Arsenic biomonitoring in the human population of Yellowknife, Dettah and Ndilo, Northwest Territories

Application of urine and toenail arsenic biomarkers

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Abstract

Arsenic is a natural but toxic element considered by the World Health Organization to be a chemical of major public health concern globally. Biomarkers are a tool used to understand exposure to chemicals (e.g., arsenic) and their impacts on the health of a population. In Yellowknife, past mining activities have resulted in a toxic legacy of arsenic impacting the surrounding environment. However, current human exposure levels in the area are largely unknown. In this thesis, I demonstrate how different biomarkers can be used to evaluate arsenic exposure and associated health effects in the human population of Yellowknife and the First Nation communities of Dettah and Ndilo.

First, I established baseline levels of arsenic exposure within the Yellowknife population by measuring urinary arsenic concentrations via speciation analysis. I also used general guidance values for urinary arsenic to assess arsenic exposure and health risks in Yellowknife compared to the general Canadian population. Children in Yellowknife had significantly higher urinary inorganic arsenic concentrations than Yellowknife adults and children in the general Canadian population. Second, I expanded on the baseline arsenic exposure levels by analyzing toenail arsenic via speciation analysis, demonstrating that toenail arsenic reflects a different exposure period than urine and may potentially indicate external environmental sources. Then, I evaluated two proteins, KIM-1 and CC16, as candidate biomarkers of effect for detecting lung and kidney impairment in children from exposure to arsenic and other contaminants (e.g., lead and manganese). Finally, I reviewed past archival human arsenic data to understand historical arsenic exposure and how past exposures compare to current levels.

I have demonstrated that different biomarkers can contribute to understanding arsenic exposure and its subsequent impacts on the Yellowknife population and other populations with similar environmental health concerns. Additionally, the results of my research contribute to public health policy and decision-making.

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List of Abbreviations

Adj. R^2	Adjusted R^2
AIC	Akaike information criterion
ANOVA	Analysis of variance
AM	Arithmetic mean
As	Arsenic
As3MT	Arsenic (III) methyltransferase
AsIII	Arsenite
AsV	Arsenate
BE	Biomonitoring equivalent
CC16	Club cell secretory protein
CHMS	Canadian Health Measures Survey
CIRNAC	Crown-Indigenous Relations and Northern Affairs Canada
COPC	Chemicals of potential concern
DMA	Dimethylarsinic acid
GM	Geometric Mean
GMOB	Giant Mine Oversight Body
GNWT	Government of Northwest Territories
KIM-1	Kidney injury molecule-1
IARC	International Agency for Research on Cancer
iAs	Inorganic arsenic
iAs-met	Inorganic-related arsenic (AsIII + AsV + MMA + DMA)
ICP-MS	Inductively coupled plasma mass spectrometry
INSPQ	Institut national de santé publique du Québec
LANSET	Laboratory for the Analysis of Natural and Synthetic Environmental Toxicants
LC-ICP-MS	Liquid chromatography inductively coupled plasma mass
ln	Natural logarithm
LOD	Limit of detection
MMA	Monomethylarsonic acid

Mn	Manganese
NSMA	North Slave Métis Alliance
NWT	Northwest Territories
Pb	Lead
RV95	Reference value
SD	Standard deviation
WHO	World Health Organization
YKDFN	Yellowknives Dene First Nations
YKHEMP	Health Effects Monitoring Program

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1. Introduction

1.1. General Introduction

Arsenic is among the top 10 chemicals of major public health concern according to the World Health Organization (WHO), predominantly due to its contamination in drinking water [1]. Elevated arsenic exposures have been identified in regions of countries such as Bangladesh, Chile, India, Mexico, Taiwan, and the United States [2–7]. While arsenic levels in Canada are generally low, certain regions may have environmental levels above background, such as in Atlantic and Northern Canada [8]. In Yellowknife, Northwest Territories (NWT), arsenic is a public concern due to the local geology and past mining legacies, most notably Giant Mine. Giant Mine was an inactive gold mine located within the city's boundary that was in operation from 1949 to 2004 [9]. Mine operations were predominantly underground as gold was extracted from arsenopyrite ores through a roasting process which resulted in arsenic trioxide as a by-product. Despite the mine's closure, it has left a toxic legacy of 237,000 tonnes of arsenic trioxide stored in its underground chambers. Consequently, the mine has been regarded as one of Canada's most expensive contaminated sites to remediate [10]. This has led to public concerns about potential exposure of arsenic and other chemicals from the mine site via surface runoff and groundwater migration [11]. Legacy-contaminated sites like Giant Mine can result in lasting impacts not only on the surrounding environment but also on the human population. Thus, it is important to establish arsenic levels in the local population to ensure that arsenic exposure does not negatively impact people's health.

