Relationship between Mercury Concentrations in Hair and Blood in Dene/Métis Communities of the Northwest Territories

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this 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.

Abstract

Background: Mercury (Hg) is a global environmental pollutant as well as a growing concern to public health. Indigenous populations in the Northwest Territories (NWT) may experience elevated Hg exposures due to higher consumption rates of fish compared to the Canadian general populations. Many biomonitoring projects are conducted to estimate human health risk caused by environmental Hg exposure; blood and hair are two of the most frequently used matrices for quantifying individual and population exposures. The association between Hg concentrations in hair and blood is often assumed to be linear with a hair-to-blood Hg concentration ratio of 250. However, this ratio varies among populations, and further research is needed to explore the relationship between Hg concentrations in hair and blood in Indigenous populations of the NWT.

Objective: The objectives of this study are to: 1) examine the relationship between Hg concentrations in hair and blood and the reliability of the widely accepted hair-to-blood ratio of 250 within this study population; 2) further improve the relationship by the application of Multiple Imputation (MI) and by the inclusion of covariates in the imputation model; 3) assess the effectiveness of MI for addressing missing data and data below limit of detection (LOD) from biomonitoring studies.

Methods: A community-based project was designed based on consultations that began in 2014. This contaminant biomonitoring project provided baseline reference Hg levels for the Sahtu region and the Dehcho region of the Northwest Territories. Blood and hair samples were collected for Hg exposure assessments. Participants were also asked to provide basic demographic information. Simple linear regression models were used to explore the relationship

between Hg concentrations in hair and blood with complete cases across communities. MI was applied to impute the missing data and the data below LOD.

Results: The association between Hg concentrations in hair and blood were found to be linear within communities. The hair-to-blood Hg concentration ratios were reported to be between 220 and 1146 for different communities from complete cases analysis (CCA). For half of the communities, these ratios from CCA were reported to be 13% to 18% less than those estimated from a simple linear regression (SLR) MI model. In comparison to the MI model based on SLR, multiple linear regression (MLR) MI models that included covariates (age, sex, and, BMI) may better address the missingness, especially for the communities yielding atypical results, e.g. the hair-to-blood ratios for one of the communities decreased from 1138 (estimated from SLR-MI model) to 994 (estimated from MLR-MI model).

Conclusions: The hair-to-blood Hg concentration ratios were generally lower when relying solely on complete cases. MI may be an effective tool to achieve less biased results by addressing the incomplete data. With the well-established linear relationships between Hg concentrations in hair and blood across communities, MI can utilize observed Hg-hair values to help recover information of Hg-blood values. Also, MI methods may be able to help extrapolate the blood levels using the current hair-to-blood ratios and segmental hair mercury levels. This approach may shed light on the seasonality of Hg exposure within Dene/Métis Communities of the Northwest Territories, Canada. The results of our study can be used to better inform strategies for biological sampling for future Hg human biomonitoring programs in the Canadian subarctic.

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1. Background & Literature Review

1.1 Background

Mercury (Hg) is a global environmental pollutant as well as a growing concern to public health (Sheehan et al., 2014). Inorganic mercury in the natural environment may be converted by microbes to methylmercury (MeHg), which is a particularly dangerous and bioaccumulative form of Hg (Driscoll et al., 2013) with significant adverse health effects on numerous biological systems and function (e.g, neurodevelopment, cardiovascular system, immune system, and central nervous system). The health hazards of Hg first attracted worldwide attention due to the outbreak of Minamata disease in Japan. The effects of Minamata disease occurred due to MeHg exposures following the consumption of contaminated fish and shellfish (Harada, 1995). These symptoms included sensory disturbance, ataxia, auditory disturbances, dysarthria, constriction of visual field, etc (Harada, 1995). Subsequent cohort studies from Faroe Islands reported that the prenatal exposure to MeHg is related to psychomotor and cognitive impairment among children (Grandjean et al., 1997). Adult exposures to MeHg may also induce adverse health effects on immune, nervous, and cardiovascular systems, although these effects are generally less consistent than those associated with prenatal exposure and tend to happen at higher levels of exposure than those that can affect neurodevelopment in the fetus (Karagas et al., 2012). The recent focus on the health effects of Hg exposure is more on chronic exposure to relatively low levels of MeHg; far lower levels than those observed during the Minamata tragedy (Ye et al., 2016). Such low dose, chronic Hg exposure is reported to be associated with subtle health effects, including higher blood pressure (Gallego-Vinas et al., 2019; Hu et al., 2018), worsening

neuronal function (Dufault et al., 2009), and neuromotor function impairment (Ohlander et al., 2016).

1.2 Mercury exposure and its health effects among Indigenous populations

There are two main types of Hg emissions: natural and anthropogenic. Natural sources of Hg include emissions from the ocean and volcanic eruptions. Anthropogenic emissions include Hg that is released from smelting, mining, and other industrial manufacturing. Studies showed that human-caused emissions have become major contributors to the Hg contamination in the Arctic regions of Canada (Donaldson et al., 2010; Kuhnlein et al., 2000). Distant anthropogenic sources of Hg can either be transported though ocean's thermohaline circulation pathways, or circulated into the air through the process of global distillation, prior to being deposited into the Arctic regions of Canada (Donaldson et al., 2010).

In humans, the primary exposure pathway to MeHg is through diet, especially through the consumption of fish, and for some populations (not for Sahtu region or Dehcho region), marine mammals (Ha et al., 2017; Risher et al., 1999). Indigenous communities sometimes experience elevated MeHg exposures due to higher consumption of fish compared to the general populations. First Nations, Métis, and Inuit are the three main groups of Indigenous peoples residing in Canada. New data from the National Household Survey (NHS) showed that among the 1,400,685 Indigenous individuals who participated in the survey, 60.8% identified themselves as First Nations, 32.3% as Métis, and 4.2% as Inuit (Turner et al., 2011). About 1 in 3 Canadians residing in Northwest Territories (NWT) were of First Nations descent, which made

them the largest ethnicity group in this area (Turner et al., 2011). Within Indigenous populations, many communities harvest food from the local environment, which is sometimes referred to as "country food" (Van Oostdam et al., 2005). Country food plays an important role in the cultural, social, and health well-being in many Indigenous populations. As country foods (some of which can have high contaminant levels) can represent a substantial proportion of Indigenous diets, Indigenous peoples sometimes experience elevated contaminant exposures relative to the general population of Canada (Van Oostdam et al., 2005).

The term bioaccumulation is defined as uptake, storage, and accumulation of organic and inorganic contaminants by organisms from their environment (Streit, 1998). Although the levels of bioaccumulation of Hg, cadmium, zinc, chromium, and lead, generally do not exceed the safe levels for human consumption in fish, the constant presence of heavy metals in concentrations near those limits considered safe for human consumption, is a reason for concern, and populations who frequently consume fish from polluted rivers should be advised on these risks (Arantes et al., 2016). As MeHg bioaccumulates in aquatic food webs, MeHg concentrations in fish can reach more than one million-fold of MeHg levels found in the waters where the fish live (Chen et al., 2007). As such, even though Hg and MeHg levels are low in the waters in such remote environments, the Hg and MeHg levels in fish can exceed human consumption guidelines (Gordon et al., 2016). The First Nations Food, Nutrition and Environment Study (FNFNES), which has been implemented across each of the ten Canadian provinces, has shown fish to be a major part of the diet for First Nations adults in the Boreal Shield/Subarctic of Canada (Chan et al., 2014, 2016, 2018). For example, mean fish consumption was reported to be 23.6 g day^{-1}, 8.0

g day^{-1} , and 20.4 g day^{-1} in the Boreal Shield/Subarctic areas of Ontario, Alberta, and Saskatchewan, respectively (Chan et al., 2014, 2016, 2018). Approximately 30% and 7% of First Nations women of child bearing age were reported to exceed the Health Canada mercury guideline of 6 μg g^{-1} in hair for the general population in the Boreal Shield/Subarctic area in Ontario and Saskatchewan, respectively (Chan et al., 2014, 2016). For the 18 First Nations communities selected from Ontario, traditional food contributed to 72% of the residents' dietary total MeHg intake, and the average dietary total MeHg exposure was reported to be 1.6 times higher on average among these First Nations populations in Ontario compared to the general Canadian populations (Juric et al., 2017). Although dietary Hg mostly comes from fish, it does not mean other dietary sources of MeHg are negligible (Horvat et al., 2003; Ysart et al., 2000). For example. within the western NWT, Bluenose caribou are capable of carrying large amount of Hg burden in the form of inorganic mercuric mercury within their kidneys (10.45 μg g^{-1}) (Larter et al., 2000).

A study involving Inuit children reported the geometric mean of hair Hg level to be 0.66 μg g^{-1}, and approximately 25% of this population had hair Hg concentrations equal to or greater than 2 μg g^{-1} (WHO reference level) (Tian et al., 2011). The assessment of contaminant and dietary nutrient interactions in the International Polar Year Inuit Health Survey reported an average Hg exposure (7.9 μg/kg/wk) exceeding the Toxicological Reference Values of 5.0 μg/kg/wk among 2074 participants from Nunavut, Nunatsiavut, and the Inuvialuit Settlement Region, with 35% of the participants above the guideline (Muckle et al., 2012). However, it is not yet clear whether these results are generalizable to Indigenous communities of the NWT, e.g. the populations

included in this study. It should also be noted that there is difference between reference values and health-based guidance value. Within human biomonitoring studies, reference values indicate the upper margin of the current background exposure of the general population (Ewers et al., 1999). On the other hand, health-based guidance values describe the level of a contaminant biomarker below which should not pose a risk to health. Based on epidemiological studies, Health Canada has established a blood Hg health-based guidance value for the general population of 20 μg L^{-1} (Health Canada, 2007; Legrand et al., 2010). Consequently, the Toxicological Reference Values describe above roughly correspond to the level of exposure that should typically result in biomarker level similar to the Health Based Guidance Value. Other reference values and health-based guidance values can be found in Appendix.

Indigenous populations can be prone to elevated MeHg exposure due to their high consumption of fish and/or marine mammals. For example, a few lakes in the NWT contained fish of certain types (e.g. Arctic char, northern pike) whose muscle Hg concentrations exceeded the estimated threshold range (0.5 to 1.0 μg g^{-1} net weight) within which adverse biological effects begin to occur (Scheuhammer et al., 2015). Although there is no regulatory guideline for concentrations in marine mammals in Canada, the Hg concentrations in some organs of marine mammals can be much higher than the commercial-sale guideline for fish. For example, studies showed that the liver, kidney, and muscle of Arctic beluga whales consistently exceeded the commercial-sale guideline for fish of 0.5 μg g^{-1} (Lockhart et al., 2005). It was reported that the mean concentrations of Hg was 10.7 μg g^{-1} in liver, 2.87 μg g^{-1} in kidney, and 1.18 μg g^{-1} in muscle for the arctic beluga whales (Lockhart et al., 2005). Hg concentrations in brain tissue of beluga

whales were lower than levels associated with neurotoxicity in mammals in general, but were sometimes high enough to cause subtle neurochemical changes that can precede overt neurotoxicity (Scheuhammer et al., 2015). Moreover, some epidemiological studies have shown negative health effects of MeHg exposure in some Indigenous populations (Harada et al., 2005; Pirkle et al., 2016; Valera et al., 2009). In a study focusing on the neurobehavioral performance of 7- to 12-year-old children from a traditional Inuit community in Qaanaaq, neuropsychological deficits were discovered to be possibly associated with Hg exposure (Weihe et al., 2002). Another study, by Beatriz Valera et al. (Valera et al., 2009), found that Hg is associated with increasing blood pressure and pulse pressure among adults from 14 Nunavik communities. Representative surveys of Nunavik Inuit and Cree adults documented associations between increasing blood Hg concentrations and cardiovascular disease (Valera et al., 2009, 2011). Moreover, the elevated levels of MeHg seen in some Indigenous communities has, on occasion, been associated with mercury-related clinical outcomes. For example, in a long-term follow-up study by M. Harada et al. (Harada et al., 2005), 85.9% of the Indigenous inhabitants in northwestern Ontario reported at least one subjective mercury-related clinical symptom, including numbness, headache, forgetfulness, dizziness, hearing difficulty, and trembling. Objective clinical symptoms related to mercury exposure were also prevalent among this study group, which was supported by 54.4% of the participants having glove and stocking-type sensory disturbance, 36.8% having difficulty in balance and walking, and 21.1% having tremor (Harada et al., 2005). Notably, these results are not generalizable to other Indigenous populations in Canada (e.g., Dene/Métis Communities of the Northwest Territories). This is because the area of northwestern Ontario was polluted with Hg from a pulp and paper mill; Minamata disease was

discovered among 45 out of 57 (78.9%) participants in study of one of the downstream First Nations communities (Harada et al., 2005). However, the Dehcho and Sahtu regions of the NWT does not have any similar point sources of industrial Hg pollution. Accordingly, Hg exposure levels are generally below the available guidance values among the Dene/Métis communities of the Dehcho region of the NWT (Ratelle et al., 2018b).

1.3 Two matrices to track mercury exposures: hair and blood

In the past decades, growing awareness of the adverse effects on human health caused by environmental Hg exposure (Guzzi et al., 2008) led to intensive human biological monitoring programs aimed at assessing levels of Hg exposure and environmental risks (Angerer et al., 2007). Biomonitoring studies were also proven to be of great value for the estimation of human health risk caused by environmental Hg exposure (Karimi et al., 2015). The Hg absorbed in the human body builds up in various types of tissue (including hair) distributed through blood. Two of the most common methods to measure the amount of mercury in the body are by measuring the mercury levels in hair and blood. For understanding MeHg exposures, blood and hair are the two most frequently used matrices for quantifying individual and population exposures (Grandjean et al., 1994). Once food is digested, MeHg is absorbed into the blood and distributed to brain and other tissues, where it can be converted to inorganic mercury (iHg) species (Clarkson et al., 2006; Guzzi et al., 2008). Hg concentrations in blood often increase with the frequency of fish intake and are usually adopted as a tracer of recent exposure to MeHg (Wilhelm et al., 2004). Together with Hg in blood, mercury accumulated in hair can also reflect MeHg exposure (Airey, 1983; Cernichiari et al., 1995b; Matsubara et al., 1985; Shao et al.,

2013). Moreover, because of the high stability of the growth rate of Hg accumulated in scalp hair (almost 1cm/month), it may be used to track Hg exposure on a time-scale ranging from weeks to months (Phelps et al., 1980).

1.3.1 Pros and cons of blood

Blood Hg concentrations are capable of reflecting cumulative exposure to Hg of all forms, including organic (Berglund et al., 2005), inorganic (Berglund et al., 2005; Bjornberg et al., 2003; Grandjean et al., 1994), and elemental Hg (Berglund et al., 2005). Particularly, MeHg and Hg^0 and can easily pass the blood-brain barrier, which makes blood mercury level a preferred option to reflect the mercury accumulation in brain (Boerleider et al., 2017; Halbach, 1985). Moreover, the blood sample can reflect recent exposures to MeHg. Blood MeHg concentrations reach a maximum within four to fourteen hours and undergo clearance to other parts of the human body after twenty to thirty hours (Boerleider et al., 2017). If the source of exposure is eliminated, follow-up blood samples can also help assure the clearance of Hg from the body (Scheepers et al., 2014). Last but not least, when clinical risks are present, blood Hg measurement is the gold standard in clinical diagnosis and medical care. For example, when patients are believed to have faced high levels of exposure and/or clinical symptoms that indicate Hg poisoning, blood Hg measurement is strongly recommended over hair Hg testing. Blood Hg is the recommended test to diagnose Hg poisoning as most Hg is present in red blood cells and blood Hg levels are raised by recent exposure, e.g. a large seafood meal may raise blood Hg levels, which then declines over subsequent weeks. In addition, blood Hg better reflects the amount of Hg that reaches target organs/tissues and is thus better to describe risk of clinical

effects at the individual levels. Hair mercury incorporates sources of inter-individual variability that are not relevant to clinical risk (e.g., deposition rates of Hg into hair). Therefore, blood testing is the preferred for assessing health risk at the individual level.

