier and the placenta (Clifton II, 2007; Goldman et al., 2001); therefore, cord blood provides a more proximal marker of fetal exposure than first trimester blood (Clifton II, 2007; Goldman et al., 2001). As previously described (Arbuckle et al., 2016; 2013), blood samples were analyzed at the Centre de Toxicologie, Institut National de Santé Publique du Québec, using inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer ELAN ICP-MS DRC II; Norwalk CT, USA). The limit of detection (LOD) for total Hg was 0.12 µg/L in first trimester blood and 0.40 µg/L for cord blood (Arbuckle et al., 2016). The LODs for total Hg concentrations in children's blood were between 0.05–0.06 µg/L.

As previously described, lead concentrations were also measured by ICP-MS in first trimester, umbilical cord, and children's whole blood with LODs of 0.1 µg/dL, 0.1 µg/dL, and 0.21 µg/dL respectively (Desrochers-Couture et al., 2018; Arbuckle et al., 2016). PBDE and PCB concentrations were measured in first trimester plasma samples (with LODs ranging from 0.005 – 0.3 µg/L) along with total lipid concentrations, as previously described (Fisher et al., 2016). Total Se concentrations were also measured by ICP-MS in child whole blood samples with a LOD of 8.15 µg/L.

6.3.3 Child IQ

Neurodevelopment visits were conducted between 36 and 47 months of age among singleton children who were born > 28 weeks gestation and without major congenital birth defects, seizures, or major neurological disorders during the perinatal period. The majority (98%) of neurodevelopment visits were conducted at the child's home by study staff. Training, monitoring, and oversight of test administration was performed by a licenced clinical psychologist. The Wechsler Preschool and Primary Scale of Intelligence–3rd Edition (WPPSI-III), short version, was used to assess cognitive function (Wechsler, 2002). Results on the five subtests (Block Design, Object Assembly, Receptive Vocabulary, Information, and Picture Naming) were used to calculate four composite scores: Full-scale IQ (FSIQ), Verbal IQ (VIQ), Performance IQ (PIQ), and General Language Composite (GLC). These composite scores were age-standardized to Canadian norms (Wechsler, 2004) with a mean of 100 and a standard deviation of 15 points. The Canadian edition of the WPSSI-III was normed and standardized based on a validation sample of 700 Canadian children whose demographic characteristics corresponded to those

reported in the 2001 Canadian census (Wechsler, 2004). In the validation sample of 2.5–7.3 year old children, composite scores had excellent internal consistencies (Cronbach's alpha = 0.90 – 0.96) and the test-retest reliability coefficients were good (intra-class correlation = 0.83 – 0.90) (Wechsler, 2004). In the MIREC-CD Plus study, examiners, parents, and children were not aware of the prenatal or child blood Hg concentrations at the time of the neurodevelopment visit.

6.3.4 Covariates

We identified covariates *a priori* from studies that have described correlates of both prenatal (Arbuckle et al., 2016) and child blood Hg (Vogel et al., 2021; Garí et al., 2013; Hrubá et al., 2012), as well as previous studies examining associations between Hg and various neurodevelopmental outcomes (Barbone et al., 2019; Jeong et al., 2017; Jacobson et al., 2015; Suzuki et al., 2010; Crump et al., 1998; Davidson et al., 1998; Grandjean et al., 1997). We developed directed acyclic graphs (DAGs) for exposure to Hg during both the prenatal and childhood time-periods (**Supplementary Figures 6.2** and **6.3**). For the prenatal exposures, covariates included the birthing parent's: age (continuous), pre-pregnancy body mass index (BMI) (continuous), parity (nulliparous vs. multiparous), level of education during early pregnancy (high school diploma or less, completed/some college or trade school, university degree), cigarette smoking status during early pregnancy (never, former, current/quit during pregnancy), alcohol use during early pregnancy (yes vs. no), place of birth (Canada vs. other), study site (random effect), and fish consumption during early pregnancy (low, moderate, high). For the sensitivity analyses, covariates also included if the birthing parent had dental fillings (zero, more than one, unknown), and whether the birthing parent had any dental filling replaced in the past 12 months (yes or no). Participant socio-demographic and lifestyle characteristics were collected through -questionnaires administered during each trimester, and child sex at birth was extracted from medical records. Participants also completed a food frequency questionnaire indicating daily, weekly, or monthly consumption of 25 species of fish during the month prior to their first trimester clinic visit. We calculated summary scores for the monthly frequency of consuming all fish species and categorized fish consumption in early pregnancy as low (0–2 times/month), moderate (3–7 times/month) and high (≥ 8 times/month). We also examined the frequency of consuming fish classified as being high in Hg (i.e., tuna, marlin, orange roughy, shark, swordfish, mackerel, escolar) (Health Canada, 2019b). Consumption of fish high in Hg was moderately correlated with the total fish consumption (consumption of the 25 species of fish) (Spearman r = 0.62). The frequency of consumption of fish high in Hg tended to increase across the categories created for total fish consumption (**Supplementary Tables 6.1A** and **6.1B**). Given these correlations and that fish consumption is not just a source of Hg but is also a source of nutrients required for optimal

neurodevelopment, we stratified fish consumption frequency according to consumption of all 25 species of fish.

For childhood Hg exposure, covariates included the birthing parent's age during pregnancy (continuous), education during pregnancy (high school diploma or less, completed/some college or trade school, university degree), parity as of early pregnancy (nulliparous vs. multiparous), birthing parent's depression at follow-up (continuous), and included the child's blood Se concentrations (continuous), duration of breastfeeding (none, < 6 months, ≥ 6 months), and a Home Observation for Measurement of the Environment (HOME) inventory score (continuous) measured at follow-up. The birthing parent's depression was assessed using the Center for Epidemiological Studies Depression Scale (CES-D), with scores ranging from 0 to 60 and higher scores indicating greater depressive symptoms (Radloff, 1977) and a derived depression score ranging from 0-10 was calculated. At follow-up, the birthing parent also completed the 55-item HOME inventory, which assessed the quality of support and stimulation that children receive at home, with a HOME total score ranging from 0–55 (Caldwell & Bradley, 2001).

6.3.5 Statistical Analysis

All analyses were conducted in SAS Enterprise Guide 7.1 (SAS institute, Cary, NC). Analyte concentrations below the LOD were substituted with one-half of the analyte and matrix-specific LOD. Missing covariate data were imputed using multiple imputation with the fully conditional specification method using a logistic model for categorical variables and a linear model for continuous variables. The proportion of missingness across covariates was less than 5% for categorical and continuous variables, with the exception of pre-pregnancy BMI (missingness for pre-pregnancy BMI was 9% and 3% for male and female children respectively) (**Table 6.1**). We used 5 imputations, which provided > 99% relative efficiency suggesting that additional imputations were not necessary. Iteration-specific parameter estimates were combined to provide appropriate variance estimation. Summary statistics (median (IQR) or n (%) as appropriate) are presented by child sex as well as stratified by fish consumption frequency categories. Hg concentrations were $\log_2$-transformed to normalize distributions and to allow for interpretation of parameter estimates as per a 2-fold increase in exposure. We used separate generalized linear mixed models to investigate the associations between Hg concentrations at each time point (first trimester, cord, and child blood) and FSIQ, VIQ, PIQ, and the GLC. These models were adjusted for confounders included in the DAGs as fixed effects, with study site included as a random effect. All models were stratified by child sex. We also explored associations of first trimester and cord blood Hg concentrations stratified by fish consumption during pregnancy and child sex. Furthermore, in the models

examining the associations between child Hg and IQ, we explored the interaction between child blood Hg and child blood Se concentrations.

As with any cohort study, one of the threats to internal validity is selection bias via differential loss to follow-up (Lash et al., 2020). We used inverse probability weighting (IPW) with stabilized weights (Seaman & White, 2013) to minimize the potential effect of differential loss to follow-up on the observed associations. We used logistic regression to derive the predicted probability of participation in the follow-up study (of those participants from the six eligible sites) based on a range of factors that may be associated with retention (Supplementary Table 2). The inverse of these predicted probabilities of follow-up participation was included as a weight in the analysis.

6.3.6 Sensitivity Analysis

We conducted several sensitivity analyses to compare the change in effect size when adjusting the models for additional exposures or covariates, or when applying additional exclusion criteria. For the models examining the associations between prenatal blood Hg concentrations (first trimester and cord blood Hg concentrations) and child IQ, we separately adjusted for: 1) first trimester or cord blood lead concentrations (separately for each time point); 2) sum of first trimester plasma PBDEs 47, 99, and 153; 3) sum of first trimester plasma PCBs 138, 153, and 180; 4) the presence of dental amalgams; and 5) having dental amalgams replaced in the past 12 months. We included plasma lipid concentration in the models containing PBDEs and PCBs. For the models examining the associations between childhood blood Hg concentrations and child IQ, we conducted separate sensitivity analyses adjusting for: 1) child blood lead concentrations; and 2) cord blood Hg concentrations. Lastly, in separate sensitivity analyses for all time-points, we ran models excluding children born preterm.

6.4 Results

6.4.1 Descriptive results

First trimester blood Hg and child blood Hg concentrations were similar when stratified by child sex, but cord blood Hg was higher in male children (**Table 6.1**). Higher first trimester and cord blood Hg concentrations were found with increasing fish consumption frequency during pregnancy in both male and female children (**Supplementary Tables 6.3A** and **6.3B**). Compared with the lowest fish consumption strata (consuming fish 0–2 times/month), Hg concentrations were 2–3 times higher in the highest fish consumption strata (consuming fish ≥ 8 times/month). Sociodemographic and other covariate data, along with IQ scores, were similar across categories of fish consumption (**Supplementary Tables 6.3A** and **6.3B**). Hg concentrations (first trimester and cord blood) and fish consumption during early

pregnancy in the analytical sample did not differ from that of the full MIREC cohort (**Supplementary Table 6.4**). On average, child IQ (FSIQ, VIQ, PIQ, and GLC) scores were slightly higher for female children compared to male children (**Table 6.1**) but were similar across categories of fish consumption during pregnancy (**Supplementary Tables 6.3A** and **6.3B**). The IQ measures were positively intercorrelated among male children (r = 0.39 to 0.86), as well as among female children (r = 0.22 to 0.84) (**Supplementary Tables 6.5A** and **6.5B)**. Hg concentrations and IQ scores stratified by relevant covariates are included in **Supplementary Tables 6.6A**, **6.6B**, **6.7A**, **6.7B**, and **6.8**.

Table 6.1: Participant characteristics stratified by child sex, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies

	Male children		**Female children**	
	n[a]	**Median (IQR) or n (%)**	**n[a]**	**Median (IQR) or n (%)**
Hg concentrations				
First trimester blood Hg (µg/L)[b]	257	0.68 (0.36 – 1.28)	270	0.60 (0.28 – 1.12)
Cord blood Hg (µg/L)[b]	214	0.91 (<LOD – 1.64)	216	0.62 (<LOD – 1.15)
Child blood Hg (µg/L)[b]	181	0.18 (<LOD – 0.52)	174	0.20 (<LOD – 0.50)
WPPSI-III				
Full-scale IQ	257	105 (96 – 113)	270	110 (100 – 117)
Verbal IQ	255	108 (98 – 116)	269	111 (103 – 119)
Performance IQ	253	100 (91 – 112)	269	103 (94 – 115)
General Language Composite	254	105 (98 – 117)	270	108 (100 – 120)
Child characteristics				
Age (months)	257	40 (37 – 44)	270	39 (37 – 43)
Breastfeeding status				
Never	18	7%	24	9%
Breastfeeding < 6 months	132	51%	113	42%
Breastfeeding ≥ 6 months	103	40%	126	47%
Missing	4	2%	7	3%
HOME Total Score	251	47 (44 – 50)	261	49 (46 – 51)
Birthing parent characteristics				
Age at delivery (years)	257	32 (29 – 36)	270	32 (30 – 36)
Parity				
0	112	44%	122	45%
1+	145	56%	148	55%
Level of education				
High school diploma or less	17	7%	10	4%
Completed/Some college/trade school	79	31%	79	29%
University degree	160	62%	180	67%
Missing	1	<1%	1	<1%

Smoking status during early pregnancy				
Never	170	66%	180	67%
Current/Quit During Pregnancy	24	9%	19	7%
Former	63	25%	71	26%
Alcohol use during early pregnancy				
Yes	117	46%	112	41%
No	140	54%	157	58%
Missing	0	0%	1	<1%
Place of birth				
Canada	216	84%	227	84%
Other	41	16%	43	16%
Monthly consumption of fish in early pregnancy				
Low (0-2 times/month)	101	39%	101	37%
Moderate (3 -7 times/month)	88	34%	93	34%
High (≥8 times/month)	68	26%	76	28%
Pre-pregnancy BMI (kg/m^2)	234	24 (22 – 28)	250	24 (21 – 27)
CES-D depression score	249	6 (3 – 9)	259	4 (2 – 7)
Additional analytes				
First Trimester blood lead (µg/dL)	256	0.62 (0.44 – 0.87)	270	0.62 (0.46 – 0.83)
First trimester blood PBDEs[c] (µg/L)	255	0.08 (0.05 – 0.16)	264	0.08 (0.04 – 0.13)
First trimester blood PCBs[d] (µg/L)	257	0.10 (0.07 – 0.15)	267	0.10 (0.06 – 0.16)
First trimester plasma lipids (g/L)	257	5.9 (5.2 – 6.8)	267	6.0 (5.4 – 6.9)
Cord blood lead (µg/dL)	214	0.83 (0.58 – 1.04)	216	0.79 (0.60 – 1.06)
Child blood lead (µg/L)	181	0.68 (0.51 – 1.00)	174	0.67 (0.47 – 0.99)
Child blood selenium (µg/L)	181	150 (142 – 158)	174	150 (142 – 158)

[a] Sample sizes may differ for continuous variables due to missing data.

[b] For male children, 8%, 25%, and 37% of the values were < LOD for the first trimester, cord, and child blood Hg concentrations respectively. For female children, 12%, 34%, and 29% of the values were < LOD for the first trimester, cord, and child blood Hg concentrations, respectively.

[c] Sum of PBDE 47, 99, and 153

[d] Sum of PCB 138, 153, and 180

WPPSI – Weschler Preschool and Primary Scale of Intelligence; HOME – Home Observation for Measurement of the Environment (HOME) inventory score; BMI – Body Mass Index; CES-D – Center for Epidemiological Studies Depression Scale

6.4.2 Prospective associations between first trimester blood Hg concentrations and child IQ

First trimester blood Hg concentrations were not associated with FSIQ, PIQ, VIQ, and GLC in either male or female children (**Tables 6.2A** and **6.2B**). However, each doubling of first trimester blood Hg concentration was associated with a 2.3-point increase PIQ in both male (β = 2.34, 95% CI = 0.52, 4.16) and female children (β = 2.37, 95% CI= 0.32, 4.42) in the highest fish consumption frequency subgroup (≥ 8 times/month). Additionally, for PIQ for male children, we observed a monotonic response across prenatal fish consumption categories, such that the associations were more strongly positive with increasing fish consumption. First trimester blood Hg concentration were also positively associated with female children's FSIQ scores (β =0.77, 95% CI= 0.31, 1.23) in the moderate frequency fish consumption (3–7 times/month) subgroup. When stratified by sex and fish consumption, associations were less precise, which may reflect the smaller sample sizes in these stratified groups (**Tables 6.2A** and **6.2B**).

Associations between first trimester blood Hg concentrations and child IQ for male children (**Supplementary Table 6.9A**) and female children (**Supplementary Table 6.9B**) remained relatively unchanged in sensitivity analyses after adjusting for first trimester blood concentrations of lead, PBDEs, or PCBs, as well as for the presence or replacement of dental amalgams, or when excluding children born preterm.

6.4.3 Prospective associations between cord blood Hg concentrations and child IQ

Overall, cord blood Hg concentrations and FSIQ, PIQ, VIQ, and GLC, were not associated for male children (**Table 6.3A**). We observed an inverse association between cord blood Hg concentrations and PIQ among male children in the low frequency of fish consumption subgroup (β = -3.27, 95% CI = -6.44, -0.09); however, the associations were positive among male children in the moderate (β = 1.08, 95% CI = -0.10, 2.26) and high (β = 3.07, 95% CI = 1.95, 4.19) fish consumption subgroup. Among participants with moderate frequency of fish consumption during pregnancy, cord blood Hg concentrations were positively associated with FSIQ (β = 1.56, 95% CI= 0.88, 2.25), VIQ (β =1.27, 95%

CI= -0.01, 2.56) and GLC (β = 2.58, 95% CI= 0.98, 4.17) in male children (**Table 6.3A**) while the associations were not seen in those with low or high prenatal fish consumption.

Among the female children, each doubling of cord blood Hg concentrations was associated with higher scores on the FSIQ (β = 1.29, 95% CI = 0.77, 1.81) and PIQ (β = 2.01, 95% CI = 1.19, 2.83) (**Table 6.3B**). When stratified by fish consumption during pregnancy, these associations were most prominent among female children with a high prenatal fish consumption. However, the pattern of association across fish consumption categories was non-monotonic in female children. Cord blood Hg concentrations were inversely associated with VIQ (β = -2.02, 95% CI= -3.04, -1.00) and GLC scores (β = -2.37, 95% CI= -4.32, -0.43) among female children with moderate prenatal fish consumption (**Table 6.3B**).

Associations between cord blood Hg and child IQ among male children (**Supplementary Table 6.10A**) and female children (**Supplementary Table 6.10B**) remained relatively unchanged in sensitivity analyses after adjusting for cord blood lead concentrations, first trimester blood concentrations of PBDEs or PCBs, as well as for the presence of or replacement of dental amalgams, or when excluding children born preterm.

Table 6.2A: Prospective associations between first trimester blood Hg concentrations and child IQ for male children and stratified by prenatal fish, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies

	Full sample		Stratified by frequency of fish consumption					
			0-2 times/month		3-7 times/month		≥8 times/month	
	n	β (95% CI)	n	β (95% CI)	N	β (95% CI)	n	β (95% CI)
Full-scale IQ	257	-0.18 (-2.30, 1.94)	101	-0.44 (-3.38, 2.49)	88	-0.48 (-3.66, 2.70)	68	1.11 (-1.39, 3.61)
Verbal IQ	255	-0.50 (-2.73, 1.73)	100	-0.10 (-2.51, 2.32)	88	-1.01 (-4.21, 2.18)	67	0.12 (-2.01, 2.24)
Performance IQ	253	0.18 (-1.67, 2.02)	100	-0.64 (-3.75, 2.48)	87	0.11 (-2.55, 2.77)	66	2.34 (0.52, 4.16)
General Language Composite	254	0.05 (-2.13, 2.23)	101	0.43 (-2.72, 3.57)	86	0.15 (-1.93, 2.23)	67	0.25 (-2.10, 2.61)

Table 6.2B: Prospective associations between first trimester blood Hg concentrations and child IQ for female children and stratified by prenatal fish consumption, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies

	Full sample		Stratified by frequency of fish consumption					
			0-2 times/month		3-7 times/month		≥8 times/month	
	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)
Full-scale IQ	270	0.44 (-0.84, 1.72)	101	0.29 (-1.32, 1.90)	93	0.77 (0.31, 1.23)	76	0.69 (-0.19, 1.58)
Verbal IQ	269	-0.09 (-1.18, 1.00)	100	0.51 (-1.55, 2.57)	93	-0.57 (-2.04, 0.91)	76	-0.68 (-2.81, 1.45)
Performance IQ	269	0.82 (-1.03, 2.66)	101	0.46 (-1.08, 2.00)	93	1.67 (-0.34, 3.68)	76	2.37 (0.32, 4.42)
General Language Composite	270	-0.74 (-2.08, 0.60)	101	-0.63 (-2.91, 1.65)	93	-0.62 (-2.49, 1.26)	76	-0.41 (-2.18, 1.36)

All models are adjusted for birthing parent age, birthing parent pre-pregnancy BMI, parity, smoking status in early pregnancy, alcohol consumption in early pregnancy, birthing parent country of birth, HOME total scores, birthing parent education, and site (random effect). Missing covariate data were imputed and models include IPW. The full sample model is additionally adjusted for the frequency of fish consumption in early pregnancy.
Regression coefficients represent a 1-point change in IQ score for each 2-fold increase in Hg concentration.

Table 6.3A: Prospective associations between cord blood Hg concentrations and child IQ for male children and stratified by prenatal fish consumption, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies

	Full Sample		Stratified by frequency of fish consumption					
			0-2 times/month		3-7 times/month		≥8 times/month	
	n	β (95% CI)	n	β (95% CI)	N	β (95% CI)	N	β (95% CI)
Full-scale IQ	214	-0.58 (-2.10, 0.93)	83	-2.81 (-5.66, 0.04)	75	1.56 (0.88, 2.25)	56	0.74 (-2.49, 3.97)
Verbal IQ	212	-0.31 (-1.23, 0.60)	82	-1.53 (-3.43, 0.38)	75	1.27 (-0.01, 2.56)	55	-0.04 (-3.94, 3.86)
Performance IQ	210	-0.79 (-2.73, 1.15)	82	-3.27 (-6.44, -0.09)	74	1.08 (-0.10, 2.26)	54	3.07 (1.95, 4.19)
General Language Composite	211	-0.89 (-1.95, 0.16)	83	-2.05 (-4.23, 0.13)	73	2.58 (0.98, 4.17)	55	-0.58 (-4.38, 3.21)

Table 6.3B: Prospective associations between cord blood Hg concentrations and child IQ for female children and stratified by prenatal fish consumption, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies

	Full Sample		Stratified by frequency of fish consumption					
			0-2 times/month		3-7 times/month		≥8 times/month	
	n	β (95% CI)	n	β (95% CI)	N	β (95% CI)	n	β (95% CI)
Full-scale IQ	216	1.29 (0.77, 1.81)	87	0.66 (-0.64, 1.97)	71	-0.32 (-1.44, 0.80)	58	1.60 (0.05, 3.15)
Verbal IQ	215	-0.01 (-0.98, 0.96)	86	0.36 (-0.74, 1.46)	71	-2.02 (-3.04, -1.00)	58	-1.24 (-3.50, 1.02)
Performance IQ	215	2.01 (1.19, 2.83)	87	1.79 (-1.29, 4.88)	70	1.22 (-0.88, 3.34)	58	2.97 (1.82, 4.12)
General Language Composite	216	0.11 (-0.99, 1.22)	87	0.87 (-0.82, 2.55)	71	-2.37 (-4.31, -0.43)	58	0.15 (-3.18, 3.48)

All models are adjusted for birthing parent age, birthing parent pre-pregnancy BMI, parity, smoking status in early pregnancy, alcohol consumption in early pregnancy, birthing parent country of birth, HOME total scores, birthing parent education, and site (random effect). Missing covariate data were imputed and models include IPW. The full sample model is additionally adjusted for the frequency of fish consumption in early pregnancy.
Regression coefficients represent a 1-point change in IQ score for each 2-fold increase in Hg concentration.

6.4.4 Concurrent associations between child blood Hg concentrations and child IQ

Child blood Hg concentrations were not associated with IQ among male children (**Table 6.4**). However, among female children, each doubling of blood Hg concentration was associated with approximately 1-point increase in FSIQ (β = 0.97, 95% CI= 0.09, 1.84), VIQ (β = 1.04, 95% CI= 0.41, 1.67), and GLC (β =1.09, 95% CI= 0.23, 1.82), but not PIQ score. We did not observe an interaction between child blood Hg and Se concentrations in either male or female children (**Supplementary Table 6.11**).

All sensitivity analyses had little impact on the results for male children (**Supplementary Table 6.12A**). Similarly, among female children (**Supplementary Table 6.12B**), adjusting for child blood lead concentrations had little impact on the results, while excluding children born preterm slightly attenuated the associations with FSIQ, VIQ, and GLC scores towards the null. Adjusting for cord blood Hg concentrations attenuated the association between child blood Hg and FSIQ towards the null, but slightly strengthened the positive associations with VIQ and GLC.

Table 6.4: Concurrent associations between child blood Hg concentrations and IQ, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Male children		**Female children**	
	n	**β (95% CI)**	**n**	**β (95% CI)**
Full-scale IQ	181	-0.14 (-1.57, 1.29)	174	0.97 (0.09, 1.84)
Verbal IQ	181	-0.21 (-1.90, 1.47)	173	1.04 (0.41, 1.67)
Performance IQ	179	-0.03 (-1.25, 1.20)	173	0.69 (-0.73, 2.11)
General Language Composite	179	0.43 (-0.98, 1.84)	174	1.03 (0.23, 1.82)

Regression coefficients represent a 1-point change in IQ score for each 2-fold increase in Hg concentration. Models are adjusted for parity, HOME total development scores, birthing parent education, breastfeeding status, birthing parent depression, child blood Se concentrations, and site (random effect). Missing covariate data were imputed and models include IPW.