Biomonitoring, defined as the measurement of chemicals or their metabolites or induced biochemicals in biological tissues, is an important tool to assess populations' exposures and health risks. For my Ph.D. thesis, I explored using different human biomarkers to assess arsenic

exposure and its potential health impact on the population of Yellowknife. The main research questions are: how can biological markers in our body be used to understand arsenic body burden? What information do different biomarkers provide, and how can they be utilized in biomonitoring/population health? Are there any biomarkers in the body (e.g., proteins, molecules) that can inform us of arsenic-related health effects? How can biomarkers be used to understand exposures of the past and present? This thesis aims to investigate the impact of arsenic exposure in the human population of Yellowknife using a biomonitoring approach, in which arsenic will be assessed through different biomarkers. Through this thesis, I aim to better understand the complex relationship between arsenic and its impact on a human population.

1.2. Background Information on Arsenic

1.2.1. General information

Arsenic (As) is a metalloid element found naturally in the Earth's crust as a component of minerals and rock [12]. It is released into the environment through both natural processes (e.g., volcanic activity and weathering) and anthropogenic activity (e.g., mining and smelting). These releases can lead to the contamination of nearby waters (ground and surface), soil, foods, and air, which can impact the surrounding ecology and populations.

In the environment, arsenic is present in organic and inorganic forms. Arsenicals are often classified based on this characteristic due to their differences in toxicity, with inorganic arsenic considered the more toxic form. Inorganic arsenic (iAs) species include trivalent arsenite (AsIII) and pentavalent arsenate (AsV), which may be found in minerals, rocks, and water [13]. Organic forms (e.g., arsenosugars, arsenolipids, arsenocholine, and arsenobetaine) are often found in nature in algae, shellfish, and fish.

1.2.2. Source and routes of exposure

The sources of human exposure are predominantly drinking water, diet, and occupational exposure. Arsenic contamination in drinking water is a global issue as an estimated 140 million people worldwide are exposed to drinking water with arsenic concentrations exceeding the WHO guideline value of 10 μg/L [14]. The primary dietary sources of arsenic are seafood and rice [15]. Seafood, in particular, is a significant source of organic arsenic species like arsenobetaine and arsenocholine. In areas with very elevated As concentrations in drinking water (e.g., >50 μg/L), contribution of other sources of exposure are often negligible [16]. Diet and other sources of exposure tend to become more significant factors the lower the concentrations of As in drinking water. For children, ingested contaminated dust or soil via hand-to-mouth contact should also be considered a source of exposure. Occupational exposure can occur in several industrial processes involving arsenic such as mining, smelting, carpentry, farming, and manufacturing of alloys, semiconductors, and electronics [17].

The main route of arsenic exposure is ingestion, mainly through drinking water and food [17]. Inhalation from As (e.g., from dust and air) can occur to a lesser extent comparatively but is a more significant factor in occupational exposure. Arsenic is poorly absorbed in the skin thus, dermal contact is not a significant route.

1.2.3. Arsenic in the human body: Absorption, Distribution, Metabolism and Excretion

1.2.3.1. Absorption

Absorption mainly occurs via the GI tract, where the small intestine will absorb ingested water-soluble As [18]. Arsenic absorption varies depending on its chemical form, with inorganic species more readily absorbed. On average, ≥ 75% of arsenicals are absorbed when ingested.

However, organic forms, such as arsenobetaine, are poorly absorbed as they have been observed to be excreted from the urine relatively unchanged [13].

1.2.3.2. Distribution

After absorption, arsenic is rapidly taken up by blood and then distributed throughout the body [19]. Despite widespread distribution, arsenic tends to accumulate in the liver, kidneys, heart, and lungs but will be cleared within hours [20]. Arsenic is rapidly cleared in most tissue, with some exceptions. Arsenic has an affinity for thiols, particularly in cysteine residues, and thus tends to deposit into keratin-rich tissue such as the skin, hair and nails, making these tissues optimal for biomonitoring [21].

