

Patterns of Fish and Whale Consumption and Concentrations of Methylmercury in Hair Among Residents of Western Canadian Arctic Communities

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Abstract

Background. Methylmercury contamination of the environment represents a substantial environmental health concern. Human exposure to methylmercury occurs primarily through consumption of fish and marine mammals. Heavily exposed subgroups include sport or subsistence fishers residing in Arctic communities. We aimed to estimate the association of fish/whale consumption patterns of Canadian Arctic subsistence fishers with the internal dose of methylmercury as measured in hair. **Methods.** This research was conducted within ongoing community projects led by the CANHelp Working Group in Aklavik and Fort McPherson, Northwest Territories and Old Crow, YT. We interviewed each participant using a fish-focused food-frequency questionnaire during September–November 2016 and collected hair samples concurrently. Methylmercury was measured in the full-length of each hair sample using gas chromatography inductively-coupled plasma-mass spectrometry. Multivariable random-effects linear regression estimated beta-coefficients and 95% confidence intervals (CIs) for the effect of fish/whale consumption on hair-methylmercury concentrations. **Results.** In total, 101 participants provided hair samples and diet data. The mean number of fish/whale species eaten by participants was 3.5 (SD:1.9). The mean hair-methylmercury concentration was 0.60µg/g (SD:0.47). Fish/whale consumption was positively associated with hair-methylmercury concentration, after adjusting for sex, hair length and use of permanent hair treatments. Hair-methylmercury concentrations among participants who consumed the most fish/whale in each season ranged from 0.30–0.50µg/g higher than those who consumed <1 meal/week. **Conclusions.** Hair-methylmercury concentrations were below the 6.0µg/g threshold for safe exposure defined by Health Canada, suggesting that fish/whale consumption patterns among participants are not increasing their risk of known serious health effects of methylmercury exposure.

Introduction

Mercury is a chemical element with three valence states: elemental mercury (Hg^0); divalent inorganic mercury compounds (Hg^{2+}); and organic compounds. Given their capacity to induce potent toxicological effects in humans, contamination of the environment with mercury compounds represents a substantial environmental health concern. For this reason, mercury has been the focus of a large body of research aimed at identifying the mechanisms through which it enters the environment, as well as pathways for human exposure and subsequent toxicological effects.

Mercury is stored in geological reservoirs within the earth's crust in its elemental form(1–3). Release from these reservoirs into surface soil, water and the atmosphere occurs through geological weathering, defined as the alteration or breakdown of rocks and minerals by mechanical and chemical processes(1,2,4,5). Weathering is a natural process resulting from changes in temperature or pressure, exposure to wind and water, or volcanic events(1,5). Anthropogenic activities of an industrial nature can accelerate weathering and the consequent release of elemental mercury from geological reservoirs(1–3,5–10). Additionally, human-caused global climate change increases the release of mercury by inducing changes to the carbon cycle that are conducive to chemical weathering(3,4,11). Other human activities, including burning fossil fuels, specific industrial processes and waste incineration, lead to direct release of mercury into the environment(1–3,6–10). Following release from geological reservoirs, mercury undergoes biogeochemical cycling that results in the formation of inorganic and organic mercury compounds(1,2,4).

When elemental mercury finds its way into aquatic systems, some of it transforms into methylmercury (MeHg), a process mediated by anaerobic organisms that involves the formation of a covalent bond between an inorganic mercury ion and a methyl group(1,2,6–8). This transformation is thought to occur most often in wetland ecosystems and the surface of lake sediments(2). As an organic compound, MeHg is lipophilic and mobile, with the capacity to enter the plasma membrane of cells and accumulate in the cytoplasm(1,2). This property has important implications for bioconcentration of MeHg in aquatic organisms and subsequent biomagnification in aquatic food chains(1,2,6–8). Specifically, the presence of the compound in the cell cytoplasm allows for transfer of MeHg between trophic levels of aquatic food chains, whereas inorganic mercury forms are predominantly membrane bound and less likely to concentrate in organisms at higher trophic levels(1). The concentration of MeHg in aquatic organisms has been shown to be greater than that of the ambient water by a factor $\geq 10^6$ (1,2).

MeHg contamination of aquatic ecosystems is considered the most abundant non-occupational source of human exposure to mercury(1,6–10). The primary source of MeHg exposure in humans is consumption of fish or fish products and marine mammals, with larger, longer-living fish posing greater risk of toxic exposure(1,6–10). The most heavily exposed human population groups include sport or subsistence fishers residing in Arctic communities(1,6,7,12). The disproportional threat posed by Arctic fish is due to greater emissions of elemental mercury in the northern hemisphere, changes to the global climate altering the mercury cycle, and more frequent consumption of species with a greater potential for high levels of organic mercury contamination(1,6,7,12). Additionally, sport and subsistence fishers do not benefit from regulatory measures that control the mercury content of commercially sold fish products.

Research on mercury exposure among Indigenous residents of the Canadian Arctic has typically focused on coastal populations that consume large amounts of marine mammals(13). This is reasonable, given the greater capacity of these large species to accumulate mercury. Residents of inland communities in the western Canadian Arctic, however, are target audiences for public health messages about fish consumption, without concurrent exposure assessments(14,15). Our preliminary ethnographic research in western Canadian Arctic communities revealed residents' concerns about mercury accumulation in their bodies and how it relates to their fish and marine mammal consumption habits. In response, we conducted this research to analyze data collected from residents of inland communities in the Canadian Arctic to: characterize fish and marine mammal consumption patterns; biochemically measure the mercury level in hair samples to ascertain individual exposure to mercury; and estimate the effect of fish and marine mammal consumption, other dietary components and participant characteristics on the internal dose of mercury.

Methods

Study Design. This mercury exposure project was an environmental health component of ongoing community-driven projects led by the Canadian North *Helicobacter pylori* (CANHelp) Working Group in western Canadian Arctic communities (www.canhelpworkinggroup.ca). The CANHelp Working Group formed during 2006-2008 in response to concerns raised by community leaders about *H. pylori* infection and gastric cancer risk. This research program is a collaborative effort, linking northern Canadian Indigenous communities, their health care providers and regional health authorities with investigators from multiple disciplines at the University of Alberta (16,17). At the invitation of community leaders, cross-sectional projects were established to describe the community health burden from *H. pylori* infection and associated disease and address community concerns. In each community, a planning committee made up of community members guided the conduct of each project and ensured that research activities were culturally appropriate and in keeping with community priorities.

Person, Place and Time. The mercury exposure project was conducted within three CANHelp Working Group community projects. The first of these projects launched in 2007 in the hamlet of Aklavik, Northwest Territories (NT) (2006 census population=590, ~92% identifying as Gwich'in [Athabaskan First Nation] or Inuvialuit [Inuit])(18,19). Projects began in 2010 in Old Crow, Yukon (YT) (2011 census population=245, ~85% identifying as Vuntut Gwich'in(20,21), and in 2012 in Fort McPherson, NT (2011 census population=844, ~90% Tetlit Gwich'in)(22). Participation in the mercury exposure project was open to all residents of these three communities during September-November 2016. Recruitment activities involved radio announcements, social media posts, flyers on community message boards, and directly contacting participants of CANHelp Working Group projects for which current contact information was available. Informed consent was obtained from all participants by providing them with an information sheet that outlined the study objectives, methods, information to be collected, benefits and potential risks of participation and confidentiality. Following review of this document, each participant was asked to fill out a consent form, confirming they had received enough information about the project and that they agree to participate. Project information sheets and consent forms were reviewed and approved by the Research Ethics Board at the University of Alberta and have been published previously(23).

Choice of Tissue for Biomarker Analysis. Evidence suggests that hair is the biological medium best suited for measuring MeHg exposure(1,10,24–36). Hair from the scalp is a commonly selected matrix for biomonitoring of MeHg exposure, because MeHg accounts for approximately 80% of the total mercury found in hair and can be measured directly(1,10,24–36). There are also several practical advantages to collecting hair samples relative urine and blood, including: chemical stability; simple and non-invasive sampling; ease in storing, transporting and archiving specimens; and relatively low cost(25,37–40).

Exposure Time Window. Among healthy individuals, estimates of the scalp hair growth rate range from 0.6 to 3.36 cm/month, with an average of 1 cm/month(37–39). The concentration of mercury measured in hair reflects exposure over the growth period of the sampled hair, typically, the past few months depending on hair length. According to input from local planning committees, residents of participating communities consume the greatest amount of aquatic species, on average, during the spring and summer seasons. For this reason, hair sample collection took place during the fall season (September-November).