Collecting blood samples from participants is invasive and requires professional staff and instruments to ensure the safety and success of the process. Also, the storage and transportation of blood samples need special conditions and are comparatively expensive (Boerleider et al., 2017). Blood samples are required to be stored at 4 degrees in the refrigerator immediately after collection, and, if blood samples are needed to be stored for a longer time, they should be frozen (Boerleider et al., 2017). On occasion, the collection of blood samples may also violate an individual's religious, cultural or ethical beliefs. Lastly, blood samples cannot shed light on retrospective exposure to Hg (Boerleider et al., 2017).

1.3.2 Pros and cons of hair

The simplicity and convenience of collection, storage, and handling of hair samples can sometimes make it a preferable method for mercury biomonitoring in remote locations (Almeida et al., 1999). Also, hair sampling is relatively non-invasive and easy to transport due to its stability. Second, total mercury (tHg) concentrations in hair provide the cumulative mid-to-long-term exposure in previous months, depending on the length of the hair sample (Bellinger et al., 2016; Cernichiari et al., 1995a; Diez et al., 2008). Hair grows at a generally consistent rate (~ 1 cm/month), making segmental analysis feasible (based on the length of hair). MeHg concentrations in the hair collected close to the scalp, combined with further segmental analysis,

allows examination of MeHg exposure history (Boerleider et al., 2017). In the segmental analysis by McDowell et al., hair samples were used to examine the peak MeHg exposure when examining the seasonal fish consumption variations (McDowell et al., 2004).

However, hair sampling has its disadvantages as well. First, hair can be contaminated by external Hg^0, which can subsequently distort the assessment results (Boerleider et al., 2017). For example, miners working in artisanal and small-scale gold mining are exposed to Hg vapors when melting a Hg^0-containing amalgam complex. As such, the absence of pretreating or washing hair sample may lead to an overestimation of internal mercury concentration. However, washing methods can also strip endogenous Hg, leading to potential underestimates of exposure. Another limitation is that, in some regions, providing hair samples may violate cultural beliefs (Veiga et al., 2004). For the members from these regions, they may not be willing to provide hair samples. In addition, hair samples may not be possible to collect from those with very short hair, shaved heads, and/or experiencing baldness. Since these factors are not uniformly distributed by age, sex, and ethnicity, these issues can introduce biases in hair Hg distributions. The hair sample is not reflective of recent Hg exposure due to the time interval between exposure and detectability (Grandjean, 1984). Moreover, the incorporation of MeHg into hair may be affected by ethnicity, age, and hair treatments (Nuttall, 2006; Yamaguchi et al., 1975).

1.3.3 The comparison of blood and hair as biological matrices

The pros and cons of blood and hair stemming from their nature as biological matrices may influence their real-world application. First, blood samples reflect the current/recent Hg exposure

(Berglund et al., 2005), while hair samples can trace the average exposure back to an earlier time (Bellinger et al., 2016; Cernichiari et al., 1995a; Diez et al., 2008). This nature of hair sample is of great value to those studies examining Hg exposure history (Boerleider et al., 2017). For example, in the segmental analysis by McDowell et al., hair was the biological matrix used to examine the peak MeHg exposure when examining the seasonal fish consumption variations (McDowell et al., 2004). Second, Hg concentrations in hair may be age dependent. For example, Paschal et al. reported that hair at 7 years of age may retain a comparatively higher weight-based mercury concentration than that at adolescence or adult ages (Paschal et al., 1989). Thus, one should be careful when using hair samples as a sole measure of mercury levels for different age groups, especially when comparisons are made across age groups. Thirdly, in comparison to hair, the blood sample result is always the gold standard for clinical diagnosis and is indicated when clinical risks are though present. For example, for those who rely on a subsistence diet that include fish and/or marine mammals, blood Hg measurement may be indicated (Liberda et al., 2014). Lastly, hair sampling may offer advantages in terms of detection rate. In this context, limit of detection (LOD) is the lowest mercury concentration that can be reliably detected from a sample with a specific instrument. If the mercury level in hair or blood is below the LOD of instrument, it is defined as undetectable. The precise level of mercury in this sample will remain unclear, all that is known is that the Hg concentration is below the LOD. The fraction of samples below the detection limit for Hg is often greater for blood than in hair because the concentration of Hg in hair is 250 times higher (on average) than that in blood. Therefore, when both hair and blood Hg levels are measured for a participant, one of the three situations are generally observed: (i) Hg-levels in both matrices are above their respective LOD values; (ii) the hair Hg-level is

above the LOD but the blood Hg-level is below the LOD; and (iii) both levels are below their respective LODs. But, based on the relative detection rates of the different matrices, these three scenarios are not equally observed. The third scenario is least likely because the detection limit for hair sample is comparatively low and Hg concentration is higher in hair than blood.

In conclusion, hair and blood are important measures of Hg exposure, but meanwhile they both have their own limitations in real-world application. Thus, if feasible, it is encouraged to measure Hg exposure through multiple matrices (like hair, blood, and urine) to benefit from their combined advantages and hence offset the limitations of individual matrices. In this case, understanding the relationship between Hg concentrations in hair and blood becomes essential. For example, in case any participant cannot or does not volunteer to provide any one of the two biological samples, the missing data can be potentially recovered through modern statistical methods according to the well-established relationship between the Hg levels in hair and blood. This relationship can also mitigate the LOD issue. Although the result of below detection limit may reassure the participants regarding their mercury results, it can pose problems for the researchers attempting to describe risk factors for exposure. This is especially true when <LOD cases accounts for a large proportion of detection results. The result of <LOD limits the extent to which researchers can make full advantage of the data, and may cause researchers issues like biased results, not enough sample size to conduct data analysis, and so on. In this way, a well-established relationship between the Hg concentrations in hair and blood can be used as a solid foundation for the estimation of <LOD and thus eases the problem.

1.4 The relationship between Hg concentrations in hair and blood

As suggested by the Joint Food and Agriculture Organization of the United Nations and the World Health Organization Expert Committee on Food Additives (JECFA), a mercury hair-to-blood ratio of 250 (Joint FAO/WHO Expert Committee on Food Additives, 2004) has been widely applied in Hg exposure and risk assessments. This hair-to-blood ratio corresponds to the findings reported by World Health Organization (WHO) (World Health Organization, 1990) and the United States Environmental Protection Agency (US EPA) (United States Environmental Protection Agency, 2001). Further, other studies have reported hair-to-blood ratios to be 270 by Johnsson et al. (Johnsson et al., 2005), 254 by Berglund et al. (Berglund et al., 2005), and 200-300 by Katz et al. (Katz et al., 1992).

There are several limitations regarding the aforementioned hair-to-blood ratio of 250. First, the predominant existing form of mercury in hair and blood is MeHg, but the ratio of 250 was primarily based on the measurement of tHg due to its convenience and comparatively low price. However, this may lead to inaccuracy of the ratio (Berglund et al., 2005; Budtz-Jorgensen et al., 2004; Legrand et al., 2010; Yaginuma-Sakurai et al., 2012) because using tHg level in whole blood as a proxy for MeHg exposure will lead to an overestimation of MeHg exposure depending on the degree of iHg exposure (Berglund et al., 2005). Second, the hair-to-blood ratio of 250 may not be applicable to different populations. For example, Yaginuma-Sakurai and his colleagues reported a mean ratio of 344±54 for 27 participants from a Japanese study (Yaginuma-Sakurai et al., 2012). In another study involving Japanse pregnant wowen (Sakamoto et al., 2007), the average hair-to-blood ratio was reported to be around 350. Also, studies have

shown that this figure can be influenced by other demographic factors, like gender, ethnicity, etc. (Budtz-Jorgensen et al., 2004; Liberda et al., 2014; Yaginuma-Sakurai et al., 2012). Liberda et al. reported that there was a systematic underestimation (-8.4%) for female and overestimation (+5.8%) for male (Liberda et al., 2014). Many studies also found that the hair-to-blood ratio might be age dependent. For example, in the same study by Liberda et al., using the blood-to-hair ratio of 250 for conversions, the blood Hg was overestimated by 12.3% in the 8-14 year old group and underestimated by (-9.8%) in the 39 years or older age group. Another study reported the median ratio to be 370 among 7 years old Faroese children, but 264 for those greater than 14 years old (Budtz-Jorgensen et al., 2004). Moreover, Budzt et al. reported that the ratio was concentration-dependent (Budtz-Jorgensen et al., 2004), and Liberda et al. discovered that the different population groups showed wide variation in regression slopes at higher concentration levels of blood Hg (Liberda et al., 2014). Thus, the ratio of 250 may not be appropriate for particular subpopulations. Third, although this hair-to-blood ratio was proven reasonable among specific groups at population level (Berglund et al., 2005; Johnsson et al., 2005; Katz et al., 1992), Liberda et al. found that this ratio may be very unreliable for the application on an individual level. In their study with 1333 subjects from nine Indigenous communities, the individual level hair-to-blood ratio was reported to be between 3 and 2845 (Liberda et al., 2014). Fourth, concentration ratios are expressed as regression coefficients where the fact that both biomarkers are affected by the imprecision of measurement is not taken into consideration (Budtz-Jorgensen et al., 2004). To be more specific, standard regression models include measurement error only in the dependent variable, and the estimated conversion factor depends on the choice of dependent variable (Brown, 1990). This also helps explain to some extent why

ratios seem to vary in different populations from Europe and America (Haxton et al., 1979; Phelps et al., 1980; Sherlock et al., 1982; M. D. Turner et al., 1980). Lastly, the effects of the time lag of hair samples may be inaccurate in subpopulations where fish consumption is highly seasonal (Murata et al., 2007).

In summary, the currently accepted association between blood and hair mercury concentration assumes linearity, with a hair-to-blood ratio of 250. However, this hair-to-blood ratio of 250 may not be applicable to Dene/Métis communities of the NWT. To the best of our knowledge, the relationship between Hg concentrations in hair and blood in the Dene/Métis communities of the NWT has not yet been examined. Therefore, a study with a focus on this relationship in the Dene/ Métis communities of the NWT is necessary. Unlike other studies (Laffont et al., 2011; Tian et al., 2011; Voegborlo et al., 2010) which collected only hair or blood sample for Hg exposure measurement, this biomonitoring project collected both samples, making it theoretically possible to explore the association between Hg concentrations in hair and blood. Although there were studies that collected both hair and blood samples, they either used hair and blood samples separately to assess Hg exposure (Bonsignore et al., 2016), or just reported the mean and median of the hair-to-blood ratios (Okati et al., 2018; Yaginuma-Sakurai et al., 2012). None of these studies tried to explore the relationship between Hg levels in hair and blood at a population level. The most similar study by Liberda et al. tried to examine the hair-to-blood Hg concentration ratios among First Nations communities in northern Québec, but their results may not be generalizable to the Indigenous communities of NWT. Therefore, this study contributes to the identified research gap by being the sole study to explore this specific relationship in the Dene/

Métis communities of the NWT. Another advantage of this biomonitoring project is its high participation rate due to its study design and recruitment process (e.g. any participant volunteering to provide only one of the two biological samples was also recruited) (Ratelle et al., 2018a).

2. Study rationale

This study mainly aims to explore the relationship between Hg-levels in hair and blood within the Dene/Métis communities of the NWT, Canada. In addition, this study will examine the validity of the widely accepted hair-to-blood ratio of 250 for the study population. To the best of our knowledge, little information on this topic is available for Indigenous populations of NWT. The most similar study, by Liberda et al., reported the hair-to-blood ratio of 250 to be unreliable for individuals from First Nations communities in Québec (Liberda et al., 2014). Then findings from our study could be utilized for further broad-scale and long-term Hg monitoring programs in these communities. Additionally, with our findings, if blood Hg concentrations can be effectively estimated from hair Hg concentrations at the population level, past blood Hg levels at the regional level (if not individual level) may also be extrapolated from retrospective hair samples.

Another study rationale is to handle the LOD issue in mercury detection. Existing methods for addressing the "non-detects" usually include discarding or replacing them with a fixed value. The deletion method is proven to be inefficient and is likely to yield biased and unreliable results due to smaller sample size and loss of information (Rubin, 2004; Schafer et al., 2002). Replacing non-detects with a fixed specific value, such as LOD/2 or LOD/$\sqrt{2}$, will also lead to biased results because all LOD cases get the same value and hence forcefully reducing the variance and yielding narrower (than expected) confidence intervals (CI) and undermining assumptions for parametric statistical tests (Nie et al., 2010). A proper method for handling these LOD cases should impute their values that belong in the range (0, LOD); such a method is more akin to the

nature of “non-detects”. Therefore, instead of discarding the information available on the overall population distribution, the incomplete data should be handled using modern statistical methods that makes use of partial data. From a data modeling perspective, our study is the first to propose the use of sophisticated statistical techniques (like Multiple Imputation) to better handle missing and LOD cases of Hg concentrations in blood and hair, thereby allowing extrapolation of missing values of Hg-concentration in blood via observed values of Hg concentration in hair, and vice versa.

3. Objectives and Hypotheses

This study will mainly examine the association between mercury concentrations in hair and blood in Dene/Métis Communities of the NWT, Canada. Our specific objectives are as follows.

- Objective 1: Explore/develop a relationship between Hg-levels in the blood and hair within the completely observed cases of this study;

  Hypothesis: The relationship between mercury concentrations in hair and blood is linear across the different communities;

- Objective 2: Improve the relationship from Objective 1 to incorporate/handle partially observed cases in this study through the use of Multiple Imputation (MI);

  Hypothesis: The information from complete cases can better help inform the simple linear imputation model involving only the hair and blood matrices.

- Objective 3: Explore the extent to which variables such as sex, age, height, and weight can help in the recovery of missing information in the blood and hair matrices;

  Hypothesis: Auxiliary/confounding variables such as the participant's age, sex, height, and weight can help improve the imputation of incomplete cases, and consequently in the assessment of the association between Hg-Blood and Hg-Hair.

4. Methods

A community-based project was designed (Ratelle et al., 2018a) based on consultations that began in 2014. This contaminant biomonitoring project aimed to provide baseline reference levels for the Sahtu region and the Dehcho region and to assess the risks and benefits of country foods consumption for contaminant and nutrient exposure (Ratelle et al., 2018b).

4.1 Participant criteria

Inclusion criteria: Community members from the Sahtu region and the Dehcho region who were six years old and older were eligible to participate, regardless of sex or ethnicity.

Exclusion criteria:

1. Children who were under the age of six;
2. Participants who were incapable of providing informed consent, including those who were under the influence of alcohol or drugs; individuals with diminished capacity to consent (e.g., those with Alzheimer's disease), as well as those minors who were not able to acquire the consent from their parent or guardian;
3. Individuals reluctant to participate or receive their results.

4.2 Ethical licences

The research team obtained the ethical licence for the study design and methods, which were reviewed and approved by the University of Waterloo Research Ethics Committee, the Aurora Research Institute, and the Stanton Territorial Health Authority for Human Research. Health

Canada ethics approval was also acquired for the additional analysis of human biological samples.

4.3 Participant recruitment

In total, six communities from the Dehcho region and three communities from Sahtu region participated in this study. Communities from the Dehcho region included: Fort Providence, Hay River Dene 1, Kakisa, West Point, Jean Marie River, and Trout Lake. Communities from the Sahtu region included Deline, Fort Good Hope, and Tulita. Details of this study were disseminated through local radio, posters, and other media appearances. Recruitment efforts, including sampling proportional to community population size, with a minimum target of 10% of the population per community, were aimed for participants to represent the sex and age distribution of the targeted population. All participants were recruited by the local coordinator and were asked to provide written consent to confirm their willingness to participate.

4.4 Data and sample collection

Participants were asked to fill in a questionnaire to provide basic personal information including sex, age, height, and weight. A confidential participant identification number was applied in place of any potentially identifying information on all forms and questionnaires.