6.5 Discussion

We examined sex-specific associations between prenatal, and child blood Hg concentrations and IQ in Canadian children. We also examined the potential for effect modification by fish consumption during pregnancy in analyses of prenatal blood Hg concentrations and child IQ as well as potential interactions between child blood Hg and Se concentrations.

6.5.1 Associations between prenatal Hg concentrations and child IQ

In male children, first trimester blood Hg concentrations and cord blood Hg concentrations were not associated with IQ but we found a monotonic positive association between both first trimester and cord blood Hg concentrations and PIQ with increasing frequency of prenatal fish consumption. At the lowest category of prenatal fish consumption, we found an inverse association between cord blood Hg concentrations and PIQ in male children, but in the highest prenatal fish consumption category the association was positive. For cord blood Hg concentrations, this represented a 6-point difference in PIQ score between the low and high frequency consumption groups.

In female children, cord blood Hg concentrations were positively associated with FSIQ and PIQ scores, but when stratified by prenatal fish consumption, positive associations were only observed for female children with the highest prenatal fish consumption. This association among the highest fish consumption group was also observed with first trimester blood Hg. For female children with moderate prenatal fish consumption, there were negative associations with VIQ and the GLC.

Across the models, associations between Hg exposures and PIQ and FSIQ seemed to follow a similar trend, while associations between Hg exposures and VIQ and GLC seemed to also follow a similar trend. Although all IQ measures were correlated with each other, the correlations between PIQ and FSIQ (male children = 0.86 and for female children = 0.80) and between VIQ and GLC (male children = 0.87 and for female children = 0.84) were the strongest. These correlations between FSIQ and the subscales (PIQ, VIQ, and GLC) likely explain some of the trends in results for FSIQ/PIQ and VIQ/GLC. VIQ is a measure of acquired knowledge, verbal reasoning, verbal comprehension, and attention to verbal stimuli, and the GLC is a measure of how quickly new receptive and expressive vocabulary skills are acquired. Whereas PIQ is a measure of fluid reasoning, spatial processing, attention to detail, and visual-motor integration, and FSIQ is a measure of global cognitive functioning.

Our finding that prenatal Hg concentrations were associated with PIQ, but not other IQ measures, is consistent with previous studies examining prenatal exposures to other contaminants, including cadmium (Jeong et al., 2015) and lead (Desrochers-Couture et al., 2018). Additionally, a study of British children showed that prenatal blood Hg concentrations

were associated with PIQ and FSIQ in models that included children with prenatal fish consumption, while prenatal blood Hg concentrations were not consistently associated with VIQ (Golding et al., 2017).

The differences in associations between prenatal Hg concentrations and child IQ when stratified by child sex were not unexpected as previous analyses from the MIREC cohort (Azar et al., 2021; Nkinsa et al., 2020; Green et al., 2019; Desrochers-Couture et al., 2018) and other cohorts (Wang et al., 2019; Llop et al., 2017; Sagiv et al., 2012) have shown sex-specific associations between prenatal exposures to a variety of environmental contaminants and child IQ. For example, in a study of 8 year-olds, Sagiv et al., (2012) primarily found adverse associations between Hg and neurobehavioral outcomes in male children while protective associations were observed in female children. However, differences in the associations between blood Hg concentrations and child cognitive development by child sex have not been seen consistently in other studies (Rothenberg et al., 2021; Wang et al., 2019; Orenstein et al., 2014), including studies assessing developmental outcomes in infants/toddlers as measured using the Bayley Scales of Infant and Toddler Development (Rothenberg et al., 2021; Wang et al., 2019).

Other studies with similar or even higher prenatal blood Hg concentrations than in the MIREC cohort have also found no associations (Davidson et al., 1998), inconsistent associations (Barbone et al., 2019; Golding et al., 2017), or positive associations (Golding et al., 2016), between prenatal blood Hg concentrations and child IQ or other cognitive outcomes. The positive associations seen in this study could be due to the nutritional benefits of fish consumption (Myers et al., 2009). Fish consumption is known to have many health benefits (Chen et al., 2022) and includes important nutrients such as omega-3 PUFA and Se (Health Canada, 2007). However, total fish consumption is a crude indicator of the intake of nutrients and contaminants from fish, as concentrations of nutrients and contaminants in fish tissue can vary widely based on the fish species consumed, preparation/cooking method, and the part of the fish species consumed (Marques et al., 2019; Sobral et al., 2018; Seves et al., 2016; Du et al., 2012).

Our results support the hypothesis that fish consumption during pregnancy may be an effect modifier of the associations between prenatal blood Hg concentrations and child IQ, in agreement with other studies (Golding et al., 2017; Jeong et al., 2017; Llop et al., 2017; Jacobson

et al., 2015; Jacobson et al., 2008). However, the direction of modification is not always consistent. For example, Golding et al., (2017) found positive associations between prenatal blood Hg concentrations and child IQ but these positive associations were only observed for children whose mothers consumed fish (as opposed to those whose mothers did not consume fish). While Jeong et al., (2017) found negative associations between prenatal blood Hg concentrations and child IQ (VIQ and FSIQ) and when stratified by fish intake, these negative associations were only observed at the highest category of fish intake. However, prenatal fish consumption was much higher on average in the Jeong et al., (2017) study from South Korea, than in the British cohort (Golding et al., 2017) and the MIREC cohort study.

Exposure to other contaminants from fish and seafood consumption, such as PCBs, may also confound the relationship between prenatal blood Hg concentrations and child IQ (Tratnik et al., 2017b; Avella-Garcia & Julvez, 2014; Counter & Buchanan, 2004). We conducted sensitivity analyses by additionally adjusting for other environmental contaminants including lead, PBDEs, and PCBs. Ultimately, this did not impact the direction, magnitude, or precision of the associations in the main models. This is consistent with previous analyses examining the potential confounding role of PCBs (Jacobson et al., 2015) and lead (Jeong et al., 2017) for the association between prenatal blood Hg concentrations and child IQ.

Additionally, the findings of null or positive associations in this study may be explained by the low levels of Hg observed in the MIREC cohort. Median first trimester blood Hg concentrations (0.68 and 0.60 µg/L for those pregnant with male and female children, respectively) were similar to blood Hg concentrations reported for women of childbearing age in the Canadian general population sampled around the same time as the MIREC cohort (Dix-Cooper & Kosatsky, 2018; Health Canada, 2011). These levels are lower than those measured in other studies of prenatal Hg exposure and child neurodevelopment where prenatal blood Hg (Golding et al., 2017; Jeong et al., 2017; Lederman et al., 2008) and/or cord blood Hg concentrations were measured (Llop et al., 2017; 2012; Tratnik et al., 2017b; Jacobson et al., 2015; Lederman et al., 2008; Grandjean et al., 1997).

6.5.2 Associations between Child Blood Mercury Concentrations and IQ

Child blood Hg concentrations in this study were generally low and similar to blood Hg concentrations reported for those aged 3–5 years of age in the Canadian general population (Health Canada, 2011). In male children, blood Hg concentrations were not associated with IQ scores. In female children, each doubling of blood Hg concentration was associated with approximately 1-point increase in FSIQ, VIQ, and GLC scores, but not PIQ score. This is consistent with our findings for prenatal Hg exposure, where we found that cord blood Hg was positively associated with FSIQ and PIQ for female children. Studies of childhood Hg exposure have found inconsistent (Myers et al., 2009), negative (dos Santos-Lima et al., 2020), or null associations (Pan et al., 2018; Davidson, et al., 2010) with child IQ. Similar to prenatal Hg exposures, it is possible that the positive associations that we observed between child blood Hg concentrations and IQ may be explained by the benefits of fish consumption. However, we did not collect data on childhood fish consumption in this cohort and are unable to examine this possibility. Future studies should explore the role of child fish consumption as a modifier of the concurrent association between blood Hg concentrations and IQ in childhood. However, childhood blood Se concentrations were included in the model as fish can be an important dietary source of Se, and prenatal Se levels have previously been associated with neurodevelopmental outcomes, including child IQ (Liu et al., 2021; Castriotta et al., 2020; Močenić et al., 2019; Amorós et al., 2018; Tratnik et al., 2017b; Kippler et al., 2016; Polanska et al., 2016; Yang et al., 2013). However, consistent with the study by Jacobson et al (2015), we found no interaction between child blood Hg and Se concentrations (**Supplementary Table 6.11**) in our models.

6.5.3 Strengths and Limitations

Strengths of the MIREC study include the longitudinal and geographically diverse study design, the availability of Hg biomarker data at multiple time points across the prenatal and childhood periods, the use of a well recognized, reliable, and validated instrument (WPPSI-III) to assess cognitive function in childhood, and the availability of data for many potential confounders, and co-exposures. An additional strength of our analysis is the use of IPW to account for potential differential loss to follow-up.

This study has some limitations. First, while the low levels of Hg exposure are a strength in terms of generalizing our findings to the Canadian population, our findings may not be generalizable to populations with higher blood Hg concentrations or more frequent fish consumption. These low levels of Hg exposure also meant that when the analyses were stratified by fish consumption during pregnancy, 51% of the cord blood Hg concentrations were below the limit of detection among participants in the strata with the lowest fish consumption. This is a limitation for the regression model in this group; however, the majority of first trimester blood Hg concentrations were above the detection limit for all three fish consumption strata, and the majority of cord blood Hg concentrations were above the detection limit for the moderate and high prenatal fish consumption strata. The higher detection frequency of Hg in the first trimester blood samples is likely due to lower LOD for first trimester samples (0.12 µg/L) vs. cord blood samples (0.40 µg/L). Both first trimester and cord blood Hg concentrations followed the same pattern across the fish consumption strata and, as expected, blood Hg concentrations increased with increased fish consumption frequency. Second, the sample size was relatively small for the models that were stratified by both child sex and fish consumption in early pregnancy. Replication with a larger sample size may be desirable. Third, data were not available for prenatal blood Se concentrations and other nutrients known to be present in some fish (such as omega-3 PUFA), or for childhood fish consumption, which precluded examining effect modification/interaction for these factors at these time points. Additionally, the prenatal fish consumption variable could be impacted by recall bias and may not reflect consumption patterns throughout pregnancy. Fourth, IQ was measured at a relatively young age in this study, and data from other caregivers were not available. Fifth, we analyzed blood total Hg and the speciation of Hg was not determined. However, in populations without high exposures to industrial or environmental sources of Hg, fish consumption is considered the major route of exposure to MeHg (Hong et al., 2012; Health Canada, 2007; Schober et al., 2003) and total blood Hg concentrations should mainly reflect MeHg exposure (Hong et al., 2012). Therefore, the use of total Hg concentrations in this study was considered suitable for the models stratified by fish consumption during pregnancy; however, there is some uncertainty regarding the main sources of Hg exposure among participants reporting no or low fish consumption during pregnancy. Dental amalgams can be a source of inorganic Hg exposure and we conducted sensitivity

analyses adjusting for the total number of dental amalgams and whether any had been replaced in the past year. The results of these additional sensitivity analyses were similar to those of the base models. Future studies could examine the speciation of Hg in blood and implications of Hg speciation for the associations between prenatal and childhood blood Hg concentrations and child IQ.

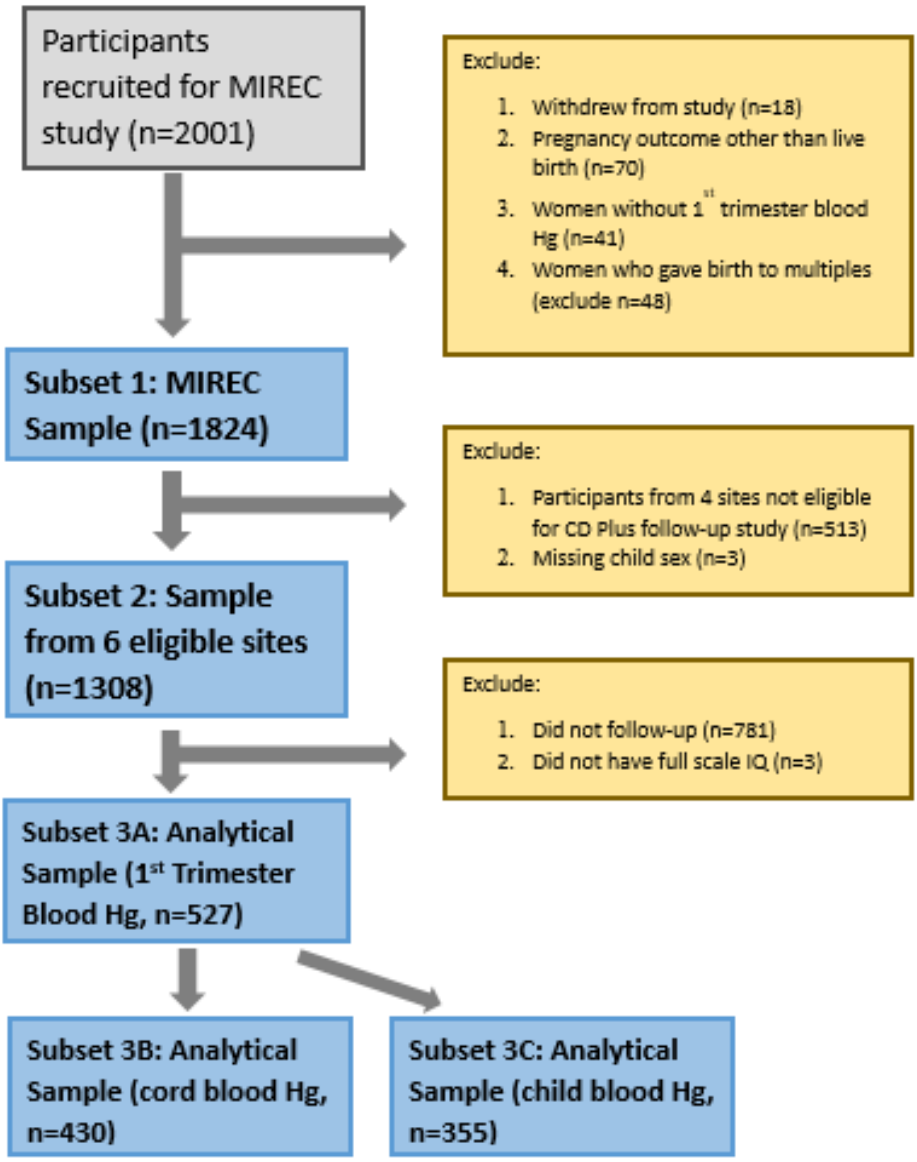
Finally, the potential for confounding is a concern of any observational epidemiological study, including the current one. To address this concern, we adjusted our analyses for a number of potential confounders based on the existing literature. We also showed that the means or proportions of covariates included in the multivariable analyses were similar across strata of fish consumption, suggesting that results from stratified analyses were not driven by differences in covariate distributions between strata. Additionally, we conducted sensitivity analyses to investigate potential confounding due to: 1) co-exposure to other environmental contaminants shown to be associated with cognitive functioning, 2) the presence of dental amalgams, and 3) prematurity. One possible unmeasured confounder is parental IQ which is plausibly associated with child IQ and possibly with fish consumption (and thereby Hg exposure). Potential confounding due to this variable is likely minimal because we adjusted our models for correlates of parental IQ including education, HOME score, smoking/alcohol use in pregnancy, and breastfeeding status. Finally, the MIREC study consists mainly of participants that are white and of higher socioeconomic status than the general Canadian population; although this sociodemographic profile limits the generalizability of our findings, it also minimizes the potential for unmeasured confounding due to socioeconomic status. Nonetheless, these factors do not eliminate the potential for unmeasured confounding and we encourage replication of these findings to evaluate the stability of these associations in different settings (i.e., across populations, geography, exposure levels, and time).

6.6 Conclusions

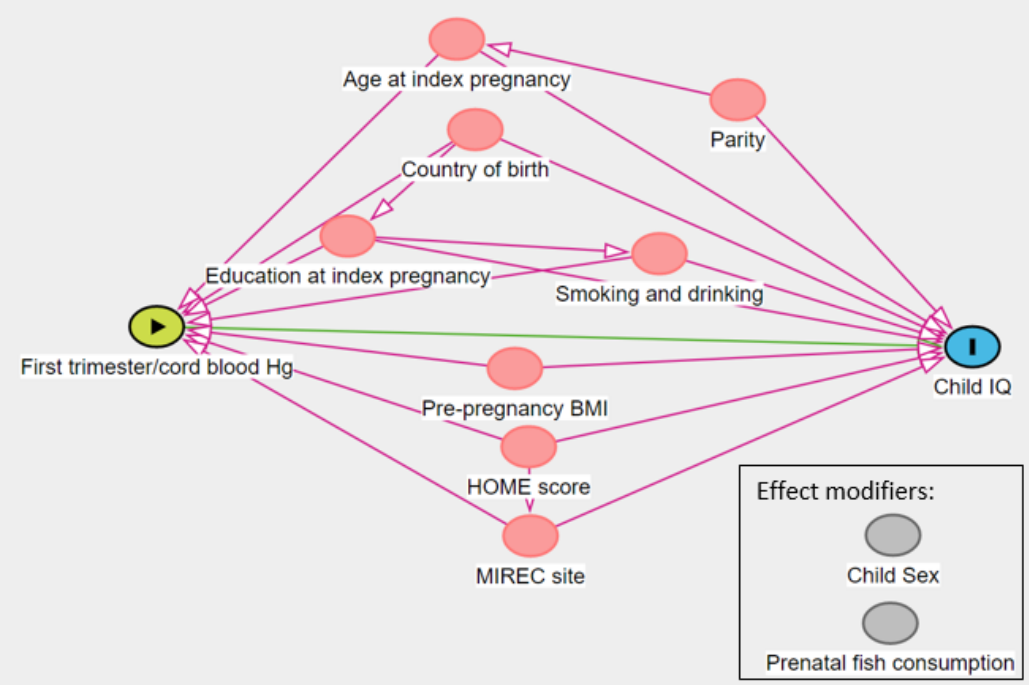
In this sample of Canadian children with low levels of Hg exposure, we observed primarily null associations between both prenatal and concurrent blood Hg concentrations and IQ in early childhood. Observed associations were inconsistent across time. We found that cord blood Hg concentrations were adversely associated with child PIQ in male children with low/no prenatal

fish consumption. Several positive associations were observed including between prenatal Hg concentrations and child PIQ in male and female children with the highest prenatal fish consumption. In female children, cord blood Hg concentrations were also positively associated with FSIQ in both the full sample and in those with the highest prenatal consumption of fish. The beneficial effects of fish consumption during pregnancy is a possible explanation for these findings. Concurrent blood Hg concentrations were positively associated with child IQ in female children. Although fish consumption is a source of exposure for Hg, it is also a source of important nutrients. Overall, these results reinforce the benefits of fish consumption (from low Hg containing fish species) during pregnancy, despite exposure to low levels of Hg.

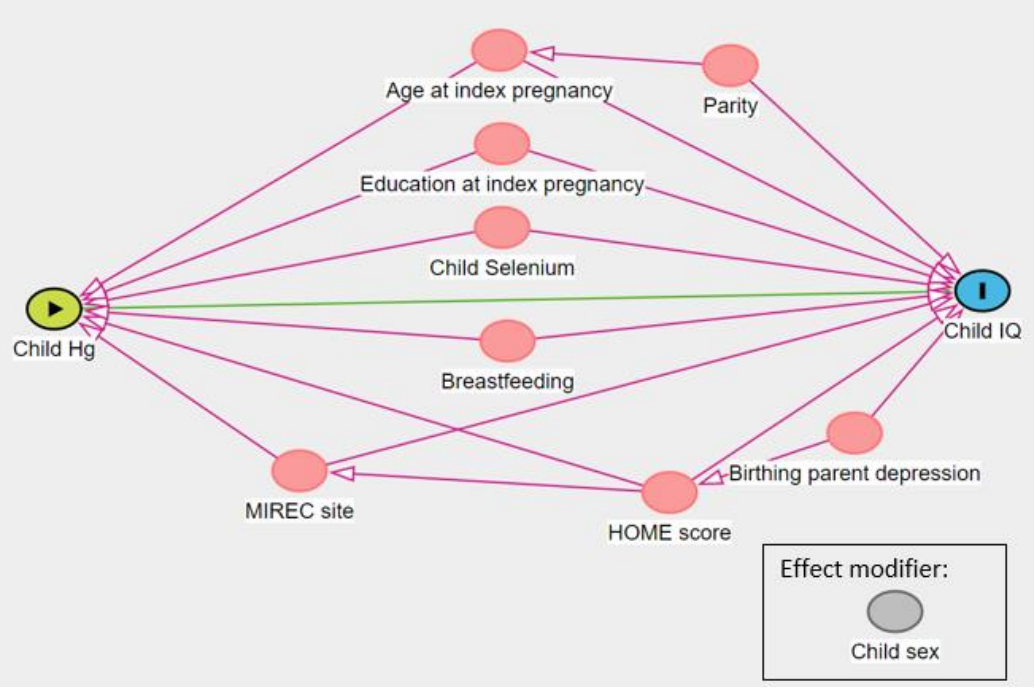
6.7 Supplementary Figures and Tables



Supplementary Figure 6.1: Flowchart of the inclusion and exclusion criteria for the final analytical samples for the associations between prenatal (first trimester or cord blood Hg concentrations) or postnatal (child blood Hg concentrations) Hg exposure and child IQ, MIREC (2008-2011) and MIREC-CD Plus (2013-2015) Studies



Supplementary Figure 6.2: Directed Acyclic Graph (DAG) of potential confounders or effect modifiers of the association between prenatal Hg exposure and child IQ, MIREC (2008-2011) and MIREC-CD Plus (2013-2015) Studies



Supplementary Figure 6.3: Directed Acyclic Graph (DAG) of potential confounders or effect modifiers of the association between child Hg exposure and child IQ, MIREC (2008-2011) and MIREC-CD Plus (2013-2015) Studies

Supplementary Table 6.1A: Frequency of prenatal consumption of fish high in Hg (male children), MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies

		Total fish consumption (25 species of fish) (n, column %)		
		0-2 times/month n=100	**3-7 times/month n = 88**	**≥8 times/month n = 68**
Consumption of fish high in Hg	**0 times/month**	61 (60%)	18 (20%)	9 (13%)
	1 times/month	35 (35%)	42 (48%)	15 (22%)
	> 1+ times/month	5 (5%)	28 (32%)	44 (65%)

Species high in Hg include tuna, marlin, orange roughy, shark, swordfish, mackerel, escolar (Health Canada, 2019b)

Supplementary Table 6.1B: Frequency of prenatal consumption of fish high in Hg (female children), MIREC (2008-2011) and MIREC-CD Plus (2013-2015) Studies

		Total fish consumption (25 species of fish) (n, column %)		
		0-2 times/month n=100	**3-7 times/month** n = 88	**≥8 times/month** n = 68
Consumption of fish high in Hg	**0 times/month**	72 (72%)	28 (30%	15 (20%)
	≤ 1 times/month	24 (24%)	35 (38%)	11 (14%)
	> 1+ times/month	3 (3%)	30 (32%)	50 (66%)
	Missing	2 (2%)	0 (0%)	0 (0%)

Species high in Hg include tuna, marlin, orange roughy, shark, swordfish, mackerel, escolar (Health Canada, 2019b)

Supplementary Table 6.2: Odds ratios and 95% confidence intervals for variables included in the inverse probability weighting (IPW) model to determine the predicted probabilities of participation at follow up (reference = did not participate at follow-up); MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies

	Odds ratio	95% confidence intervals	
Birthing parent's age	1.03	1.00	1.06
Pre-pregnancy BMI[a] (ref= under/normal weight)			
Obese	1.16	0.87	1.54
Overweight	0.94	0.73	1.20
Missing	1.10	0.77	1.55
Parity (≥1 vs 0)	0.98	0.86	1.12
Smoking status (Current vs Never)	0.76	0.56	1.01
Smoking status (Former vs Never)	1.05	0.85	1.31
Alcohol use during early pregnancy (Yes vs No)	0.80	0.27	2.38
Alcohol use during early pregnancy (Missing vs No)	1.91	0.22	16.89
Birthing parent's country of birth (Other vs Canada)	0.96	0.80	1.13
Fish consumption in early pregnancy (≥8 times/month vs 0-2 times/month)	1.14	0.94	1.38
Fish consumption in early pregnancy (3-7 times/month vs 0-2 times/month)	0.96	0.80	1.14
Birthing parent's education (≤ High school vs University)	0.67	0.46	0.95
Birthing parent's education (At least some college vs University)	1.29	1.02	1.62
Child sex (Male vs Female)	0.86	0.76	0.98
Provided a breastmilk sample (Yes vs No)	1.22	1.07	1.39
Participated in MIREC ID follow-up study (Yes vs No)	2.29	2.02	2.61
Preterm birth (Yes vs No)	0.83	0.67	1.03
Pregnancy complication (Yes vs No)	0.82	0.36	1.71
Labour (Induced vs Spontaneous)	0.95	0.77	1.19
Labour (No labour vs Spontaneous)	1.00	0.78	1.29
NICU Stay (Yes vs No)	1.21	0.92	1.59
Married (No vs Yes)	1.00	0.87	1.16
Number of moves during pregnancy (1+ vs None)	1.02	0.77	1.34
Location (Rural vs Urban)	0.96	0.75	1.23
Occupation (Education vs Unemployed)	1.03	0.76	1.40
Occupation (Healthcare vs Unemployed)	0.99	0.76	1.30
Occupation (Office worker vs Unemployed)	0.94	0.74	1.19
Occupation (Other position vs Unemployed)	1.31	0.90	1.90
Occupation (Sales vs Unemployed)	0.80	0.48	1.32

[a] Based on World Health Organization classification with cut-offs at 25 and 30 kg/m^2 (WHO, 2010)
Model fit statistics: Cox-snell R-square= 0.17, Tjur's R-square = 0.18
MIREC-ID: Maternal Infant Research on Environmental Chemicals – Infant Development study
Pregnancy complication – includes complications related to preeclampsia/gestational hypertension such as disseminated intravascular coagulation, pulmonary edema, convulsions – eclampsia transfusion, elevated liver enzyme levels, and low platelet count ($<50x10^9/L$)

Supplementary Table 6.3A: Descriptive statistics stratified by prenatal fish consumption for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Low Fish Consumption		Moderate Fish Consumption		High Fish Consumption	
	n[a]	Median (IQR) or n (%)	n[a]	Median (IQR) or n (%)	n[a]	Median (IQR) or n (%)
Hg concentrations						
First Trimester blood Hg (µg/L)[b]	101	0.40 (0.18 - 0.72)	88	0.93 (0.52 – 1.52)	68	0.99 (0.61 – 1.77)
Cord blood Hg (µg/L)[b]	83	<LOD (<LOD – 0.96)	75	1.12 (0.58 – 1.95)	56	1.41 (0.90 – 1.91)
WPPSI-III						
Full-scale IQ	101	105 (92 – 113)	88	105 (96 – 104)	68	104 (94 – 111)
Verbal IQ	100	108 (98 – 116)	88	108 (98 – 115)	67	106 (95 – 114)
Performance IQ	100	100 (90 – 112)	87	100 (94 – 112)	66	103 (94 – 112)
General Language Composite	101	105 (98 – 117)	86	108 (98 – 117)	67	102 (95 – 114)
Child characteristics						
Age (months)	101	40 (37 – 44)	88	40 (38 – 45)	68	39 (37 – 45)
Breastfeeding status						
Never	7	7%	7	8%	4	6%
Breastfeeding < 6 months	51	50%	43	49%	38	56%
Breastfeeding ≥ 6 months	40	40%	38	43%	25	37%
Missing	3	3%	0	0%	1	1%
HOME Total Score	98	48 (44 – 50)	86	47 (45 – 50)	67	48 (46 – 50)
Birthing parent characteristics						
Age at delivery (years)	101	32 (28 – 35)	88	33 (29 – 37)	68	33 (30 – 37)
Parity						
0	45	45%	45	51%	22	32%
1+	56	55%	43	49%	46	68%
Level of education						
High school diploma or less	6	6%	7	8%	4	6%
Some college/trade school	42	42%	23	26%	14	21%
University degree	53	52%	58	66%	49	72%
Missing	0	0%	0	0%	1	1%
Smoking status during early pregnancy						
Never	63	62%	64	73%	43	63%

Current/Quit During Pregnancy	15	15%	7	8%	2	3%
Former	23	23%	17	19%	23	34%
Alcohol use during early pregnancy						
Yes	42	42%	43	49%	32	47%
No	59	58%	45	51%	36	53%
Place of birth						
Canada	94	93%	74	84%	48	71%
Other	7	7%	14	16%	20	29%
Pre-pregnancy BMI (kg/m^2)	92	25 (22 – 30)	82	23 (21 – 27)	60	24 (21 – 26)
CES-D depression score	95	6 (4 – 9)	88	5 (3 – 8)	66	6 (2 – 9)

[a] Sample sizes may differ for continuous variables due to missing data

WPPSI-III – Wechsler Preschool and Primary Scale of Intelligence, third edition; HOME – Home Observation for Measurement of the Environment (HOME) inventory score; BMI – Body Mass Index; CES-D – Center for Epidemiological Studies Depression Scale

[b] For male children whose with low prenatal fish consumption, approximately 18%, and 51% of the values were <LOD for the first trimester, and cord blood Hg concentrations respectively. For male children with moderate prenatal fish consumption, approximately 0%, and 16% of the values were <LOD for the first trimester, and cord blood Hg concentrations respectively. For male children with high prenatal fish consumption, approximately 8%, and 7% of the values were <LOD for the first trimester, and cord blood Hg concentrations respectively.

Supplementary Table 6.3B: Descriptive statistics stratified by prenatal fish consumption for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Low Fish Consumption		Moderate Fish Consumption		High Fish Consumption	
	n[a]	**Median (IQR) or n (%)**	**n[a]**	**Median (IQR) or n (%)**	**n[a]**	**Median (IQR) or n (%)**
		Hg concentrations				
First Trimester blood Hg (µg/L)	101	0.26 (<LOD – 0.66)	93	0.66 (0.40 – 1.12)	76	0.99 (0.56 – 1.76)
Cord blood Hg (µg/L)	87	<LOD (<LOD – 0.70)	71	0.82 (LOD – 1.18)	58	1.09 (0.58 – 1.77)
		WPPSI-III				
Full-scale IQ	101	108 (100 – 115)	93	110 (103 – 119)	76	110 (102 – 119)
Verbal IQ	100	108 (103 – 119)	93	114 (103 – 119)	76	111 (105 – 119)
Performance IQ	101	103 (94 – 112)	92	103 (94 – 112)	76	106 (94 – 115)
General Language Composite	101	108 (100 – 117)	93	111 (102 – 120)	76	111 (100 – 120)
		Child characteristics				
Age (months)	101	39 (37 – 42)	93	39 (37 – 43)	76	39 (37 – 43)
Breastfeeding status						
Never	9	9%	9	19%	6	8%
Breastfeeding < 6 months	40	40%	42	45%	31	41%
Breastfeeding ≥ 6 months	49	49%	40	43%	37	49%
Missing	3	3%	2	2%	2	3%
HOME Total Score	97	48 (46 – 51)	92	49 (46 – 50)	72	49 (46 – 51)
		Birthing parent characteristics				
Age at delivery (years)	101	31 (28 – 35)	93	33 (30 – 36)	76	33 (30 – 37)
Parity						
0	51	50%	38	41%	33	43%
1+	50	50%	55	59%	43	57%
Level of education						
High school diploma or less	2	2%	4	4%	4	5%
Some college/trade school	35	35%	23	25%	21	28%
University degree	64	63%	65	70%	51	67%
Missing	0	0%	1	1%	0	0%
Smoking status during early pregnancy						
Never	70	69%	60	65%	50	66%

Current/Quit During Pregnancy	6	6%	7	8%	6	8%
Former	25	25%	26	28%	20	26%
Alcohol use during early pregnancy						
Yes	36	36%	43	46%	33	43%
No	64	63%	50	54%	43	57%
Missing	1	1%	0	0%	0	0%
Place of birth						
Canada	88	87%	79	85%	60	79%
Other	13	13%	20	22%	16	21%
Pre-pregnancy BMI (kg/m^2)	97	24 (21 – 29)	83	23 (21 – 26)	70	24 (22 – 26)
CES-Ddepression score	98	4 (3 – 7)	90	4 (1 – 8)	71	5 (3 – 8)

[a] Sample sizes may differ for continuous variables due to missing data

WPPSI-III – Wechsler Preschool and Primary Scale of Intelligence, third edition; HOME – Home Observation for Measurement of the Environment (HOME) inventory score; BMI – Body Mass Index; CES-D – Center for Epidemiological Studies Depression Scale

[b] For female children with low prenatal fish consumption, approximately 18%, and 51% of the values were <LOD for the first trimester, and cord blood Hg concentrations respectively. For female children with moderate prenatal fish consumption, approximately 0%, and 24% of the values were <LOD for the first trimester, and cord blood Hg concentrations respectively. For female children with high prenatal fish consumption, approximately 0%, and 16% of the values were <LOD for the first trimester, and cord blood Hg concentrations respectively.

Supplementary Table 6.4: Comparison of Hg concentrations and prenatal fish consumption in the full MIREC cohort sample versus the analytical sample, MIREC study (2008–2011)

	Analytical sample		Full sample	
	n	**Median (IQR) or n%**	**n**	**Median (IQR) or n%**
	Hg Concentrations			
First Trimester blood Hg (µg/L)	527	0.66 (0.32, 1.22)	1824	0.70 (0.32, 1.36)
Cord blood Hg (µg/L)	430	0.74 (0.32, 1.44)	1359	0.80 (0.34, 1.56)
	Monthly consumption of fish in early pregnancy			
Low (0-2 times/month)	202	38%	710	39%
Moderate (3 -7 times/month)	181	34%	619	34%
High (≥8 times/month)	144	27%	495	27%

Supplementary Table 6.5A: Pearson correlations among IQ scales for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Full-scale IQ	Performance IQ	Verbal IQ
Performance IQ	0.86		
Verbal IQ	0.84	0.44	
General Language Composite	0.74	0.39	0.87

Supplementary Table 6.5B: Pearson correlations among IQ scales for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Full-scale IQ	Performance IQ	Verbal IQ
Performance IQ	0.80		
Verbal IQ	0.77	0.25	
General Language Composite	0.65	0.22	0.84

Supplementary Table 6.6A: IQ measures stratified by covariates for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Full-scale IQ		Verbal IQ		Performance IQ		General Language Composite	
	N	**Median (IQR)**	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**n**	**Median (IQR)**
Child Characteristics								
Age (months)								
<38 months	68	103 (96 – 112)	68	106 (98 – 116)	67	103 (94 – 106)	68	102 (97 – 117)
38 – <42 months	85	106 (96 – 111)	83	108 (98 – 116)	83	97 (91 – 112)	84	108 (95 – 117)
≥ 42 months	104	106 (94 – 115)	104	108 (98 – 114)	103	103 (91 – 115)	102	105 (98 – 117)
Breastfeeding status								
Never	18	104 (91- 110)	18	99 (92 – 108)	17	106 (94 – 112)	18	99 (93 – 105)
Breastfeeding < 6 months	132	103 (92 – 112)	131	106 (98 – 116)	131	100 (91 – 109)	129	105 (95 – 114)
Breastfeeding ≥ 6 months	103	108 (98 – 119)	102	111 (100 – 119)	101	103 (91 – 118)	103	108 (98 – 120)
Home Total Score								
<46	86	98 (89 – 108)	85	100 (92 – 111)	84	94 (88 – 106)	83	102 (93 – 111)
46 – 49	98	107 (98 – 117)	98	108 (100 – 116)	97	103 (91 – 115)	98	108 (98 – 117)
≥ 50	73	108 (103 – 117)	72	113 (103 – 121)	72	103 (94 – 117)	73	111 (100 – 120)
Birthing parent characteristics								
Age at delivery (years)								
≤ 30	95	108 (100 – 115)	94	110 (100 – 116)	95	103 (94 – 118)	94	108 (100 – 117)
31 -35	132	103 (94 – 111)	131	106 (98 – 114)	128	102 (91 – 112)	131	105 (95 – 117)
≥ 36	11	108 (89 – 121)	11	114 (95 – 119)	11	97 (88 – 115)	11	123 (98 – 126)
Parity								
0	112	107 (98 – 115)	111	111 (100 – 116)	110	103 (94 – 115)	111	111 (100 – 117)
1+	145	103 (92 – 113)	144	106 (95 – 114)	143	100 (91 – 112)	143	102 (95 – 114)
Level of education								
High school diploma or less	17	103 (91 – 108)	17	100 (92 – 114)	16	94 (91 – 103)	16	104 (90 – 114)
Completed/Some college/trade school	79	98 (91 – 111)	77	103 (95 – 111)	78	97 (88 – 109)	78	102 (93 – 111)
University degree	160	108 (100 – 117)	160	111 (100 – 116)	158	103 (94 – 115)	159	108 (98 – 120)
Smoking status during early pregnancy								
Never	170	105 (94 – 113)	169	108 (98 – 116)	166	100 (91 – 112)	168	105 (95 – 117)

Current/Quit During Pregnancy	24	103 (91 – 109)	24	106 (95 – 113)	24	96 (90 – 109)	23	102 (98 – 114)
Former	63	106 (98 – 115)	62	108 (98 – 114)	63	103 (91 – 115)	63	108 (98 – 117)
Alcohol use during early pregnancy								
Yes	117	108 (98 – 113)	117	108 (100 – 116)	116	102 (94 – 112)	114	108 (98 – 120)
No	140	103 (92 – 113)	138	106 (98 – 114)	137	100 (91 – 112)	140	105 (95 – 114)
Place of birth								
Canada	216	105 (96 – 114)	214	108 (98 – 116)	213	100 (91 – 112)	214	105 (98 – 117)
Other	41	100 (92 – 111)	41	100 (92 – 111)	40	102 (93 – 112)	40	101 (90 – 113)
Monthly consumption of fish in early pregnancy								
Low (0-2 times/month)	101	105 (92 – 113)	100	108 (98 – 116)	100	100 (90 – 112)	101	105 (98 – 117)
Moderate (3 -7 times/month)	88	108 (98 – 115)	88	108 (98 – 115)	87	100 (94 – 112)	86	108 (98 – 117)
High (≥8 times/month)	68	104 (94 – 111)	67	106 (95 – 114)	66	103 (94 – 112)	67	102 (95 – 114)
Pre-pregnancy BMI								
Under/normal weight (<25)	133	106 (98 – 115)	132	110 (100 – 116)	131	100 (91 – 112)	131	108 (98 – 117)
Overweight (25 – <30)	56	109 (93 – 115)	55	108 (98 – 119)	54	103 (94 – 115)	56	105 (98 – 119)
Obese (≥30)	45	102 (91 – 111)	45	103 (92 – 114)	45	100 (91 – 112)	44	104 (97 – 114)
CES-D depression score								
<4	81	108 (100 – 115)	80	111 (100 – 116)	80	103 (94 – 114)	80	108 (98 – 117)
4 – 6	77	105 (92 – 113)	76	108 (98 – 115)	75	97 (91 – 112)	76	105 (98 – 117)
≥7	99	103 (91 – 113)	99	103 (95 – 114)	98	97 (88 – 112)	98	105 (95 – 114)

Supplementary Table 6.6B: IQ measures stratified by covariates for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Full-scale IQ		Verbal IQ		Performance IQ		General Language Composite	
	n	**Median (IQR)**	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**n**	**Median (IQR)**
Child Characteristics								
Age (months)								
<38 months	90	108 (100 – 117)	89	108 (103 – 119)	90	106 (94 – 115)	90	102 (100 – 120)
38 – <42 months	98	108 (98 – 117)	98	111 (103 – 119)	97	103 (91 – 112)	98	108 (100 – 120)
≥ 42 months	82	110 (105 – 119)	82	114 (106 – 122)	82	106 (97 – 115)	82	111 (102 – 120)
Breastfeeding status								
Never	24	105 (96 – 110)	23	108 (98 – 111)	23	103 (94 – 112)	24	101 (99 – 108)
Breastfeeding < 6 months	113	110 (98 – 117)	113	111 (103 – 119)	113	103 (94 – 112)	113	108 (100 – 117)
Breastfeeding ≥ 6 months	126	110 (103 – 119)	126	113 (106 – 119)	126	106 (97 – 115)	126	111 (100 – 120)
Home Total Score								
<46	70	106 (98 – 115)	70	108 (100 – 116)	70	103 (91 – 112)	70	102 (98 – 117)
46 – 49	97	108 (102 – 119)	96	113 (106 – 119)	96	103 (95 – 115)	97	111 (102 – 117)
≥ 50	103	111 (106 – 120)	103	114 (103 – 122)	103	109 (97 – 115)	103	111 (102 – 120)
Birthing parent characteristics								
Age at delivery (years)								
≤ 30	92	108 (102 – 115)	92	108 (103 – 116)	91	103 (94 – 115)	92	107 (100 – 117)
31 -35	139	110 (98 – 117)	138	114 (103 – 119)	139	106 (94 – 112)	139	111 (100 – 120)
≥ 36	20	110 (103 – 121)	20	111 (106 – 122)	20	108 (94 – 120)	20	107 (101 – 123)
Parity								
0	122	111 (100 – 119)	122	111 (103 – 122)	121	109 (94 – 115)	122	111 (100 – 123)
1+	148	108 (100 – 115)	147	111 (103 – 119)	148	103 (94 – 112)	148	108 (100 – 117)
Level of education								
High school diploma or less	10	96 (89 – 108)	10	100 (98 – 108)	10	93 (85 – 97)	10	98 (93 – 111)
Completed/Some college/trade school	79	106 (98 – 113)	79	106 (100 – 114)	79	103 (94 – 112)	79	102 (98 – 111)
University degree	180	111 (103 – 119)	179	114 (106 – 122)	179	106 (94 – 115)	180	111 (102 – 120)
Smoking status during early pregnancy								
Never	180	110 (102 – 117)	179	111 (103 – 119)	180	103 (94 – 115)	180	108 (100 – 120)

Current/Quit During Pregnancy	19	108 (98 – 117)	19	108 (103 – 114)	19	100 (88 – 115)	19	102 (102 – 111)
Former	71	110 (98 – 117)	71	111 (103 – 122)	70	109 (94 – 112)	71	108 (100 – 117)
Alcohol use during early pregnancy								
Yes	112	110 (100 – 117)	112	111 (103 – 119)	111	106 (94 – 115)	112	108 (100 – 120)
No	157	110 (100 – 117)	156	111 (103 – 119)	157	103 (94 – 112)	157	108 (100 – 117)
Place of birth								
Canada	227	110 (100 – 117)	226	111 (103 – 119)	226	106 (94 – 115)	227	108 (100 – 120)
Other	43	110 (100 – 117)	43	111 (103 – 119)	43	103 (94 – 112)	43	108 (100 – 117)
Monthly consumption of fish in early pregnancy								
Low (0-2 times/month)	101	108 (100 – 115)	100	108 (103 – 119)	101	103 (94 – 112)	101	108 (100 – 117)
Moderate (3 -7 times/month)	93	110 (103 – 119)	93	114 (103 – 119)	92	103 (94 – 112)	93	111 (102 – 120)
High (≥8 times/month)	76	110 (102 – 119)	76	111 (105 – 119)	76	106 (94 – 115)	76	111 (100 – 120)
Pre-pregnancy BMI								
Under/normal weight (<25)	159	110 (103 – 119)	159	114 (103 – 122)	158	109 (94 – 115)	159	111 (102 – 120)
Overweight (25 – <30)	52	108 (97 – 116)	51	111 (100 – 119)	52	103 (94 – 112)	52	107 (99 – 116)
Obese (≥30)	39	106 (98 – 111)	39	108 (103 – 114)	39	103 (91 – 112)	39	108 (100 – 111)
CES-D depression score								
<4	111	110 (102 – 119)	111	111 (103 – 119)	110	105 (94 – 115)	111	108 (100 – 120)
4 – 6	81	108 (98 – 115)	81	108 (103 – 119)	81	103 (91 – 112)	81	108 (100 – 117)
≥7	78	109 (105 – 117)	77	114 (103 – 119)	78	109 (97 – 115)	78	110 (100 – 120)

Supplementary Table 6.7A: Prenatal blood Hg concentrations (first trimester blood and cord blood Hg concentrations) for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies

	First Trimester Blood Hg Concentrations (µg/L)		Cord Blood Hg Concentrations (µg/L)	
	n	**Median (IQR)**	**n**	**Median (IQR)**
	Birthing parent characteristics			
Age at delivery (years)				
≤ 30	95	0.56 (0.32 – 0.96)	80	0.72 (<LOD – 1.39)
31 -35	132	0.83 (0.44 – 1.43)	108	1.12 (0.46 – 1.92)
≥ 36	11	0.72 (0.36 – 1.06)	10	1.41 (0.58 – 2.21)
Parity				
0	112	0.73 (0.35 – 1.43)	87	1.00 (0.40 – 1.69)
1+	145	0.62 (0.40 – 1.20)	127	0.82 (<LOD– 1.62)
Level of education				
High school diploma or less	17	0.56 (0.30 – 1.00)	16	0.90 (<LOD – 1.74)
Completed/Some college/trade school	79	0.58 (0.26 – 0.96)	70	0.60 (<LOD – 1.10)
University degree	160	0.74 (0.43 – 1.44)	127	1.12 (0.44 – 1.93)
Smoking status during early pregnancy				
Never	170	0.72 (0.36 – 1.36)	139	0.90 (0.42 – 1.73)
Current/Quit During Pregnancy	24	0.43 (0.20 – 0.81)	20	0.51 (<LOD – 1.33)
Former	63	0.66 (0.42 – 1.22)	55	1.00 (<LOD – 1.56)
Alcohol use during early pregnancy				
Yes	117	0.76 (0.44 – 1.38)	96	1.01 (0.44 – 1.83)
No	140	0.60 (0.32 – 1.21)	118	0.78 (<LOD– 1.50)
Place of birth				
Canada	216	0.61 (0.34 – 1.20)	183	0.82 (<LOD – 1.50)
Other	41	0.98 (0.68 – 1.69)	31	1.66 (0.76 – 2.21)
Pre-pregnancy BMI				
Under/normal weight (<25)	133	0.74 (0.40 – 1.36)	112	1.04 (0.45 – 1.72)
Overweight (25 – <30)	56	0.67 (0.39 – 1.38)	46	0.93 (<LOD– 1.69)
Obese (≥30)	45	0.52 (0.20 – 0.78)	37	0.60 (0.40 – 1.00)

Supplementary Table 6.7B: Prenatal blood Hg concentrations (first trimester blood and cord blood Hg concentrations) for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies

	First Trimester Blood Hg Concentrations (µg/L)		Cord Blood Hg Concentrations (µg/L)	
	n	**Median (IQR)**	**n**	**Median (IQR)**
		Birthing parent characteristics		
Age at delivery (years)				
≤ 30	92	0.38 (0.18 – 0.72)	79	0.42 (<LOD – 0.74)
31 -35	139	0.66 (0.36 – 1.40)	106	0.82 (<LOD – 1.34)
≥ 36	20	1.02 (0.61 – 1.50)	14	0.96 (0.46 – 1.32)
Parity				
0	122	0.70 (0.26 – 1.32)	99	0.66 (<LOD – 1.16)
1+	148	0.56 (0.31 – 1.04)	117	0.60 (<LOD – 1.10)
Level of education				
High school diploma or less	10	0.41 (0.30 – 0.72)	10	0.32 (<LOD – 0.54)
Completed/Some college/trade school	79	0.52 (0.24 – 0.96)	65	0.48 (<LOD – 1.04)
University degree	180	0.68 (0.31 – 1.37)	140	0.72 (<LOD – 1.28)
Smoking status during early pregnancy				
Never	180	0.57 (0.27 – 1.04)	142	0.60 (<LOD – 1.10)
Current/Quit During Pregnancy	19	0.56 (0.24 – 1.04)	17	0.60 (<LOD– 1.08)
Former	71	0.66 (0.32 – 1.42)	57	0.72 (<LOD– 1.26)
Alcohol use during early pregnancy				
Yes	112	0.72 (0.33 – 1.50)	86	0.77 (<LOD – 1.40)
No	157	0.56 (0.24 – 0.98)	129	0.58 (<LOD – 1.04)
Place of birth				
Canada	227	0.56 (0.26 – 1.04)	183	0.58 (<LOD – 1.08)
Other	43	0.94 (0.42 – 1.75)	33	1.02 (0.50 – 1.84)
Pre-pregnancy BMI				
Under/normal weight (<25)	159	0.70 (0.32 – 1.42)	125	0.76 (<LOD – 1.32)
Overweight (25 – <30)	52	0.56 (0.36 – 0.95)	45	0.50 (<LOD – 1.00)
Obese (≥30)	39	0.34 (0.06 – 0.82)	32	0.32 (<LOD – 0.64)

Supplementary Table 6.8: Child blood Hg concentrations stratified by covariates for male and female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies

	Child Blood Hg Concentrations (µg/L)			
	Male children		Female children	
	n	Median (IQR)	n	Median (IQR)
Child Characteristics				
Age (months)				
<38 months	43	0.20 (0.06 – 0.42)	65	0.16 (0.06 – 0.32)
38 – <42 months	61	0.20 (0.06 – 0.50)	64	0.20 (0.06 – 0.58)
≥ 42 months	77	0.16 (0.05 – 0.64)	45	0.20 (0.05 – 0.56)
Breastfeeding status				
Never	13	0.20 (0.06 – 0.42)	16	0.06 (0.05 – 0.19)
Breastfeeding < 6 months	98	0.19 (0.05 – 0.52)	80	0.20 (0.06 – 0.51)
Breastfeeding ≥ 6 months	66	0.19 (0.05 – 0.62)	74	0.24 (0.14 – 0.56)
Home Total Score				
<46	60	0.16 (0.05 – 0.50)	47	0.18 (0.05 – 0.38)
46 – 49	74	0.20 (0.05 – 0.64)	60	0.20 (0.06 – 0.66)
≥ 50	47	0.20 (0.05 – 0.42)	67	0.20 (0.06 – 0.42)
Birthing parent characteristics				
Age at delivery (years)				
≤ 30	68	0.20 (0.06 – 0.59)	57	0.16 (0.06 – 0.38)
31 -35	89	0.16 (0.05 – 0.50)	93	0.20 (0.06 – 0.50)
≥ 36	10	0.10 (0.05 – 0.46)	12	0.36 (0.11 – 0.67)
Parity				
0	73	0.20 (0.06 – 0.56)	83	0.20 (0.06 – 0.56)
1+	108	0.14 (0.05 – 0.50)	91	0.20 (0.06 – 0.42)
Level of education				
High school diploma or less	13	0.18 (0.12 – 0.46)	6	0.09 (0.05 – 0.14)
Completed/Some college/trade school	53	0.10 (0.05 – 0.20)	50	0.18 (0.06 – 0.34)
University degree	114	0.20 (0.05 – 0.66)	118	0.20 (0.06 – 0.56)
Place of birth				
Canada	146	0.16 (0.05 – 0.50)	147	0.18 (0.06 – 0.38)
Other	35	0.34 (0.06 – 0.80)	27	0.50 (0.18 – 0.94)
Pre-pregnancy BMI				
Under/normal weight (<25)	93	0.20 (0.05 – 0.60)	101	0.26 (0.06 – 0.58)
Overweight (25 – <30)	39	0.16 (0.05 – 0.64)	33	0.18 (0.06 – 0.32)
Obese (≥30)	30	0.10 (0.05 – 0.30)	25	0.14 (0.06 – 0.20)
CES-D depression score				
<4	57	0.20 (0.05 – 0.74)	62	0.20 (0.12 – 0.56)
4 – 6	53	0.18 (0.05 – 0.42)	60	0.16 (0.06 – 0.39)
≥7	71	0.18 (0.05 – 0.60)	52	0.20 (0.06 – 0.47)

Supplementary Table 6.9A: Sensitivity analyses for associations between first trimester blood Hg concentrations and IQ for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Full-scale IQ	Verbal IQ	Performance IQ	General Language Composite
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Main Model	-0.18 (-2.30, 1.94)	-0.50 (-2.73, 1.73)	0.18 (-1.67, 2.02)	0.05 (-2.13, 2.23)
Includes first trimester blood Pb[a]	-0.49 (-2.55, 1.56)	-0.72 (-2.78, 1.34)	-0.10 (-2.05, 1.84)	-0.29 (-2.58, 1.99)
Includes first trimester blood PBDEs[a]	-0.25 (-2.07, 1.56)	-0.51 (-2.46, 1.44)	0.07 (-1.56, 1.70)	-0.00 (-1.86, 1.85)
Includes first trimester blood PCBs[a]	-0.23 (-2.17, 1.70)	-0.50 (-2.59, 1.59)	0.10 (-1.53, 1.73)	0.05 (-2.11, 2.21)
Includes variable has dental amalgams	-0.15 (-2.42, 2.12)	-0.50 (-2.77, 1.77)	0.26 (-1.75, 2.27)	0.09 (-2.31, 2.48)
Includes variable had dental amalgams replaced in past 12 months	-0.21 (-2.37, 1.94)	-0.57 (-2.74, 1.61)	0.23 (-1.69, 2.14)	0.06 (-2.25, 2.38)
Excluding pre-term births	-0.21 (-2.33, 1.92)	-0.54 (-2.76, 1.69)	0.12 (-1.86, 2.10)	-0.24 (-2.46, 1.99)

Models are adjusted for birthing parent age, pre-pregnancy BMI, parity, smoking status in early pregnancy, alcohol consumption in the three months before the first trimester visit, birthing parent country of birth, HOME total development scores, birthing parent education, the frequency of fish consumption in early pregnancy, and site (random effect). Models for PBDEs and PCBs are additionally adjusted for birthing parent blood lipids.
Regression coefficients represent a 1-point change in IQ score for each 2-fold increase in Hg exposure
Summed PBDEs represent the sum of PBDEs 47, 99, and 153
Summed PCBs represent the sum of PCBs 138, 153, and 180
[a] First trimester blood lead, lipids, summed PBDEs, and summed PCBs were log transformed (log_2 scale)

Supplementary Table 6.9B: Sensitivity analyses for associations between first trimester blood Hg concentrations and child IQ for sensitivity analyses for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Full-scale IQ	Verbal IQ	Performance IQ	General Language Composite
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Main Model	0.44 (-0.84, 1.72)	-0.09 (-1.18, 1.00)	0.82 (-1.03, 2.66)	-0.74 (-2.08, 0.60)
Includes first trimester blood Pb[a]	0.50 (-0.76, 1.75)	0.09 (-1.02, 1.21)	0.78 (-1.01, 2.57)	-0.57 (-1.95, 0.82)
Includes first trimester blood PBDEs[a]	0.48 (-0.98, 1.94)	0.05 (-1.07, 1.17)	0.70 (-1.45, 2.84)	-0.59 (-2.04, 0.86)
Includes first trimester blood PCBs[a]	0.40 (-0.84, 1.63)	-0.09 (-1.23, 1.05)	0.72 (-1.01, 2.46)	-0.75 (-2.18, 0.69)
Includes variable has dental amalgams	0.55 (-0.65, 1.74)	0.07 (-0.85, 1.00)	0.87 (-1.01, 2.74)	-0.56 (-1.69, 0.58)
Includes variable had dental amalgams replaced in past 12 months	0.45 (-0.83, 1.73)	-0.10 (-1.20, 1.00)	0.86 (-1.03, 2.74)	-0.76 (-2.12, 0.60)
Excluding pre-term births	0.29 (-1.09, 1.67)	-0.11 (-1.41, 1.18)	0.58 (-1.42, 2.58)	-0.70 (-2.30, 0.89)

Models are adjusted for birthing parent age, birthing parent pre-pregnancy BMI, parity, smoking status in early pregnancy, alcohol consumption in the three months before the first trimester visit, birthing parent country of birth, HOME total development scores, birthing parent education, the frequency of fish consumption in early pregnancy, and site (random effect). Models for PBDEs and PCBs are additionally adjusted for birthing parent blood lipids.
Regression coefficients represent a 1-point change in IQ score for each 2-fold increase in Hg exposure
PBDEs represent the sum of PBDEs 47, 99, and 153
PCBs represent the sum of PCBs 138, 153, and 180
[a] first trimester blood lead, lipids, summed PBDEs, and summed PCBs were log transformed (log_2 scale)

Supplementary Table 6.10A Sensitivity analyses for associations between cord blood Hg concentrations and child IQ for sensitivity analyses for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Full-scale IQ	Verbal IQ	Performance IQ	General Language Composite
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Main Model	-0.58 (-2.10, 0.93)	-0.31 (-1.23, 0.60)	-0.79 (-2.73, 1.15)	-0.89 (-1.95, 0.16)
Includes cord blood Pb[a]	-0.37 (-1.78, 1.04)	-0.04 (-0.98, 0.90)	-0.65 (-2.57, 1.27)	-0.66 (-1.70, 0.37)
Includes first trimester blood PBDEs[a]	-0.65 (-1.94, 0.64)	-0.34 (-0.99, 0.31)	-0.91 (-2.76, 0.95)	-0.95 (-1.94, 0.03)
Includes first trimester blood PCBs[a]	-0.72 (-2.15, 0.71)	-0.38 (-1.14, 0.38)	-0.97 (-2.98, 1.05)	-0.94 (-1.97, 0.10)
Includes variable has dental amalgams	-0.62 (-2.14, 0.91)	-0.32 (-1.22, 0.58)	-0.85 (-2.85, 1.15)	-0.90 (-1.90, 0.09)
Includes variable had dental amalgams replaced in past 12 months	-0.59 (-2.15, 0.97)	-0.32 (-1.31, 0.68)	-0.78 (-2.71, 1.15)	-0.89 (-1.94, 0.17)
Excluding pre-term births	-0.43 (-1.81, 0.96)	-0.22 (-1.18, 0.73)	-0.62 (-2.35, 1.10)	-1.00 (-2.05, 0.06)

Models are adjusted for birthing parent age, birthing parent pre-pregnancy BMI, parity, smoking status in early pregnancy, alcohol consumption in the three months before the first trimester visit, birthing parent country of birth, HOME total development scores, birthing parent education, the frequency of fish consumption in early pregnancy, and site (random effect). Models for PBDEs and PCBs are additionally adjusted for birthing parent blood lipids.
Regression coefficients represent a 1-point change in IQ score for each 2-fold increase in Hg exposure
PBDEs represent the sum of PBDEs 47, 99, and 153
PCBs represent the sum of PCBs 138, 153, and 180
[a] Cord blood lead, and first trimester lipids, summed PBDEs, and summed PCBs were log transformed (log_2 scale)

Supplementary Table 6.10B: Sensitivity analyses for associations between cord blood Hg concentrations and child IQ for sensitivity analyses for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) studies

	Full-scale IQ	Verbal IQ	Performance IQ	General Language Composite
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Main Model	1.29 (0.77, 1.81)	-0.01 (-0.98, 0.96)	2.01 (1.19, 2.83)	0.11 (-0.99, 1.22)
Includes cord blood Pb[a]	1.39 (0.58, 2.21)	0.16 (-1.05, 1.37)	2.03 (1.20, 2.86)	0.20 (-1.14, 1.55)
Includes first trimester blood PBDEs[a]	1.40 (1.05, 1.74)	0.06 (-0.87, 0.99)	2.01 (1.05, 2.97)	0.15 (-0.91, 1.21)
Includes first trimester blood PCBs[a]	1.39 (0.84, 1.95)	0.08 (-0.96, 1.13)	2.08 (1.24, 2.92)	0.21 (-1.02, 1.43)
Includes variable has dental amalgams	1.30 (0.70, 1.92)	0.09 (-0.90, 1.08)	1.97 (1.17, 2.76)	0.24 (-0.83, 1.32)
Includes variable had dental amalgams replaced in past 12 months	1.29 (0.77, 1.81)	-0.00 (-0.99, 0.99)	1.97 (1.02, 2.92)	0.15 (-0.99, 1.30)
Excluding pre-term births	1.32 (0.70, 1.93)	0.14 (-0.84, 1.11)	1.95 (1.17, 2.72)	0.30 (-0.83, 1.42)

Models are adjusted for birthing parent age, pre-pregnancy BMI, parity, smoking status in early pregnancy, alcohol consumption in the three months before the first trimester visit, birthing parent country of birth, HOME total development scores, birthing parent education, and the frequency of fish consumption in early pregnancy, and site (random effect). Models for PBDEs and PCBs are additionally adjusted for birthing parent blood lipids.

Regression coefficients represent a 1-point change in IQ score for each 2-fold increase in Hg exposure

PBDEs represent the sum of PBDEs 47, 99, and 153

PCBs represent the sum of PCBs 138, 153, and 180

[a] Cord blood lead, and first trimester lipids, summed PBDEs, and summed PCBs were log transformed (log_2 scale)

Supplementary Table 6.11: Regression coefficients and 95% CI for associations between the child blood Hg and selenium interaction term and child IQ, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Male children		Female children	
	n	**β (95% CI)**	**n**	**β (95% CI)**
Full-scale IQ	181	-0.02 (-0.12, 0.07)	174	0.01 (-0.01, 0.02)
Verbal IQ	181	-0.03 (-0.10, 0.05)	173	-0.01 (-0.05, 0.04)
Performance IQ	179	-0.02 (-0.11, 0.07)	173	0.03 (-0.03, 0.08)
General Language Composite	179	-0.04 (-0.14, 0.06)	174	-0.02 (-0.07, 0.04)

Models are adjusted for child age, parity, HOME total development scores, birthing parent education, breastfeeding status/duration, birthing parent depression, child blood selenium concentrations, child blood Hg concentrations, child sex, and site (random effect). Child blood Hg concentrations were $\log_2$ transformed and child blood selenium concentrations are in μg/L.

Supplementary Table 6.12A: Regression coefficients and 95% CI for associations between child blood Hg concentrations and child IQ for sensitivity analyses for male children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Full-scale IQ	Verbal IQ	Performance IQ	General Language Composite
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Main Model	-0.14 (-1.57, 1.29)	-0.21 (-1.90, 1.47)	-0.03 (-1.25, 1.20)	0.43 (-0.98, 1.84)
Includes child blood Pb[a]	-0.14 (-1.58, 1.31)	-0.20 (-1.81, 1.41)	-0.03 (-1.21, 1.15)	0.43 (-0.90, 1.75)
Includes cord blood Hg[a]	-0.00 (-2.03, 2.02)	0.20 (-1.61, 2.01)	-0.27 (-2.42, 1.89)	0.96 (-0.70, 2.62)
Excludes preterm births	0.25 (-1.07, 1.56)	0.14 (-1.36, 1.64)	0.29 (-1.01, 1.58)	0.72 (-0.50, 1.94)

Models are adjusted for birthing parent age, parity, HOME total development scores, birthing parent education, breastfeeding status/duration, birthing parent depression, child blood selenium concentrations, and site (random effect).
Regression coefficients represent a 1-point change in IQ score for each 2-fold increase in Hg exposure
[a]Child blood lead and cord blood Hg concentrations were $\log_2$ transformed

Supplementary Table 6.12B: Regression coefficients and 95% CI for associations between child blood Hg concentrations and child IQ for sensitivity analyses for female children, MIREC (2008–2011) and MIREC-CD Plus (2013–2015) Studies

	Full-scale IQ	Verbal IQ	Performance IQ	General Language Composite
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Main Model	0.97 (0.09, 1.84)	1.04 (0.41, 1.67)	0.69 (-0.73, 2.11)	1.03 (0.23, 1.82)
Includes child blood Pb[a]	0.97 (0.07, 1.87)	1.05 (0.45, 1.64)	0.70 (-0.76, 2.15)	1.03 (0.27, 1.80)
Includes cord blood Hg[a]	0.86 (-0.34, 2.05)	1.32 (0.42, 2.21)	0.42 (-1.28, 2.12)	1.55 (0.35, 2.75)
Excludespreterm births	0.87 (-0.07, 1.82)	0.88 (0.18, 1.58)	0.72 (-0.83, 2.26)	0.95 (0.16, 1.74)

Models are adjusted for birthing parent age, parity, HOME total development scores, birthing parent education, birthing parent depression, child blood selenium concentrations, and site (random effect).
Regression coefficients represent a 1-point change in IQ score for each 2-fold increase in Hg exposure
[a] Child blood lead and cord blood Hg concentrations were $\log_2$ transformed

Chapter 7: Conclusions

7.1 Overview

This thesis used targeted biomonitoring to examine Hg exposures, in sub-populations in Canada. Collectively, this work sought to better understand exposures to Hg in potentially disproportionately affected sub-populations. The two sub-populations whose Hg exposures were examined were 1) Dene/Métis communities in northern Canada, and 2) pregnant women and children in Canada. This thesis focused on northern Indigenous communities, as these communities frequently consume locally harvested freshwater fish, and elevated levels of Hg have been measured in some local freshwater species of fish from some local northern waterbodies. Those who frequently consume fish and/or consume fish with elevated Hg concentrations, could be exposed to elevated concentrations of Hg. Therefore, Hg exposures, through the use of Hg biomarkers (hair and blood Hg concentrations), their determinants (including the consumption of fish), and exposure estimates (using fish analyte and consumption data), were examined in northern Indigenous communities, including Dene/Métis communities of the Northwest Territories. This thesis also focused on pregnant women and children in Canada, as early life stages are particularly sensitive to the neurotoxic effects of Hg as their nervous systems are undergoing rapid development. Therefore, the associations between prenatal and childhood Hg exposures and child neurodevelopment (as measured by child IQ), and whether these associations were modified by prenatal fish consumption, were examined.

The main findings of this thesis were:

- After cooking, less than 100% of the Hg in northern freshwater fish is bioaccessible
- Hair Hg concentrations were higher on average in the Dene/Métis communities in northern Canada that were examined compared to hair Hg concentrations measured in the Canadian general population through the CHMS
- However, blood Hg concentrations in these communities were similar to those measured in the Canadian general population

- Hair to blood Hg ratios were much higher in these communities (geometric mean ratio 619:1 for the Dehcho region and 1220:1 for the Sahtú) than those measured in the Canadian General population (geometric mean ratio of 293; Singh et al., 2023)
    - This difference could be explained by seasonal variations in mercury biomarker levels (which was observed in a hair segmental analysis), due to seasonal differences in diet
    - Seasonal changes in hair mercury concentrations did seem to follow a similar pattern to seasonal changes in reported total fish consumption frequency
- Although the consumption of fish was identified as a determinant of Hg exposure for this Canadian sub-population (Dene/Métis communities in northern Canada), fish consumption, and the consumption of other traditional foods, were identified as determinants of biomarker levels of the important nutrients Se and omega-3 PUFA
- Exposure assessment models for Hg, Se, and EPA+DHA from the consumption of locally harvested freshwater fish from the Dehcho and Sahtú regions of the NWT confirmed that even though fish Hg concentrations are elevated in some fish species in certain waterbodies in northern Canada, Hg exposures remain relatively low for this sub-population
- At similar levels of Hg exposures, the associations between low level prenatal and childhood Hg exposures and child IQ were examined in the second sub-population included in this thesis: pregnant women and children in Canada
    - Higher cord blood Hg concentrations were associated with lower PIQ in male children for those in the sub-group with the lowest prenatal fish consumption
    - Progressively stronger positive associations were observed with PIQ in male children for the moderate and high prenatal fish consumption subgroups
    - Cord blood Hg concentrations were positively associated with female children's FSIQ and PIQ, however, when stratified, these positive associations were only observed in the highest fish consumption subgroup

    - Among female children, higher child blood Hg concentrations were associated with an approximately 1-point increase in FSIQ, VIQ, and GLC

- Positive associations between prenatal/childhood Hg exposures and child IQ may be due to the beneficial effects of prenatal fish consumption
    - The findings from this research also highlighted the importance of considering the potential benefits of fish consumption, and the modifying role prenatal fish consumption can play, when the associations between early life Hg exposures and child neurodevelopment are examined

Overall, this thesis fills important gaps in our knowledge about fish consumption and Hg exposures in potentially disproportionately affected sub-populations in Canada. This work can be further summarized by several key findings for each research chapter.

7.2 Key Findings

7.2.1 Key Findings – Chapter 2

Chapter 2 of this thesis focused on determining the bioaccessiblity of Hg in freshwater fish harvested in northern Canada. The key finding of this chapter was that total Hg bioaccessibility in these freshwater northern fish was on average less than 100% (which is commonly assumed in exposure assessments) and differed by fish species, location/waterbody, tissue type (muscle versus liver), and preparation method (raw versus cooked). Hg bioaccessibility in raw fish muscle samples on average ranged between 56-96% across the fish species and waterbodies examined and was on average 39% in the fish liver samples analyzed. Cooking fish samples (pan-fried in water) resulted in increased Hg concentrations (likely due to moisture loss during the cooking process) but lower percent bioaccessiblity, resulting in lower bioaccessible concentrations of Hg (on average 32% lower) compared to raw fish. This research fills a large knowledge gap on the bioaccessiblity of Hg in northern freshwater fish species and to the best of my knowledge is the first time Hg bioaccessibility has been measured in the liver (raw and cooked) of freshwater fish.

7.2.2 Key Findings – Chapter 3

Chapter 3 of this thesis focused on examining Hg concentrations in hair and blood samples in community members from the Dehcho and Sahtú regions of the Northwest Territories, determining and comparing hair to blood Hg concentration ratios for these regions, and conducting a retrospective hair segmental Hg analysis and comparing hair Hg concentrations to reported fish consumption over the past year. The first key finding of this chapter was that hair and blood Hg concentrations were relatively low in these communities, and that hair to blood Hg concentration ratios differed between the Dehcho and Sahtú regions, and differed from the commonly accepted ratio of 250:1. The vast majority of hair and blood Hg concentrations were below relevant health-based guidance values and were generally similar to levels measured in the Canadian general population through the CHMS. Geometric mean hair to blood Hg concentration ratios were 619:1 for the Dehcho region and 1120:1 for the Sahtú region, which was much higher than the 250:1 ratio derived by the WHO (WHO, 1990). Using the 250:1 ratio to estimate blood Hg concentrations from hair Hg concentrations would substantially over-estimate Hg exposures in the studied regions. The second key finding of this chapter was that hair Hg concentrations, from the hair segmental analysis of the long hair samples, varied over time and seemed to follow the same trends as total fish consumption frequencies reported by the participants. Hair Hg concentrations were highest on average in the hair segments estimated to be from fall/early winter (September/October, and November/December), and lowest in those estimated to be from spring (March/April, and May/June). Reported total fish intake frequency was highest in late summer, and lowest in winter. Although there was a lag between the time-period with the highest fish consumption and the time-period with the highest hair Hg concentrations, they appeared to follow the same seasonal pattern.

7.2.3 Key Findings – Chapter 4

Chapter 4 further examined the hair and blood Hg concentrations from participants from the Dehcho region and Sahtú region of the Northwest Territories, and from the community of Old Crow from the Yukon, as well as blood Se, and plasma omega-3 PUFA concentrations (specifically EPA+DHA concentrations). This chapter determined whether several demographic, biometric, and dietary factors could explain the variability of blood Hg, hair Hg, blood Se, and plasma EPA+DHA concentrations within and between these northern Canadian regions. The key

finding of this chapter was that the consumption of at least one species of fish, and the consumption of waterfowl, were included in final models to explain hair and blood Hg concentrations, blood Se concentrations, and plasma EPA+DHA concentrations. The findings of this chapter highlight the importance of considering nutrients such as Se, and omega-3 PUFA, when examining Hg exposures, as their biomarker levels can have common determinants.

7.2.4 Key Findings – Chapter 5

Chapter 5 used all of the information generated from the previous chapters to construct, refine, and interpret dietary exposure assessment models for Hg, Se, and EPA+DHA, for participants from the Dehcho and Sahtú regions of the Northwest Territories, from the consumption of the four most commonly consumed local freshwater fish species. The key finding of this chapter was that although the consumption of locally harvested freshwater fish is a source of Hg exposure for people in these regions, people's Hg exposure has stayed relatively low, and these fish are an important source of essential nutrients. The majority of Hg exposure estimates from the models were below relevant TDIs. Refining the models by including lake-specific fish Hg concentration data, on top of species-specific fish Hg concentration data, and species-specific Hg bioaccessibility data, resulted in lower Hg exposure estimates which seemed most similar to the Hg biomarker concentrations in terms of percent exceedances (of TDIs or health-based guidance values). Model estimates for Se and EPA+DHA indicated that the included freshwater fish species were an important source of these essential nutrients for these regions.

7.2.5 Key Findings – Chapter 6

Chapter 6 focused on examining the associations between prenatal and childhood Hg exposures and child IQ in participants of the Canadian MIREC birth cohort study. Analyses with prenatal Hg exposures were stratified by prenatal fish consumption to determine if this modified the associations. One key finding was that for male children cord blood Hg concentrations were negatively associated with PIQ in the group with the lowest prenatal fish consumption, but progressively stronger positive associations were seen in the moderate and high prenatal fish consumption groups. Cord blood Hg concentrations were positively associated with FSIQ, and PIQ, in female children; however, when stratified, these associations only persisted in the group

with the highest prenatal fish consumption. These findings suggest that the benefits of prenatal fish consumption may override the negative effects of prenatal Hg exposure, at the low levels of Hg exposure typical in Canada.