Hair Sample Collection. The procedures for collecting hair samples were adapted from protocols outlined by the United States Centers for Disease Control (CDC) for use in the National Health and Nutrition Examination Survey (NHANES)(41). We collected all hair samples from the occipital region of the scalp using stainless steel shears, obtaining a minimum of 120 mg of hair from each participant to allow for duplication of the laboratory measurements for quality assurance/quality control (QA/QC) purposes. To ensure enough hair was obtained from each participant, we used a high precision digital scale to weigh the sample immediately following collection. Given that hair length determines the exposure period represented in the strand, we also used a ruler to measure hair length (in cm) before transferring samples into a zip closable plastic bag and applying a label specifying the sample ID number, collection date, sample weight and hair length. Additionally, we recorded information on use of permanent hair treatments, including hair dye or permanent waves, and time since the most recent treatment.

Laboratory Analysis of Samples. The collected hair samples were analyzed by the University of Alberta Biogeochemical Analytical Service Laboratory (BASL). This lab has been accredited by the Canadian Association for Laboratory Accreditation (CALA) as meeting ISO/IEC 17025 standards for the performance of specific tests. MeHg was measured in the full-length of each hair sample using gas chromatography inductively coupled plasma-mass spectrometry (GC-ICP-MS)(42,43). Quality control methods employed by the lab included the use of reference material 1AEA-085 for MeHg, total mercury and other trace elements in hair. Single point calibration was applied, and the calibration standard was analyzed in 4 replicates. The relative standard deviation for the ratio of Hg isotope 201:202 was considered acceptable if the value was less than 5%. If the value was greater than 5%, the calibration was repeated. Instrument and method blanks and a second source reference material were also used to monitor contamination with MeHg, accuracy and instrumental drift during analysis. These were incorporated into the analysis at a frequency of 1 per batch of approximately 30 samples. The instrument was re-calibrated if the second source reference material measurements were outside of the 80-120% recovery range. Additionally, water samples were spiked with a known quantity of enriched MeHg isotope ($\text{CH}_3^{201}\text{Hg}$) as an internal standard. Finally, laboratory duplicates were performed at a frequency of 1 per 5 samples. For added quality assurance, we divided approximately 10% of the samples and submitted them to the lab as separate individuals. Lab personnel were blinded to all participant characteristics, including age, sex and the amount and types of aquatic species consumed.

Fish and Marine Mammal Consumption Data. We designed a population-appropriate Food Frequency Questionnaire (FFQ) focused on fish and marine mammal consumption. Community input guided the selection of included fish species and incorporation of familiar names and descriptions for locally harvested fish, to ensure respondents had a clear understanding of each FFQ item. Planning committee members identified Beluga Whale (*D. leucas*) as the only regularly consumed marine mammal. The FFQ measured consumption frequencies as average number of times each type of fish or whale was consumed per week (we will refer to food consumption events as “meals”). The FFQ did not include portion size to reduce the burden on participants and because validation studies have shown that attempting to ascertain portion size does not appreciably improve overall characterization of diet, because most people have poor recall of portion sizes(44). To capture seasonal variability in consumption, the FFQ asked respondents to specify the time of year in

which they typically harvest each aquatic species they reported consuming. The FFQ then asked respondents the typical number of meals per week of each species during the time of year they are harvested. Since it is common for community members to preserve harvested fish by drying, freezing or smoking the meat, the FFQ asked respondents to report the frequency of consuming each species during other parts of the year. Given the potential for preparation methods to alter the bioavailability of mercury in consumed fish, the FFQ also asked participants to specify how they typically prepare each type of fish/whale for eating and the parts they consume (45). Most participants were able to identify the specific species they consumed; pictures were available for those who were unsure. The potential for the overall composition of an individual's diet and intake of specific nutrients to directly or indirectly influence the toxicokinetic properties of MeHg has been described in the scientific literature(46). For this reason, the FFQ collected data on other dietary components, including average weekly intake of: fruit, fresh fruit juice, raw and cooked vegetables, fresh or packaged milk and yogurt.

Exposure Definition. Fish/whale consumption, as ascertained by the FFQ, constituted the source of mercury exposure examined for this analysis. The structure of the FFQ permitted the creation of separate variables representing the usual frequency of consuming each reported species in units of average meals per week in each of the four seasons. Additionally, we generated season-specific variables to represent total fish/whale meals per week by summing the weekly number of meals in each season across species. Input from community planning committees indicated that there was too much variation across seasons in the frequency and types of fish/whale consumed for valid use of a year-round average.

Outcome Definition. The outcome for this analysis was the MeHg concentration measured in hair samples in units of $\mu\text{g/g}$ on a continuous scale. Guidelines generated by Health Canada for interpreting the degree of risk associated with hair-mercury levels provide perspective for interpreting values(47): hair mercury concentrations of $\leq 6 \mu\text{g/g}$ are considered acceptable for adult males and females who are not pregnant or breastfeeding(47); among children under 12 years and women who are pregnant, breastfeeding or of reproductive age, concentrations $\leq 2 \mu\text{g/g}$ are considered acceptable(47).

Statistical Analysis. The goal of the statistical analysis was to estimate the association between fish/whale meals per week and hair MeHg concentration among participants. We constructed a multivariable linear regression model to estimate beta coefficients and 95% confidence intervals (CIs) as measures of the association between characteristics of interest and hair MeHg concentration ($\mu\text{g/g}$). Because participants residing in the same community cannot be assumed to be independent with respect to study variables, the model included clustering in communities as a random effect, giving each community its own intercept. This approach allows each community to have its own baseline value of hair-mercury concentration in the intercept, to which the effects of all covariates are added. The standard deviation (SD) of the random community effect measures the extent to which baseline values across communities deviate from the population mean of all communities combined and represents the magnitude of the effect of clustering in communities. The magnitude of the community effect depends on the extent to which covariates in the model explain differences in mean MeHg concentration across communities.

To avoid assuming that the relationships between increasing consumption of food items and hair mercury levels were linear, each variable was converted to a categorical format. When possible, category boundaries were defined so that there was no more than a two-fold increase in number of servings within a category(44). The purpose of this was to generate categories within which the effect of interest does not vary substantially(44,48). If data were too sparse to permit the use of optimal category boundaries, adjacent categories were collapsed to improve statistical precision. To confirm whether these variables could be modeled as continuous, the linearity of the relationship of hair MeHg concentration with the continuous form of each variable was assessed using a lowess plot (bandwidth: 0.80). The presence of a trend in the relationship between fish/whale consumption frequency and hair MeHg concentrations ($\mu\text{g/g}$) was detected using an extension of the Wilcoxon rank-sum test for testing trends over ordered groups that incorporates a correction for ties(49).

We used purposeful selection, as proposed by Hosmer and Lemeshow (2000), to identify the best set of adjustment variables for each of the season-specific exposure variables (50). This method follows a change-in-estimate approach, with variable selection decisions based on the extent to which each potential covariate influences the magnitude of exposure effects of interest(48,50): All potential covariates were included in a multivariable random effects model and subsequently removed one at a time. If the coefficient of any independent variable changed by $\geq 10\%$ with the removal of a given covariate, the removed variable was included in the final model(48,50). Variables considered for inclusion in the model were: age, sex, use of permanent hair treatments, the proportion of consumed fish/whale species harvested from the ocean or local rivers, the proportion of consumed species usually prepared by cooking (versus eaten raw, dried or smoked), and other dietary frequencies, including fruits and vegetables, dairy products or regular use of dietary supplements. Hair length was automatically included in the final model to account for variation in the exposure period represented in hair strands of different lengths.

Community Effect. Although data were insufficient for estimating species-specific effects, some of this effect was likely picked up by the random effect, given the considerable variation in fish/whale species consumed across communities. A sensitivity analysis explored the extent to which variation in species consumed by participants from different communities explained the residual variation. We inspected community-specific patterns of fish/whale consumption, for each season separately, to identify species most likely to discriminate between communities based on the relative frequencies of their intake. We included species-specific and total fish/whale consumption frequencies in the same model if the correlation coefficients were < 0.7 (48,50). The variables representing intake of the selected species were then added to the model for each season, to quantify changes in the residual variation across communities as measured by the SD. Given that data were limited, the linearity of the relationship between consumption of each species-specific consumption variable and hair mercury concentration was assessed to see whether they could be modeled as continuous.