Blood and hair were only collected from those who consented to provide biological samples. The blood collection process was conducted by registered nurses. Blood was collected from the median antecubital vein of the anterior arm of participants, stored in a metal-free plastic tube,

and stored at 4 degrees in portable coolers until laboratory analysis. The hair sample was collected with sterilized scissors under the guideline established by the previous study and stored in a polyethylene bag.

4.5 Laboratory analysis

The hair samples were analyzed for total mercury in the laboratory of Dr. Brian Branfireun (Biotron Analytical Services, University of Western Ontario). In this study, the 2 cm of hair most proximal to the scalp was utilized, and the LOD was 0.01 μg g^{-1}. The blood samples were analyzed in the laboratory of Michele Bouchard in the Department of Environment and Occupational Health at the University of Montreal, and the LOD was 0.05 μg L^{-1}.

4.6 Variables of interest

Hg concentrations in blood and hair were treated as continuous variables in this study. As age, sex, and Body Mass Index (BMI in kg m^{-2}) were also reported to as potential covariates for the association between Hg levels in hair and blood (Bonsignore et al., 2016; Budtz-Jorgensen et al., 2004; Liberda et al., 2014). Sex was treated as categorical variable; age and BMI were treated as continuous variables. BMI was calculated based on measured height and weight as follow: $BMI = (weight)/[height]^2$.

4.7 Data analysis

Multiple Imputation is one of the modern statistical methods for handling missing data in various applications. Instead of replacing each missing value with a single value, Rubin's MI procedure

replaces each missing value with a set of plausible values that represent the uncertainty about the unknown/missing value (Rubin, 1987). Each of the imputed data sets is individually analyzed by utilizing standard procedures for complete data (for example, simple linear regression, multiple linear regression, logistic regression, etc) and the results are combined for inference for the parameter of interest. Specifically, MI is performed in three stages: (i) imputation stage; (ii) analysis stage; and (iii) combining stage. In the imputation stage, multiple "complete" versions (say *m*) of the original incomplete data are created, and each version is a result of a random draw from the posterior predictive distribution of missing data given the complete data. In analysis stage, each of the *m* imputed data sets is analyzed with the complete data procedures to yield a set of *m* parameter point and variance estimates. In the final combing stage, the *m* point and variance estimates for the parameter of interest are combined to produce an overall inference for the parameter of interest.

In our analyses, observations are categorized as follow:

- Complete cases: individuals whose hair and blood Hg-levels are both detected, i.e., above the LOD; analysis based on these complete cases will be referred to as Complete Case Analysis (CCA).
- Partially observed cases: (i) participants whose Hair-Hg levels are detected while Blood-Hg levels are not detectable (i.e., below the LOD); (ii) cases that have either blood or hair missing (but not both). Participants whose hair-Hg levels and blood-Hg levels are both not detectable were excluded from this study.

For Objective 1, we first performed exploratory data analysis which makes use of histograms, scatter plots, correlations, region stratified analysis, linear regression, coefficient of determination, etc. The term "anomaly" was used in this study to describe the atypical results from the exploratory data analysis. Then, we built simple linear regression models to explore the relationship between Hg-Blood level (independent variable) and Hg-Hair level (dependent variable) across communities with complete cases only.

For Objective 2, we improved on the relationship from Objective 1 by using MI to better handle incomplete cases (i.e. partially observed and <LOD cases). MI is a technique that helps recover information of incomplete cases by borrowing information from observed cases. In this study, incomplete cases for blood are imputed (m-times) under a conditional (on the observed value of hair) truncated normal distribution*. We considered m=1000 imputations to yield 1000 imputed data sets, where their analysis results were combined using Rubin's rules (Rubin, 2004) as needed. We also provide empirical estimates (like median, IQR and other percentiles) which do not require normality.

For Objective 3, we improved our imputation model of Objective 2 by incorporating confounding variables — participant's age, sex and BMI in the imputation model. This implies the change in the imputation model from Objective 2; specifically, the imputation model in Objective 2 is a Simple Linear Regression (SLR) imputation model, whereas in Objective 3 it is Multiple Linear Regression (MLR) imputation model.

* The missing cases were imputed from a truncated normal distribution with limits 0 and 35; while the <LOD cases has limits of 0 and 0.03.

A significance level of 0.05 was utilized for all statistical analyses. All analyses were conducted in R and/or SAS. The sample size of complete case for one community was very low (less than 5), thus this community was excluded from data analysis. In this study, two communities were combined and analyzed as a whole community because of their proximity and shared characteristics . Therefore, the results of a total of 7 communities rather than 9 were reported in this study. In addition, detailed information pertaining to sample size of the seven communities were intentionally excluded to protect individual and community anonymity and privacy.

5. Results

5.1 Exploratory data analysis

Table 1: Overall summary statistics for Hg concentrations in hair and blood in Dene/Métis communities of the Northwest Territories, Canada

variable	mean (std)	median (IQR)	lower quartile	upper quartile	no. of detected cases	no. of incomplete cases
blood (μg L^{-1})	2.54 (4.16)	1.20 (2.05)	0.5	2.55	104	335
hair (μg g^{-1})	0.91 (1.05)	0.51 (0.94)	0.25	1.19	420	19

According to **Table 1**, in total, 439 participants were included in this study. Of the 335 participants with incomplete data for blood Hg levels, 170 had blood Hg concentrations below detection limit and 165 refused to provide blood samples. Of the 19 participants with incomplete data for blood Hg levels, 4 had hair Hg concentrations below detection limit and 15 chose not to provide blood samples. These 19 cases also showed Hg levels in blood missing or below LOD, which means that the precise values for both Hg levels in blood and hair were not available for these 19 cases. Therefore, these 19 cases were excluded from all data analyses. The mean and median in **Table 1** were all calculated with detected Hg levels only.

The summary statistics of variables of interest for our objectives are reported in **Table 2**. This table stated that the average age was around 40 for all the communities, and the percentage of male was around 50% across communities. In the overall study population, the proportion of blood Hg level below LOD for the study sample was 26.2%, whereas at the community level this figure could be as high as 43.8% (community B). Overall, the proportion of missing data was 55.1%, and this figure ranged from 18.8% to 69.1% at a community level. The reasons why

missingness of data varied so substantially among communities remains to be explored. Community F had a relatively small sample size among all the communities (although not reported due to confidentiality reasons). As a consequence, this percent missingness figure may be greatly and randomly influenced by the very few participants recruited in this study. With respect to the health based guidance values that Canada follows, the average Hg levels in blood and hair were below the threshold values of 20 μg L^{-1} and 6 μg g^{-1} (Statistics Canada, 2013), respectively. However, since the proportions of missingness/<LOD were very high for most of the communities and varied across communities, average Hg levels between communities would be difficult to directly compare. For example, the percentage of <LOD was 0% for community E, while this figure could be as high as 43.8% (for community B).

Table 2: Summary statistics for Hg concentrations in hair and blood in Dene/Métis communities of the Northwest Territories, Canada, stratified by community

community	age[1]	sex (male)	blood Hg level <LOD	blood missing data[2]	blood Hg level[1]	hair Hg level[1]
A	46 (20)	56.7%	13.4%	67.2%	4.28 (4.66)	1.43 (1.23)
B	43 (23)	40.6%	43.8%	45.3%	1.66 (1.88)	0.90 (0.97)
C	41 (18)	53.2%	15.8%	69.1%	2.56 (3.19)	0.73 (0.85)
D	40 (19)	42.6%	38.3%	48.9%	2.12 (1.81)	0.59 (0.68)
E	44 (24)	57.1%	0%	57.1%	1.42 (2.08)	0.68 (0.98)
F	43 (23)	62.5%	37.5%	18.8%	8.56 (11.02)	1.38 (2.35)
G	43 (22)	49.4%	37.6%	40.0%	0.93 (0.55)	0.91 (0.84)
total	42 (21)	49.4%	26.2%	55.1%	2.54 (4.16)	0.91 (1.05)

[1] Presented in the form of mean (standard deviation)
[2] The percentage of participants who provided a hair sample but not a blood sample

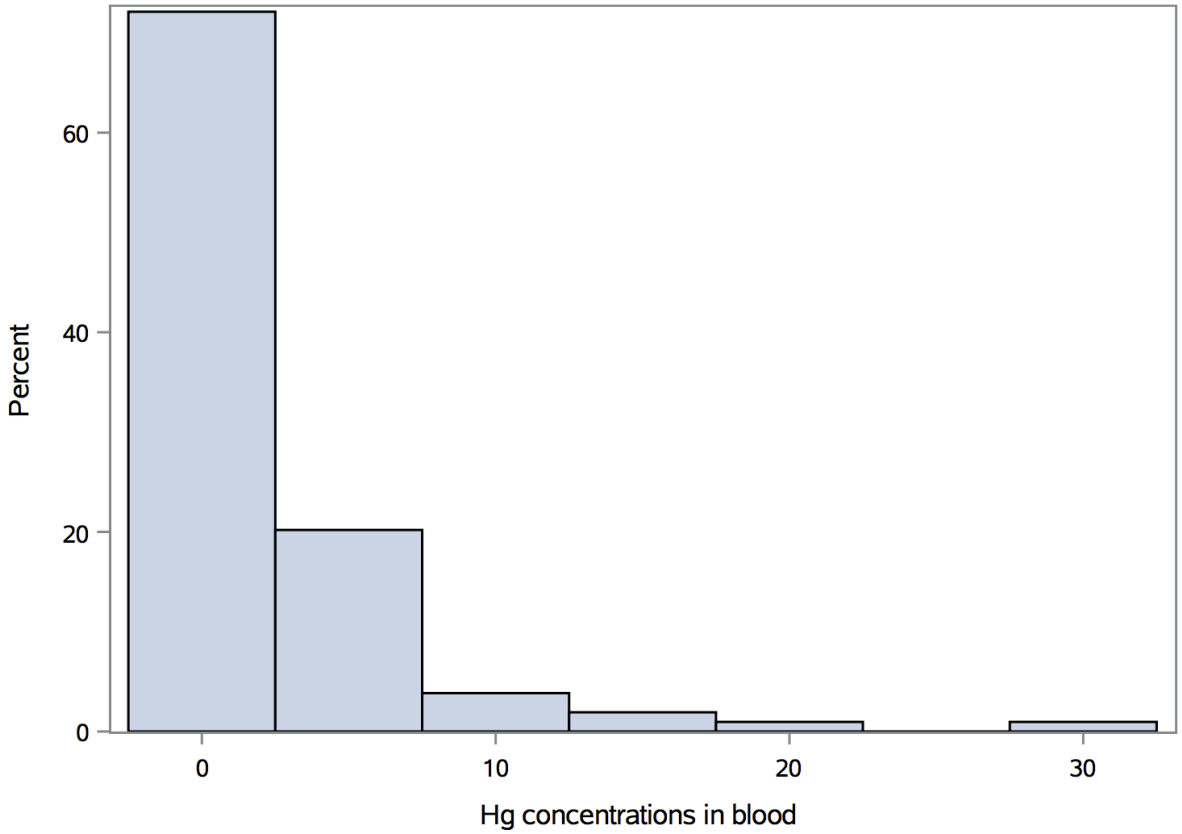

Figure 1: The distribution of Hg concentrations in blood among participants from Dene/Métis communities of the Northwest Territories, Canada

In **Figure 1**, the distribution of Hg concentrations in blood was right skewed, with the majority of the values distributed between 0.05 to 10 μg L^{-1}. There were very few potential outliers to the far right, which is expected because very few participants would have been exposed to particularly high levels of Hg. The same pattern appeared in the distribution of Hg concentrations in hair in **Figure 2**, with the majority of the values ranging between 0.01 and 4 μg g^{-1} and only very few potential outliers to the far right.

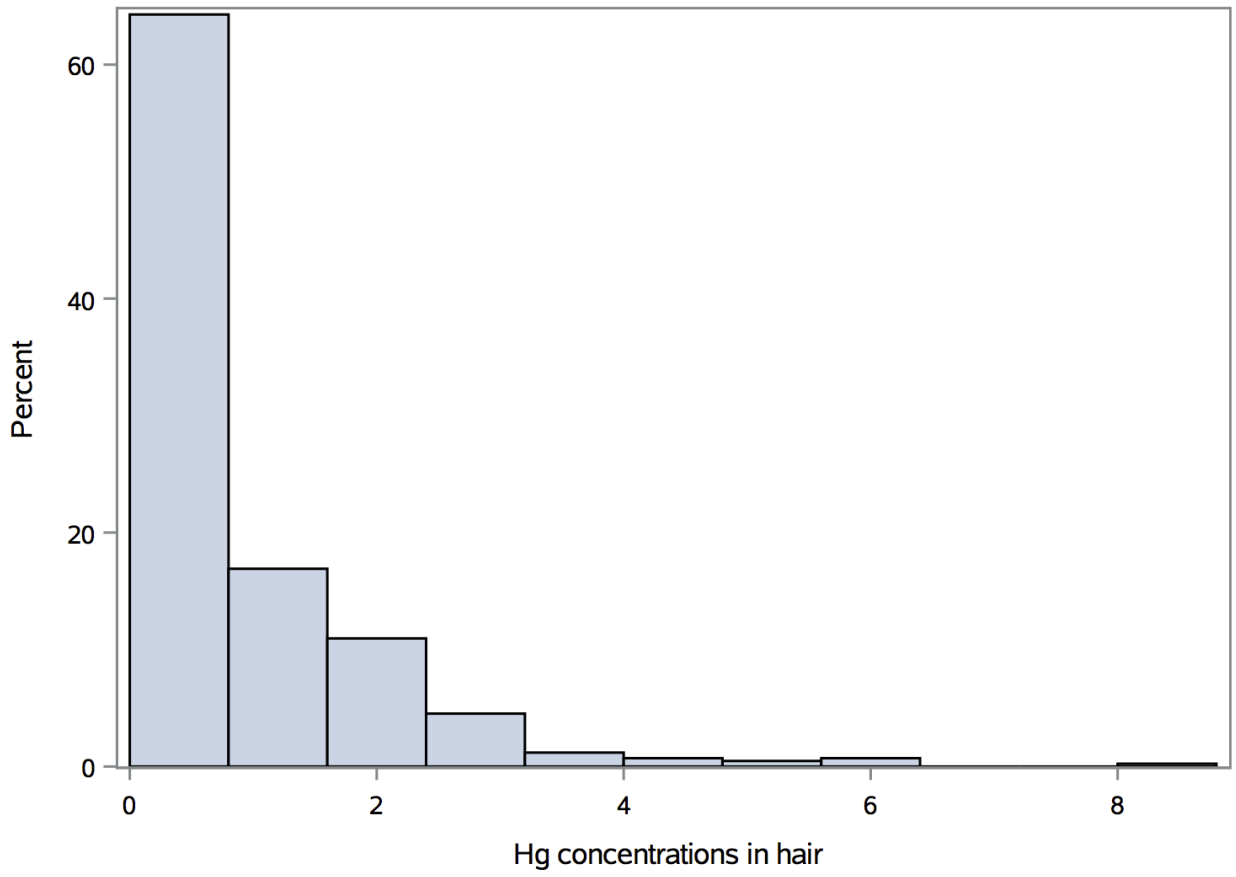

Figure 2: The distribution of Hg concentrations in hair among participants from Dene/Métis communities of the Northwest Territories, Canada

For the Hg concentration in hair, the median value in males were higher than females, but the data for males had a higher spread (variance) than females (**Figure 3**). Since there was overlapping IQR of the box plots, hair Hg levels between sexes appeared similar. A similar pattern was found in blood Hg levels (**Figure 4**). The overlap in the two box plots (**Figure 4**) was (again) inconclusive for difference in the Hg concentrations in hair between males and females. To protect the confidentiality of information, the dots above the health based guidance values (20 µg L^{-1} for blood and 6 µg g^{-1} for hair Hg levels, respectively) (Statistics Canada, 2013) were removed in **Figure 3** and **Figure 4**.