7.3 Implications for Public Health Sciences

The findings of this thesis have implications for the field of public health sciences at a regional and territorial level for Indigenous communities in northern Canada, and more generally for pregnant women and children across the country. Although there was concern that Hg exposures could be elevated in the Dene/Métis communities included in this thesis, blood Hg concentrations remained relatively low and similar to levels reported for the Canadian general population measured through the CHMS. Results from the northern biomonitoring projects also appeared to be similar to those observed in the pregnant participants in the MIREC cohort study. For example, median blood Hg concentrations ranged between <LOD – 1.11 μg/L and 95th percentile values ranged between 2.98-5.63 μg/L, across the three northern regions examined (Dehcho region, Sahtú region, and Old Crow). In the pregnant participants in MIREC, the median was 0.70 μg/L and 95th percentile was 2.81 μg/L for first trimester blood Hg concentrations (Arbuckle et al., 2016). Therefore, although some of the determinants of Hg exposure (especially dietary determinants like the consumption of specific fish species) may differ between these sub-populations, the magnitude of exposure was similar. This has implications for future messaging and research directions for both sub-populations.

The results of the Mackenzie River Valley and Old Crow human biomonitoring projects have been returned to the communities and individuals that took part in the studies. These projects were designed and implemented in consultation with these communities to address their concerns about environmental contaminants in their traditional foods, including Hg in locally harvested freshwater fish. At an individual level, participants received their personal results for the biomarker levels of contaminants and nutrients measured in the samples they provided in the form of an individual results letter. For the vast majority of participants, the results of the biomonitoring component of the study confirmed that their levels of exposure were similar or lower to exposures typically seen in the Canadian general population (measured in the CHMS) and other indigenous populations (measured in the FNBI) and that their levels of exposure to

contaminants were below health-based guidance values (when available). This included hair and blood Hg concentrations (described in Chapters 3 and 4 of this thesis), blood Se concentrations (described in Chapter 4 of this thesis), and plasma omega-3 PUFA concentrations (described in Chapter 4 of this thesis). For the small number of participants with contaminant levels above health-based guidance levels, follow-up was offered and additional testing was performed and individuals were provided with advice on how to reduce their contaminant levels. Reports were generated at a community and project-level to return and present aggregate data from these studies to the communities. The results of these projects, including those presented in this thesis, have important implications for both public health sciences researchers in these regions, and those who use knowledge from the field of public health science such as public health agencies and policy makers. For example, the biomarker levels for both nutrients and contaminants provide important baseline data for the communities in these regions that can be used by public health science researchers in future studies to determine if nutrient and contaminant intakes in these sub-populations are changing over time. This is an important consideration for Hg exposures, as climate change may impact concentrations of Hg in these local fish in the future. These data can also be used by policymakers, such as the territorial government and health agencies, to potentially refine messages and consumption notices about Hg and fish consumption.

This thesis also provides information that can be used to guide decision making around fish consumption at a community and individual level. The results from Chapters 3-6 highlight that although fish is a source of Hg exposure, it is also an important source of essential nutrients. Chapter 3 demonstrated that hair Hg concentrations seemed to follow the same seasonal trends as was observed for reported total fish consumption frequency in the Dehcho and Sahtú regions of the Northwest Territories and Chapter 4 confirmed that the consumption of at least one species of fish was associated with higher hair and blood Hg concentrations. Chapter 4 also showed that the consumption of at least one species of fish was associated with higher blood Se and plasma EPA+DHA concentrations and Chapter 5 demonstrated that intakes of Se and EPA+DHA estimated from the consumption of four freshwater fish species contributed towards recommended daily intakes for these nutrients. Additionally, Chapter 6 demonstrated how the consumption of fish during pregnancy modified the associations between prenatal exposures to

Hg and child neurodevelopment. Collectively, the results of this thesis indicate that the consumption of fish, especially those that are low in Hg, should continue to be encouraged. This is inline with current messaging about fish consumption from public health agencies across Canada. Examples of some of the Canadian public health agencies that have messages about fish consumption and Hg are included in **Table 7.1**. However, the results from this thesis can be used to refine these public health messages, including those at the territorial level.

Table 7.1: Examples of some of the health messages released about fish consumption and Hg at various levels in Canada

Public Health Agency/ Government Agency	Level	Summary of Key Message	Source
Government of Canada	National	• Canadians should limit their consumption of fresh/frozen Tuna, Shark, Swordfish, Escolar, Marlin, and Orange Roughy. There is specific consumption advice related to these fish for children and those who are pregnant. • There are no recommended consumption limits for other fish. • Consumption recommendations for wild-caught fish (non-commercially sold) are given at the provincial and territorial level	Mercury in Fish – Questions and Answers https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/chemical-contaminants/environmental-contaminants/mercury/mercury-fish-questions-answers.html#ca2
Government of Canada	National	• Canada's food guide recommends the consumption of a variety of healthy foods daily which includes fish	Canada's Food Guide https://food-guide.canada.ca/en/
Government of the Northwest Territories	Territorial	• Eating fish is part of a healthy diet • Predatory fish tend to contain more contaminants • Pregnant women should still eat fish but those that are lower in the food chain and low in Hg (like Whitefish and Arctic Char) • General consumption guidelines are given for specific fish species (Lake Trout, Northern Pike, Walleye) • Lake-specific advice and consumption notices are also available for specific lakes	General Fish Consumption Guidelines for the NWT https://www.hss.gov.nt.ca/sites/hss/files/general-fish-consumption-guidelines-nwt.pdf
Yukon Government	Territorial	• Fish consumption has many benefits • Yukon fish are a healthy food choice	Mercury in Yukon Fish https://yukon.ca/sites/yukon.ca/files/hss/hss-mercury-yukon-fish.pdf

		• Guidance is presented for Lake Trout and Burbot/Loche including guidance specific to women of childbearing age and children • General advice should be followed unless there is lake-specific consumption advice	
Ottawa Public Health	Regional	• Advice for women, children and families • Eating fish is good for health but you should choose to eat those fish that are low in mercury and should avoid/only rarely eat those high in Hg especially for those who are pregnant, breastfeeding, or children	A Guide to Eating Fish for Women, Children, and Families https://www.ottawapublichealth.ca/en/public-health-topics/resources/Documents/fish_women_children_en.pdf

At the territorial level, previously the measurement of elevated levels of Hg concentrations in some species of fish from some lakes, resulted in the release of consumption notices to protect communities (DHHS, 2012). The findings of this thesis, as well as a report (included in Appendix A) that examined the impact of changing fish harvest locations on estimated Hg intakes, can provide useful information to policymakers within the territorial government that can be used when evaluating existing fish consumption notices. For example, the report (Appendix A) found that increasing the proportion of fish coming from waterbodies considered to have moderate (geometric mean fish Hg concentrations between 0.4 – 0.5 ppm) or high (geometric mean fish Hg concentrations >0.5 ppm) fish Hg concentrations compared to only consuming fish from low Hg waterbodies (geometric mean fish Hg concentrations of <0.4 ppm) did increase estimated Hg intakes but the majority of mean estimated intakes remained below relevant TDIs (Packull-McCormick et al., 2020b). However, in the models, increasing fish consumption from moderate/high fish Hg lakes had a larger impact on women of childbearing age and children when examining the percent of estimated intakes exceeding TDIs (as this sub-population has a lower TDI due to the increased sensitivity of early life stages to the neurotoxic effects of Hg). This report suggested that based on the types of fish reported eaten in the FFQ, frequently harvesting fish from lakes with low Hg levels (including some with consumption notices) and occasionally harvesting fish from lakes with moderate Hg levels appears unlikely to affect typical Hg exposures. However, it is particularly important that messages and recommendations regarding fish Hg and the fish species that are most safe to eat reach women of child-bearing age and children. This report suggested that site-specific consumption notices and messages seemed most warranted for waterbodies with particularly high Hg levels (ie. geometric mean fish Hg concentrations >0.5 ppm). This information could be used in conjunction with the Hg bioaccessibility data generated in this thesis (Chapter 2), the refined models (Chapter 5), and new fish analyte data from ongoing fish monitoring projects, to refine and perhaps remove some of the fish consumption notices in these regions as Hg exposures have remained relatively low. The exposure assessment models (Chapter 5) presented in this thesis can also be further refined as more fish analyte data becomes available and can be used to model different scenarios such as if fish analyte concentrations change in the future or if consumption patterns change (such as if some consumption notices were changed or removed). Overall, the results from this thesis could

provide the justification necessary to re-evaluate current consumption notices for locally caught freshwater fish and can provide important information for this re-assessment.

Additionally, the findings of this thesis could have implications for ongoing environmental monitoring programs in these regions. The most commonly consumed fish species and the waterbodies that participants most commonly reported getting their fish from were identified in this thesis (Chapter 2 and 5). This could guide ongoing fish monitoring programs as to which fish species and lakes to prioritize for sample collection. The four most commonly consumed fish species were Lake Whitefish (consumed by 87% of participants), Lake Trout (62%), Northern Pike (44%), and Walleye (34%), although this did vary by region. In both regions Lake Whitefish (92% Dehcho, 76% Sahtú), and Lake Trout (54% and 75%) were commonly consumed, but Northern Pike (60% Dehcho, 16% Sahtú) and Walleye (49% Dehcho, and 5% Sahtú) had much lower consumption in the Sahtú region relative to the Dehcho region. The eleven waterbodies identified for the participating communities were: Great Slave Lake, the Mackenzie River, Kakisa Lake, Willow Lake, Jean Marie River, Kelly Lake, Ekali Lake, Tathlina Lake, Trout Lake, Great Bear Lake, and Colville Lake. However, the waterbodies commonly reported did vary by community so additional studies could be conducted to identify important waterbodies for other communities in these regions who did not take part in the biomonitoring study. As well, the surprising finding that the consumption of locally harvested waterfowl (Chapter 4) was included in the final models to explain the variability in hair Hg, blood Hg, blood Se, and plasma EPA+DHA data, has important implications for ongoing environmental monitoring programs. There is a gap in the literature for concentrations of Hg, Se, and omega-3 PUFA concentrations in waterfowl, particularly for these regions, which limits the ability to determine to what extent these foods may be contributing to the intake of Hg, Se, and EPA+DHA. The wild caught birds included in the analyses and considered “waterfowl” for the purpose of this thesis were those with a piscivorous or omnivorous diet as included: black duck (*Anas rubripes*), mallard (*Anas platyrhynchos*), fish duck/merganser (*Mergus merganser*), oldsquaw/long-tailed duck (*Clangula hyemalis*), canvasback (*Aythya valisineria*), pintail (*Anas acuta*), and white-winged scoter (*Melanitta deglandi*). Ongoing sampling projects could collect and analyze samples of these birds and include them in future Hg risk assessments.

More broadly, the work of this thesis has important implications for the field of public health sciences in Canada. It highlights how the use of focused biomonitoring projects can address gaps in knowledge about the exposure levels and risks of populations in remote Canadian regions who are not covered by the national biomonitoring study the CHMS, (Chapter 3-4), and vulnerable life stages (pregnancy and childhood, Chapter 6). This work supports national messaging about the benefits of fish consumption, of those species low in Hg, including in northern Indigenous populations, and those who are pregnant. These results also provide important insight that can be used when designing and implementing future biomonitoring projects. For example, the knowledge that hair to blood Hg concentration ratios (Chapter 3) differ by region and differ substantially from the established 250:1 ratio (even given the variation that was reported around that ratio; WHO 1990) in the Dene/Métis communities included in these studies, can be used to support decision making in future studies when researchers are deciding which biological samples to collect. The seasonal patterns in fish consumption and Hg biomarker concentrations identified in this thesis can inform the timing of future study collection dates. For example, should communities wish to complete follow-up biomonitoring work during the time frame with potentially the highest Hg exposures in northern regions, sampling for blood and hair Hg concentrations should be conducted between late summer and early fall when the higher average blood Hg concentrations would also be reflected in hair Hg concentrations.

This thesis also has implications for international research into the effects of Hg exposure on neurodevelopment and international efforts to reduce global mercury pollution such as the Minamata Convention on Mercury. Although associations between prenatal and childhood Hg exposures and child neurodevelopment have been examined in several international studies (Jeong et al., 2017; Jacobson et al., 2015; Suzuki et al., 2010; Crump et al., 1998; Grandjean et al., 1997), these studies have found inconsistent results and were generally at higher levels of exposure than those seen in Canada. In this thesis, the associations between prenatal and childhood Hg exposures and child IQ were explored in a Canadian sample (with blood Hg concentrations similar to those measured in the Canadian general population and the northern biomonitoring projects described in this thesis). These results add to the international findings that prenatal Hg exposures are associated with child IQ, even at the low levels seen in Canada, but that prenatal fish consumption may be an important modifier of these associations. However,

data were not available on the children's consumption of fish, so childhood fish consumption as a potential modifier of the associations between childhood Hg exposure and child IQ could not be examined. Future studies examining the associations between childhood exposure to Hg and child neurodevelopment should include a dietary survey, such as an FFQ, to assess the children's fish consumption during their early childhood and should examine the potential modifying effect of childhood fish consumption on associations between childhood Hg exposure and child neurodevelopmental outcomes like child IQ. Collectively, this thesis provides valuable information about fish consumption and Hg exposures in Canada for researchers, stakeholders, and policymakers. It also highlights the importance of global work towards decreasing mercury pollution through the Minamata Convention on Hg (UNEP, 2013).

7.4 Contributions to the Literature

This thesis makes several important contributions to the literature. Firstly, Chapter 2 fills a gap in the literature for Hg bioaccessibility in northern freshwater fish species. The majority of published Hg bioaccessibility data is for marine fish species, especially those harvested in coastal locations with a relatively mild climate (Afonso et al., 2015a; Matos et al., 2015; Calatayud et al., 2012). Hg bioaccessibility is also more commonly reported in raw fish samples (Liao et al., 2020; Costa et al., 2015; Ouédraogo & Amyot, 2011), while in northern Canada locally caught fish are normally cooked or otherwise prepared before consumption (Ratelle et al., 2020). Chapter 2 of this thesis fills this gap in the literature by reporting Hg bioaccessibility in the muscle of five freshwater fish species commonly consumed in northern Canada, both raw and cooked (for a subset of the samples, pan-fried in water). Additionally, to the best of my knowledge, for the first time, Hg bioaccessibility is reported in the liver of a freshwater fish (Burbot), both raw and cooked. This information fills an important gap, and this data can be used in future Hg risk assessments in northern Canada.

Secondly, this thesis fills a gap in the literature on the determinants of variability in hair Hg, blood Hg, blood Se, and plasma EPA+DHA concentrations in northern inland Indigenous communities. Although other studies of southern populations and coastal populations have demonstrated that several demographic, biometric, and dietary factors, are associated with these biomarker concentrations (Caspersen et al., 2019; Gaxiola-Robles et al., 2014; Gari et al., 2013;

Jenssen et al., 2012), the determinants of these biomarker concentrations have not been examined for non-coastal Indigenous communities in northern Canada. Chapters 3 and 4 of this thesis filled this gap by demonstrating that hair Hg concentrations seemed to follow the same trends as total fish consumption, and final models to explain variability in hair Hg, blood Hg, blood Se, and plasma EPA+DHA concentrations contained several potential explanatory factors including the consumption of at least one species of fish, and the consumption of waterfowl. Chapter 3 also showed that hair to blood Hg concentration ratios differ by region, and Chapter 4 indicated that several of the biomarkers were correlated with each other.

Thirdly, this thesis filled a gap in the literature for a site-specific Hg exposure assessment for the Dehcho and Sahtú regions of the Northwest Territories that also considered nutrient intakes (Se and EPA+DHA), and whether the inclusion of lake-specific and Hg bioaccessibility data would strengthen the link between exposure estimates and biomarker-based measures of internal dose. Previously, Siedlikowski et al. (2016) found that the inclusion of bioaccessibility data did not significantly alter the parameters of their exposure assessment models. In Chapter 5 of this thesis, although the inclusion of lake-specific data, and Hg bioaccessibility data (only applicable for Hg exposure estimates), did not change the strength of the associations between the exposure estimates and biomarker concentrations, Hg exposure estimates in the model with both lake-specific and Hg bioaccessibility data were lower and corresponded better with the Hg biomarker concentrations (in terms of percent exceedance of TDIs versus health-based guidance values). This chapter also provided model estimates and a model template that can be used and further refined in the future to understand exposures to contaminants and nutrients from the consumption of freshwater fish in these regions.

Finally, this thesis filled the gap in the literature on whether there are associations between prenatal and childhood Hg exposures and child IQ at the low levels of exposure typically seen in Canada. The majority of studies of Hg and child IQ have been conducted in populations with higher levels of Hg exposure than typically seen in the Canadian general population (dos Santos-Lima et al., 2020; Jeong et al., 2017; Jacobson et al., 2015; Suzuki et al., 2010; Myers et al., 2009; Crump et al., 1998; Grandjean et al., 1997). The biomonitoring projects in the Northwest Territories and Yukon examined in this study were cross-sectional in nature, did not examine health or developmental outcomes, and did not specifically seek to include

pregnant women, so could not be used to address this research gap. However, levels of Hg exposure in these studies were similar to those measured in the Canadian general population (CHMS), and similar to those measured in the MIREC cohort study. As the MIREC study had similar exposure levels, was a longitudinal study, collected data on health and developmental outcomes, and specifically recruited pregnant individuals, this study provided a unique opportunity to examine this research question and fill the gap in the literature. The results of the analysis of the MIREC data (Chapter 5) not only addressed the gap in the literature on the prenatal associations between low levels of Hg exposure and child IQ in Canadian children, it also examined associations with concurrent childhood Hg exposure levels. Not many studies have measured Hg at multiple time points to directly compare the impacts of prenatal and childhood exposures on cognitive function (Hsi et al., 2014; Davidson, et al., 2010; Plusquellec et al., 2010).

7.5 Limitations

7.5.1 Sample Size

There were several challenges to conducting the research described in this thesis and consequently some limitations. Sample size was a consistent issue across the studies included and examined in this thesis and this limited some of the statistical analyses that were possible. However, every effort was made to have an adequate sample size during the data collection phase, and statistical methods were then adapted based on the final sample size that was available.

For the Mackenzie River Valley and Old Crow human biomonitoring projects, these studies and data collection took place in remote regions in northern Canada. Several of the communities that took part in the study were fly in only and compared to the population sizes in communities in southern Canada, the populations in these communities were small, limiting the number of participants that could be recruited. To address this issue during the recruitment stage, in communities with a population of 100 or less, all community members were invited to participate, and in communities with over 100 members, participants were recruited by random sampling (by contacting a random sample of households) and by including anyone who came to the clinic and volunteered to participate (Ratelle et al., 2018b). However, the resulting sample

size was not large enough for some of the communities to conduct statistical analyses at the community-level. Therefore, at the analysis stage, due to the low sample size, all analyses were conducted either at the region-level, or pooled across regions (Chapter 3, 4, and 5). Therefore, although there were likely community differences in terms of the frequency of consumption of certain traditional foods and harvest locations, data in this thesis could only be presented at the regional level. Additionally, within Chapter 4, sample size was an issue that limited the number of factors (dietary and other factors, and interaction terms) that could be included in the explanatory models. In order to increase the sample size for these analyses, data from the Old Crow biomonitoring project was included and pooled with the data from the Mackenzie Valley, NWT, biomonitoring project. Moreover, although the data could not be presented at the community-level, weights were calculated and included at the community-level for the locations participants harvested their fish for the site-specific exposure assessment models in Chapter 5. Finally, for the blood Hg concentrations examined and used within Chapters 3-5, the detection of Hg in blood samples was an issue that limited the sample size available. For the Dehcho and Sahtú regions, 61% of blood Hg concentrations were <LOD. In order to maintain the sample size for summary statistics within Chapters 3-5, blood Hg values <LOD were imputed at half the LOD (this method of imputation was chosen to maintain comparability with the CHMS, as this method of imputation is also used for summary statistics in CHMS reports). For the analysis of hair:blood Hg ratios in Chapter 3, a complete case analysis (which only included those participants with a blood Hg concentration >LOD) was used as too many (>50%) blood Hg concentrations were <LOD to use the imputed data in the statistical analysis. However, to maintain the sample size for the statistical analyses in Chapter 4, and since blood Hg was a dependent/outcome variable in the analyses, a Tobit regression model was used for the blood Hg models in Chapter 4. The use of a Tobit regression model allowed all of the blood Hg data (even that <LOD) to be included in the analysis as the blood Hg concentrations <LOD were treated as left-censored data in the model. Therefore, the main limitation of the relatively small sample sizes in the northern biomonitoring studies was that the data could only be presented at a regional-level, rather than a community-level, although exposures and determinants for Hg, Se, and EPA+DHA may have varied at a community-level as well.

Sample size was also an issue in the MIREC cohort study included in this thesis (Chapter 6). Although 2001 participants were recruited into the original MIREC cohort, only 527 participants were included in the MIREC-CD plus follow-up study. This loss to follow-up is a common issue with longitudinal studies. To maintain sample size, for those with missing covariate data, multiple imputation was used, however, the analyses were restricted to those with complete exposure (1st trimester, cord, or child, blood Hg concentrations) and outcome data (FSIQ, PIQ, VIQ, or GLC). Additionally, loss to follow-up was a concern, as differential loss to follow-up could result in selection bias which would be a threat to the internal validity of the study (Lash et al., 2020). To address this, inverse probability weighting (IPW) with stabilized weights (Seaman & White, 2013) was used to minimize the potential effect of differential loss to follow-up on the observed associations. However, the low sample size still meant that when analyses were stratified by both child sex and prenatal fish consumption, the associations were less precise.

7.5.2 Use of Food Frequency Questionnaire Data

An additional potential limitation of this thesis was that these research chapters all utilized data collected from FFQs. Although FFQs are commonly used for the collection of food consumption frequency data, they can be subject to recall bias as they rely on participant memory to recall their usual food consumption in the past, and food consumption frequencies from FFQs can be over or under-estimated (Ratelle et al., 2020; Rodrigo et al., 2015). However, FFQs are considered to have reasonable relative reliability of food estimates and can provide estimates of usual intakes (Rendo-Urteaga et al., 2020; Vézina-Im et al., 2016). It should be noted that the FFQs used in the northern biomonitoring projects were refined for use in these regions (Ratelle et al., 2020a).

For the northern biomonitoring projects, the FFQs that were used focused on the consumption of locally harvested traditional foods over the past year including the frequency of consumption for each food, the preparation/cooking methods used, and a typical portion size consumed for that type of food. FFQs were chosen to examine traditional food consumption in these regions as FFQs can provide estimates of usual intakes over extended periods of time (in this case the past year) (Kolahdooz et al., 2014), they are relatively easy and inexpensive to use and administer (Kolahdooz et al., 2014), and they could be adapted from and compared to previous FFQs administered in these regions (Ratelle et al., 2020a). Comparatively, a single 24-

hour recall dietary survey, although perhaps more accurate for absolute estimates of dietary intakes, cannot be used to estimate usual intakes especially for traditional foods whose consumption can vary throughout the year. Therefore, data from the FFQs administered in the northern biomonitoring projects were used rather than data from the 24-hour recall. However, uncertainty around the dietary data collected from the FFQs, especially in regard to the consumption frequencies (with concerns about under or over-estimates), is a limitation of this thesis. This is particularly a limitation in Chapter 5, where reported consumption frequencies were used to calculate absolute values for intake rates of four fish species. In order to somewhat mitigate this, total fish intake rates were capped at a maximum of 870 grams/week (equivalent to approximately 7 fish meals a week) to reduce overestimates of fish consumption. A similar adjustment was made to the models presented in the report in Appendix A. Other uses of the FFQ data from the northern biomonitoring projects relied more on relative measures of fish consumption. For example, for the analysis in Chapter 4, the consumption of traditional foods were compared relative to each other as a %consuming (percent of participants reporting consuming that food) and within the models they were used as binary variables (consumed or not consumed) rather than using consumption frequencies. This was also the case when making decisions about which fish species to focus on and include in Chapters 2-5. The FFQ data was used in this way, as FFQs are considered to have reasonable relative reliability (Rendo-Urteaga et al., 2020; Vézina-Im et al., 2016).

A final limitation of the FFQs used in the northern biomonitoring projects, is that they did not fully capture the seasonal variability in traditional food consumption and accessing of harvest locations. Seasonal variation in the total consumption of fish could be assessed, as questions were included that asked about total fish consumption frequency across bi-monthly time periods, and this was used in Chapter 2. However, the FFQs only asked about the frequency of consumption of specific fish species on average for the whole year. For the commonly consumed fish species, information about the seasonal variation in consumption could explain some of the seasonal variation that was observed in the hair Hg concentrations (Chapter 2). Additionally, including a ranking of commonly accessed harvest locations in future FFQs could be used to further refine the exposure assessment models in Chapter 5. To address the limitations of the FFQs used in the northern biomonitoring projects, future northern biomonitoring studies could

include additional questions on the dietary surveys they administer to gather information about the seasonal variation in the consumption of specific fish species and harvest locations they use to further refine exposure assessments for these regions.