Bias Analysis. Given the potential for MeHg measurement error to produce outcome misclassification, we conducted a quantitative bias analysis using the measured hair mercury concentrations among duplicated samples as parameters. The percent change between analyses of the same participant's hair was calculated. To achieve this, the value obtained during the repeat analysis of an individual's sample was subtracted from the originally measured value

and the difference was divided by the originally measured value. For participants with more than 2 measurements, the largest difference between measured concentrations was used. To quantify the extent to which measurement error influenced inferences drawn from this analysis, the originally measured MeHg value was adjusted in two ways. First the overall mean percent change and the proportion of the repeated measurements that increased or decreased in value were used to estimate the magnitude of measurement error and frequency of change in either direction in the entire study population. Second, the mean percent change between repeated measurements and the proportion that increased or decreased were stratified by participant characteristics to apply stratum-specific estimates of the magnitude and direction of measurement error to corresponding subsets of participants, selecting at random the participants assigned increasing or decreasing MeHg concentrations. All analyses were repeated using the adjusted MeHg concentrations as outcome variables.

Results

In the three communities combined, 101 participants provided hair samples and diet data (42 from Aklavik, NT; 32 from Old Crow, YT; and 24 from Fort McPherson, NT). The mean age was 52 (SD: 15.7; Range: 10-86) years. Participants were nearly all Indigenous, predominantly identifying as either Gwich'in (60%; 60/101) or Inuvialuit (30%; 30/101). A small proportion of participants were of European descent (6%; 6/101) but had been residing in the community for at least 5 years. The study population was disproportionately female (63%; 64/101), none pregnant or breastfeeding at the time of data collection. Assuming an average growth rate of 1 cm/month, the exposure periods represented in the collected hair samples ranged from approximately 3 weeks to almost 9 years (median: 1.1 year; IQR: 2.1 years).

Almost all participants (96%; 97/101) reported eating fish or marine mammals in the past 12 months. The data obtained from the fish-focused FFQ was consistent with input from community planning committees, which identified the summer as the main season during which community members consume fish/whale. However, there was considerable variation by species and community (Figure 1). Table 1 shows the 17 aquatic species participants reported consuming in the previous 12 months. The fish species consumed by the largest proportion of participants was Broad Whitefish (*C.nasus*) (83%), followed by Inconnu (*S.nelma*) (42%) and Dolly Varden (*S.malma*) (33%). A large proportion of participants also ate Beluga Whale (*D.leucas*) (42%), with 71% of those who reported eating Beluga Whale in the past 12 months were from Aklavik, NT (30/42), the community with the largest proportion of Inuit residents.

Table 1: Fish and marine mammal species consumed at least once in the past 12 months by community, 101 western Canadian Arctic residents, 2016

Species		Proportion that Consumed Each Species					
		in the Past 12 Months					
		Aklavik (n=45)		Old Crow (n=32)		Fort McPherson (n=24)	
		n	%	n	%	n	%
Scientific Name	Common Name						
Salmonidae Family							
<i>Salvelinus alpinus</i>	Arctic Char	11	24	3	9	1	4
<i>Salvelinus malma</i>	Dolly Varden	30	67	0	0	3	13
<i>Salvelinus namaycush</i>	Lake Trout	1	2	0	0	5	21
<i>Coregonus nasus</i>	Broad Whitefish	36	80	26	81	22	92
<i>Coregonus clupeaformis</i>	Lake Whitefish	2	4	5	16	0	0
<i>Coregonus autumnalis</i>	Arctic Cisco	18	40	0	0	1	4
<i>Oncorhynchus tshawytscha</i>	Chinook Salmon	6	13	25	78	1	4
<i>Oncorhynchus keta</i>	Chum Salmon	1	2	7	22	3	13
<i>Oncorhynchus kisutch</i>	Coho Salmon	3	7	5	16	0	0
<i>Oncorhynchus nerka</i>	Sockeye Salmon	0	0	4	13	1	4
<i>Oncorhynchus gorbuscha</i>	Pink Salmon	2	4	0	0	2	8
<i>Thymallus arcticus</i>	Arctic Grayling	0	0	9	28	0	0
<i>Stenodus nelma</i>	Inconnu	24	53	1	3	17	71
Lotidae Family							
<i>Lota Lota</i>	Burbot	12	27	7	22	10	42
Osmeridae Family							
<i>Thaleichthys pacificus</i>	Eulachon	0	0	1	3	0	0
Percidae Family							
<i>Sander vitreus</i>	Walleye	1	2	0	0	0	0
Monodontidae Family							
<i>Delphinapterus leucas</i>	Beluga Whale	30	67	8	25	4	17

Table 2 shows the five most frequent species consumed ≥ 1 time/week by community and season. The mean number of different species eaten by participants was 3.5 (SD: 1.9; Range: 0-9). The main waterways and sites from which participants reported harvesting fish and whale are shown in Figure 2. On average, participants reported harvesting most of the species they consume from local rivers, followed by the ocean and nearby lakes. The mean

proportions of harvesting sites for reported species consumed were: rivers 66.7% (SD: 32.9%; Range: 0-100); the ocean 21.7% (SD: 27.4; Range: 0-100); and lakes 1.8% (SD: 8.2; Range: 0-50). The mean proportion of consumed species purchased from the store was 2.0% (SD: 7.6; Range: 0-33).

Table 2: Five most frequent aquatic species consumed at least once per week by season and community, 101 western Canadian Arctic residents, 2016

Season	Aklavik, NT (n=45)		Old Crow, YT (n=32)		Fort McPherson, NT (n=24)	
	Species	n %	Species	n %	Species	n %
Winter	<i>S. nelma</i>	9 20	<i>C. nasus</i>	8 25	<i>C. nasus</i>	9 38
	<i>C. nasus</i>	8 18	<i>O. tshawytscha</i>	6 19	<i>S. nelma</i>	5 21
	<i>D. leucas</i>	7 16	<i>C. clupearformis</i>	3 9		
	<i>S. malma</i>	5 11	<i>O. kisutch</i>	2 6		
	<i>C. autumnalis</i>	4 9	<i>T. arcticus</i>	2 6		
Spring	<i>S. nelma</i>	8 18	<i>C. nasus</i>	8 25	<i>C. nasus</i>	11 46
	<i>D. leucas</i>	8 18	<i>O. tshawytscha</i>	7 22	<i>S. nelma</i>	6 25
	<i>C. nasus</i>	7 16	<i>C. clupearformis</i>	4 13		
	<i>S. malma</i>	5 11	<i>O. kisutch</i>	2 6		
	<i>C. autumnalis</i>	4 9	<i>T. arcticus</i>	2 6		
Summer	<i>D. leucas</i>	17 38	<i>O. tshawytscha</i>	20 63	<i>C. nasus</i>	14 58
	<i>S. malma</i>	14 31	<i>C. nasus</i>	9 28	<i>S. nelma</i>	10 42
	<i>C. nasus</i>	14 31	<i>O. keta</i>	3 9	<i>S. aplinus</i>	1 4
	<i>C. autumnalis</i>	13 29	<i>S. aplinus</i>	2 6	<i>D. leucas</i>	1 4
	<i>S. nelma</i>	10 22	<i>O. kisutch</i>	2 6		
			<i>T. arcticus</i>	2 6		
			<i>C. nasus</i>	9 28	<i>C. nasus</i>	15 63
Fall	<i>S. nelma</i>	8 18	<i>O. tshawytscha</i>	7 22	<i>S. nelma</i>	8 33
	<i>D. leucas</i>	6 13	<i>O. keta</i>	5 16	<i>L. Lota</i>	5 21
	<i>S. malma</i>	6 13	<i>L. Lota</i>	4 13	<i>S. aplinus</i>	1 4
	<i>C. autumnalis</i>	4 9	<i>C. clupearformis</i>	3 9	<i>S. malma</i>	1 4
			<i>T. arcticus</i>	3 9		

Among participants from all communities combined, the mean concentration of MeHg in hair samples was 0.60 µg/g (SD: 0.47; Range: 0.059-2.07). This varied slightly across communities, with mean values from Aklavik, NT, Old Crow, YT and Fort McPherson, NT of 0.51 µg/g (SD: 0.44; Range: 0.06-2.07), 0.54 µg/g (SD: 0.35; Range: 0.11-1.51) and 0.84 µg/g (SD: 0.58; Range: 0.06-1.90), respectively. The distributions of MeHg in hair samples across the entire study population and stratified by community are shown in Figure 3. Mean hair mercury levels (µg/g) ± SD stratified by population characteristics are shown in Tables 3 and 4. No participants had hair mercury levels that exceeded the exposure maximum defined by Health Canada.