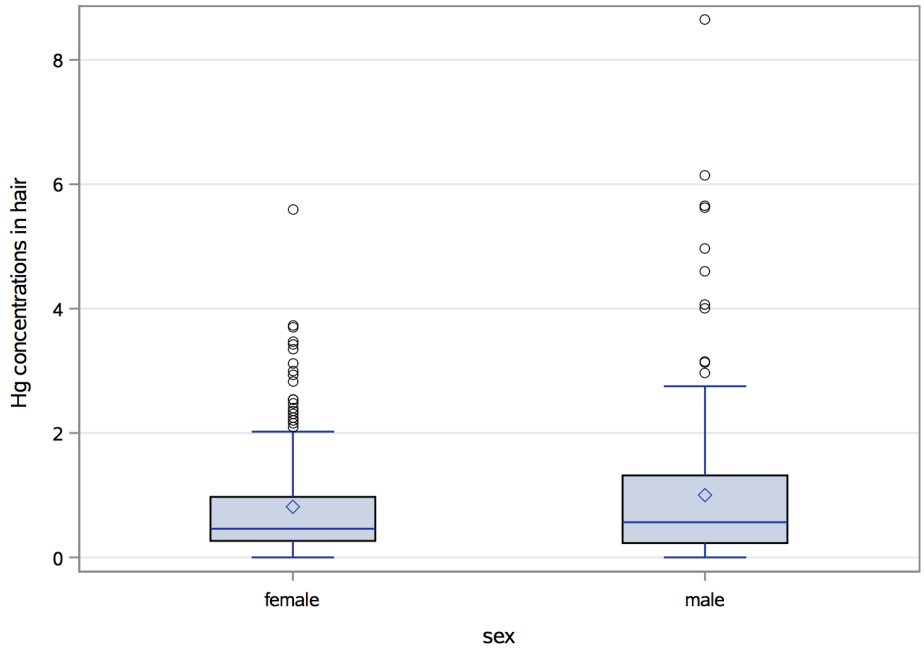

Figure 3: Comparison of Hg concentrations in hair between male and female participants from Dene/Métis communities of the Northwest Territories, Canada

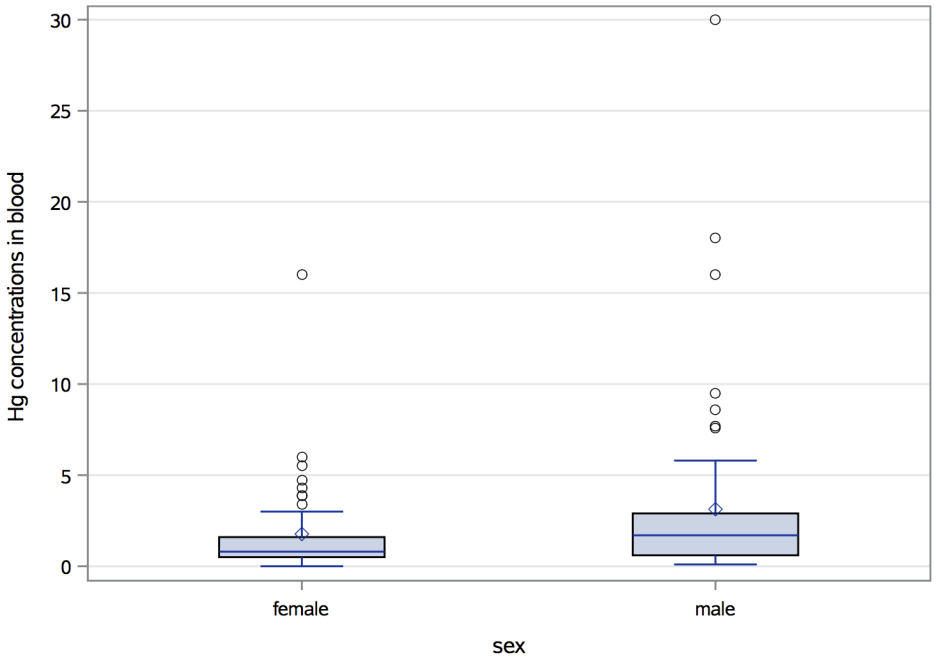

Figure 4: Comparison of Hg concentrations in blood between male and female participants from Dene/Métis communities of the Northwest Territories, Canada

Figure 5 suggests a positive association between age and hair Hg levels, which aligns with other literature (Budtz-Jorgensen et al., 2004). This positive association was also observed in the log-scale of age and hair Hg levels (see Appendix). Similarly, the positive association between the age and Hg concentrations in blood was observed in **Figure 6**. These figures suggest that age is an important confounding variable in the study examining the relationship between hair and blood Hg levels. These findings aligned with existing literature that has reported age as an important factor associated Hg levels in hair and blood (Budtz-Jorgensen et al., 2004).

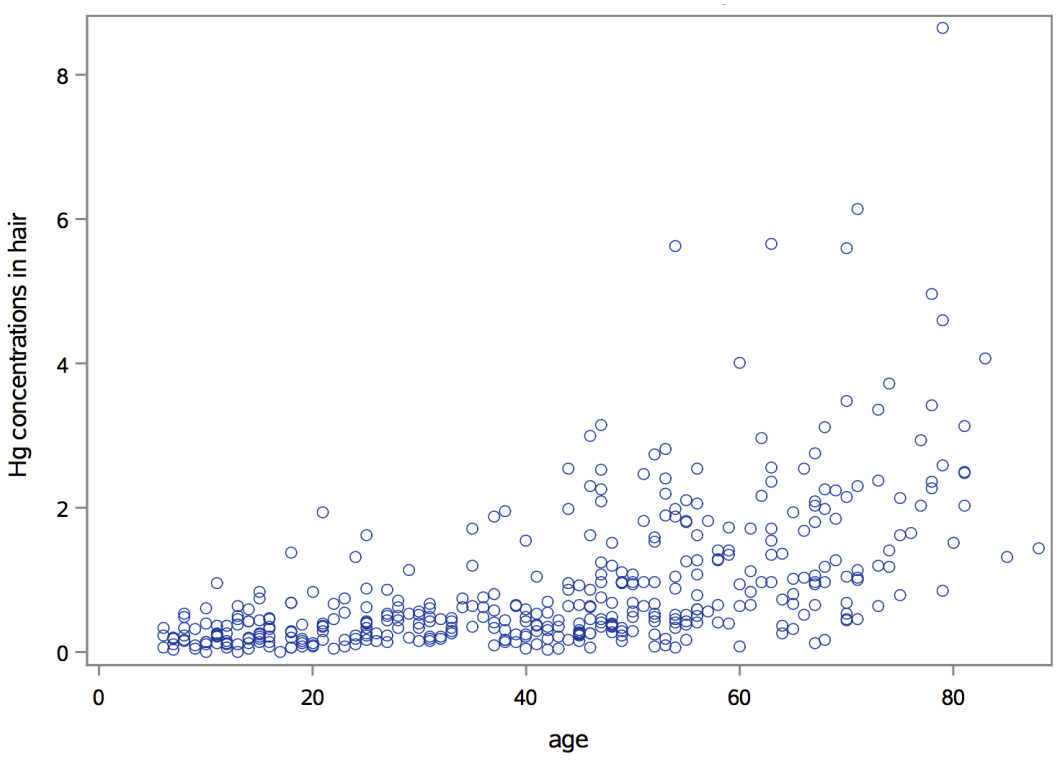

Figure 5: The relationship between age and Hg concentrations in hair among participants from Dene/Métis communities of the Northwest Territories, Canada

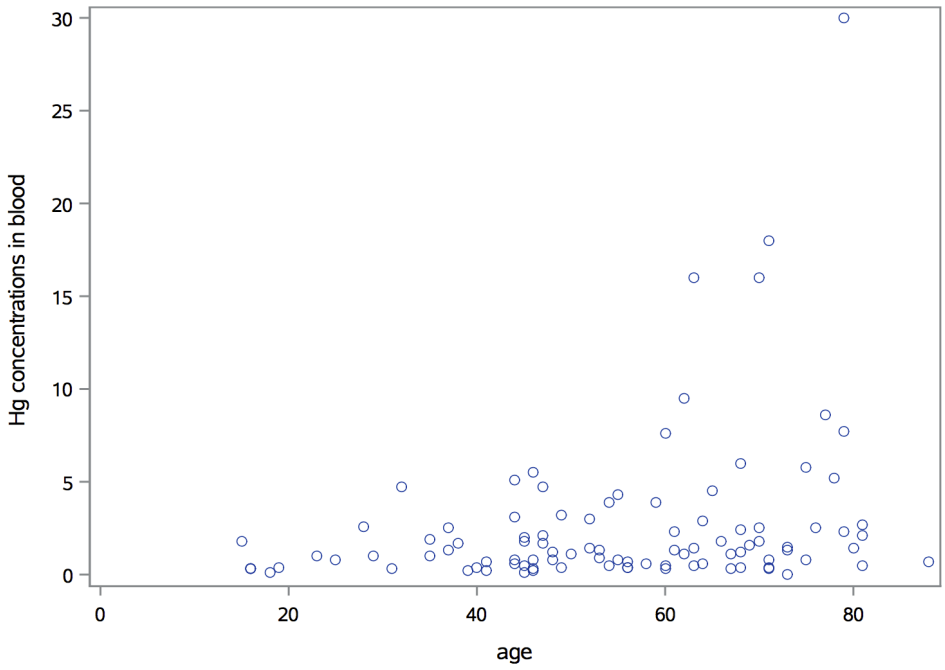

Figure 6: The relationship between age and Hg concentrations in blood among participants from Dene/Métis communities of the Northwest Territories, Canada

In our stratified (by community) exploratory analyses, we found that the distributions of Hg concentrations in hair were right skewed, with the majority of the values distributed between 0.01 to 4 μg g^{-1}. A right skewed distribution of Hg concentrations in hair was expected because very few participants would have high levels of Hg exposure. There seemed to be a few probable outliers for communities C, E, and F (**Figure 7**). However, the distributions for some communities (especially community D) were observed to have lower skewness than that for other communities, which could be caused by the sparsity of the data due to incompleteness.

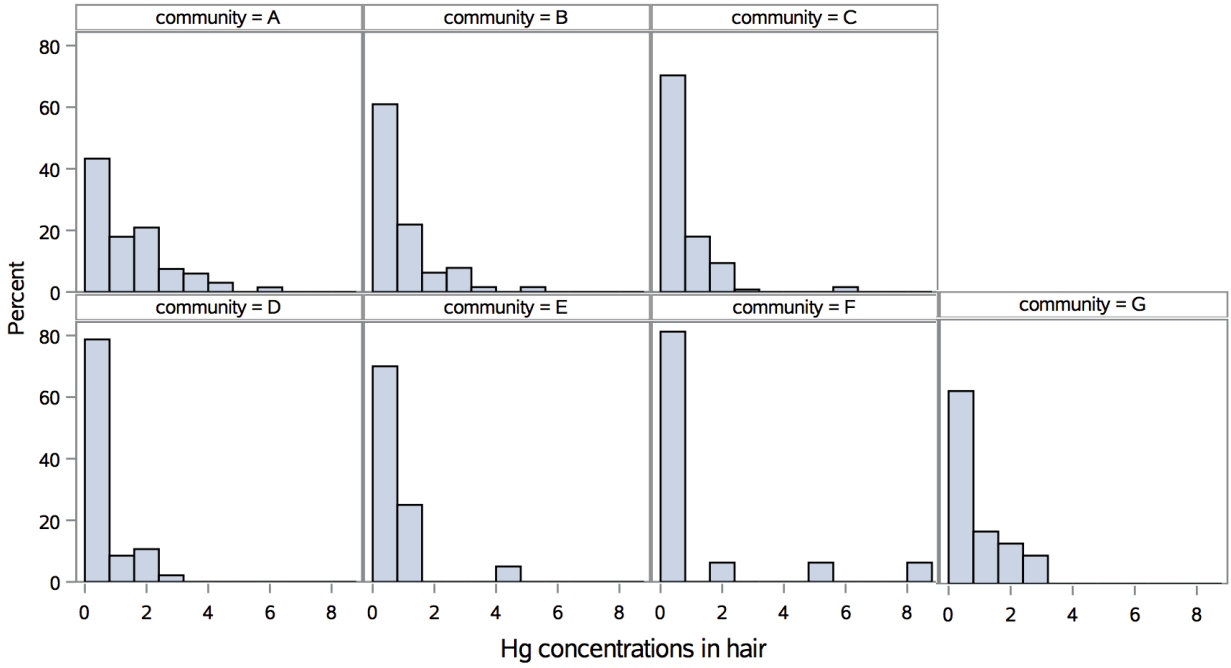

Figure 7: The distribution of hair Hg levels among participants from Dene/Métis communities of the Northwest Territories, Canada, stratified by community

Most of the distributions of blood Hg levels by community were also observed to be right skewed with a few probable outliers in **Figure 8**, However, similar to differences in the distributions of hair Hg levels by community observed in **Figure 7**, the distributions for some communities, (especially community G with no probable outliers) were also observed to have greater skewness than that for other communities, which could be caused by the data sparsity due to incompleteness. Moreover, the peak (mode) of the distribution for community A in **Figure 8** was not observed between the interval starting from 0.05, which was atypical of results from other communities. The distribution disparity across communities might be caused by the differences in the data missingness and/or that the concentrations levels varied across the communities of this study. More anomalies were observed in **Figure 8** in comparison to **Figure**

7, which also aligned with the fact that there were more incomplete data for blood Hg levels than hair Hg levels.

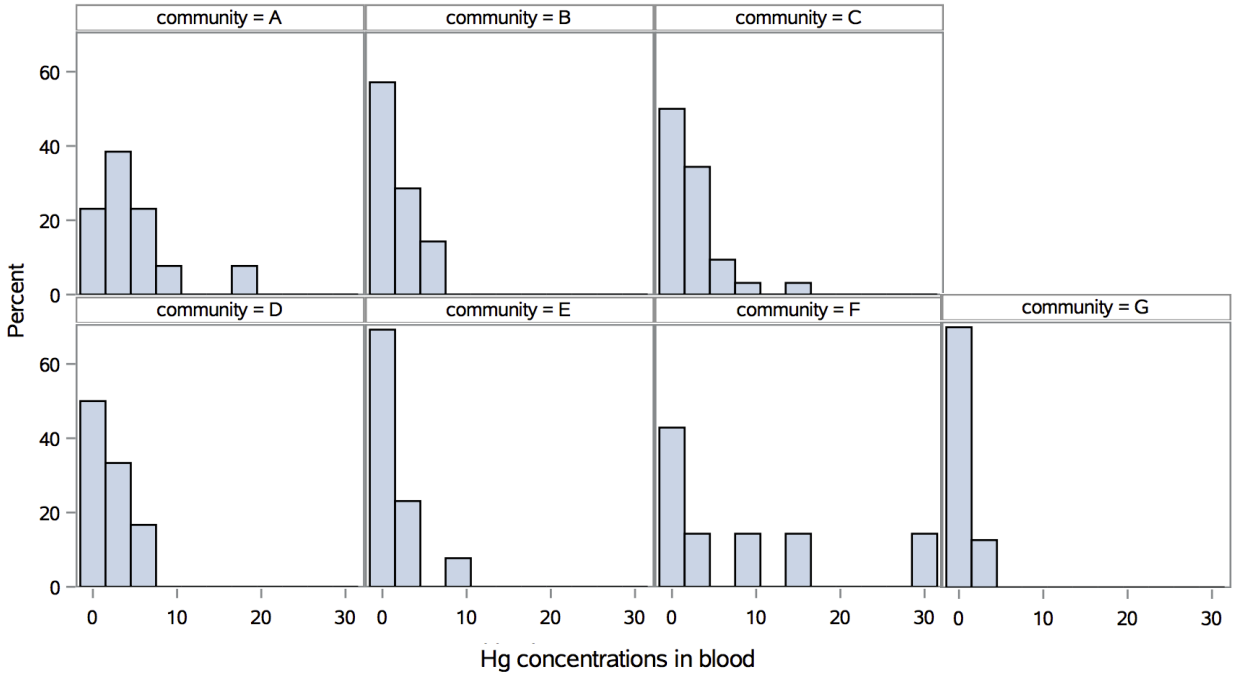

Figure 8: The distribution of blood Hg levels among participants from Dene/Métis communities of the Northwest Territories, Canada, stratified by community

In our exploration of the relationship between hair and blood, the scatter plot (regardless of community) showed a positive but non-linear relationship (**Figure 9**). Although the factors associated with the hair-to-blood ratios remain to be explored, different slopes from CCA were observed across communities (**Figure 10**). As a consequence, if the relationship was explored within the whole study population, different slopes being combined together would lead to a non-linear trend.