Additionally, data from a FFQ was also used in Chapter 6, in the analyses to determine if prenatal fish consumption modified the associations between prenatal Hg exposures and child IQ. However, again instead of relying on absolute estimates on fish consumption from the FFQ, the FFQ data were used to create three sub-groups based on relative total fish consumption. These three groups were those considered to have low, moderate, or high fish consumption relative to each other with these categories representing those who reported consuming fish 0-2 times/month, 3-7 times/month, and 8 or more times/month, respectively. Additionally, the total consumption of fish and the consumption of fish considered high in Hg, reported in the FFQ administered as part of the MIREC study, were compared and found to have similar trends. Therefore, although a reliance on FFQ data was a potential source of uncertainty throughout this thesis, and a limitation, several steps were taken to minimize this issue.

7.5.3 Use of Total Hg Concentrations/Lack of Hg Speciation Data

Throughout this thesis, total Hg concentrations were used for both biomarker concentrations and for fish analyte data, which is an additional limitation of this study. In Chapter 2 of this thesis, when examining Hg bioaccessibility, total Hg concentrations were measured in the raw and cooked fish samples, and total Hg bioaccessibility was measured. Hg speciation was not included in the analysis due to inadequate sample mass and other challenges as this research took place in between Covid-19 related lab shutdowns (see **Section 7.5.6**). Additionally, with the current *in vitro* gastrointestinal digestion method, it would not be possible to measure the speciation of the non-bioaccessible fraction of the gastrointestinal digest (as it was lost within the GMF filters) so a mass balance for the mercury species would not be possible. Therefore, given the limited sample mass available (additional limitations and recommendations related to the limited sample mass available for the fish samples used in this thesis are described in more depth in subsequent sections of this thesis), the focus of the analyses in Chapter 2 was on total Hg. Similarly, as only limited MeHg data was available in the literature for northern freshwater fish, total Hg concentration data was also used in the construction of the MeHg exposure assessment models in Chapter 5 and MeHg estimates were based on assuming

100% of total Hg in fish muscle was MeHg. Although the lack of speciation data for Hg concentrations in the fish samples and data used in this thesis was a limitation, it has been previously reported that the majority of Hg in fish is typically in the form of MeHg (Arroyo-Abad et al., 2016; Kuwabara et al., 2007; Storelli et al., 2005). The percent of Hg that is in the form of MeHg can vary between and within fish species (Health Canada, 2007; Yamashita et al., 2005) and between tissue types (Arroyo-Abad et al., 2016; Yamashita et al., 2005), however, assuming total Hg in fish samples is 100% MeHg would result in conservative estimates for MeHg exposures in this thesis and this could be further refined in future studies.

Similarly, in chapters of this thesis that reported on and/or used Hg biomarker concentrations (in hair and/or blood), total Hg concentrations were used. For Hg biomarker concentrations in this thesis, the assumption was made that the majority of total Hg in hair and blood was in the form of MeHg. As fish consumption is considered the major route of exposure to MeHg for populations without high exposures to industrial or environmental sources of Hg (Hong et al., 2012; Health Canada, 2007; Schober et al., 2003), total blood Hg/total hair Hg concentrations should mainly reflect methylmercury exposure (Hong et al., 2012) supporting this assumption. However, to address this limitation in Chapter 6, and due to the availability of information about participants dental history, sensitivity analyses were conducted by adjusting the models for the total number of dental amalgams participants had and whether any had been replaced in the past year, as dental amalgams can be a source of inorganic Hg exposure. Therefore, although the assumption was made that 100% of total Hg was in the form of MeHg for both fish tissue samples and human blood and hair samples, the majority of Hg in these matrices is expected to be MeHg.

7.5.4 Availability of Fish Analyte Data

An additional limitation of this study was that the refinement of the models in Chapter 5 relied on fish analyte data and there was little available data for some of the analytes and waterbodies of interest. Fish analyte data for the models (Chapter 5) was extracted from the literature, from peer-reviewed publications and published reports, and was combined with data shared from researchers currently conducting fish sampling programs in northern Canada, including Dr. Heidi Swanson and Dr. Marlene Evans. However, even with the data from all of these sources, there were still some gaps in the dataset. For example, for Se and omega-3 PUFA,

there was a lack of waterbody-specific data for the four most commonly consumed fish species for several of the most commonly reported waterbodies. For example, Se concentration data was not available for at least one of the four most commonly consumed fish species for ten out of the eleven waterbodies examined, and of these, lake-specific Se concentration data was not available for any of the four fish species for two of the eleven waterbodies. Waterbody-specific fish omega-3 PUFA concentration data was not available for at least one fish species for ten out of the eleven waterbodies examined, and of these, the data was not available for any of the four fish species for six of the eleven waterbodies. To address this in the models, when species and waterbody-specific analyte data was not available, species-specific data was used for that waterbody. However, as data becomes available in the future for these species and waterbody combinations, these exposure assessments can be further refined.

7.5.5 Availability of Bioaccessibility Data

The lack of bioaccessibility data for the commonly consumed northern freshwater fish species examined in this thesis, for any of the analytes, in raw or cooked tissue, also presented a challenge and limitation to this thesis, especially for the refinement of the exposure assessment models in Chapter 6. In general, there is a lack of bioaccessibility data in the literature for freshwater fish species, particularly those from northern regions. To begin to fill this gap, total Hg bioaccessibility was measured in the muscle of five freshwater fish species (in Chapter 2 of this thesis) in both raw and cooked samples. Samples were obtained for this work from the research projects of Dr. Heidi Swanson and Mary Gamberg. The number of samples analyzed for raw Hg bioaccessibility (n=111) was relatively large and represented samples from the muscle of 5 commonly consumed freshwater fish species (and the liver from one species) harvested from several lakes in northern Canada. However, the size of these fish samples and total mass available was fairly small for the majority of the samples, which limited the number of analyses that samples could undergo. Therefore, only a relatively small sub-set of the samples had sufficient mass after raw Hg bioaccessibility testing, to also undergo cooking and analysis for cooked Hg bioaccessibility (n = 41). Lake Whitefish (n=4) and Lake Trout (n=2) had an especially small number of samples with adequate mass for cooking and cooked Hg bioaccessibility testing. Interruptions due to the Covid-19 pandemic also limited the number of analyses that could be conducted (discussed in more depth in **section 7.5.5**). Additionally, this

meant that only one cooking/preparation method (pan frying in water) was examined in this thesis. However, many different preparation methods are reported as being used by local communities including smoking, drying, roasting/baking, and in soup (Ratelle et al., 2020). Cooking did have a significant effect on the total Hg bioaccessibility of the samples examined, so future work should examine the effects of other common preparation methods on total Hg bioaccessibility. Furthermore, bioaccessibility of Se, and EPA+DHA, was not measured in this thesis and is not available for these northern freshwater fish. Collectively, this lack of bioaccessibility data for Hg, Se, and EPA+DHA impacted the refinement of the exposure assessments in Chapter 5 of this the thesis and is an important limitation. This meant that the exposure assessment models for Se and EPA+DHA (Chapter 5) could not be refined with the inclusion of bioaccessibility data. However, using the data generated in Chapter 2, the Hg exposure assessment model could be refined with the inclusion of Hg bioaccessibility data specific to the fish species commonly consumed in these northern regions (although it should be noted that raw bioaccessibility data were used rather than cooked due to the small sample sizes for Lake Whitefish and Lake Trout). In the future, when additional bioaccessibility data becomes available, the Hg, Se, and EPA+DHA estimates presented in this thesis can be further refined.

7.5.6 Consequences of the Covid-19 Pandemic

It should also be noted that some of the research described in this thesis was conducted during the Covid-19 pandemic which posed unique challenges and contributed to some of the limitations of this thesis. Fortunately, human biological sample collection, biological sample analysis, and dietary data collection, had been completed for the: Mackenzie River Valley NWT Human Biomonitoring project, the Old Crow Biomonitoring Yukon Human Biomonitoring Project, the MIREC cohort study, and the MIREC-CD Plus follow-up study before the Covid-19 pandemic. Fish samples included in this project had also been collected prior to the pandemic. However, Hg bioaccessibility testing in fish samples had just begun in the Fall of 2019, and the first round of bioaccessibility testing, of fish muscle samples from three fish species, from one lake, was completed in Winter 2020 just prior to shutdowns and stay at home orders due to the Covid-19 pandemic. This first round of bioaccessibility testing only included the gastric phase of the *in vitro* gastrointestinal model due to difficulties obtaining a key reagent for the intestinal phase of the model. Due to the Covid-19 pandemic, the laboratory facilities where this work took

place were closed and all laboratory work was suspended. The laboratory remained closed for over six months and when lab work resumed after that period, it was conducted under restrictions. As the Hg bioaccessibility data presented in this thesis (Chapter 2), was required for the refinement of the Hg exposure assessment model presented in Chapter 6, the lab closure somewhat delayed the completion of this thesis. It also meant that when lab work could resume, the lab work needed to be completed on a tighter schedule. This tighter time frame and pandemic related restrictions on occupancy limits and hours in the laboratory, along with small masses of the available fish samples, somewhat limited the analyses that were completed. As previously described, the small sample size of cooked Hg bioaccessibility data, the use of only one cooking method, and the lack of Se and EPA+DHA bioaccessibility data, are limitations of this thesis. However, the laboratory shut-down provided an opportunity to continue searching for the missing reagent needed for the intestinal phase of the *in vitro* gastrointestinal model. This compound was able to be obtained, so when the lab work resumed, Hg bioaccessibility was measured using the full gastrointestinal digestion model which is a strength of this thesis.

The pandemic, through travel restrictions, also impacted the ability of the research team to communicate results of the northern biomonitoring projects to participants, northern community members, and stakeholders. The return of results in some cases were delayed, but in other cases new methods of returning results were developed and used such as virtual meetings and presentations, hybrid meetings/workshops, and through the development of plain language reading materials (ie. summaries and factsheets). This process is still continuing. Results were also shared with the scientific community and policymakers in new formats such as virtual meetings/video calls, and virtual or hybrid conferences. In some cases, the increased use of video conferencing software also presented new opportunities for collaboration and knowledge transfer. This included the unique opportunity to collaborate with Health Canada, fully remotely, that allowed for the project included as Chapter 6 of this thesis to occur. Therefore, in many ways the Covid-19 pandemic provided several unique challenges and contributed to limitations in this thesis, but also presented several unique opportunities.

7.6 Recommendations and Future Directions

Based on the work and results summarized in this thesis, there are several proposed recommendations and suggestions for future work. These recommendations fall under three themes: 1) recommendations concerning fish consumption; 2) recommendations to fill remaining data gaps; and 3) recommendations for future biomonitoring studies.

7.6.1 Recommendations Concerning Fish Consumption

The main recommendation for this thesis is that the consumption of fish, especially those species low in Hg, should continue to be encouraged throughout Canada. This applies to both sub-populations examined in this thesis: 1) northern Indigenous communities and 2) pregnant women and children. Across all chapters of this thesis, it is clear that the consumption of fish has important nutritional and health benefits. For example, in Chapter 6, prenatal fish consumption is suggested to be a modifier of the associations between prenatal Hg exposures and child IQ, even at low levels of Hg exposure. In Chapter 5, model estimates indicated that the consumption of four locally harvested fish species were an important source of Se, and EPA+DHA for northern Indigenous communities. It is recommended that the consumption of commercially sold fish in Canada, especially those designated as low in Hg and high in omega-3 PUFA, continue to be encouraged for the general Canadian population. The consumption of fish low in Hg and high in EPA+DHA should also continue to be encouraged during pregnancy. However, future studies should seek to further clarify how prenatal fish consumption and prenatal Hg exposure impact child neurodevelopment. Additionally, for northern Indigenous communities, it is recommended that the consumption of fish, especially those low in Hg like Lake Whitefish, and other traditional foods continue to be encouraged. However, levels of contaminants (including contaminants of emerging concern) should continue to be monitored in traditional foods, and in northern populations, as climate change may impact the levels of contaminants in biota in the Canadian arctic and sub-arctic. Risk assessments for these Canadian sub-populations should continue to take into account not just the risk of Hg exposure, but also the nutritional benefits from fish consumption. Future work could include using the research from this thesis to support messaging and policies around environmental chemicals, specifically Hg, in Canada. Although fish consumption is a source of Hg exposure, the consumption of fish, especially those low in Hg, should continue to be encouraged due to the nutritional benefits.

7.6.2 Recommendations to Fill Remaining Data Gaps

This thesis also contains several recommendations to fill outstanding gaps in the literature and datasets. Firstly, it is recommended that the bioaccessibility dataset for northern freshwater fish should be expanded and that Hg bioaccessibility data should be included in Hg exposure assessments in northern Canada. As demonstrated in Chapter 2, total Hg in northern freshwater fish differs by species, waterbody, tissue type, and cooking/preparation method and as demonstrated in Chapter 5, the inclusion of Hg bioaccessibility data in exposure assessment models results in lower Hg exposure estimates which were more inline with Hg biomarker levels. Future work could include expanding the Hg bioaccessibility dataset generated in this thesis to include additional commonly consumed freshwater fish species (Chapter 4) from the lakes participants commonly reported getting fish from (Chapter 5). This could also include expanding the dataset to include additional common preparation and cooking methods, measuring the bioaccessibility of nutrients, such as Se and omega-3 PUFA, and the speciation of Hg. In order to do this future work, it is recommended that fish sampling projects collect larger pieces of fish for analysis when possible, so multiple analyses and cooking methods can be performed on each sample.

It is also recommended that additional analyte data, especially Se and omega-3 PUFA data (and potentially Hg speciation/MeHg concentration data), should be collected for fish from lakes local communities commonly report getting their fish from. As demonstrated by Chapter 4 and 5, locally caught freshwater fish can be an important dietary source of Se and omega-3 PUFA. Additionally, the inclusion of lake-specific Hg analyte data in the exposure assessment models in Chapter 5 reduced the Hg exposure estimates and, along with the inclusion of Hg bioaccessibility data, made the model estimates more similar to Hg biomarker levels. However, lake-specific Se and EPA+DHA fish concentration data were not available for many of the waterbodies that communities reported getting their fish from. Future work could focus on collecting and analyzing fish samples from the waterbodies participants commonly reported harvesting their fish from to fill this gap.

Finally, it is recommended that analyte data be collected for locally harvested waterfowl. As identified in Chapter 4, waterfowl consumption may explain some of the variation in biomarker levels of Hg, Se, and EPA+DHA. However, data on analyte concentrations in

waterfowl from these regions is limited. Additionally, as identified in Chapter 4, waterfowl consumption may be important to examine in relation to Hg, Se, and EPA+DHA biomarker levels. Future work could include the collection and analysis of samples of waterfowl to further explore this finding. This could be included in ongoing traditional food sampling projects in these regions.

7.6.3 Recommendations for Future Biomonitoring Studies

This thesis also has recommendations for future biomonitoring projects. Firstly, human biomonitoring projects in northern regions should take into account seasonal variation in Hg exposures and fish (and other traditional food) consumption. Based on the findings of Chapter 3, in order to sample during the time period with the highest potential Hg exposures, it is recommended that hair and blood samples for Hg analysis should be collected during the late summer/early fall. It is also recommended that both hair and blood Hg samples are collected in future biomonitoring project, when possible, as hair to blood Hg concentration ratios may differ between northern regions and may differ compared to the Canadian general population and other global populations.

It is also recommended that follow-up studies be conducted in these northern regions to determine if exposures to environmental contaminants and nutrients, and the diets of these populations, are changing over time. Climate change has the potential to impact access to traditional foods including fish in northern Canada (Proverbs, et al., 2020) and levels of environmental contaminants, such as Hg, in those foods (Rohonczy et al., 2020). Additionally, it is recommended that future biomonitoring studies examining Hg biomarker levels, also examine nutrient biomarker levels, like Se and EPA+DHA, as they can be correlated and have common dietary sources. Future studies examining the associations between Hg exposures and health outcomes, including studies of child neurodevelopment, should take into account the nutritional benefits of fish consumption and the potential modifying role fish consumption can play.

7.7 Summary

Collectively, this thesis emphasizes that although fish consumption can be a source of Hg exposure for the Canadian general population and northern Indigenous communities, it is also a source of important nutrients. This thesis fills an important gap in the literature on the levels,

determinants, and dietary intakes of Hg, Se, and omega-3 PUFA in First Nations communities in northern Canada. This is also unique in that the dataset included and generated by this thesis includes co-located, and site-specific: human dietary intake data, human biomarker concentration data, fish tissue concentrations (for a contaminant and essential nutrients), and contaminant (Hg) bioaccessibility data, which is found in few, if any, other theses. Furthermore, this thesis provides evidence of the importance of fish consumption as a modifier of the associations between early life exposures to low levels of Hg and child IQ. Future work in northern regions should include the collection of additional waterbody-specific fish Se and omega-3 PUFA concentration and bioaccessibility data. Future exposure assessments and epidemiological studies on Hg exposure should take into account the important nutritional benefits of fish consumption. The consumption of fish, of those species that are considered low in Hg, should continue to be encouraged for populations in Canada.

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Appendix

Report: Estimating the Impact of Freshwater Fish Harvest Location on Mercury Exposures in Dene Communities of the Northwest Territories

December 2020

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Summary:

- The research described in this report investigated how mercury levels of Dene communities of the Northwest Territories could change if people increased their use of fish from waterbodies with elevated fish mercury levels.
- Models predicting population mercury exposure were created for five age/sex categories (under 18 years, women 18-39 years, men 18-39 years, 40-59 years, and over 60 years) according to seven fish consumption scenarios.
    - **Model 1**: 100% of fish were from lakes with low fish mercury without any current consumption notices
    - **Model 2**: 50% of fish were from lakes with low fish mercury without any current consumption notices with the remaining of 50% from lakes with current consumption notices but also with relatively low fish mercury.
    - **Model 3**: 100% of fish were from lakes with low fish mercury, regardless of whether those lakes have a current consumption notice.
    - **Model 4**: 75% of fish were from lakes with low fish mercury, 25% from lakes with moderate fish mercury
    - **Model 5**: 50% of fish were from lakes with low fish mercury, 50% from lakes with moderate fish mercury
    - **Model 6**: 75% of fish were from lakes with low fish mercury, 25% of fish were from lakes with high fish mercury
    - **Model 7**: 50% of fish were from lakes with low fish mercury, 50% of fish were from lakes with high fish mercury
- In all models, the majority of estimated exposures were below relevant tolerable daily intakes (TDIs) for methylmercury
- Because children (under 18 years) and women of childbearing age (18-39 years) are most sensitive to mercury, it is especially important that these groups eat fish with low mercury levels.
- Based on the types of fish that people report eating in the NWT, occasionally harvesting fish from lakes with moderate mercury levels appears unlikely to affect typical mercury exposures. Communities should continue to harvest most of their fish from lakes with low levels of fish mercury.

- General messages and recommendations about the fish species that are most safe to eat may have the largest impact on people's mercury exposure.
    - It is particularly important for such messages and recommendations to focus on women of child-bearing age and children.
    - Site-specific messages appear most warranted for lakes and waterbodies with high fish mercury.

1.0 Introduction

Fish mercury concentrations have been tested in waterbodies of the Northwest Territories via core monitoring and community-based monitoring for the past ~30 years (NCP, 2003). Elevated mercury levels in some fish species led to the development of consumption notices that suggest people limit their consumption of specific predatory fish species from some lakes in the Northwest Territories (NWT, 2016). Waterbodies with consumption notices include several lakes in the Dehcho and Sahtú regions. To address community concerns regarding the health risks posed by fish mercury levels, a human contaminant biomonitoring project was launched in nine communities of the Northwest Territories in 2016 (Ratelle et al., 2018a, Ratelle et al., 2018b). This biomonitoring project included the collection of demographic data, human dietary data (via food surveys), risk communication data (via surveys on preferences for, and behaviours from, health messaging), and human biological samples (via hair and blood sampling) from 2016-2018.

Mercury concentrations were measured in hair (443 participants) and blood (276 participants) samples from participants that ranged from 6 to 88 years of age. Results from this work showed most participants had hair and blood mercury levels that were below health-based guidance values (Ratelle et al., 2019). Therefore, even though mercury levels are occasionally elevated in specific fish species from certain lakes in the Northwest Territories, most people's exposures in the Dehcho and Sahtú region have remained low. These results helped reinforce that the benefits of fish consumption tend to outweigh the risk of contaminant exposure in the Northwest Territories.

The research described in this report investigated how mercury exposures in Dene communities of the Northwest Territories could change if people increased their use of fish from waterbodies with elevated fish mercury levels.

2.0 Methods

Models that estimate distributions of mercury exposure were created using fish consumption data collected from the Dehcho and Sahtú regions of the Northwest Territories for the four most commonly consumed fish species: Lake Whitefish, Lake Trout, Northern Pike, and Walleye. Mercury concentrations for these fish species from lakes in the area of interest were also collated for these analyses. Each model was created using Oracle Crystal Ball (Release 11.1.2.4.600) software (**Figure 1.0**).

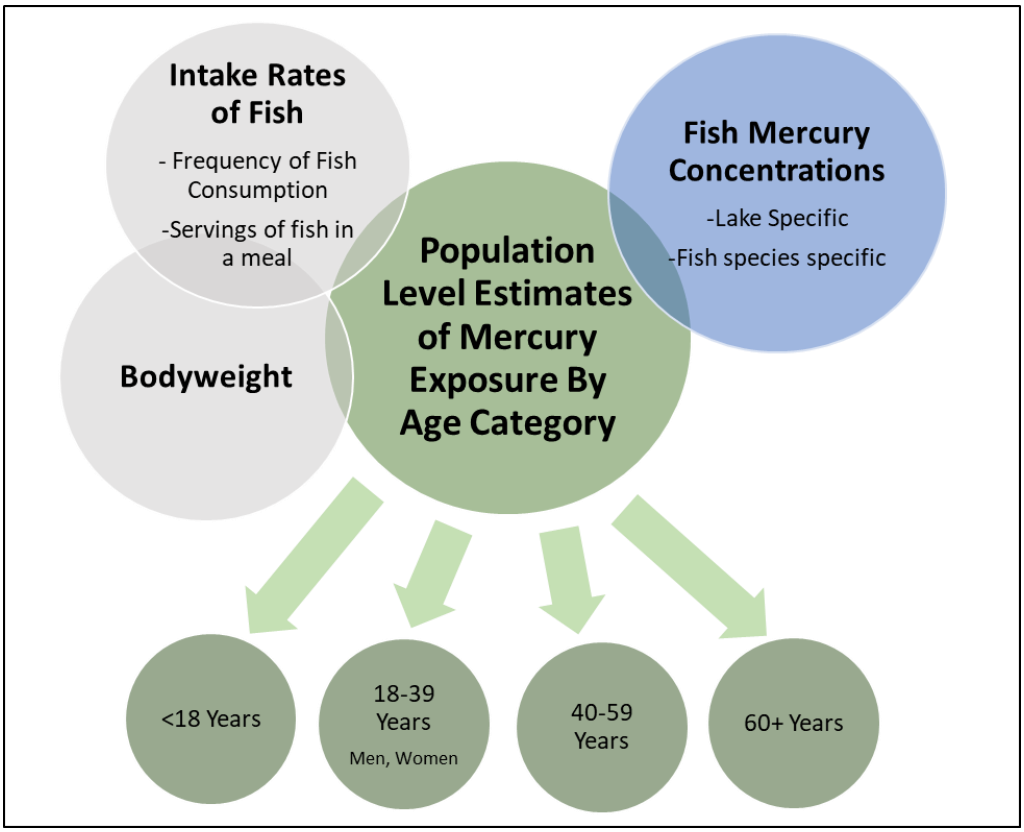


Figure 1.0: Types of human data (represented with grey circles) and fish data (represented with a blue circle) that were included in the model

2.1 Human Data

Data on fish consumption included in this report reflect results of Food Frequency Questionnaires (FFQ) that were completed by people who participated in the contaminant biomonitoring project (Ratelle et al., 2020). The FFQ included questions about which fish were eaten by participants, the frequency of fish consumption over the past year (in times per week), and how many servings were eaten in a typical meal. These data were used to calculate an intake

rate for each participant, which was expressed as grams per week (for each species). These fish intake rates, which were stratified by age/sex category were then used in mercury exposure models (see **Figure 2.0**). Summary statistics for intake rates by age category are included in **Appendix I** in **Supplementary Table 1.0**. Due to overestimates of consumption in the FFQ (Ratelle et al., 2020), FFQ data were capped at the 95th percentile. Also, maximum fish consumption (of all species) was set to 7 meals per week. Bodyweight was included in the models to standardize mercury exposure. Aggregate statistics on bodyweight (by age category) are shown in **Appendix I** in **Supplementary Table 2.0**.