Table 3: Distribution of demographic characteristics and stratum-specific mean MeHg concentrations (µg/g) by community, 101 western Canadian Arctic residents, 2016

characteristics	Total (n=101)		Aklavik, NT (n=45)		Old Crow, YT (n=32)		Fort McPherson, NT (n=24)	
	n (%)	Mean ± SD	n (%)	Mean ± SD	n (%)	Mean ± SD	n (%)	Mean ± SD
	9 (9)	0.258 ± 0.205	5 (11)	0.167 ± 0.080	4 (12.5)	0.372 ± 0.269	0 (0)	-
	15 (15)	0.449 ± 0.600	8 (18)	0.504 ± 0.695	4 (12.5)	0.526 ± 0.661	3 (12.5)	0.196 ± 0.233
	14 (14)	0.486 ± 0.341	8 (18)	0.414 ± 0.348	4 (12.5)	0.579 ± 0.354	2 (8)	0.586 ± 0.441
	35 (35)	0.791 ± 0.515	14 (31)	0.603 ± 0.345	7 (22)	0.571 ± 0.441	14 (58)	1.089 ± 0.570
	17 (17)	0.574 ± 0.307	6 (13)	0.781 ± 0.427	9 (28)	0.469 ± 0.156	2 (8)	0.424 ± 0.004
	11 (11)	0.641 ± 0.391	4 (9)	0.421 ± 0.357	4 (12.5)	0.777 ± 0.265	3 (12.5)	0.752 ± 0.561
	37 (37)	0.742 ± 0.508	13 (29)	0.529 ± 0.462	17 (53)	0.681 ± 0.403	7 (29)	1.282 ± 0.490
	64 (63)	0.514 ± 0.424	32 (71)	0.504 ± 0.432	15 (47)	0.377 ± 0.196	17 (71)	0.655 ± 0.523

Table 4: Distribution of permanent hair treatment use and stratum-specific MeHg concentrations ($\mu\text{g/g}$) by community, 101 western Canadian Arctic residents, 2016

Uses	Total (n=101)		Aklavik, NT (n=45)		Old Crow, YT (n=32)		Fort McPherson, NT (n=24)	
	n (%)	Mean \pm SD	n (%)	Mean \pm SD	n (%)	Mean \pm SD	n (%)	Mean \pm SD
	74 (73)	0.630 \pm 0.479	33 (73)	0.483 \pm 0.372	20 (62.5)	0.624 \pm 0.400	21 (87.5)	0.869 \pm 0.607
	27 (27)	0.507 \pm 0.429	12 (27)	0.589 \pm 0.590	12 (37.5)	0.397 \pm 0.205	3 (12.5)	0.621 \pm 0.341
	95 (94)	0.605 \pm 0.476	45 (100)	0.511 \pm 0.436	31 (97)	0.547 \pm 0.356	19 (79)	0.923 \pm 0.611
	6 (6)	0.478 \pm 0.298	0 (0)	-	1 (3)	0.275	5 (21)	0.518 \pm 0.314
	70 (69)	0.636 \pm 0.486	33 (73)	0.483 \pm 0.372	20 (62.5)	0.624 \pm 0.399	17 (71)	0.947 \pm 0.635
	31 (31)	0.512 \pm 0.417	12 (27)	0.589 \pm 0.590	12 (37.5)	0.397 \pm 0.205	7 (29)	0.575 \pm 0.323

Because, data were insufficient for estimation of species-specific effects on hair mercury levels, this analysis was limited to effects of total fish/whale consumption. Table 5 shows MeHg levels stratified by total fish/whale consumption frequency across seasons. The strong correlations between season-specific fish/whale consumption variables prohibited including them in the same model, so season-specific effects were estimated in separate models. Model building procedures yielded the same set of adjustment variables for fish/whale consumption in each season: sex, hair length, use of hair dye or other permanent hair treatments, and the proportion of fish/whale meals usually prepared by cooking. There was no evidence of statistical interaction between fish/whale consumption and any of the model covariates. Visual inspection of the lowess plots representing the locally weighted regression of MeHg concentration on exposure variables for each season showed that the relationships were not sufficiently linear to justify modeling exposures as continuous variables.

Table 5: Hair MeHg concentrations ($\mu\text{g/g}$) within categories of fish/whale consumption by season and community, 101 western Canadian Arctic residents, 2016

Intake Category	Total (N=101)		Aklavik, NWT (N=45)		Old Crow, YT (N=32)		Fort McPherson, NWT (N=24)	
	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD
1 meal/week	55	0.512 \pm 0.463	23	0.408 \pm 0.387	17	0.398 \pm 0.238	15	0.801 \pm 0.634
2 meals/week	27	0.613 \pm 0.380	13	0.571 \pm 0.350	9	0.669 \pm 0.424	5	0.624 \pm 0.448
3 meals/week	19	0.822 \pm 0.535	9	0.689 \pm 0.613	6	0.741 \pm 0.398	4	1.244 \pm 0.382
1 meal/week	51	0.409 \pm 0.343	23	0.343 \pm 0.301	16	0.373 \pm 0.240	12	0.585 \pm 0.481
2 meals/week	28	0.708 \pm 0.455	12	0.568 \pm 0.365	10	0.701 \pm 0.412	6	0.999 \pm 0.609
3 meals/week	22	0.893 \pm 0.547	10	0.829 \pm 0.595	6	0.708 \pm 0.349	6	1.184 \pm 0.588
1 meal/week	28	0.443 \pm 0.381	11	0.373 \pm 0.326	8	0.413 \pm 0.279	9	0.554 \pm 0.517
2 meals/week	24	0.524 \pm 0.493	11	0.375 \pm 0.375	9	0.341 \pm 0.215	4	1.343 \pm 0.444
4 meals/week	18	0.616 \pm 0.451	9	0.600 \pm 0.437	4	0.423 \pm 0.156	5	0.799 \pm 0.620
5 meals/week	31	0.784 \pm 0.482	14	0.669 \pm 0.521	11	0.834 \pm 0.374	6	0.961 \pm 0.577
1 meal/week	45	0.419 \pm 0.358	23	0.369 \pm 0.331	14	0.412 \pm 0.261	8	0.574 \pm 0.549
2 meals/week	17	0.589 \pm 0.491	8	0.499 \pm 0.347	6	0.411 \pm 0.338	3	1.182 \pm 0.753
4 meals/week	23	0.792 \pm 0.467	8	0.633 \pm 0.460	8	0.868 \pm 0.408	7	0.888 \pm 0.554
5 meals/week	16	0.830 \pm 0.539	6	0.910 \pm 0.646	4	0.514 \pm 0.143	6	0.961 \pm 0.577

Each season-specific model showed that hair MeHg concentration increased with increasing fish/whale consumption frequency (p -values for trend ranging from <0.0001 to 0.005). Tables 6-9 show multivariable random effects regression results. Hair mercury concentrations among participants who had the highest level of fish/whale consumption in each season ranged from 0.30 to 0.50 $\mu\text{g/g}$ higher than those who consumed <1 meal/week. In the model for each season, the magnitude of the effect of each consumption category decreased slightly following adjustment for covariates. The magnitude of the change in hair MeHg concentration corresponding to the contrast of ≥ 3 vs. <1 meal/week was highest for intake during the spring (β : 0.40 ; 95%CI: 0.20 , 0.60). Conversely, the magnitude of this effect was lowest for intake during the winter (β : 0.28 ; 95%CI: 0.07 , 0.50). Tables 6-9 also show the intercepts from these models, representing the expected mean concentration of mercury ($\mu\text{g/g}$) if all covariates are at their reference level, and corresponding SDs, representing the variation in these values associated with clustering in communities, which reflect residual clustering in each model, suggesting that variation in baseline hair-mercury concentrations across communities is not fully explained by the variables in the model.