1.2.3.3. Metabolism

Overall, metabolism occurs through a series of reductions and methylation reactions where arsenic is biotransformed into various intermediates and metabolites, as displayed in Figure 1.1. [22]. The first reaction is the reduction of AsV to AsIII. This is followed by a series of sequential oxidative methylation reactions. AsIII is first methylated to pentavalent intermediate monomethylarsonic acid (MMA^{V}) by arsenic (III) methyltransferase (AS3MT) with methyl donor S-adenosyl methionine (SAM) as a conjugate. MMA^{V} is then reduced to monomethylarsonous acid (MMA^{III}) via glutathione-S-transferase omega-1 (GSTO`) with reduced glutathione (GSH) as an electron donor. The second sequence of methylation and reduction reactions follows to generate dimethylarsinic acid (DMA^{V}) and dimethylarsinous acid (DMA^{III}), respectively. This process is often incomplete resulting in the excretion of inorganic arsenic (i.e., AsIII and AsV) and intermediate metabolite (i.e., MMA) along with the final metabolic product DMA. Thus, it is important to measure the different arsenic species, to understand iAs exposure in the body.

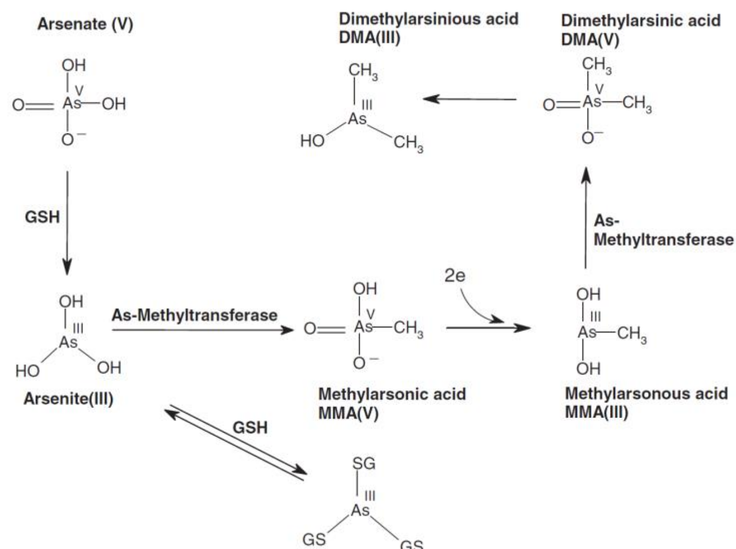

Figure 1.1. Schematic mechanism pathway for inorganic arsenic metabolism (Jomova et al., 2011).

1.2.3.4. Excretion

Excretion occurs mainly via the renal route; thus, arsenic is primarily excreted in the urine, making it an apt indicator of arsenic body burden [23]. Excretion is relatively rapid as the half-life for arsenic is 4-5 days. The urinary inorganic arsenic profile is typically 10-30% AsIII+AsV, 10-20% MMA, and 60-80% DMA [22]. A high % of MMA in urine often reflects metabolic inefficiencies [24]. Urinary concentrations of organic arsenic (e.g., arsenobetaine and arsenocholine) are highly dependent on diet. These species are poorly absorbed, and ingested organic species will be excreted relatively unchanged. As such, urine is a suitable biological matrix for measuring arsenic, though the different species must be considered when assessing toxicity.

1.2.4. Toxicology

Arsenic exerts its toxicity by interacting with a diverse range of mechanisms thus generating numerous health outcomes [20]. Toxicity varies with arsenic species, with inorganic arsenic (i.e., AsIII and AsV) being regarded as more toxic than organic arsenic (e.g.,

arsenobetaine and arsenocholine). Within inorganic arsenic species, AsIII is considered more toxic than AsV. Metabolism of arsenic had been previously regarded as a detoxification mechanism as sequential methylation produces progressively less toxic and reactive metabolites which are more readily excreted [25]. However, studies have shown that methylated trivalent forms of arsenic metabolites (MMA and DMA) may have cytotoxic and genotoxic effects as potent, if not more, than inorganic arsenic [26–30]. Thus, MMA and DMA concentrations should be considered when assessing inorganic exposure. However, there are no currently established guideline values for methylated arsenic concentrations in humans.

Although the adverse effects of arsenic toxicity have been well-documented, the exact mechanisms and associated pathways are yet to be clearly understood [23]. Nonetheless, arsenic has been observed to inhibit up to 200 enzymes, most of which are involved with metabolism, disrupting key cellular pathways and processes [18]. Proposed toxic mechanisms are linked to the chemical properties of inorganic arsenic. AsV is chemically and structurally similar to phosphate. Thus, it can replace phosphate in ATP, disrupting processes in cellular respiration [31]. Meanwhile, AsIII has an affinity for sulfhydryl groups. Therefore, it may inhibit enzymes by readily reacting to thiol-containing active sites. Additional proposed mechanistic actions include oxidative stress, epigenetic interaction, altered DNA methylation, and signal transduction [32].