When observing the same data by community, we saw a simple and expected (from existing literature) linear relationship (**Figure 10**). However, these plots suggested different slopes and intercepts across communities; for example, community B had the greatest intercept and community G had the steepest slope. These differences might be a result of intrinsic imparity between the communities, but the slopes and intercepts might also be influenced by the missingness of data. For example, the steepest slope for community G could be partially explained by the atypical distribution of blood Hg levels observed in **Figure 8**. The distribution of blood Hg levels for community G was atypical. For example, none of the participants were observed with Hg levels in blood over 5 μg L^{-1}, but a value of over 5 μg L^{-1} in blood Hg levels was observed in all other communities. As was discussed above, the atypical blood Hg levels observed in community G might be caused by the sparsity of data due to missingness. Given the aforementioned anomalies, results of CCA are highly influenced by extreme values in blood and hair because of some influential points which deviated from the linear regression lines. For example, the slope for community C might be greatly influenced by the specific point at the top right. The potential influence of incomplete data on the reliability of CCA results further emphasizes the importance of MI in the study.

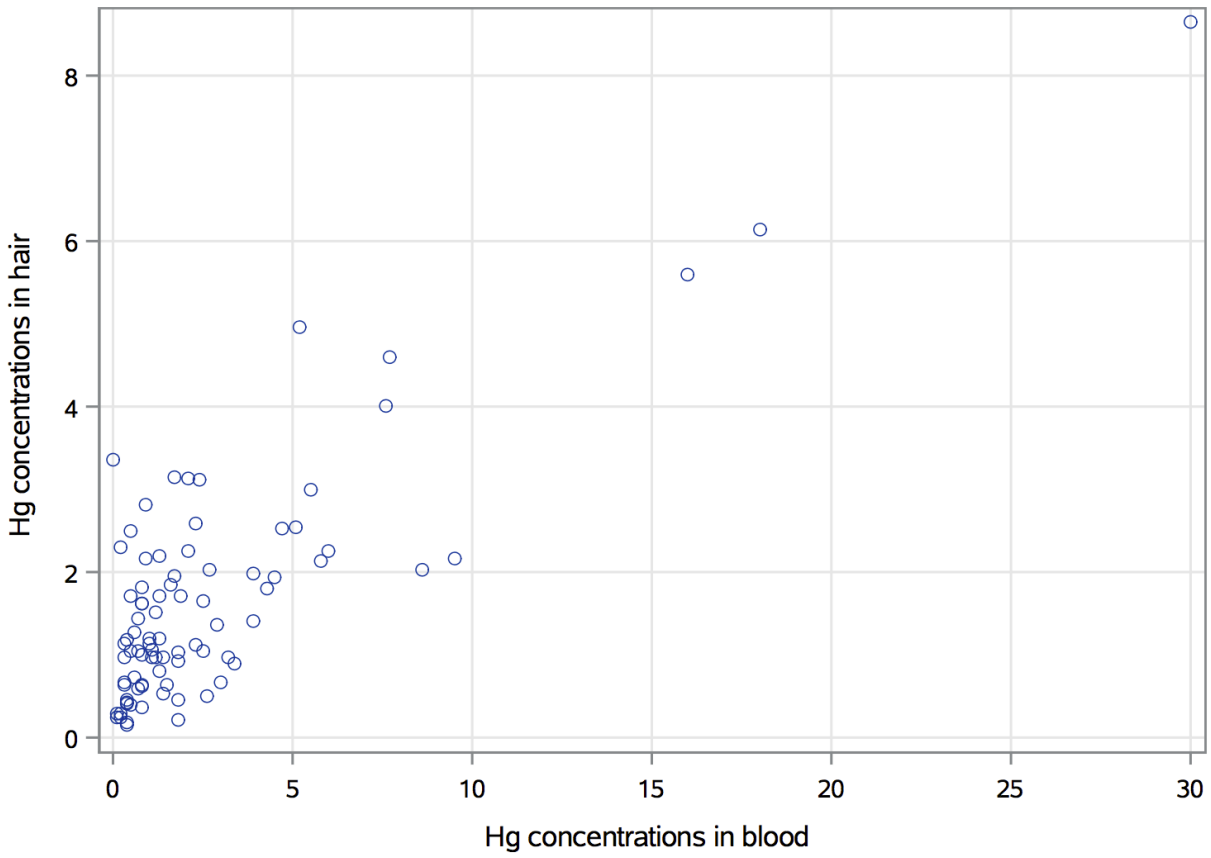

Figure 9: The relationship between Hg concentrations in blood and hair among complete cases from Dene/Métis communities of the Northwest Territories, Canada

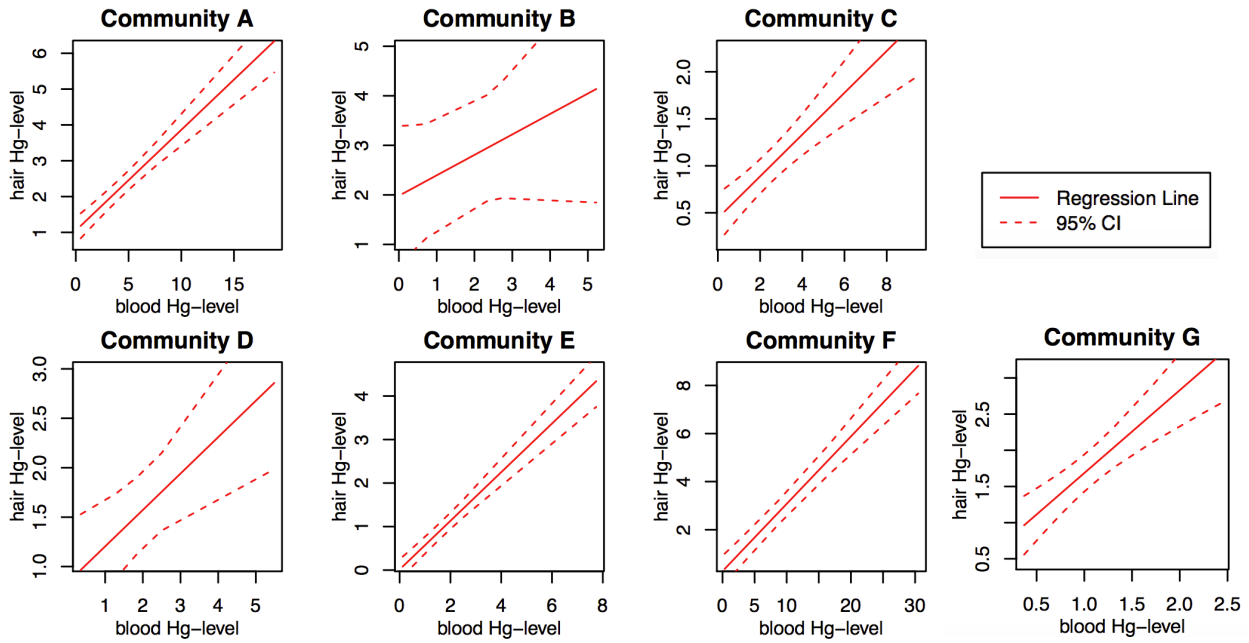

Figure 10: The relationship between Hg concentrations in blood and hair among complete cases from Dene/Métis communities of the Northwest Territories, Canada, stratified by community

Note: The dots representing the individuals were removed in order to protect the confidentiality of information.

5.2 Results for simple linear regression model from CCA

Table 3: Statistics and estimated hair-to-blood Hg level ratios from the simple linear regression model between Hg concentrations in hair and blood among complete cases from Dene/Métis communities of the Northwest Territories, Canada, stratified by community

community	intercept (95%CI)	slope (95%CI)	R^2	p value	hair-to-blood Hg level ratio
A	1.051 (0.684, 1.418)	0.281 (0.223, 0.339)	0.913	<0.0001	281
B	1.982 (0.569, 3.395)	0.413 (-0.171, 0.997)	0.398	0.1362	413
C	0.443 (0.181, 0.705)	0.222 (0.142, 0.302)	0.640	<0.0001	222
D	0.836 (0.209, 1.462)	0.368 (0.135, 0.602)	0.828	0.0120	368
E	0.013 (-0.222, 0.248)	0.559 (0.468, 0.650)	0.949	<0.0001	559
F	0.273 (-0.372, 0.918)	0.280 (0.233, 0.328)	0.979	<0.0001	280
G	0.540 (-0.001, 1.087)	1.146 (0.689, 1.602)	0.623	0.0001	1146

From **Table 3**, we can see that in the CCA there were significant linear relationships between Hg concentrations in hair and blood for all communities except community B ($p=0.1362>0.05$). All intercepts were reported to be positive, ranging from 0.013 to 1.982, and three of them (community E, F, and G) were not statistically different from 0. The slopes for community E and G were reported to be statistically different from the other communities. However, the slopes for the communities other than communities E and G were not significantly different from each other. Again, these intercepts and slopes could be artifact because of the small sample size of complete cases, e.g. these results were highly influenced by extreme values due to sparse data. The hair-to-blood ratios calculated based on coefficient were around 250 (between 220 to 281) for communities A, C, and F. Meanwhile, for communities B, E and G, this ratio values were at least 1.5 times greater than 250. However, the comparison between these results might not be very reliable due to the potential difference in the incomplete data across communities. As the

CCA only analyzed the data above LOD, the ratios estimated from CCA were believed to be biased and relatively unreliable. In addition, the proportions of data below detection limit varied across communities, ranging from 0% to 43.8%. Therefore, the extent to which the ratios from CCA were biased or influenced by <LOD was also believed to vary across communities. However, whether the ratios were systematically underestimated or overestimated was unknown. This is because the ratios were influenced by the Hg levels in hair and blood simultaneously. Also, beside <LOD data, there were missing data in our study. In comparison to the <LOD data, there were more uncertainties of the missing data, in terms of the range of the possible values and the reasons why they were missing. In conclusion, although these results were assumed to be subject to bias, it is difficult to determine how and to what extent these ratios estimated from CCA were biased. In this study, MI did not yield any unreasonable results even though the percentage of unavailable data was very high. However, it is difficult to determine the maximum level of data missingness that MI could handle because it may be influenced by many factors spontaneously, e.g. the representativeness of the complete data and the sample size the completely observed cases. Future studies can examine the performance of MI under different conditions. The R^2 values for communities A, E, and F were very high (above 0.900), and this figure for community B was only 0.398. The influential points might also lead to situations where certain communities had lower R^2 values, especially for the communities with a very small sample size of complete cases, such as community B. Again, these R^2 values estimated from CCA are also likely to be biased and less reliable due to the incomplete data.

5.3 Results for Objective 2: MI using a simple linear imputation model with Hg levels in hair and blood only

Table 4: Comparing R^2 results of CCA and the Empirical R^2 results of SLR analysis model between Hg levels in hair and blood using data sets imputed by the SLR imputation model with Hg levels in blood and hair, stratified by community

community	R^2 from CCA	Empirical statistics from SLR imputation model (Objective 2)	
		median R^2	(25th percentile R^2, 75th percentile R^2)
A	0.913	0.821	(0.783, 0.850)
B	0.398	0.112	(0.055, 0.196)
C	0.640	0.760	(0.717, 0.794)
D	0.828	0.657	(0.508, 0.743)
E	0.949	0.921	(0.902, 0.933)
F	0.979	0.970	(0.960, 0.976)
G	0.623	0.627	(0.584, 0.667)

In comparison to CCA, the sample size after imputation (Objective 2) increased at least 2 times for communities A to G, respectively (detailed information about the sample size was not disclosed in order to protect the confidentiality of communities/participants). The minimum and maximum values of the imputed data in Objective 2 can be found in the Appendix. No extreme or unreasonable values were found within the 1000 imputed datasets. Extreme or unreasonable values were defined as the ones that were below 0 or exceeded the maximum values of the Hg levels within the whole study population.

For community B, the median R^2 from MI was very low (0.112). The MI results for community B was less reliable probably because the association between Hg concentrations in hair and

blood, explained via a line, is insufficient. For other communities, MI median R^2 values were very close to or slightly greater than the R^2 from CCA.

Table 5: Estimates for the SLR analysis model between Hg levels in hair and blood using data sets imputed by the SLR imputation model between Hg levels in blood and hair, stratified by community

community	parameter	PE[1] (95%CI)	SE[2]	t-statistic	p-value[3]	hair-to-blood ratio
A	intercept	0.525 (0.330, 0.720)	0.096	5.465	0.000	328
	slope	0.328 (0.273, 0.383)	0.027	12.090	0.000	
B	intercept	0.671 (0.318, 1.024)	0.170	3.937	0.001	259
	slope	0.259 (-0.109, 0.627)	0.177	1.465	0.157	
C	intercept	0.195 (0.085, 0.305)	0.055	3.548	0.000	270
	slope	0.270 (0.224, 0.316)	0.023	11.799	0.000	
D	intercept	0.253 (0.057, 0.449)	0.089	2.858	0.016	422
	slope	0.422 (0.166, 0.678)	0.116	3.642	0.004	
E	intercept	-0.014 (-0.208, 0.180)	0.089	-0.151	0.882	549
	slope	0.549 (0.460, 0.638)	0.041	13.513	0.000	
F	intercept	0.207 (-0.112, 0.526)	0.144	1.433	0.182	279
	slope	0.279 (0.244, 0.314)	0.016	17.981	0.000	
G	intercept	0.359 (0.194, 0.524)	0.082	4.368	0.000	1138
	slope	1.138 (0.881, 1.395)	0.128	8.890	0.000	

[1]PE denote the point estimate obtained from MI using Rubin's combining rules on imputed data
[2]SE denote the standard error obtained from MI using Rubin's combining rules on imputed data
[3]The p-values used the denominator degrees of freedom estimates as provided by Barnard and Rubin (Barnard et al., 1999)

Table 5 shows results based on an SLR imputation model that used Hg levels in blood and hair only to create imputed data sets. Blood Hg-levels were positively and significantly associated

with hair Hg-levels for all communities except community B. The intercept for community E was negative (-0.014) but not statistically different from zero (p=0.882). For the communities other than community E, the intercepts were all positive, and these intercepts were all statistically significant from 0 (except community F). The slopes for community G were found to be statistically different from the slopes for all other 6 communities (except community B), and the slope for community E was statistically different from the slopes for 5 out of 6 communities (except community B). For communities A, C, and D, the hair-to-blood ratios estimated from CCA were reported to be 14% (281 versus 328), 18% (222 versus 270), and 13% (368 versus 422) lower in comparison to MI model, respectively. For communities E, F, and G, the slopes estimated from CCA were very close to that from MI models. After being compared to the results from MI models, the CCA results for communities E, F, and G seemed to be less unreliable than the CCA results for communities A, C, and D. However, even for communities E, F, and G, MI was still helpful because it largely increased the sample size for data analysis and yielded narrower CI in comparison to CCA. Thus, MI was still recommended for all the communities, even the results from MI were close to that from CCA.

From **Figure 11**, we can see that, the intercepts from MI model were greater than that from CCA for community A, B, C, D, and G. For communities A, C, and D, the slopes for the regression lines from MI model were steeper than that from CCA, indicating that the hair-to-blood ratios from CCA were smaller than that of these communities. For communities E, F, and G, the slopes for the regressions lines from MI model were almost parallel to those from CCA. For these communities, the hair-to-blood ratios from CCA were very close to the figures from MI model.