Table 6: Random effects regression of hair MeHg concentrations ($\mu\text{g/g}$) on fish/whale consumption frequency during the spring season, 101 western Canadian Arctic residents, 2016

	Unadjusted ∞		Adjusted Φ		p-value for Trend
	β	95% CI	β	95% CI	
Sex	Reference		Reference		
Female	-0.250	-0.430, -0.070	-0.054	-0.248, 0.141	
Hair Length (cm)	-0.006	-0.0103, -0.0017	-0.004	-0.009, 0.0003	
Use of Perm	Reference		Reference		
Proportion Cooked	-0.124	-0.314, 0.0664	-0.169	-0.334, -0.004	
Fish Consumption Frequency	0.004	0.00013, 0.0076	0.003	0.00004, 0.007	
Spring Consumption	Reference		Reference		
1 meal/week	0.302	0.115, 0.489	0.258	0.076, 0.439	
2 meals/week	0.476	0.274, 0.679	0.406	0.204, 0.609	0.000
Random intercept for community from adjusted model: SD 0.131 (95%CI: 0.045, 0.379)					

Each model included a random intercept for the effect of clustering in communities

Adjusted for sex, hair length, use of hair dyes or permanent treatments, the proportion of fish meals usually prepared by cooking, and fish/whale consumption frequency in the spring

Table 7: Random effects regression of hair MeHg concentrations ($\mu\text{g/g}$) on fish/whale consumption during the summer season, 101 western Canadian Arctic residents, 2016

	Unadjusted ∞		Adjusted Φ		p-value for Trend
	β	95% CI	β	95% CI	
Sex	Reference		Reference		
Female	-0.250	-0.430, -0.070	-0.144	-0.341, 0.053	
Hair Length (cm)	-0.006	-0.0103, -0.0017	-0.004	-0.009, 0.0002	
Use of Perm	Reference		Reference		
Proportion Cooked	-0.124	-0.314, 0.0664	-0.157	-0.333, 0.019	
Fish Consumption Frequency	0.004	0.00013, 0.0076	0.004	0.0009, 0.008	
Summer Consumption	Reference		Reference		
1 meal/week	0.120	-0.113, 0.354	0.044	-0.183, 0.270	
2 meals/week	0.187	-0.0669, 0.593	0.183	-0.065, 0.432	
4 meals/week	0.374	0.156, 0.593	0.320	0.114, 0.526	0.001
Random intercept for community from adjusted model: SD 0.147 (95%CI: 0.053, 0.409)					

Each model included a random intercept for the effect of clustering in communities

Model covariates: sex, hair length, use of hair dyes or permanent treatments, the proportion of fish meals usually prepared by cooking, and fish/whale consumption frequency in the summer

Table 8: Random effects regression of hair MeHg concentrations ($\mu\text{g/g}$) on fish/whale consumption during the fall season, 101 western Canadian Arctic residents, 2016

	Unadjusted ∞		Adjusted Φ		p-value for Trend
	β	95% CI	β	95% CI	
Sex	Reference		Reference		
Female	-0.250	-0.430, -0.070	-0.093	-0.291, 0.105	
Hair Length (cm)	-0.006	-0.0103, -0.0017	-0.004	-0.009, 0.0005	
Use of Perm	Reference		Reference		
Proportion Cooked	-0.124	-0.314, 0.0664	-0.220	-0.394, -0.046	
Fish Consumption Frequency	0.004	0.00013, 0.0076	0.004	0.0002, 0.007	
Fall Consumption	Reference		Reference		
1 meal/week	0.170	-0.0663, 0.406	0.150	-0.069, 0.369	
2 meals/week	0.358	0.144, 0.572	0.323	0.113, 0.534	
5 meals/week	0.387	0.144, 0.631	0.376	0.143, 0.610	0.000
Random intercept for community from adjusted model: SD 0.097 (95%CI: 0.025, 0.369)					

Each model included a random intercept for the effect of clustering in communities

Model covariates: sex, hair length, use of hair dyes or permanent treatments, the proportion of fish meals usually prepared by cooking, and fish/whale consumption frequency in the fall

Table 9: Random effects regression of hair MeHg concentrations ($\mu\text{g/g}$) on fish/whale consumption frequency during the winter season, 101 western Canadian Arctic residents, 2016

	Unadjusted ∞		Adjusted Φ		p-value for Trend
	β	95% CI	β	95% CI	
Sex	Reference		Reference		
Female	-0.250	-0.430, -0.070	-0.131	-0.332, 0.070	
Hair Length (cm)	-0.006	-0.0103, -0.0017	-0.004	-0.009, 0.0004	
Use of Perm	Reference		Reference		
Yes	-0.124	-0.314, 0.0664	-0.165	-0.339, 0.009	
Proportion Cooked	0.004	0.00013, 0.0076	0.004	0.0009, 0.008	
Winter	Reference		Reference		
1 meal/week	0.123	-0.0777, 0.323	0.112	-0.077, 0.301	
2 meals/week	0.326	0.0992, 0.552	0.284	0.071, 0.497	0.005
3 meals/week					
Random intercept for community from adjusted model: SD 0.143 (95%CI: 0.050, 0.405)					
Each model included a random intercept for the effect of clustering in communities					

Model covariates: sex, hair length, use of hair dyes or permanent treatments, the proportion of fish meals usually prepared by cooking, and fish/whale consumption frequency in the winter

Table 10 shows the distribution of ascertained diet components overall and by community. Few participants reported consuming more than 2 daily servings of fruit and vegetables. Conversely, a large proportion of participants (45%; 45/101) reported consuming dairy products more than once per day. Of 101 participants, 40 reported regular use of dietary supplements (vitamins calcium, fish oil, omega-3 and fibre). Table 10 also shows MeHg concentrations within intake categories of dietary components. Inspection of these distributions does not reveal clear patterns of association between intake frequencies and hair MeHg levels, consistent with results of the model building procedures, which did not identify these dietary factors as important confounders or effect-measure modifiers of the relationship under investigation in the study population. It should be noted, however, that the observed

Table 10: Hair MeHg concentrations ($\mu\text{g/g}$) within categories of year-round average dietary component intake by community, 101 western Canadian Arctic residents, 2016

Total		Aklavik, NWT		Old Crow, YT		Fort McPherson, NWT (N=24)	
(N=101)		(N=45)		(N=32)			
n (%)	Mean \pm SD	n (%)	Mean \pm SD	n (%)	Mean \pm SD	n (%)	Mean \pm SD
20 (20)	0.689 \pm 0.517	11 (24)	0.530 \pm 0.417	5 (16)	0.476 \pm 0.183	4 (17)	1.391 \pm 0.493
27 (27)	0.611 \pm 0.509	12 (27)	0.490 \pm 0.393	5 (16)	0.512 \pm 0.441	10 (42)	0.807 \pm 0.637
30 (30)	0.531 \pm 0.399	13 (29)	0.358 \pm 0.284	11 (34)	0.667 \pm 0.443	6 (25)	0.654 \pm 0.448
24 (24)	0.589 \pm 0.470	9 (20)	0.736 \pm 0.630	11 (34)	0.450 \pm 0.266	4 (17)	0.640 \pm 0.512
29 (29)	0.610 \pm 0.504	13 (29)	0.531 \pm 0.398	10 (31)	0.502 \pm 0.430	6 (25)	0.964 \pm 0.716
14 (14)	0.672 \pm 0.418	9 (20)	0.590 \pm 0.399	1 (3)	0.593	4 (17)	0.875 \pm 0.503
13 (13)	0.252 \pm 0.156	9 (20)	0.231 \pm 0.132	2 (6)	0.369 \pm 0.253	2 (8)	0.229 \pm 0.240
45 (45)	0.666 \pm 0.485	14 (31)	0.622 \pm 0.561	19 (59)	0.573 \pm 0.338	12 (50)	0.864 \pm 0.567
62 (61)	0.577 \pm 0.466	33 (73)	0.458 \pm 0.361	19 (59)	0.592 \pm 0.436	10 (42)	0.940 \pm 0.655
39 (39)	0.630 \pm 0.473	12 (27)	0.657 \pm 0.590	13 (41)	0.460 \pm 0.160	14 (58)	0.765 \pm 0.536

Hair length was inversely correlated with MeHg concentration, with 1 cm increases in length corresponding to slight reductions in $\mu\text{g/g}$ of mercury after adjusting for sex, permanent hair treatment use, fish/whale consumption and the proportion of fish/whale meals prepared by cooking (tables 6-9). Multivariable random effects regression yielded evidence of reduced hair mercury concentration among those who reported recent use of permanent hair treatments relative to those with untreated hair (tables 6-9). Figure 4 shows the adjusted effects of consuming different quantities of fish/whale in each season on hair mercury concentration, stratified by use of permanent hair treatments. These graphs show that within categories of fish/whale intake, participants who used permanent hair treatments had lower hair mercury concentrations relative to those who did not use such treatments.