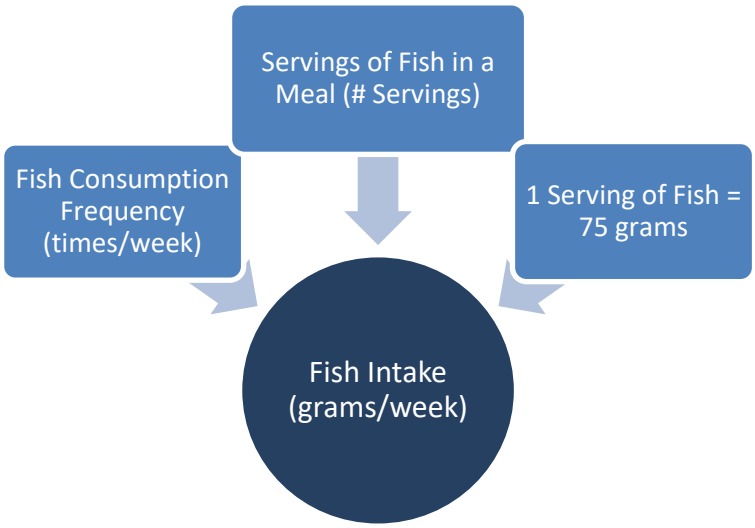


Figure 2.0: Information to Calculate Fish Intake Rates for the Model

2.2 Fish Data

Fish mercury concentrations from lakes of the Northwest Territories were gathered from peer-reviewed publications, regional reports, and raw data shared by northern partners. Specific lakes, species, sample sizes and sources of the fish mercury data included in this report are included in **Appendix I** of this report in **Supplementary Table 3.0**. The fish data included in the models and described in this report represent sampled collected and reported between 1996 – 2015. Approximately 22% of the samples were collected in the past ten years. Geometric mean mercury concentrations were used to construct categories for the lakes (low, moderate, high).

2.3 Model Set

To examine how people's exposure levels to mercury would change if they got more fish from lakes with higher fish mercury levels, seven models were constructed using the categories described below. Models differed according to how much of the fish mercury data came from each lake category (low, moderate, or high fish mercury. These seven models therefore represented seven different scenarios of fish consumption, as summarized in **Figure 3.0**.

Table 1.0: Lake Categories and Criteria

Classification	Category	Fish Mercury	Example Lake
Low Fish Mercury	**Category 1a**	Geometric mean of fish mercury less than 0.4 µg/g. No consumption notice.	Kakisa Lake: • Northern pike • Walleye
	Category1b	Geometric mean of fish mercury less than 0.4 µg/g. Consumption notice.	Ekali Lake: • Northern Pike • Walleye Trout Lake: • Lake Trout • Walleye
	Category 1	Geometric mean of fish mercury less than 0.4 µg/g. Category 1a and 1b combined.	
Moderate Fish Mercury	**Category 2**	Geometric mean of fish mercury between 0.4 and 0.5 µg/g	Kelly Lake: • Lake Trout
High Fish Mercury	**Category 3**	Geometric mean of fish mercury above 0.5 µg/g.	Sanguez Lake: • Northern Pike • Walleye Kelly Lake: • Northern Pike

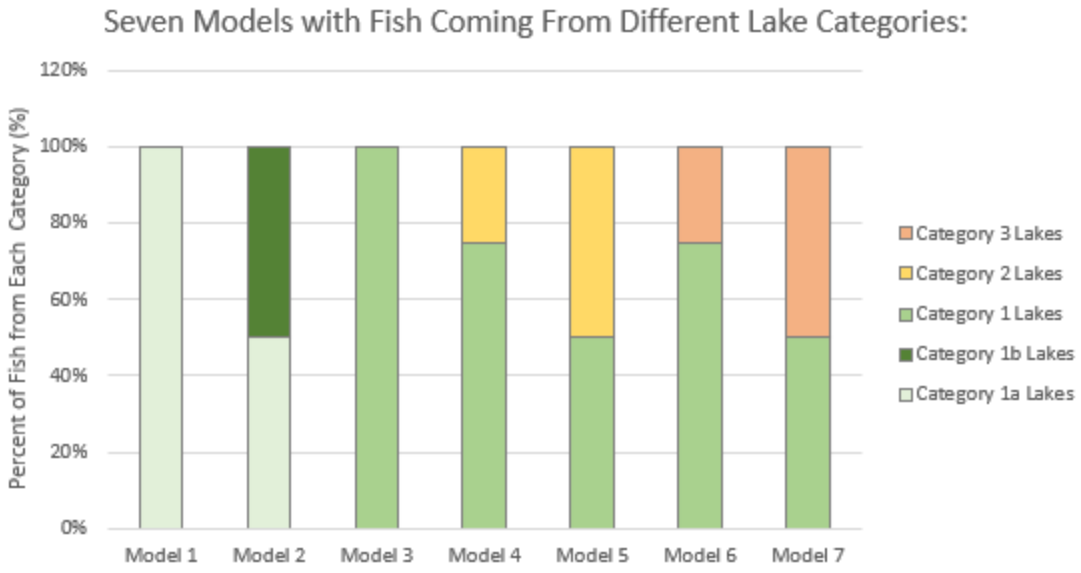


Figure 3.0: The percent of fish by lake category included in seven mercury exposure models.

Summary statistics for fish mercury distributions used for each of the seven models are summarised in **Appendix I** in **Supplementary Table 5.0**. Since all Lake Whitefish data fell into Category 1a, fish mercury levels for Lake Whitefish remained the same across Models 1 – 7. In contrast, fish mercury levels differed among models for each of Lake Trout, Northern Pike, and Walleye.

2.4 Risk Analysis

Mercury exposure distributions were compared to the toxicological reference values of methylmercury:

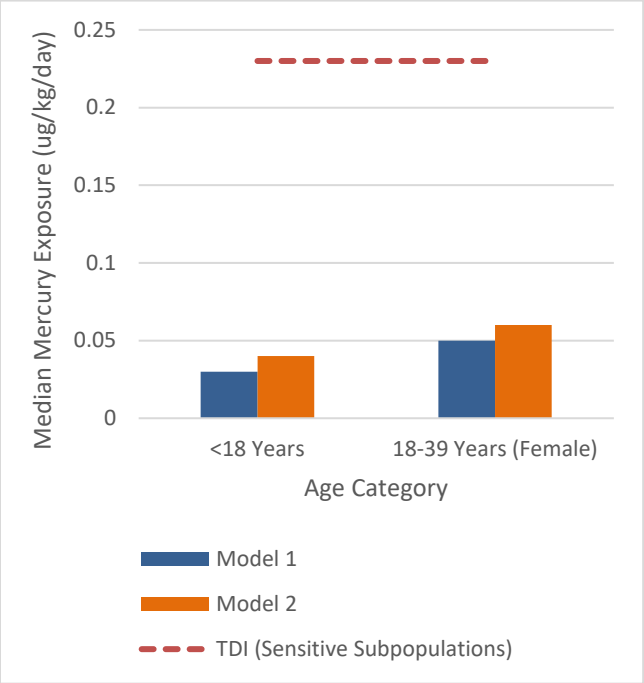
- Males 18+ years; Females 40+ years: A methylmercury Tolerable Daily Intake (TDI) of 0.47 µg/kg/day was used (Health Canada, 2007)
- Children; Females 18-39 years: A methylmercury TDI of 0.23 µg/kg /day was used (WHO, 2003)

3.0 Results and Discussion

3.1 Fish Consumption from "Low Fish Mercury" Category Lakes

Comparing Models 1 and 2 explores how mercury exposures may change if people harvested up to 50% of their predatory fish from lakes with relatively low fish mercury levels

(e.g., geometric mean Hg less than 0.4 µg/g) but for which there is a current consumption notice. In **Figure 4.1,** the median mercury exposures are compared against each of the methylmercury TDIs.



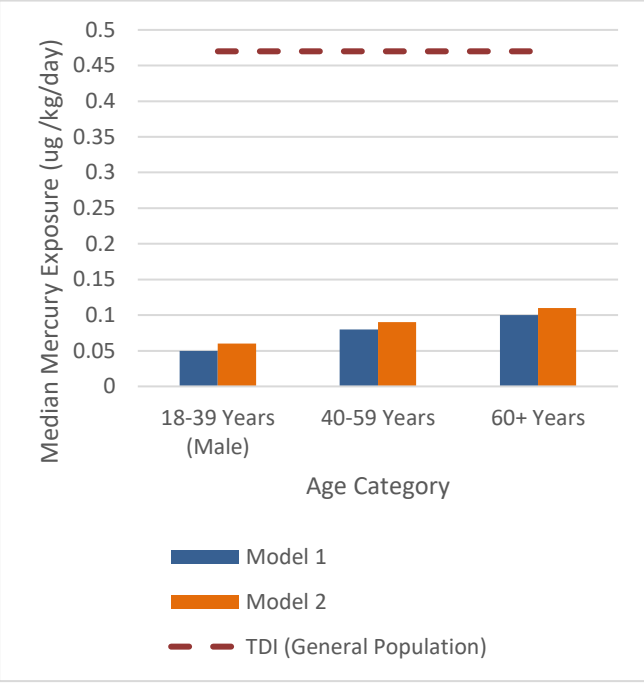


Figure 4.1: Median estimated mercury exposure under Models 1 and 2 by Age Category. *Model 1:* 100% of fish mercury data from Category 1a Lakes. *Model 2*: 50% of fish mercury data from Category 1a lakes with the other 50% from Category 1b Lakes.

In **Figure 4.2** the percent of exposures exceeding the methylmercury TDI (by age category) are shown for Model 1 and Model 2. Additional summary statistics are included in **Appendix II** in **Supplementary Table 6.0**.

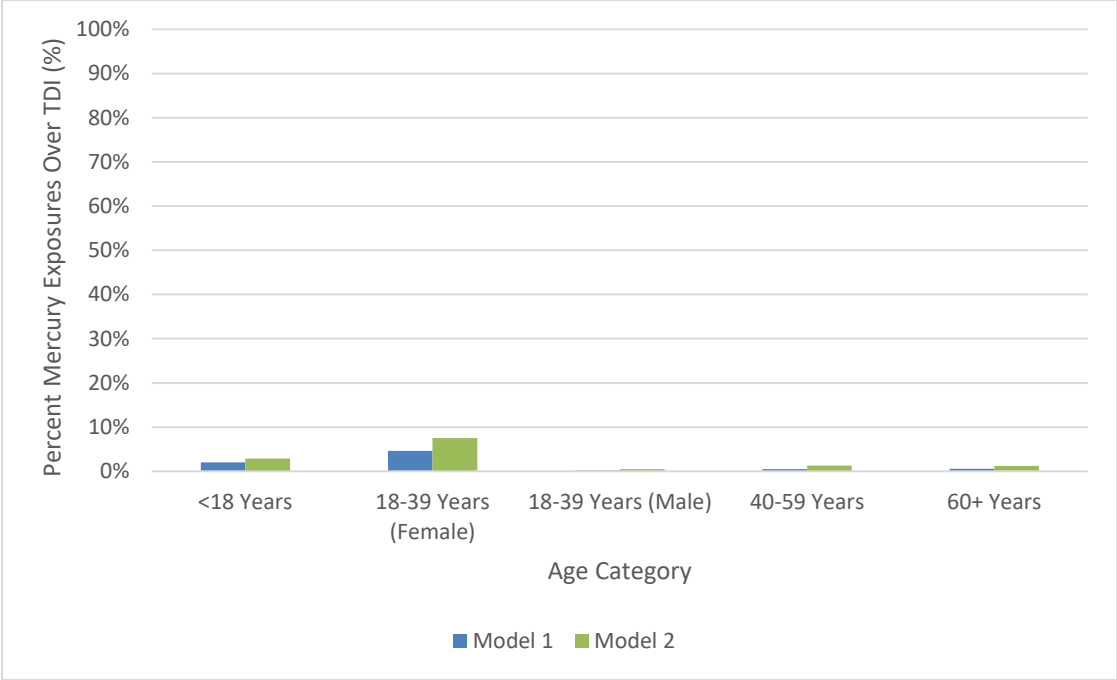


Figure 4.2: Percent of mercury exposures (by Age Category) from Models 1 and 2 that exceed mercury TDIs. *Model 1:* 100% of fish mercury data from Category 1a Lakes. *Model 2*: 50% of fish mercury data from Category 1a lakes with the other 50% from Category 1b Lakes.

For both Models 1 and 2, the majority of estimated mercury exposures fell below Health Canada's TDI for methylmercury.

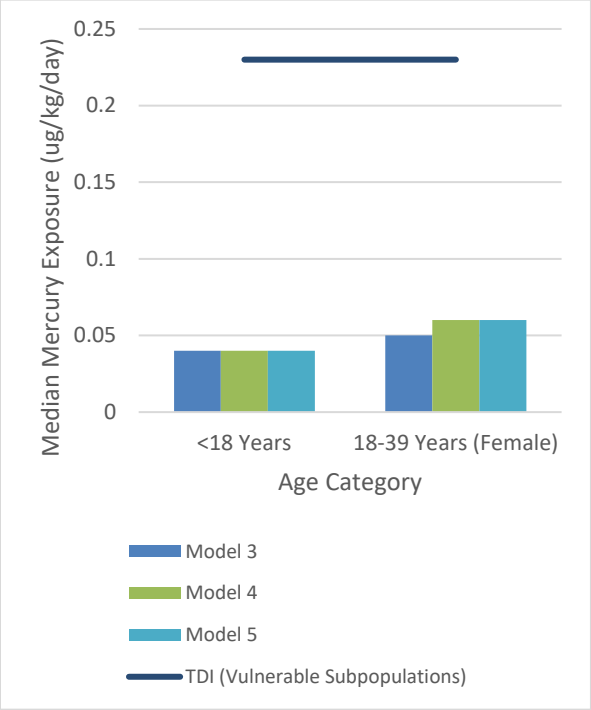
- Median estimated doses for all age categories in both models were well below the relevant TDIs for methylmercury (**Figure 4.1**).
- Median mercury exposures were lowest for those under 18 years of age, and increased with age (**Figure 4.1**).
- When up to 50% fish mercury data came from Category 1b lakes, median mercury exposures remained similar and far below methylmercury TDIs **(Figure 4.1)**.
- The vast majority (99%) of mercury exposures for adult males fell below the TDI for methylmercury. For children and women of child-bearing age, between 92.5 – 98.0% of modeled results were below the TDI for methylmercury **(Figure 4.2)**. Differences between age/sex categories were observed because children and women of child-bearing age are more sensitive to mercury.

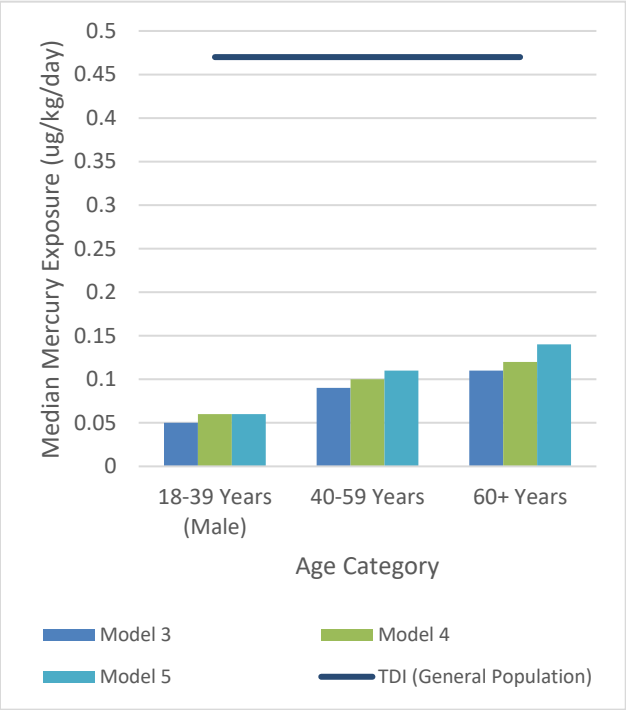
Percent exceedances of TDIs were predicted to range from 0.6% in the 60+ years of age demographic to 2.9% for women 18-39 years of age. Given the similarity in results between

Models 1 and 2, fish mercury results from all waterbodies with geometric mean mercury less than 0.4 µg/g were pooled into Category 1 to compare against each of the next mercury exposure models (i.e., Models 3-7).

3.2 Consuming More Fish from "Moderate Fish Mercury" Category Lakes

Comparing Models 3, 4, and 5 explores how mercury exposures may change if people harvested up to 50% of their predatory fish from lakes with moderate fish mercury levels (e.g., geometric mean fish mercury between 0.4 and 0.5 µg/g). In **Figure 5.1,** the median mercury exposures are compared against relevant TDIs for methylmercury; that is, for the general





population, and for vulnerable sub-populations (children and women of child-bearing age).

Figure 5.1: Median estimated mercury exposure under Models 3 – 5 by Age Category. *Model 3:* 100% of fish mercury data from Category 1 lakes (a + b combined). *Model 4*: 75% of fish mercury data from Category 1 lakes with the remaining 25% from Category 2 lakes. *Model 5*: 50% of fish mercury data from Category 1 lakes with the remaining 50% from Category 2 lakes.

In **Figure 5.2** the percent of exposures exceeding the methylmercury TDI (by age category) are shown for Model 3, 4, and 5. Additional summary statistics are included in **Appendix II** in **Supplementary Table** 7**.0**.

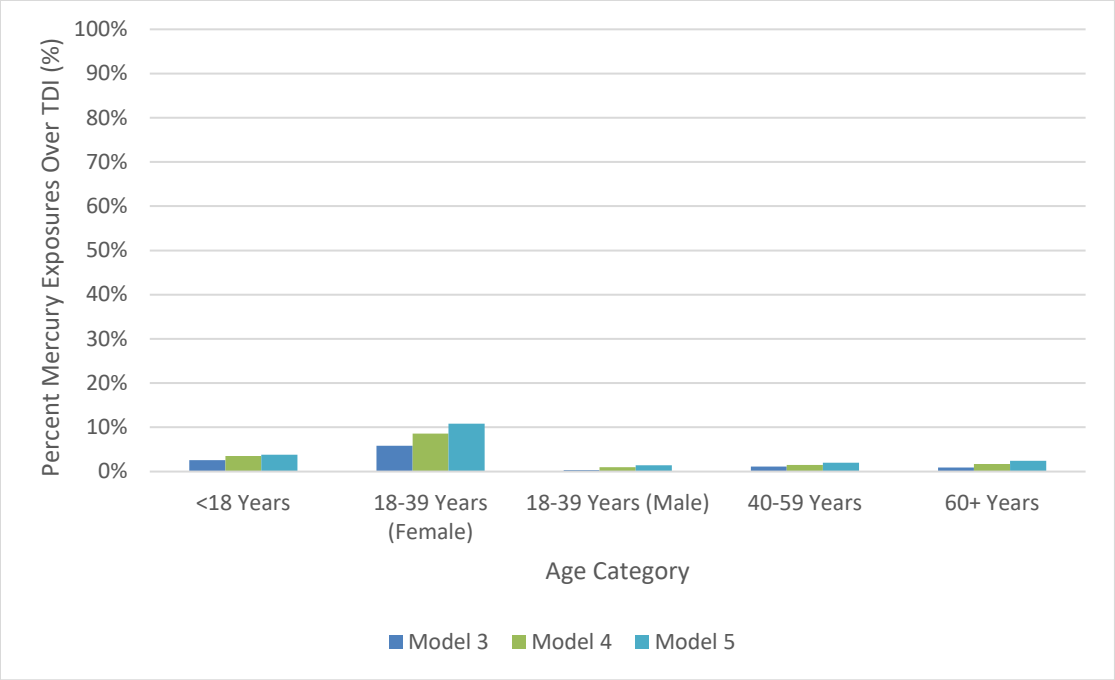


Figure 5.2: Percent of mercury exposures (by Age Category) from Models 3 – 5 that exceed mercury TDIs. *Model 3:* 100% of fish mercury data from Category 1 lakes (a + b combined). *Model 4*: 75% of fish mercury data from Category 1 lakes with the remaining 25% from Category 2 lakes. *Model 5*: 50% of fish mercury data from Category 1 lakes with the remaining 50% from Category 2 lakes.

For Models 3, 4, and 5, the majority of estimated exposures still fell below the TDIs for methylmercury.

- Median estimated exposures for all age categories across all three models were wellbelow relevant TDIs for methylmercury (**Figure 5.1**).
- The majority (at least 89%) of mercury exposure estimates fell below the relevant TDIs for methylmercury **(Figure 5.2)**.
- When 25% - 50% of fish mercury data came from Category 2 lakes:
    - Median mercury exposures were increased slightly in Models 4 and 5 **(Figure 5.1)**.
    - At least 95% of exposure estimates consistently fell below methylmercury TDIs for four of the five Age Categories (**Figure 5.2)**. Because they are more sensitive to methylmercury, these percentages ranged between 89.2 – 91.4% for women of child-bearing age.

- When 50% of fish mercury data came from Category 2 lakes, 10.8% of exposure estimates for women of childbearing age (18-39 years of age) exceeded the methylmercury TDI (**Figure 5.2**).

Between Models 3 and 4 (i.e., with up to 25% of fish mercury data coming from lakes with moderate fish mercury levels), percent exceedances of TDI may increase between 0.4% (40-59 years of age) and 2.8% (women 18-39 years of age). Between Models 3 and 5 (i.e., with up to 50% of fish mercury data coming from moderate mercury lakes), percent exceedances may increase between 0.9% (40-59 years of age) and 5.0% (women 18-39 years of age).

3.3 Consuming More Fish from "High Fish Mercury" Category Lakes

Comparing Models 3, 6, and 7 explores how mercury exposures may change if people harvest up to 50% of their predatory fish from lakes with relatively high fish mercury levels (e.g., geometric mean Hg above 0.5 µg/g). In **Figure 6.1** the median mercury exposures are compared against relevant TDIs for methylmercury.

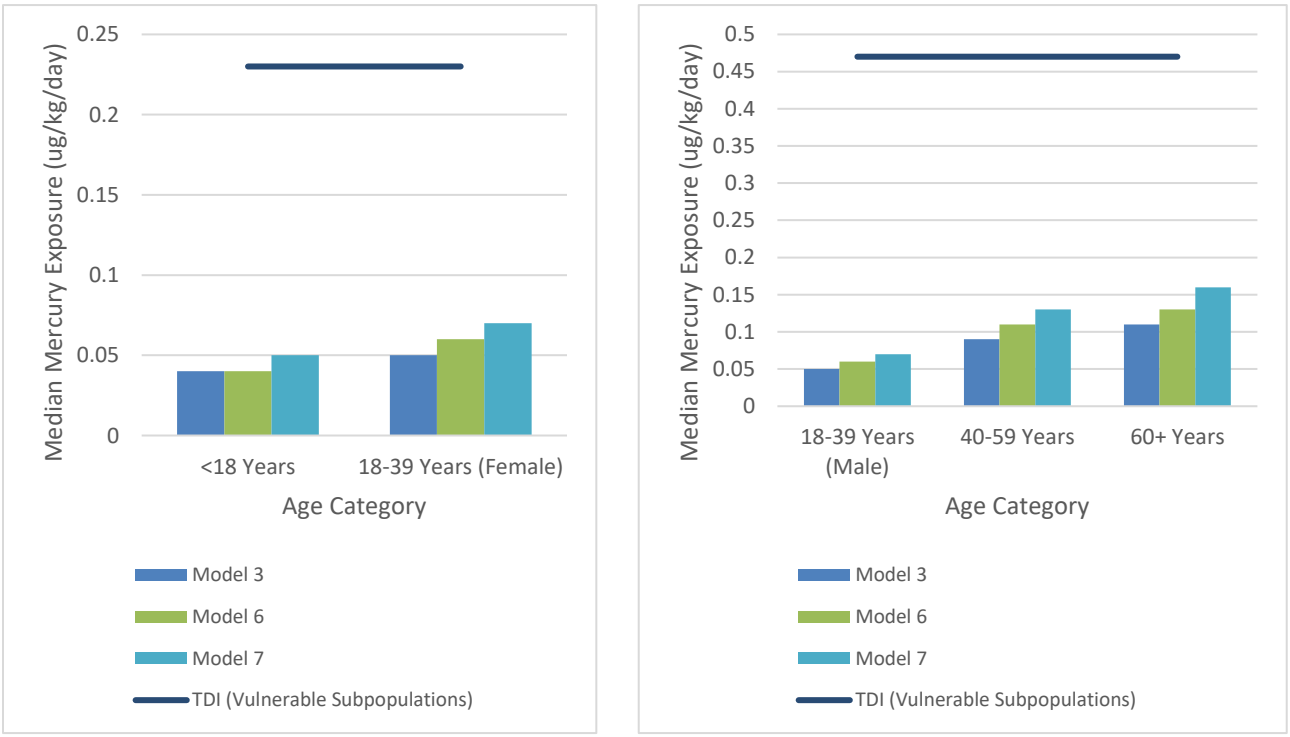


Figure 6.1: Median estimated mercury exposures under Models 3, 6, and 7 by Age Category. *Model 3:* 100% of fish mercury data from Category 1 lakes (a + b combined). *Model 6:* 75% of fish mercury data from Category 1 lakes

with the remaining 25% from Category 3 lakes. *Model 7*: 50% of fish mercury data from Category 1 lakes and 50% from Category 3 lakes.