For communities E and F, the CCA regression lines were within the 95% CI of the MI regression lines, respectively. **Figure 11** clearly demonstrates the superiority of MI over CCA (when it comes to better utilization of incomplete cases) because MI (i) utilizes the incomplete cases (unlike CCA which ignores them); (ii) provides less biased results (through informative extrapolation of incomplete cases); and (iii) provides narrower confidence intervals (compared to CCA) which allows for reliable inference for population parameter of interest. Hence, in our study, MI results are recommended over CCA results.

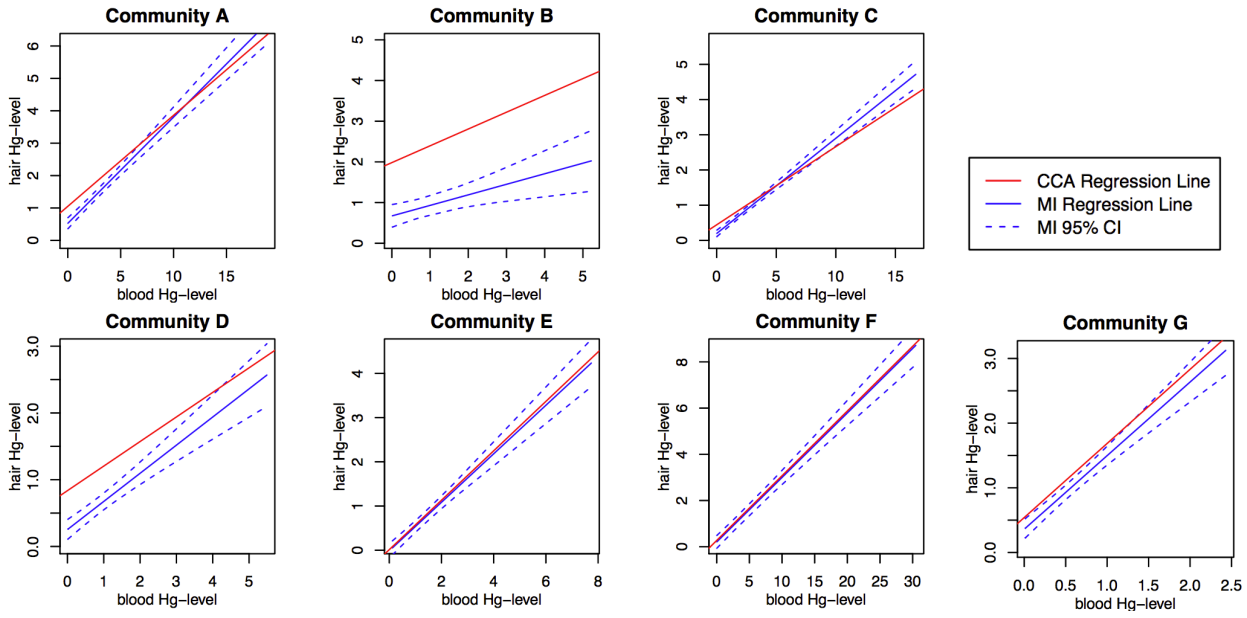

Figure 11: Comparison of the fitted regression lines from CCA and the SLR analysis model between Hg levels in hair and blood using data sets imputed by the SLR imputation model with Hg levels in blood and hair, stratified by community

Note: The dots representing the individuals were removed in order to protect the confidentiality of information.

5.4 Results for Objective 3: MI using a multiple linear imputation model with Hg levels in blood, age, sex, and BMI

Table 6: Comparing R2 results of CCA and the Empirical R2 results of SLR analysis model between Hg levels in hair and blood using data sets imputed by the MLR imputation model with Hg levels in blood and hair, sex, age, and BMI, stratified by community.

community	R^2 from CCA	Empirical statistics from SLR imputation model (Objective 3)	
		median R^2	(25th percentile R^2, 75th percentile R^2)
A	0.913	0.785	(0.719, 0.830)
C	0.640	0.774	(0.731, 0.806)
E	0.949	0.932	(0.915, 0.941)
G	0.623	0.463	(0.394, 0.524)

In **Table 6**, the results for communities B, D, and F were not reported due to extremely low degrees of freedom (between 1 and 2 for these communities). For other communities, the R^2 values for the MI models in Objective 3 were smaller than the figures from CCA or the MI models in Objective 2. However, the R^2 from CCA can be highly influenced by extreme values when data are sparse. In comparison, MI results are less susceptible to extreme value influence because it accounts for the uncertainty of missing values in the imputation, thereby mitigating the impact of extreme values. The minimum and maximum values of the imputed data on Objective 3 can be found in Appendix. No extreme or unreasonable values were found within the 1000 imputed datasets.

Table 7: Estimates for the SLR analysis model between Hg levels in hair and blood using data sets imputed by the MLR imputation model with Hg concentrations in hair and blood, sex, age, and BMI, stratified by community

community	parameter	PE[1] (95%CI)	SE[2]	t-statistic	p-value[3]	hair-to-blood ratio
A	intercept	0.544 (0.295, 0.793)	0.120	4.536	0.000	300
	slope	0.300 (0.223, 0.377)	0.037	7.993	0.000	
C	intercept	0.204 (0.081, 0.327)	0.061	3.321	0.002	243
	slope	0.243 (0.197, 0.289)	0.023	10.699	0.000	
E	intercept	-0.006 (-0.222, 0.210)	0.098	-0.058	0.954	552
	slope	0.552 (0.459, 0.645)	0.042	12.978	0.000	
G	intercept	0.467 (0.193, 0.741)	0.134	3.479	0.002	994
	slope	0.994 (0.549, 1.439)	0.217	4.579	0.000	

[1]PE denote the point estimate obtained from MI using Rubin's combining rules on imputed data
[2]SE denote the standard error obtained from MI using Rubin's combining rules on imputed data
[3]The p-values used the denominator degrees of freedom estimates as provided by Barnard and Rubin (Barnard et al., 1999)

Table 7 shows results based on an MLR imputation model that used Hg levels in hair and blood, age, sex, and BMI to create imputed data sets. The slope for community E was negative but not statistically different from 0 (p=0.954). For the communities other than community E, the slopes were all positive, ranging from 0.204 to 0.544, and were statistically different from 0. Hg concentrations in blood were all positively and significantly associated with Hg levels in hair for all communities. For communities A and C, the hair-to-blood ratios for MI models were greater than that from CCA, which was consistent with the findings in Objective 2. Moreover, after MI imputation, the ratios for community G were less extreme in comparison to the results from CCA and Objective 2, indicating that an MLR imputation model (with known confounders) might be able to better recover missing information than a SLR imputation model (as in Objective 2).

From **Figure 12**, we can see that, for communities A and C, the slopes for MI models were slightly steeper than the slopes from CCA, suggesting that the hair-to-blood ratios from CCA were smaller for these communities. The CCA regression line was within the 95% CI of the MI regression line for community E. A less steeper slope was observed for community G, indicating that the hair-to-blood ratio was less extreme after MI in Objective 3. For community G, covariates better helped MI to address the missingness.

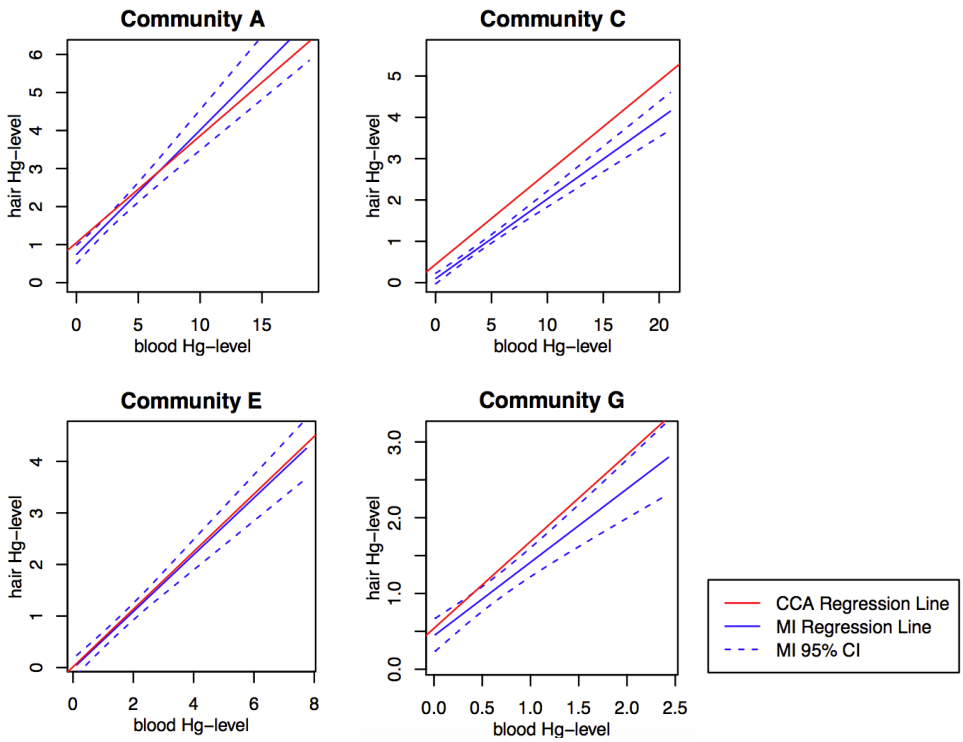

Figure 12: Comparison of the fitted regression lines from CCA and the SLR analysis model between Hg levels in hair and blood using data sets imputed by the MLR imputation model with Hg levels in blood and hair, sex, age, and BMI, stratified by community

Note: The dots representing the individuals were removed in order to protect the confidentiality of information.

6. Discussion

Human biomonitoring is an important tool to quantify environmental exposure to contaminants and assess health risk, supporting the identification and characterization of contaminant exposure and nutrient status. In remote Indigenous communities of NWT, Canada, the ongoing reliance on country food (especially fish) may influence people's exposure to Hg, and biomonitoring projects are thus needed to monitor the change in Hg exposure among these populations. Although similar studies are not scarce, previous studies have not yet fully examined the relationships between Hg concentrations in hair and blood within different Indigenous communities of NWT or how to achieve a more reliable relationship by addressing incomplete data. Understanding this relationship is of great importance because it can be used to improve the strategy of biological sampling and therefore facilitate the long-term large-scale biomonitoring projects that can detect early health risks in such populations.

Previous studies have shown that the relationship between hair and blood Hg-levels is linear (Budtz-Jorgensen et al., 2004; Liberda et al., 2014), with a hair-to-blood Hg concentration ratio of 250 (Joint FAO/WHO Expert Committee on Food Additives, 2004; United States Environmental Protection Agency, 2001; World Health Organization, 1990). Our findings from CCA also suggested a linear relationship between hair and blood Hg-levels when stratified by communities. Of the seven communities sampled, three communities reported hair-to-blood Hg concentration ratios (from CCA) to be around 250 (222 to 280), which was consistent with the findings from WHO and US EPA (United States Environmental Protection Agency, 2001; World Health Organization, 1990). The ratios from CCA for other three communities in this study were

greater than 250 (between 368 and 559), which was also in line with the results from other literature (Liberda et al., 2014; Yaginuma-Sakurai et al., 2012). However, one community in this study reported the ratio (from CCA) to be 1146, which was much greater than the figure reported by other studies (Liberda et al., 2014; United States Environmental Protection Agency, 2001; World Health Organization, 1990; Yaginuma-Sakurai et al., 2012). Since the sample size of complete cases was not large at a community level in this study, the ratios were likely to be influenced by a few participants with extreme values of Hg concentrations. For example, after excluding the participant with most extreme values of Hg levels (the participant with the highest Hg concentration in blood) from community C, the ratio from CCA for community C was changed (in a biased way) from 222 to 278. In addition, it is sometimes difficult to compare the results in the current study to previously published studies due to improved detection limits (Dumont et al., 1998). Also, the detailed information pertaining to the missingness of data, e.g. the proportion of the <LOD or missing data, was sometimes not reported in previous studies (Budtz-Jorgensen et al., 2004; Liberda et al., 2014; Yaginuma-Sakurai et al., 2012). Last, the methods to handle incomplete data also varied in different studies. All these differences greatly complicate the task of comparing the ratios from different studies.

The intercepts for communities A, B, C, and D were positive and statistically different from 0 in **Table 3**, which was not expected (when the blood Hg levels equal to zero, the hair Hg levels theoretically equal to zero). Also, many of the intercepts are statistically different from each other in this study. These could be caused by several reasons. First, the hair samples in our study could, at least in theory, be contaminated by external Hg. Follow-up analysis is warranted to

eliminate this possibility. Second, it may also be related to biological reasons. Intercepts not equaling to zero were reported by many of the studies which explored the association between Hg concentrations in hair and blood (Bonsignore et al., 2016; Budtz-Jorgensen et al., 2004; Liberda et al., 2014). However, from biological perspective, Hg levels in both blood and hair can never theoretically reach zero. The Hg metabolism in the body may also be different under such extreme condition, i.e. when the Hg levels are close to 0 or extremely low. The relationship between Hg levels in hair and blood may also not be linear when either one is approaching zero. Therefore, whether we can use a linear relationship to estimate hair Hg levels when forcing blood Hg levels to be zero needs to be explored. Another possible reason is related to the differences in seasonal patterns of fish consumption across communities. For example, for communities where fish consumption is particularly seasonally dependent, blood Hg levels may appear to taper more quickly than hair Hg levels due to the time lag of hair Hg accumulation. As a consequence, these communities may yield a higher intercept. On the flip side, for communities with a fish consumption pattern that is less seasonally variant, blood Hg would likely decrease less with time, lowering the impact of time lag on the intercept. Further studies can examine this topic from a biological perspective. Beside intercepts being different from 0, some intercepts were also statistically different from each other across communities in **Table 3**.

In this study, associations between Hg levels in hair and blood were found to be linear by communities but non-linear for the whole study sample. This suggests that, when researchers are exploring objectives similar to ours, they should consider stratification. Through stratification by community, the CCA yielded hair-to-blood ratios ranging widely from 250 to 1146. This finding

indicates that the WHO ratio of 250 may not be applicable to all the Indigenous populations of NWT, Canada. For long-term Hg exposure biomonitoring projects amongst Indigenous populations, researchers should strive to establish site-specific ratios after stratifying dataset according to community feedback. In addition, particular care is warranted when atypical hair-to-blood ratios are noted. For example, in this study, the ratio was reported to be over 1000 for one community, which was much higher than the results of any other communities in this research. Individual level risks could be particularly overestimated in communities with such high hair-to-blood ratios. The wide range of hair-to-blood ratios across Indigenous communities of NWT also indicates that any attempts to group or combine subpopulations for further analysis on the ratios should be done with caution. Grouping communities with vastly different hair-to-blood ratios may lead to a non-linear relationship between Hg levels in hair and blood for the whole population. However, such attempts can be worthy in the biomonitoring projects amongst Indigenous communities of NWT. Clustering subgroups or communities can be one of the initial steps to form hypotheses about which factors are most important in determining or influencing the relationship between Hg concentrations in hair and blood within those Indigenous communities. However, the communities to be clustered should have similar intercepts and slopes in order not to violate the assumption of linear relationship for the linear regression model. Future research can combine subgroups or communities based on data driven analysis, or information on dietary patterns (especially fish consumption), geography of environments across subgroups or communities, etc.

To better utilize hair analysis to assist in the Hg measurement in blood for the biomonitoring projects among Indigenous populations of NWT, it is crucial to first establish a more reliable and robust relationship between Hg levels in hair and blood. In other biomonitoring studies, the <LOD cases are usually handled via case or record deletion or replacement by a fixed value (such as 1/2 of the LOD). However, the method of deletion is not recommended because it results in loss of resources used to collect data and possibly causes biased. The replacement by a fixed value grossly underestimates the variance of an estimator, and a fixed value cannot take into account the uncertainty about the prediction of the missing values. Both of these widely used methods may yield biased or unreliable results within biomonitoring projects, although the extent to which those results are biased or unreliable may vary across different cases. A better alternative to handle incomplete data is via Multiple Imputation, which does not use a fixed value for any missing cell in the data and accounts for additional uncertainty (i.e. variance) due to missingness. In addition, MI will increase the sample size for analysis and consequently decrease the width of confidence intervals. This study contributes to this identified research gap by being the sole study that utilized MI to address the incomplete data in a Hg exposure biomonitoring project.