Tables 6-9 show SDs and 95% CIs representing the random effect for clustering in communities. Visual comparison of community-specific patterns of fish/whale intake (table 2) revealed the following species as having the most divergent consumption patterns across communities: Beluga Whale (*D.leucas*), Arctic Grayling (*T.arcticus*), Chinook Salmon (*O.tshawytscha*) and Burbot (*L.lota*). Assessment of the linearity of the relationships between intake frequency and hair MeHg concentration indicated that each of these variables could be modeled as continuous. Table 11 shows the SDs and 95% CIs representing residual variation across communities for listed sets of covariates. The largest reduction in the SD was observed in the fall model, with the addition of Chinook Salmon (*O.tshawytscha*) and Arctic Grayling (*T.arcticus*) intake modeled as continuous variables. Variation across communities

increased with the inclusion of beluga whale consumption in all models, likely because whale consumption was almost exclusively reported by residents of Aklavik, NT. Inclusion of this variable highlighted a strong association between Beluga Whale (*D.leucas*) consumption and hair MeHg concentration. The beta coefficients (95% CI) showing the increase in hair MeHg concentration for each one-meal-per-week increase in beluga whale intake in the spring, summer, fall and winter seasons were 0.2 (95%CI: 0.01, 0.4), 0.04 (95%CI: -0.05, 0.1), 0.3 (95%CI: 0.05, 0.5) and 0.3 (95%CI: -0.04, 0.6), respectively.

Of the 22 samples selected at random in the lab for duplicate MeHg concentration measurement, 4 came from individuals also among the 10 selected at random as blind duplicates, yielding 28 participants with duplicate measurements; the median percent change in MeHg concentration between measurements was 14.67% (IQR: 10.75) with 36% (10/28) having second values that were higher than the initial value. The maximum percent change of 159% corresponded to a woman with hair that had been dyed one month before sample collection. Excluding this extreme outlier, the mean percent change in MeHg concentration between measurements was 15.84% (SD: 9.95; Range: 3.32 - 43.77). We performed a bias analysis using MeHg values adjusted by the mean percent change observed in the validation subsample after excluding the outlier; we increased by 15.84% the values of 36% of the study population selected at random and decreased by the same amount the values of the remaining 64%. The mean of the adjusted MeHg values was 0.58 µg/g (SD: 0.47; Range: 0.05-2.05).

Table 11: Sensitivity analysis: Relationship of selected variables to residual between-community heterogeneity, 101 western Canadian Arctic residents, 2016

Model	SD	95%CI
Spring		
Model 1: Sex, Hair Length, Proportion Cooked, Permanent Hair		
Treatments, Total Fish/Whale Consumption in Spring	0.131	0.045, 0.379
Model 1 + Beluga Whale Consumption	0.144	0.053, 0.393
Model 1 + Arctic Grayling Consumption	0.124	0.042, 0.370
Model 1 + Chinook Salmon Consumption	0.116	0.037, 0.363
Model 1 + Chinook Salmon & Arctic Grayling Consumption	0.115	0.037, 0.361
Summer		
Model 2: Sex, Hair Length, Proportion Cooked, Permanent Hair		
Treatments, Total Fish/Whale Consumption in Summer	0.156	0.057, 0.423
Model 2 + Beluga Whale Consumption	0.159	0.059, 0.426
Model 2 + Chinook Salmon Consumption	0.155	0.054, 0.445
Model 2 + Arctic Grayling Consumption	0.150	0.054, 0.413
Model 2 + Chinook Salmon & Arctic Grayling Consumption	0.154	0.054, 0.442
Fall		
Model 3: Sex, Hair Length, Proportion Cooked, Permanent Hair		
Treatments, Total Fish/Whale Consumption in Fall	0.105	0.030, 0.371
Model 3 + Burbot Consumption	0.109	0.032, 0.371
Model 3 + Beluga Whale Consumption	0.119	0.038, 0.368
Model 3 + Arctic Grayling Consumption	0.092	0.022, 0.382
Model 3 + Chinook Salmon Consumption	0.070	0.010, 0.473
Model 3 + Chinook Salmon & Arctic Grayling Consumption	0.064	0.008, 0.534
Winter		
Model 4: Sex, Hair Length, Proportion Cooked, Permanent Hair		
Treatments, Total Fish/Whale Consumption in Winter	0.151	0.054, 0.417
Model 4 + Burbot Consumption	0.146	0.052, 0.411
Model 4 + Arctic Grayling Consumption	0.146	0.052, 0.410
Model 4 + Chinook Salmon Consumption	0.140	0.048, 0.404
Model 4 + Chinook Salmon & Arctic Grayling Consumption	0.139	0.048, 0.403
Model 4 + Chinook Salmon, Arctic Grayling & Burbot Consumption	0.133	0.045, 0.394

We inspected the mean percent change in repeat measurements by participant characteristics within the validation subsample and identified use of hair dyes or permanent hair treatments as possibly related to the magnitude of the difference between repeated measurements. The mean percent change among those who reported recent use of hair dye or other permanent hair treatments was 38.5 (SD: 59.5), compared to 16.2 (SD: 10.4) among those who did not. Among those who used permanent hair treatments, 50% had second values that were higher than initial values, while among those with untreated hair, 32% had second values that were higher than initial values. We performed a second bias analysis using MeHg values adjusted by the observed mean percent change by hair treatment status, increasing by 38.5% the values of 50% of those with treated hair selected at random and decreasing by the same amount the values of the other 50%, and increasing by 16.2% the values of 32% of those with untreated hair selected at random and decreasing by the same amount the remaining participants with untreated hair. This adjustment resulted in a mean MeHg concentration of 0.57 µg/g (SD: 0.50; Range: 0.05-2.87). Under each of these scenarios, all participants remained at levels below those thought to pose serious health risks. Table 12 compares results of regression models using the originally measured MeHg concentrations to results of models using values adjusted for measurement error. These comparisons indicate that error in laboratory measurement of MeHg is not likely to have impacted inferences drawn from this analysis.

Sensitivity Analysis: Multivariable random effects regression of hair MeHg concentrations (µg/g) on fish/whale intake, comparing models using measured MeHg values adjusted for the degree of variability in MeHg measurements observed in subsets of the study population, 101 western Canadian Arctic residents

/week)	Original MeHg Measurement			Adjusted MeHg Measurement 1 \square			Adjusted MeHg Measurement 2 \square		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
	<i>Ref</i>			<i>Ref</i>			<i>Ref</i>		
	0.258	0.076, 0.439		0.231	0.048, 0.413		0.288	0.085, 0.4900	
	0.406	0.204, 0.609	0.000	0.377	0.174, 0.579	0.000	0.444	0.220, 0.668	0.000
	<i>Ref</i>			<i>Ref</i>			<i>Ref</i>		
	0.044	-0.183, 0.270		0.017	-0.207, 0.241		0.082	-0.167, 0.331	
	0.183	-0.065, 0.432		0.104	-0.133, 0.342		0.059	-0.204, 0.323	
	0.320	0.114, 0.526	0.001	0.293	0.089, 0.498	0.001	0.365	0.138, 0.592	0.001
	<i>Ref</i>			<i>Ref</i>			<i>Ref</i>		
	0.150	-0.069, 0.369		0.135	-0.089, 0.358		0.185	-0.064, 0.434	
	0.323	0.113, 0.534		0.216	0.006, 0.427		0.180	-0.053, 0.414	
	0.376	0.143, 0.610	0.000	0.274	0.041, 0.507	0.000	0.400	0.142, 0.658	0.000
	<i>Ref</i>			<i>Ref</i>			<i>Ref</i>		
	0.112	-0.077, 0.301		0.087	-0.099, 0.274		0.107	-0.103, 0.317	
	0.284	0.071, 0.497	0.005	0.291	0.081, 0.501	0.004	0.302	0.066, 0.538	0.008

entrations adjusted by the mean percent change of 15.8% estimated among 27 individuals with repeat measurements (excluding 1 outlier). MeHg concentration was increased by 15.8% for a random subset of participants and decreased by 15.8% for the remaining 64%, based on the distribution of increased or decreased values in the validation subset.

- MeHg concentrations adjusted according to reported use of permanent hair treatments: Among participants who used hair treatments, 50% were increased by the 38.5% mean percent change observed among 6 participants in the validation subset with reported use of permanent hair treatments; among those who did not use permanent hair treatments, 32% were increased by the 16.2% mean percent change observed among 22 participants in the validation subset who did not use permanent hair treatments.