In **Figure 6.2** the percent of exposures exceeding the methylmercury TDI (by age category) are shown for Model 3, 6, and 7. Additional summary statistics are included in **Appendix II** in **Supplementary Table 8.0**.

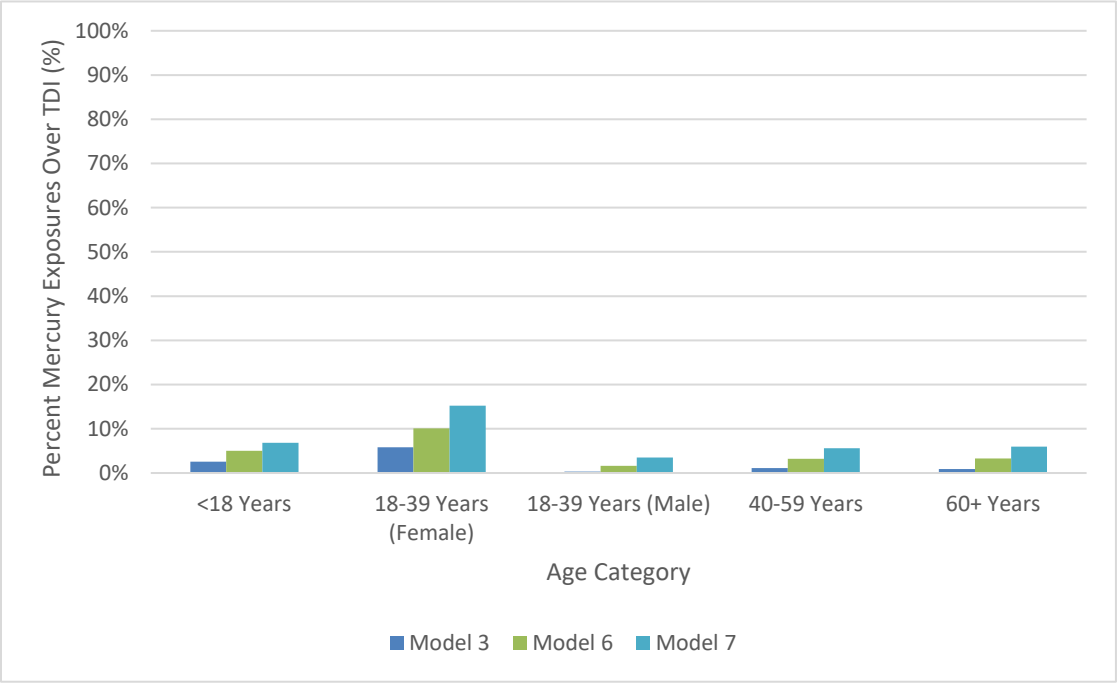


Figure 6.2: Percent of mercury exposures (by Age Category) from Models 3, 6, and 7. *Model 3:* 100% of fish mercury data from Category 1 lakes (a + b combined). *Model 6:* 75% of fish mercury data from Category 1 lakes with the remaining 25% from Category 3 lakes. *Model 7*: 50% of fish mercury data from Category 1 lakes and 50% from Category 3 lakes.

For Models 3, 6, and 7, the majority of estimated exposures still fell below the TDIs for methylmercury.

- Median estimated exposures for all age categories across all three models were well below relevant TDIs for methylmercury (**Figure 6.1**).
- The majority (at least 85%) of mercury exposure estimates fell below the relevant TDIs for methylmercury (**Figure 6.2**).
- When 25% - 50% of fish mercury data came from Category 3 lakes:

- Between 5.0 – 6.8% of mercury exposure estimates for children exceeded the TDI. Similarly, because of their sensitivity to methylmercury, between 10.1 – 15.2% of estimates for women of childbearing age exceeded the TDI (**Figure 6.2**).

- When 50% of fish mercury data came from Category 3 lakes, exceedance rates were above 5% for four of five Age Categories. The key exception to this was for men 18 – 39 years of age (**Figure 6.2**).
    - For men 18 – 39 years of age, exceedance rates were consistently 3.5% or less.

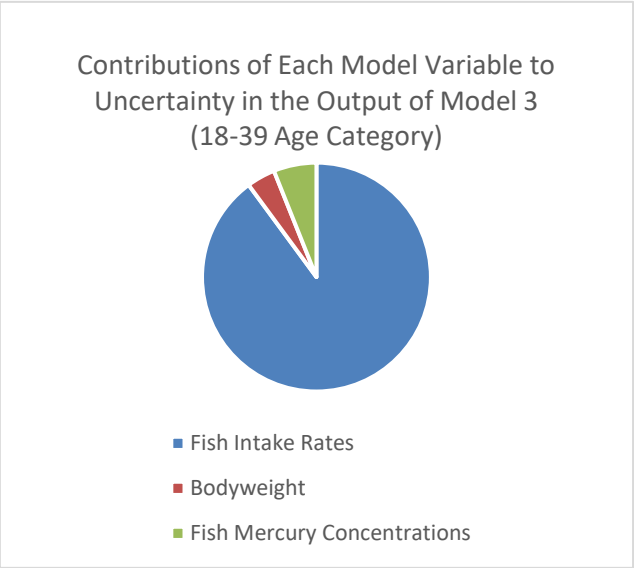
Between Models 3 and 6 (i.e., with up to 25% of predatory fish coming from Category 3 lakes), percent exceedances may increase between 2.1% (40-59 years of age) and 4.3% (women 18-39 years of age). Between Models 3 and 7 (i.e., with up to 50% of predatory fish coming Category 3 lakes), percent exceedances may increase between 4.2% (<18 years of age) and 9.4% (women 18-39 years of age).

3.4 Sensitivity Analyses

For each of the models, sensitivity analyses were also conducted to see which variables included in the model contributed the most to the uncertainty in estimated total mercury doses for each age category and each model.

- In each model and for every age group, the main factors that contributed to variation in methylmercury exposure were the intake rates of the four fish species.
- The relative importance of the intake rates of each fish species varied between the age categories and models.
- Other factors (such as body weight and fish mercury levels) contributed less to the variation in methylmercury exposure estimates.

The different exposure factors were combined into categories (e.g., fish intake rates and fish mercury concentration data) for visual representations. As an example, **Figure 7.0** shows the contribution to uncertainty in estimated mercury doses for the 18-39 age group from Model 3 and Model 7.



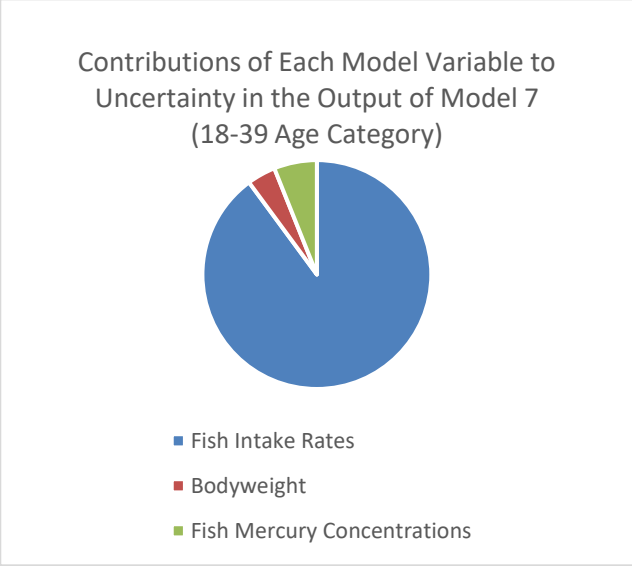


Figure 7.0: Comparison of the Sensitivity Analyses for Model 3 versus Model 7 for the 18-39 Age Group - Contributions of Each Model Variable to Uncertainty in the Outputs

When comparing a model with only low mercury fish data (Model 3) to a model with 50% fish mercury data from high mercury lakes (Model 7) as shown in **Figure 7.0**:

- Human intake rates of fish were the major contributor to uncertainty in the estimated mercury doses in both models
- Body weight and fish mercury concentrations contributed only a small fraction to the uncertainty in estimated mercury doses

The results of the sensitivity analyses suggest that messages and recommendations about the fish species that are most safe to eat may have the largest impact on people's mercury exposure.

4.0 Conclusions

1) Estimated mercury exposures were generally lowest in the youngest age group and increased with age category.

2) In all models, the majority of estimated mercury exposures were below relevant TDIs. Based on the types of fish that people reported eating in the NWT, occasional harvesting of fish from

lakes with moderate mercury levels appears unlikely to affect typical mercury exposures. Communities should continue to harvest most of their fish from lakes with low levels of fish mercury.

- Because children (under 18 years) and women of childbearing age (18-39 years) are most sensitive to mercury, it is especially important for the fish they eat to have low mercury levels.

3) Some lakes with consumption notices in the Northwest Territories have relatively low fish mercury levels. Harvesting fish from such lakes appears unlikely to affect typical mercury exposures.

- When 50% of fish came from lakes with low fish mercury (but with consumption notices), median exposures remained similar and far below methylmercury TDIs.
- Future work using updated fish mercury data will examine the usefulness and impacts of such lake-specific consumption notices on mercury exposures in the Northwest Territories.

4) When more fish mercury data came from lake categories with higher mercury concentrations, the estimated mercury exposures consistently increased for each age category.

- The highest estimated mercury exposures were seen in Model 7 when 50% of the fish mercury data came from the highest mercury lake category, Lake Category 3
- When 50% of fish came from Category 3 Lakes, between 5 – 15% of the estimated mercury exposures exceeded the TDIs.

5) General messages and recommendations about the fish species that are most safe to eat may have the largest impact on most people's mercury exposure. These results also indicate:

- The importance of messages and recommendations regarding fish mercury reaching women of child-bearing age.
- Site-specific messages appear most warranted for lakes and waterbodies with particularly high fish mercury levels (e.g., geometric mean fish mercury over 0.5 ppm).

Input Data for the Models

Appendix Table 1: Summary Statistics for Fish Intake Rates for the Top Four Consumed Species by Age Group from the Food Frequency Questionnaire

		Recorded Intake Rates (grams/week)			
Fish Species	**Summary Statistics**	**Age <18 Year**	**Age 18 to 39**	**Age 40 to 59**	**Age 60+**
Lake Whitefish	**Mean**	112	95	185	198
	Standard Deviation	169	102	207	261
	Median	60	60	113	113
	75th percentile	112.5	168.75	225	225
	95th percentile	379*	295*	607*	638*
	Maximum	750	450	975	1463
Lake Trout	**Mean**	36	96	78	164
	Standard Deviation	63	168	122	282
	Median	0	38	56	64
	75th percentile	56	75	84	169
	95th percentile	124*	450*	225*	857*
	Maximum	300	900	731	1463
Northern Pike	**Mean**	29	56	73	67
	Standard Deviation	45	146	112	99
	Median	0	0	19	0
	75th percentile	60	42	113	113
	95th percentile	118*	253*	300*	225*
	Maximum	169	900	600	510
Walleye	**Mean**	30	47	62	50
	Standard Deviation	71	111	125	96
	Median	0	0	0	0
	75th percentile	19	57	61	64
	95th percentile	185*	253*	231*	205*
	Maximum	338	600	750	510

*As described in the methods, intake rates were capped at the 95th percentile (amounts reported by participants that were above the 95th percentile were imputed at the 95th percentile)

Appendix Table 2: Summary Statistics for Defining Distribution for Bodyweight as a Model Input Variable

	Bodyweight (Kg)			
Age Category	**<18 Years Old**	**18 to 39 Years Old**	**40 to 59 Years Old**	**60+ Years Old**
Min	19.0	48.3	46.3	53.1
Mean	57.9	77.4	78.5	79.2
Standard Deviation	20.7	16.9	19.7	15.0

Appendix Table 3: Sources of Fish Mercury Data Included in the Models

Type of Data Source	Lakes	Fish Species (Sample Size)	Year(s) Data is from	Citation (If Available)
Published Literature – M. Sc. Thesis	Mustard Lake, Trout Lake, Ekali Lake, Gargan Lake, Kakisa Lake, McGill Lake, Sanguez Lake, and Tathlina Lake	Lake Trout (51), Lake Whitefish (68), Northern Pike (85), and Walleye (61)	2013 - 2015	Laird, M. (2017). Dietary Exposure Assessment and Contaminants Biomonitoring in the Dehcho Region, Northwest Territories: Exploring the Relationship Between Mercury Exposure, Omega-3 Fatty Acid Status, and Fish Consumption (Master's thesis, University of Waterloo).
Published Literature – Report	Aubry Lake, Bandy Lake, Colville Lake, Kelly Lake, Lac Belot,, Loon Lake, Mahoney Lake, Manuel Lake, Mirror Lake, Rorey Lake, Tagatui Lake, and Turton Lake	Lake Trout (624), Lake Whitefish (285), Northern Pike (117)	1996 - 2000	Stewart, D. B., Taptuna, W. E. F., Lockhart, W. L., & Low, G. (2003). Biological data from experimental fisheries at special harvesting areas in the Sahtú Dene and Métis settlement area, NT: Volume 2. Lakes near the communities of Colville Lake, Fort Good Hope, Norman Wells, and Tulita. Can. Data Rep. Fish. Aquat. Sci./Rapp. Stat. Can. Sci. Halieut. Aquat., (1126), 108.
Raw Data from a Partner Organisation – Sahtú Renewable Resources Board	Great Bear Lake	Lake Trout (9), Lake Whitefish (11), and Walleye (6)	2013	

Appendix Table 4: Summary Statistics of Fish Mercury Concentrations by Species and Lake Category

		Mercury Concentrations (µg/g)			
Lake Category	**Summary Statistics**	**Lake Whitefish**	**Lake Trout**	**Northern Pike**	**Walleye**
Category 1a (geomean for Hg data for the fish species below 0.4 µg/g and no fish consumption notice emitted)	**Geometric Mean**	0.13	0.20	0.20	0.23
	Arithmetic Mean	0.18	0.23	0.24	0.28
	Standard Deviation	0.13	0.12	0.17	0.20
	Min	0.01	0.02	0.04	0.11
	Max	0.62	0.99	0.91	0.67
	Sample size	364	475	86	8
Category 1b (geomean for Hg for the fish species below 0.4 µg/g and a consumption notice has been emitted)	**Geometric Mean**		0.31	0.33	0.23
	Arithmetic Mean		0.34	0.42	0.29
	Standard Deviation		0.15	0.32	0.20
	Min		0.21	0.10	0.04
	Max		0.64	1.43	0.85
	Sample size		12	51	30
Category 1 (geomean for Hg data for the fish species below 0.4 µg/g)	**Geometric Mean**	0.13	0.20	0.24	0.23
	Arithmetic Mean	0.18	0.23	0.31	0.29
	Standard Deviation	0.13	0.12	0.25	0.20
	Min	0.01	0.02	0.04	0.04
	Max	0.62	0.99	1.43	0.85
	Sample size	364	487	137	38
Category 2 (geomean for Hg between 0.4 and 0.5 µg/g for the species)	**Geometric Mean**		0.44	0.43	
	Arithmetic Mean		0.47	0.47	
	Standard Deviation		0.17	0.24	
	Min		0.22	0.14	
	Max		1.19	1.76	
	Sample size		79	42	
Category 3 (geomean for Hg above 0.5 µg/g	**Geometric Mean**		0.58	0.72	0.69
	Arithmetic Mean		0.60	0.89	0.73
	Standard Deviation		0.20	0.68	0.27

for the fish species)	**Min**		0.30	0.29	0.32
	Max		1.65	3.12	1.43
	Sample size		118	23	29

Appendix Table 5: Fish Mercury Distributions for the Models to Estimate Mercury Exposure

		Mercury Concentrations (µg/g)			
Model	**Fish Mercury Distribution Summary Statistics**	**Lake Whitefish**	**Lake Trout**	**Northern Pike**	**Walleye**
Model 1	**Arithmetic Mean**	0.17	0.23	0.23	0.25
	Standard Deviation	0.11	0.12	0.14	0.11
	Min	0.02	0.04	0.05	0.11
	Max	0.62	0.97	0.91	0.67
Model 2	**Arithmetic Mean**	0.17	0.27	0.31	0.26
	Standard Deviation	0.11	0.11	0.21	0.14
	Min	0.02	0.04	0.05	0.06
	Max	0.62	0.96	1.43	0.85
Model 3	**Arithmetic Mean**	0.17	0.23	0.30	0.27
	Standard Deviation	0.11	0.12	0.21	0.15
	Min	0.02	0.05	0.05	0.05
	Max	0.62	0.98	1.43	0.85
Model 4	**Arithmetic Mean**	0.17	0.29	0.34	0.27
	Standard Deviation	0.11	0.16	0.22	0.15
	Min	0.02	0.05	0.05	0.05
	Max	0.62	1.19	1.72	0.85
Model 5	**Arithmetic Mean**	0.17	0.35	0.38	0.27
	Standard Deviation	0.11	0.18	0.23	0.15
	Min	0.02	0.05	0.05	0.05
	Max	0.62	1.19	1.72	0.85
Model 6	**Arithmetic Mean**	0.17	0.32	0.44	0.38
	Standard Deviation	0.11	0.21	0.38	0.25
	Min	0.02	0.04	0.05	0.06
	Max	0.62	1.64	3.11	1.43
Model 7	**Arithmetic Mean**	0.17	0.41	0.57	0.49
	Standard Deviation	0.11	0.24	0.46	0.29
	Min	0.02	0.05	0.05	0.06
	Max	0.62	1.62	3.11	1.43

Output Data from the Models

Appendix Table 6: Summary Statistics for Estimated Total Mercury Doses from Model 1 and Model 2

		Estimated Total Mercury Dose (µg/kg of bodyweight/day)			
	Summary Statistics	**<18 Years Old**	**18 to 39 Years Old**	**40 to 59 Years Old**	**60+ Years Old**
Mode1 1 (100% Fish Mercury Concentrations from Category 1a Lakes)	**Arithmetic Mean**	0.05	0.07	0.11	0.12
	Standard Deviation	0.06	0.08	0.09	0.09
	Median	0.03	0.05	0.08	0.10
	75th percentile	0.07	0.10	0.15	0.16
	90th percentile	0.13	0.17	0.22	0.23
	95th percentile	0.17	0.22	0.28	0.29
	%Above 0.23 TDI	2.0%	4.6%		
	%Above 0.47 TDI		0.2%	0.5%	0.6%
Model 2 (50% Fish Mercury Concentrations from Category 1a Lakes and 50% from Category 1b Lakes)	**Arithmetic Mean**	0.06	0.08	0.12	0.14
	Standard Deviation	0.07	0.09	0.10	0.10
	Median	0.04	0.06	0.09	0.11
	75th percentile	0.08	0.12	0.16	0.18
	90th percentile	0.14	0.21	0.25	0.27
	95th percentile	0.19	0.26	0.31	0.33
	%Above 0.23 TDI	2.9%	7.5%	NA[a]	NA[a]
	%Above 0.47 TDI	NA[b]	0.5%	1.3%	1.2%

* Since all of the Lake Whitefish data came from lakes classified as Category 1a Lakes, for model 2 the Lake Whitefish data was still from 100% Category 1a data

[a] Not Applicable. The TDI of 0.23 µg/kg/d is applicable only to women of childbearing age and children.

[b] Not Applicable. The TDI of 0.47 µg/kg/d is not sufficiently protective for children. Instead, the TDI of 0.23 µg/kg/d is recommended for this age group.

Appendix Table 7: Summary Statistics for Estimated Total Mercury Doses for Models 3, 4 and 5

		Estimated Total Mercury Dose (µg/kg of bodyweight/day)			
	Summary Statistics	**<18 Years Old**	**18 to 39 Years Old**	**40 to 59 Years Old**	**60+ Years Old**
Model 3 (100% Fish Mercury Concentrations from Category 1 Lakes)	**Arithmetic Mean**	0.06	0.08	0.11	0.13
	Standard Deviation	0.06	0.08	0.10	0.10
	Median	0.04	0.05	0.09	0.11
	75th percentile	0.08	0.11	0.16	0.17
	90th percentile	0.14	0.18	0.24	0.25
	95th percentile	0.18	0.24	0.31	0.31
	%Above 0.23 TDI	2.6%	5.8%		
	%Above 0.47 TDI		0.3%	1.1%	0.9%
Model 4 (75% Fish Mercury Concentrations from Category 1 Lakes and 25% from Category 2 Lakes)	**Arithmetic Mean**	0.06	0.09	0.13	0.15
	Standard Deviation	0.07	0.10	0.11	0.11
	Median	0.04	0.06	0.10	0.12
	75th percentile	0.09	0.12	0.18	0.20
	90th percentile	0.15	0.21	0.27	0.29
	95th percentile	0.20	0.28	0.34	0.36
	%Above 0.23 TDI	3.5%	8.6%		
	%Above 0.47 TDI		1.0%	1.5%	1.7%
Model 5 (50% Fish Mercury Concentrations from Category 1 Lakes and 50% from Category 2 Lakes)	**Arithmetic Mean**	0.07	0.10	0.14	0.16
	Standard Deviation	0.07	0.11	0.12	0.12
	Median	0.04	0.06	0.11	0.14
	75th percentile	0.09	0.13	0.19	0.22
	90th percentile	0.16	0.24	0.29	0.31
	95th percentile	0.21	0.31	0.37	0.39
	%Above 0.23 TDI	3.8%	10.8%	NA[a]	NA[a]
	%Above 0.47 TDI	NA[b]	1.4%	2.0%	2.4%

* Since all of the Lake Whitefish data came from lakes classified as Category 1a Lakes, all models the Lake Whitefish data was still from 100% Category 1a data

[a] Not Applicable. The TDI of 0.23 µg/kg/d is applicable only to women of childbearing age and children.

[b] Not Applicable. The TDI of 0.47 µg/kg/d is not sufficiently protective for children. Instead, the TDI of 0.23 µg/kg/d is recommended for this age group.

Appendix Table 8: Summary Statistics for Estimated Total Mercury Doses from Model 6 and Model 7

		Estimated Total Mercury Dose (µg/kg of bodyweight/day)			
	Summary Statistics	**<18 Years Old**	**18 to 39 Years Old**	**40 to 59 Years Old**	**60+ Years Old**
Model 6 (75% Fish Mercury Concentrations from Category 1 Lakes and 25% from Category 3 Lakes)	**Arithmetic Mean**	0.07	0.10	0.14	0.16
	Standard Deviation	0.08	0.12	0.14	0.13
	Median	0.04	0.06	0.11	0.13
	75th percentile	0.09	0.13	0.19	0.22
	90th percentile	0.17	0.23	0.31	0.33
	95th percentile	0.23	0.31	0.40	0.42
	%Above 0.23 TDI	5.0%	10.1%		
	%Above 0.47 TDI		1.6%	3.2%	3.3%
Model 7 (50% Fish Mercury Concentrations from Category 1 Lakes and 50% from Category 3 Lakes)	**Arithmetic Mean**	0.08	0.12	0.17	0.19
	Standard Deviation	0.10	0.15	0.16	0.16
	Median	0.05	0.07	0.13	0.16
	75th percentile	0.11	0.16	0.23	0.26
	90th percentile	0.19	0.29	0.38	0.39
	95th percentile	0.27	0.41	0.49	0.50
	%Above 0.23 TDI	6.8%	15.2%	NA[a]	NA[a]
	%Above 0.47 TDI	NA[b]	3.5%	5.6%	6.0%

* Since all of the Lake Whitefish data came from lakes classified as Category 1a Lakes, all models the Lake Whitefish data was still from 100% Category 1a data

[a] Not Applicable. The TDI of 0.23 µg/kg/d is applicable only to women of childbearing age and children.

[b] Not Applicable. The TDI of 0.47 µg/kg/d is not sufficiently protective for children. Instead, the TDI of 0.23 µg/kg/d is recommended for this age group.