Most implementations of MI assume data are missing completely at random (MCAR) or missing at random (MAR) (White et al., 2010). Data are rarely missing completely at random in a real-world research, and our study is assumed not to be an exception. Missing at random is more plausible in this study because the reasons why the participants refused to provide their blood samples seemed not to be associated with the blood Hg levels. In future biomonitoring projects,

there may be possibility that the data will be missing not at random (MNAR). In biomonitoring studies of this topic, MNAR refers to situations where participants refuse to provide biological samples, and such missingness is associated with Hg level itself. For example, participants who eat a lot of wild-harvested fish (and potentially have higher levels of Hg) may have been on the land rather than in the community during the sample collection period. If the data are not missing at random, MI will yield biased results and will not be recommended (Hughes et al., 2019). As a consequence, researchers should define the missing data mechanism before the data imputation method is determined.

Although the results from CCA were considered biased due to missingness, the extent to which they were considered biased and how they were biased were different across communities. For example, in **Figure 11**, the intercepts from CCA were greater in comparison to the intercepts from MI models for communities B and D, respectively. For other communities, the intercepts appeared to be less unreliable and not too different from the intercepts from the MI models. For communities E, F, and G, the slopes from CCA were almost parallel to those from MI models and thus were considered to be reliable. For communities A, C, and D, the slopes from CCA were steeper than those from MI models, which indicated that the ratios from CCA were smaller in comparison to MI models. These results in our study were consistent with the findings from another study which reported that there are situations where CCA analyses are unbiased (Hughes et al., 2019). However, MI is also discovered to be valid for all MAR situations (no matter whether CCA results are reliable) and has the potential to reduce bias and improve precision (Hughes et al., 2019). Thus, MI was preferred over CCA in our study, no matter whether the

CCA results were close to the MI analyses or not. Using MI in our objective 2, we found that the hair-to-blood ratios from CCA were 13% to 18% smaller in comparison to the estimates from MI for half of the communities studied. This finding reveals that the hair-to-blood ratios yielded from CCA are possibly biased in this study. Therefore, it is recommended to apply techniques, e.g. MI, to recover the incomplete data before the estimation or the application of the hair-to-blood ratio.

The hair-to-blood ratios for community G were reported to be 1146 from CCA and 1138 from the MI model in Objective 2, much greater than the ratios for other communities. However, in Objective 3, this ratio for community G was reported to be 994, less extreme than the ratios reported by CCA or the MI model in Objective 2. It implied that MI model with covariates might better address anomalies in missing or incomplete data than MI model based on a simple linear regression model. However, it should also be noted that, although the ratio yielded from Objective 3 was 13% lower than that from CCA, this decrease is not necessarily biologically significant. This ratio of 994 estimated from Objective 3 was still considered atypical. As such, using the generally-assumed ratio of 250 would be particularly problematic for this community. Further examination is needed to explore the reasons why this community yielded such an atypical ratio. From a biological perspective, the Hg concentrations in hair and blood can be influenced by age (Clarkson, 1997; Dickman et al., 1998; Nakagawa, 1995; Pellizzari et al., 1999), sex (Benson et al., 1972; Kosatsky et al., 2000; Mortada et al., 2002; Olivero et al., 2002), and BMI (Gao et al., 2018; Moon, 2017). Humans accumulate Hg in their body through the consumption of fish and other marine organisms (Clarkson, 1997): a positive relationship

between Hg levels and age has been reported by several studies (Dickman et al., 1998; Nakagawa, 1995; Pellizzari et al., 1999). A consistent positive association between BMI and Hg levels was also observed (Gao et al., 2018; Moon, 2017). Studies have also demonstrated possible differences in the toxicokinetics of MeHg between males and females in the blood (Grandjean et al., 1997), and experimental data has also proven interactions between MeHg and sex hormones (Hirayama et al., 1987). Studies in kidney donors showed a three times higher renal Hg levels in females (Barregard et al., 1999), and a higher urinary Hg excretion in females than males was also observed in another study (Bates, 2006). Higher hair Hg levels in females than in males have been reported in many researches (Benson et al., 1972), but this finding was inconsistent across studies (Kosatsky et al., 2000; Mortada et al., 2002; Olivero et al., 2002). Failure to take into account the effect of fish consumption (with stratification for fish consumption frequency) and of other potential confounders may have biased the results of most of the studies that examined sex-specific differences in Hg levels, so no definitive conclusion can be drawn so far (Elhamri et al., 2007). In addition, the dependence of Hg concentrations on age is also possibly related to the greater fish consumption in males, which leads to an input rate higher than the Hg excretion rate with consequent age-related accumulation (Elhamri et al., 2007). Therefore, fish consumption frequency may an important factor influencing these relationships and thus is recommended to be collected and analyzed in all such studies of mercury exposure. Information pertaining to fish consumption frequency can be collected by questionnaire. In addition, fish consumption amount and Hg concentrations in fish are both promising factors to be explored in the association between Hg levels in hair and blood. The information of these variables can also be collected and incorporated into future studies. In this

study, for communities A, C, and E, covariates including age, sex, and BMI did not provide much information in the imputation of missing or incomplete data in Objective 3. However, this does not imply that covariates cannot further improve the imputation of partially observed cases. In this study, covariates' contribution to the imputation may be limited by the small sample size of complete cases at the community level for our study population or the absence of other variables, e.g. fish consumption frequency. In this biomonitoring project, selenium concentrations in blood were also collected. Biological interaction between MeHg and selenium was observed in human bodies, and selenium and Hg are assumed to have an effect on each other's bioavailability (Raymond et al., 2004). Therefore, selenium concentration is a potential confounder to explore in future studies. The role of covariates/confounders in the imputation model can be further explored in a study with a larger sample size of complete cases. Future studies can also examine other relevant variables from the biological and toxicology perspectives and explore their potential contribution to the imputation model of MI.

Given the aforementioned contribution from use of MI, it is not without restrictions and limitations. In this study, the sample size of complete cases was approximately 10 for several communities: MI is not a suitable solution for handling incomplete data for communities with such a small sample size†. If a complex imputation is proposed, researchers should ensure that the sample size of CCA is large enough to provide reliable estimates to begin the imputation process of recovering information for the incomplete cases. Our results found the imputation to be stable when the degree of freedom (to estimate error variance) was at least 4. Since the sample

† The small sample size results in small degrees of freedom to reliably estimate the variance.

size at a community level is not supposed to be too small for a broad-scale Hg exposure biomonitoring project, MI technique oughts to address missingness of data more effectively than CCA in those projects. Another limitation is about the assumption regarding extreme or unreasonable values in this study. Although there were no imputed values that exceeded the maximum values of the Hg levels within the whole study population, such values were considered unreasonable in this study. However, this assumption remains to be tested in future study.

With a more reliable relationship established between Hg levels in hair and blood, longitudinal Hg analysis may be possibly conducted on hair data alone. The strategy of biological sampling for the biomonitoring projects in these specific populations should adjust to the fact that people's Hg exposure levels in NWT remain typically low (Ratelle et al., 2018b). Compared to blood sampling, hair is more efficient, practical, and economical in long-term large-scale biomonitoring projects, especially among populations under comparatively low risk to Hg poisoning. Nevertheless, blood samples are still necessary and important because the estimation of blood Hg level based on hair Hg concentration is not reliable at the individual level (Liberda et al., 2014). In addition, blood Hg level can provide helpful and accurate information when it comes to specific clinical purposes, such as diagnosis of Hg toxicity, etc. Thus, when it comes to human safety concerns, especially for those who rely on a subsistence diet that contains fish, blood Hg measurements should be included as part of a rigorous biomonitoring project (Liberda et al., 2014). In this biomonitoring project, Hg concentrations in urine were also collected. Urinary tHg levels mainly reflect exposure to Hg^0 and are suitable to assess long-term tHg

exposure to Hg^0 vapor (Kristensen et al., 2013). Thus, urine sampling may be more suitable to estimate the Hg exposure among populations who are frequently exposed to an elemental or inorganic source of Hg, like small-scale gold miners (Kristensen et al., 2013; Schulz et al., 2007). Thus, urine Hg levels were not analyzed in this study as this study mainly focused on more general populations.

Ethical concerns pertaining to data recovery should also be taken into consideration. This is not a major issue for this study because the imputed values were exclusively reported in aggregate via data summary and results/analyses. Moreover, the data were recovered only for those who offered consent to provide information regarding their Hg exposure. For example, in this study, the blood Hg levels were only imputed (not recovered) based on the value of hair Hg levels, and vice versa. The imputation was done in this study also because it can help us better understand and answer related questions, e.g. how people are exposed to mercury in these communities and what can be done to keep exposures low. These questions warrant understanding the determinants and sources of exposure in depth. However, the missingness in the data makes it a lot more difficult to explore these topics. Therefore, MI was necessary in our investigation. Nevertheless, data imputation would pose ethical issues should it be used to generate sensitive personal information, (e.g. income, ethnicity, health status) that they do not consent to provide. The sensitive information is missing at the first place possibly because the participants do not want to provide any relevant information. Researchers should be careful about potential ethical issues when it comes to the recovery/imputation of sensitive information.

To the best of our knowledge, this study is the first research to apply MI technique to address the incomplete data in a Hg exposure biomonitoring project within Indigenous populations of NWT. In our biomonitoring project, the proportion of blood Hg level below LOD was reported to be 26.2% (on average) and the proportion of incomplete data was 81.3% for the whole study sample. The results of this study provided baseline information about the LOD issue in biomonitoring projects in Indigenous communities of NWT. In addition, we applied MI technique to several communities, which allowed us to examine the reliability of this method in different situations. These findings can also provide initial and preliminary guidelines for further application of MI technique in future biomonitoring projects.

Though this study has provided numerous insights, there are limitations. First, this study did not include all potential confounders. The possible confounders included in our research were sex, age, BMI, and region. Other factors that we did not include can be fish consumption, cultural customs, hair colour, etc, which still remain to be explored in future studies. As was discussed above, the information pertaining to diet behaviour, especially fish consumption frequency is recommended to be incorporated in future studies (this biomonitoring project had collected the data of fish consumption frequency). Fish consumption frequency is also recommended as one of the follow-up investigations for our biomonitoring research in the NWT using the data collected in 2016-2018. In addition, for the age variable, study reported that the median of hair-to-blood ratios were different between 7-year-old individuals and those who are greater than 14 years old (Budtz-Jorgensen et al., 2004). This is because the average blood Hg level doubled between the ages of 7 and 14 years old, but the Hg level in hair only increased by a little over 50% (Budtz-

Jorgensen et al., 2004). In this study, only those who were greater than age 6 were recruited, thus we did not have enough participants in the 7-year-old group for further analysis. Also, as stated previously, the hair-to-blood ratio calculated based on the slope regression coefficient fails to take measurement error in both matrices and thus relies on the choice of the dependent variable, which may cause inaccurate results for the imputation model (but not for the analysis model). Structural Equational Modeling (SEM) is suggested to address this issue. However, in this study, there may not be enough variables or sufficient sample size to successfully assess unobservable 'latent' constructs. Therefore, a linear regression model was used as a fundamental method to explore the Hg levels in hair and blood in this study.

7. Conclusions

This thesis project established linear relationships between Hg concentrations in hair and blood across communities in Dehcho and Sahtu regions of NWT. The hair-to-blood ratios estimated from CCA ranged from around 250 to as extreme as 1146 across communities. Multiple imputation technique could be an effective tool to address the incomplete data and achieve less biased results in this study. For most communities, MI increased the sample size by at least twice when compared to CCA. The ratios estimated from CCA were also found to be 13 to 18% smaller than the ratios from MI models for half of the communities. In addition, MI model with covariates could better address the missingness, especially for the communities yielding atypical results. However, MI does require some assumptions for its application in our study, which includes (but is not limited to) linearity, consideration on degrees of freedom in the imputation model, inclusion of auxiliary variables, etc. Further research can explore these assumptions of MI for application in biomonitoring projects.

In summary, with the well established linear relationships between Hg concentrations in hair and blood across communities, future biomonitoring projects can better utilize hair analysis to assist in the Hg measurement in blood, thus decreasing the demand for the expensive and invasive procedure of blood sample collection. In this study, the proportion of incomplete data was found to be very high in all communities. Without addressing the incomplete data appropriately, the results will be biased. MI can be a promising method to handle the missing data and the data below detection limit. With MI, we may be able to achieve less unreliable or biased results in the biomonitoring projects. In addition, MI can be particularly necessary in those studies that aim to

extrapolate the past blood levels by hair-to-blood ratios and segmental hair mercury levels. In these studies, to achieve less biased results, a more reliable relationship between Hg concentrations in hair and blood is essential and vital. Thus, the MI technique can be very helpful and necessary in some human biomonitoring projects.

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9. Appendix

Table 1: Mercury reference guidelines

Organization	Matrices	Reference Level	Description
WHO (WHO/ UNEP DTIE Chemicals Branch, 2008)	blood	5 to 10 μg L^{-1}	The normal mean concentration of total Hg among individuals with no consumption of contaminated fish
WHO (WHO/ UNEP DTIE Chemicals Branch, 2008)	hair	1-2 μg g^{-1}	The normal mean concentration of total Hg among individuals with no consumption of contaminated fish
Health Canada (Health Canada, 1979)	blood	5 μg L^{-1}	Normal concentrations of Hg
Health Canada (Health Canada, 1979)	hair	10 μg g^{-1}	Normal concentration of Hg
Health Canada (Health Canada, 1979)	blood	100 μg L^{-1}	The maximum safe concentration limit
Health Canada (Health Canada, 1979)	blood	200 μg L^{-1}	Associated with the onset of irreversible neurological symptoms
Health Canada (Legrand et al. 2010)	blood	8 μg L^{-1}	A harmonized provisional interim blood guidance value based on the existing provisional Tolerable Daily Intake for children, pregnant women, and women of childbearing age

Table 2: Diagnostic of the imputed data for the Hg levels in blood

community	(min, max)* in Objective 2	(min, max)* in Objective 3
A	(0, 20)	(0, 25)
B	(0, 30)	NA
C	(0, 30)	(0, 35)
D	(0, 20)	NA
E	(0, 10)	(0, 10)
F	(0, 35)	NA
G	(0, 5)	(0, 5)

*(min, max) was reported in an increment of 5 in order to protect the privacy of data

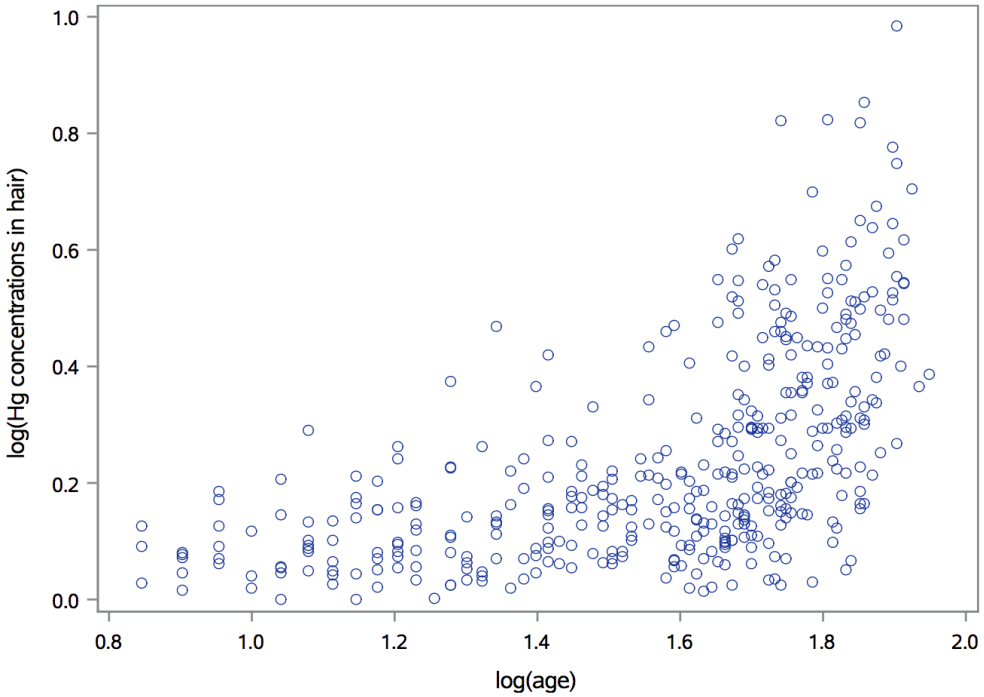

Figure 1: The relationship between log-transformed age and log-transformed Hg concentrations in hair among participants from Dene/Métis communities of the Northwest Territories, Canada