Discussion

Overall, MeHg concentrations measured in hair samples obtained from participants were all in the range at which MeHg is not expected to cause serious health effects. Participants in this study reported consuming a wide variety of aquatic species, the frequency of which varied by season and community. In each season, increasing fish/whale consumption was associated with increasing MeHg concentration in hair. Variation across communities was partially explained by the specific types of fish consumed; in particular, accounting for Chinook Salmon (*O.tshawytscha*) and Arctic Grayling (*T.arcticus*) consumption reduced the residual heterogeneity measured in multivariable random effects models and consumption of Beluga Whale (*D.leucas*) was strongly associated with increased hair-mercury concentration. These analyses showed that use of permanent hair treatments influences hair MeHg measurements; however, data were insufficient for precise estimation of this effect.

Our findings are consistent with the literature pertaining to the relationship between intake of aquatic species and internal dose of mercury as measured in hair(26,32,51–89). Among 66 studies of this relationship identified by systematic review, 44 (67%) showed evidence that increasing fish consumption was associated with increasing hair mercury concentrations (23). Additionally, the nonlinear shape of this relationship we observed in the present study is not unexpected. The systematic review showed that the shape of the relationship between fish consumption frequency and hair mercury level was not always linear and varied across populations. As well, our identification of factors associated with the reliability of hair-mercury measurements are consistent with the literature. Specifically, our observation that the use of dyes and other permanent hair treatments were associated with the greatest percent-change between repeated measurements is consistent with evidence suggesting that cuticle damage impacts the retention of compounds, given the potential for treatment induced damage to differentially impact strands within the same region of the head(90). The small number of repeated analyses conducted on samples from participants who used permanent hair treatments (n=6) should, however, be noted.

Our analyses were limited by insufficient data for precise estimation of some effects of interest on hair mercury levels. This research would have benefited from estimation of the effects of individual species that participants reported consuming. Greater statistical power would also facilitate investigation of modifying effects of dietary factors. Finally, results indicated that use of permanent hair treatments was associated with greater percent-change in repeated measurements of the same individual's sample.

Our data showed hair length to be inversely correlated with MeHg concentration, after adjusting for other participant characteristics. Because samples were collected in the fall, this finding may reflect seasonal variation in fish/whale consumption among participants, whose intake was considerably greater in summer than in other seasons (figure 1). This inverse relationship is consistent with the assumption that measurements from hair long enough to have grown year-round have lower average hair MeHg concentration because these concentrations reflect seasons during which consumption was less frequent

relative to measurements from shorter hair that grew exclusively during summer months. Additionally, there was a wide range of hair lengths among the collected samples, representing diverse exposure periods (median: 1.1 years; IQR: 2.1 years). Although hair length was included as a covariate in multivariable regression models, this may not have adequately controlled for the differential relationship between hair of various lengths and fish consumption in each of the seasons. Also, given the potential for hair growth rates to vary across individuals(38,91), the exposure time windows represented in hair samples cannot be classified perfectly. For this reason, experts on the use of hair samples for toxicological analysis have cautioned that exposures occurring close to the time at which samples are collected or those occurring more than 1 year prior to collection may not be reliably represented in the hair sample for all members of a study population(38,91). An additional consideration is the extent to which measurements in distal ends of strands that have been growing for extended periods of time can be considered reliable. Although MeHg remains chemically stable in hair relative to other tissues, a proportion of MeHg deposited in hair may be released over time, particularly if the cuticle sustains damage(38,90,91).

A major strength of this research is the strong partnerships between scientists and members of participating Indigenous communities. Community planning committees provided key input on fish/whale consumption practices in the study population, the development of the fish-focused FFQ, and the timing of hair sample collection. This led to collection of hair samples following the season of greatest exposure and incorporation of commonly used names for aquatic species, which likely improved participants' ability to provide accurate consumption data. The accuracy of consumption data is evident in the consistency between our results and the existing body of evidence on fish/whale consumption and hair mercury concentration; for example, the strong association between Beluga Whale (*D.leucas*) consumption and MeHg is expected due to reported MeHg levels in large marine mammals (13,92).

Conclusions

This mercury exposure project revealed that a large proportion of western Arctic Canadians regularly consume a wide range of fish species, as well as Beluga Whale. Increased fish/whale consumption in each season was associated with increased hair MeHg concentration. Overall, however, hair MeHg concentrations were low, indicating that fish consumption practices among participating residents of western Arctic Canadian communities are not placing them at elevated risk of serious health outcomes associated with mercury exposure.

Declarations

Ethics Approval and Consent to Participate. Informed consent was obtained from all participants by providing them with an information sheet that outlined the study objectives, methods, information to be collected, benefits and potential risks of participation and confidentiality. Following review of this document, each participant was asked to fill out a consent form, confirming they had received enough information about the project and that they agree to participate. Project information sheets and consent forms were reviewed and approved by the Research Ethics Board at the University of Alberta and have been published previously(23).

Consent for Publication. Not Applicable

Availability of Data and Materials. All data collected and created in partnership with Indigenous communities is considered confidential, sensitive and vulnerable to misappropriation. Data cannot be shared without the expressed permission of the communities who participated in the research. Researchers who are interested in the data can send a proposal to the corresponding author for consideration by community planning committees.

Competing Interests. The authors have no competing interests to declare.

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Author's Contributions. This work was completed as part of EW's PhD dissertation. EW designed the study with input from YY SG and KG. EW collected and analyzed the data and wrote the manuscript. YY, SG and KG reviewed the write-up and KG edited the manuscript.

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Figures

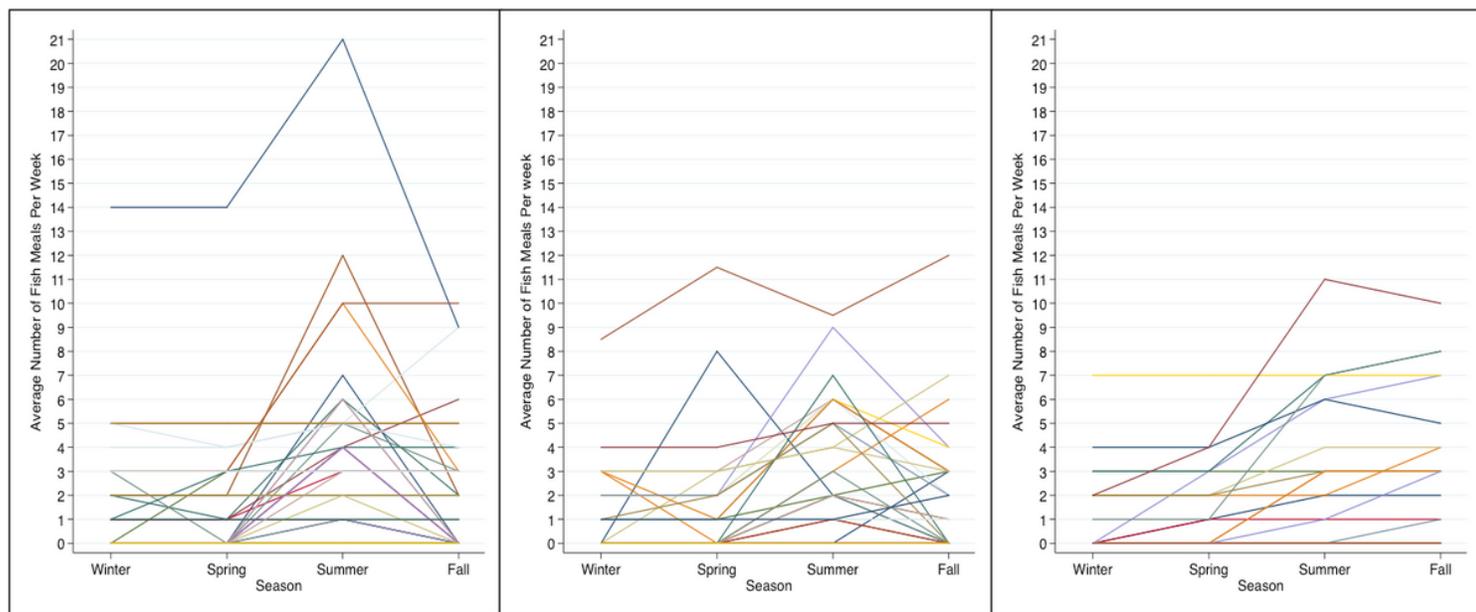


Figure 1
Average number of fish/whale meals per week by season among participants from Aklavik, Northwest Territories (left; n=45), Old Crow, Yukon (middle; n=32) and Fort McPherson, Northwest Territories (Right; n=24), 2016. Each Line represents an individual.