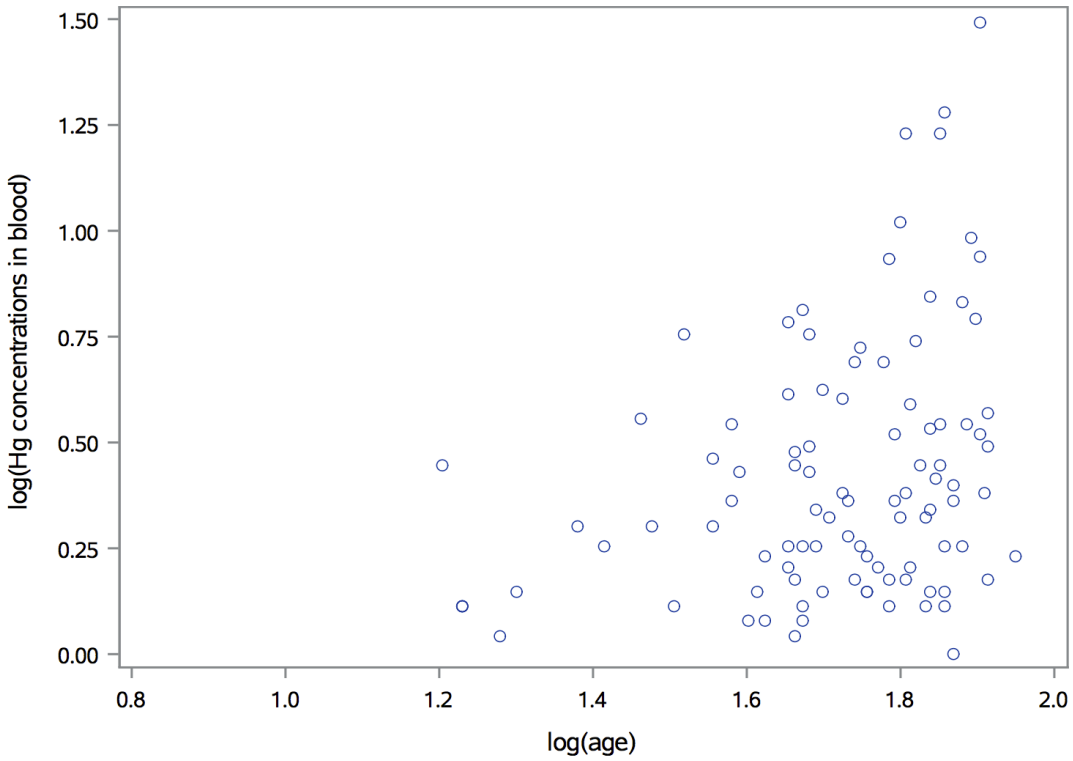

Figure 2: The relationship between log-transformed age and log-transformed Hg concentrations in blood among participants from Dene/Métis communities of the Northwest Territories, Canada

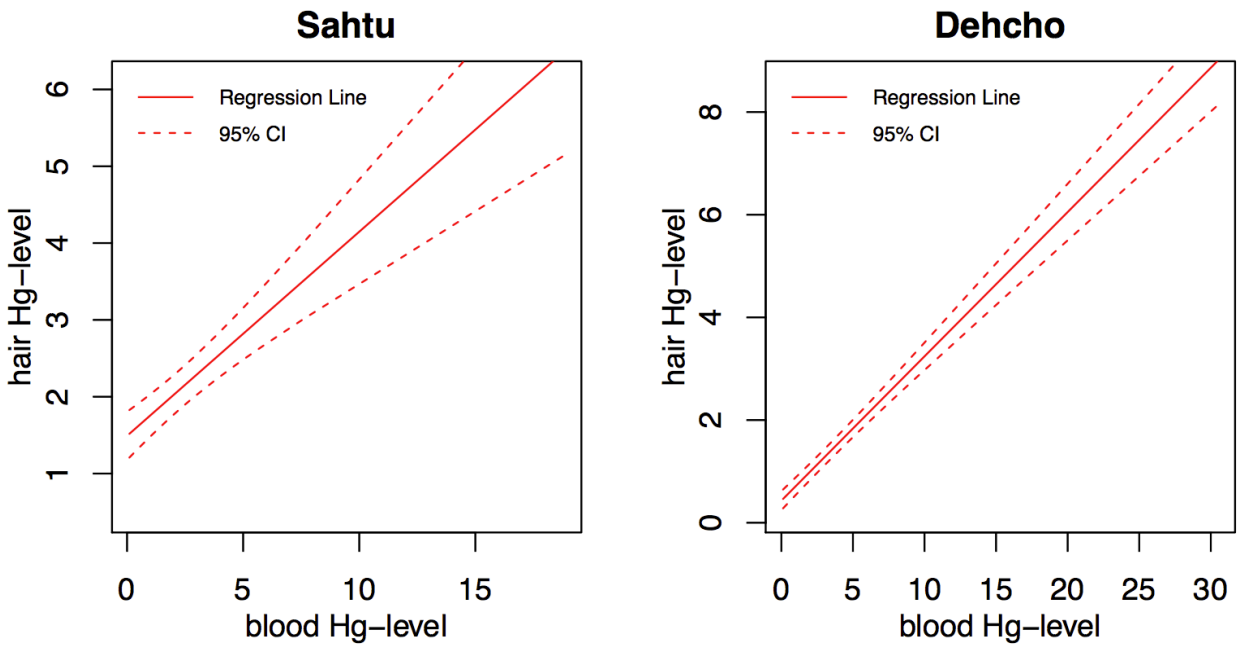

Figure 3: The relationship between Hg concentrations in blood and hair among complete cases from Dene/Métis communities of the Northwest Territories, Canada, stratified by region

Note: The dots representing the individuals were removed in order to protect the confidentiality of information.

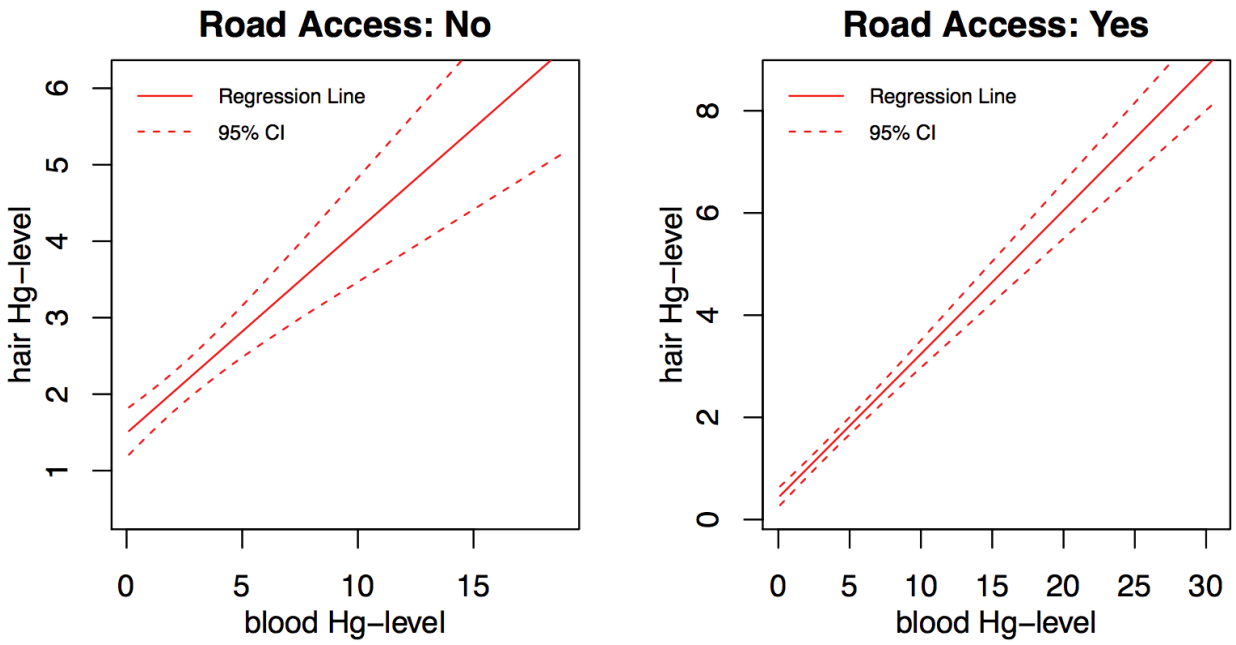

Figure 4: The relationship between Hg concentrations in blood and hair among complete cases from Dene/Métis communities of the Northwest Territories, Canada, stratified by road access

Note: The dots representing the individuals were removed in order to protect the confidentiality of information.

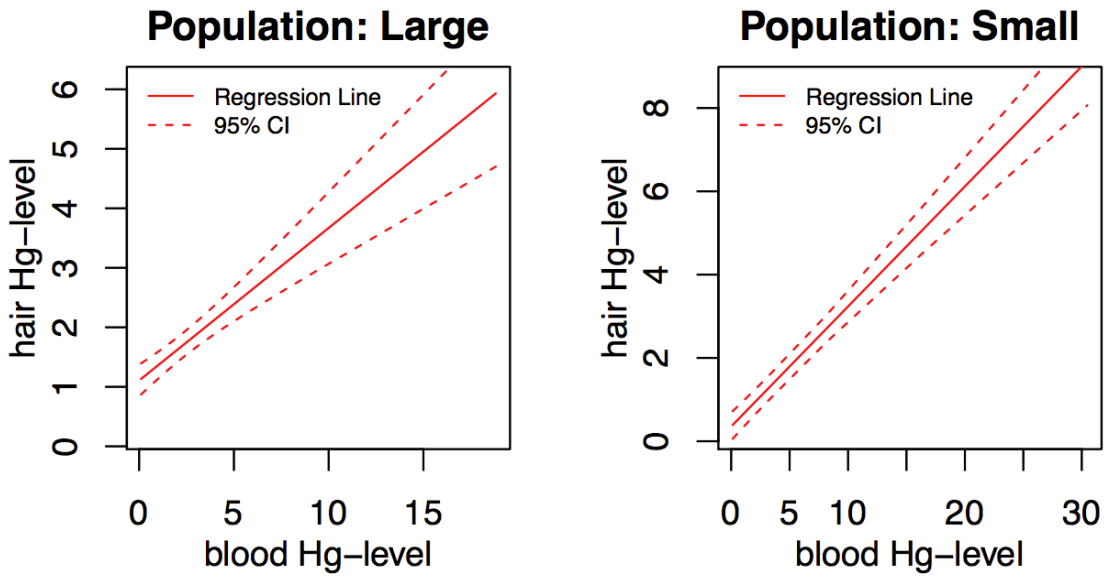

Figure 5: The relationship between Hg concentrations in blood and hair among complete cases from Dene/Métis communities of the Northwest Territories, Canada, stratified by population size
Note: The dots representing the individuals were removed in order to protect the confidentiality of information.

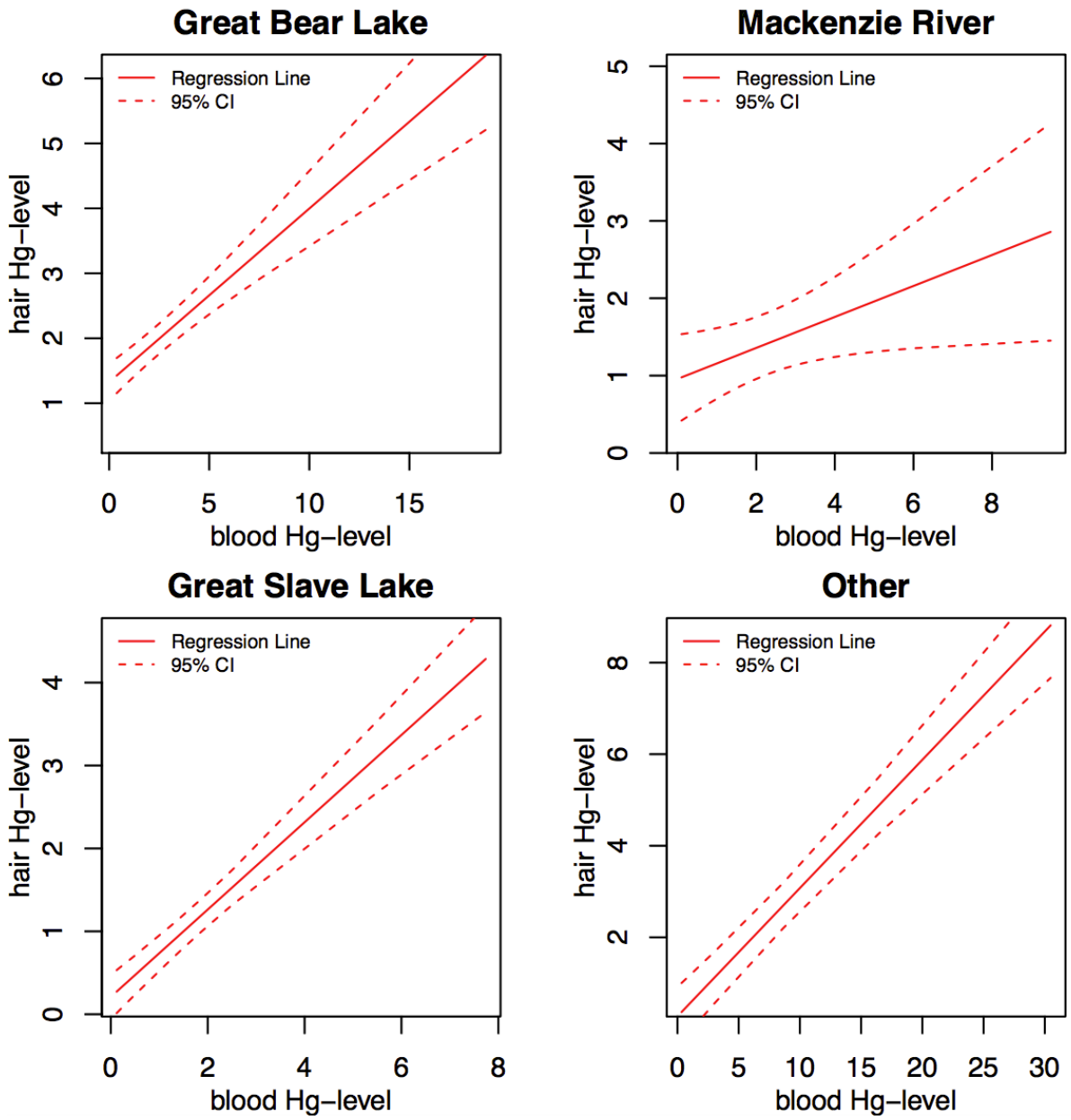

Figure 6: The relationship between Hg concentrations in blood and hair among complete cases from Dene/Métis communities of the Northwest Territories, Canada, stratified by waterbody profile

Note: The dots representing the individuals were removed in order to protect the confidentiality of information.