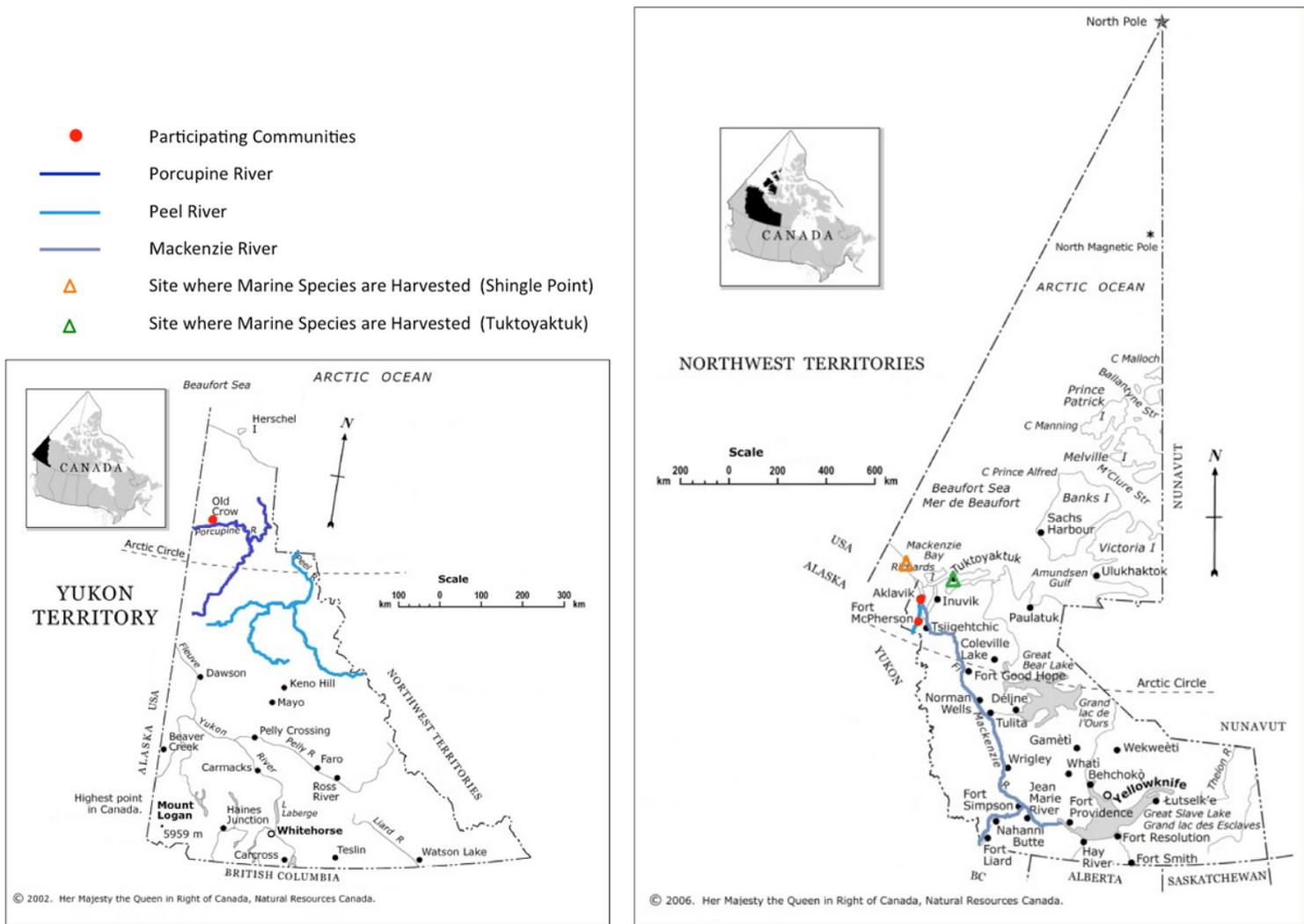


Figure 2

Maps showing the locations of the participating communities and main waterways and sites from which participants harvest aquatic species in the Northwest Territories and Yukon.

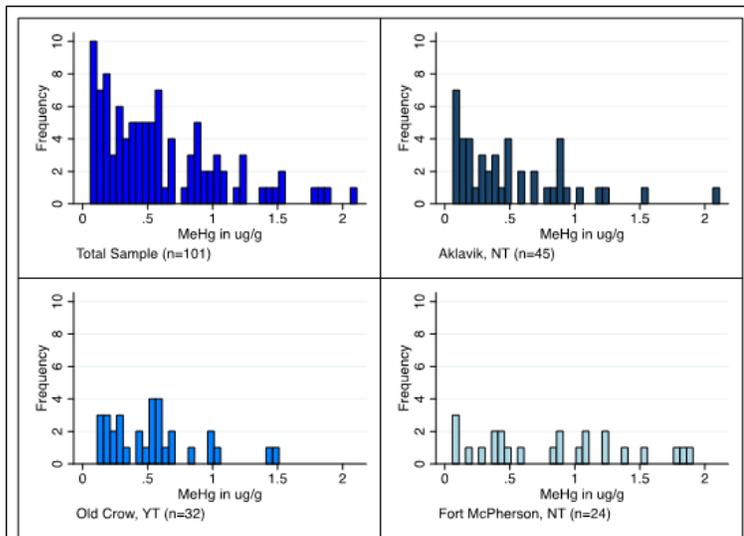


Figure 3

Distribution of MeHg measurements ($\mu\text{g/g}$) in hair samples among 101 western Canadian Arctic residents by community, 2016.

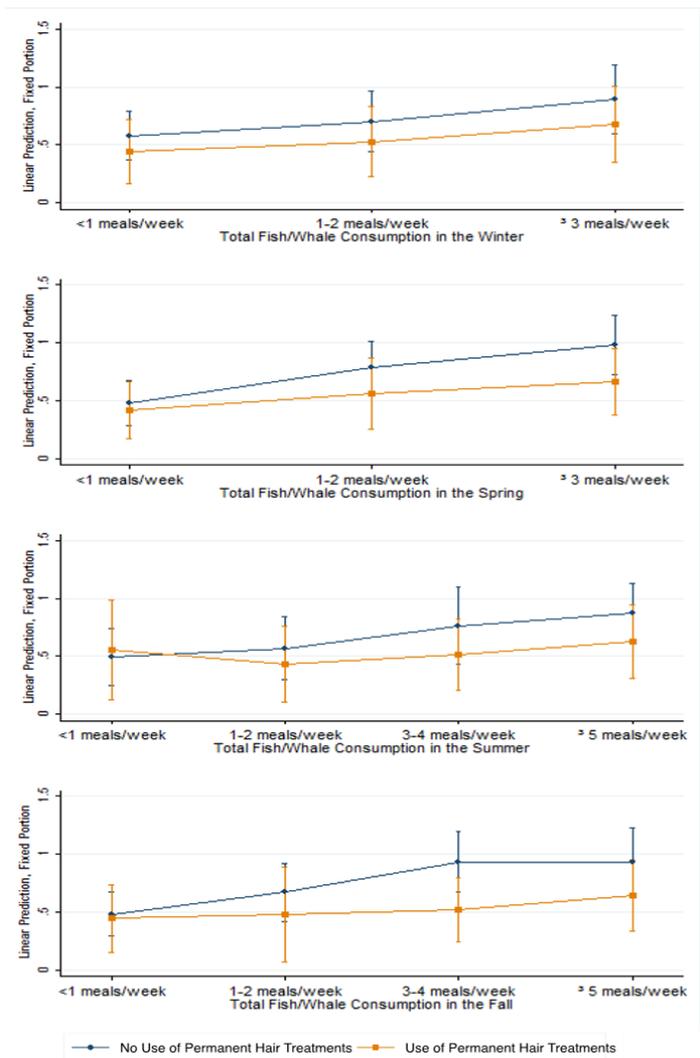


Figure 4
 Hair MeHg levels (µg/g) for different categories of fish/whale consumption frequency stratified by use of permanent hair treatments, adjusted for sex, proportion of fish/whale meals usually prepared by cooking and hair length