1.2.5. Health Effects of Arsenic

Arsenic exerts its toxicity by interacting with a diverse range of mechanisms, thus resulting in numerous health outcomes. Acute and chronic exposure will generate different outcomes. In the case of this thesis, the effects of chronic exposure are of greater concern.

Chronic exposure to relatively moderate to high doses of inorganic arsenic has been associated with diseases and symptoms affecting most body systems and major organs. The

skin is often the first organ to display the effects of chronic arsenic toxicity. Arsenic has an affinity for thiol and subsequently, keratin-rich tissue making the skin a target organ [33]. A hallmark symptom of chronic arsenic toxicity is distinctive skin lesions on the extremities, particularly on the palms of the hand and soles of the feet. The skin lesions often manifest in distinct "raindrop" patterns [34]. These may occur after a minimum exposure of approximately five years and can be a precursor to skin cancer. Arsenic also affects respiratory, gastrointestinal, neurological, cardiovascular, renal, and lymphatic systems, impacting key organs like the liver, kidneys, bladder, and lungs. Notable disease outcomes include Blackfoot Disease [35], liver disease [36], decreased lung function [37], neurodevelopment [38], diabetes mellitus [27], kidney disorders [39], and cardiovascular disease [40].

Inorganic arsenic has also been classified by the International Agency for Research on Cancer (IARC) as a Class-I human carcinogen [16]. Moderate to elevated levels of chronic exposures have been associated with an increased risk of lung, skin, bladder, and kidney cancers in arsenic-endemic areas [41–45]. Arsenic-induced cancers may have delayed occurrence, manifesting years later, even after exposure has ceased. Latencies for cancers are generally 35 years, although skin cancer may have a shorter latency of 20 years [46].

1.3. Biomonitoring and Biomarkers

Given the public health risks of chronic arsenic exposure, biomarkers are a prominent tool in biomonitoring and risk assessment. Biomarkers are quantifiable indicators within an organism that reflect a particular event, process, or state. In biomonitoring, biomarkers provide information about the activity or presence of the chemical of interest within the body. There are three general types of biomarkers: exposure, effect, and susceptibility [47]. Each type reflects a different aspect of the biological fate of arsenic. For the scope of this thesis, I examined the use

of different biomarkers of exposure and effect to assess arsenic exposure and subsequent health impacts.

1.3.1 Biomarkers of Exposure

Biomarkers of exposure indicate the internal dose of the chemical present in the body, which reflects how much an individual has been exposed to a certain chemical [6]. Several matrices are useful biomarkers to measure the internal dose of arsenic: urine, hair, and nail (finger and toenail). Samples of these biomarkers are taken and measured for the chemicals in question, which indicate the exposure level.

Arsenic is distributed in the body via blood and predominantly excreted in the urine, thus, both matrices are plausible biomarkers. Other plausible biomarkers are hair and nails as these tissues are rich in keratin which contain thiols groups [48]. Urine is the biomarker most frequently used for measuring arsenic exposure. Generally, urinary and blood concentrations act as biomarkers of recent exposures, while fingernail and toenail levels reflect longer exposures. Ingested arsenic and its metabolites are cleared in the blood within 2-3 hours and excreted in the urine, reflecting an exposure of up to 5 days [49]. Nails typically reflect an exposure of 2-3 months, and toenails reflect longer exposures of 6-12 months as toenails grow more slowly [50].

Based on a review of the literature and the characteristics of the study population in Yellowknife, toenails and urine are the most suitable to assess exposure. Blood is excluded from this monitoring program due to its invasive nature, which is not ideal given the size and age ranges of the target population. Toenails were chosen over fingernails because they reflect a more extended exposure period due to a slower growth rate. Moreover, toenails are less exposed to outdoor contaminants (e.g., dust, soil) compared to hair or fingernails, thus, are less likely to contain exogenous concentrations which may interfere with measuring the internal dose [16].

Overall, urine and toenails are apt exposure biomarkers because of their accessibility, non-invasive nature, and validity. Furthermore, they reflect different exposure lengths, with urine reflecting a few days and several months for urine. Using both biomarkers can improve the understanding of arsenic exposure as each biomarker can provide different information. Thus, both biomarkers are used in this thesis. Past studies have evaluated the validity of the chosen biomarkers, finding significant correlations between their concentrations and environmental exposure concentrations such as in drinking water [51–53], household dust [54–56], soil [54,56] and diet [57]. Though it should be noted that there is a large focus in the existing literature on drinking water and diet to a lesser extent, shaping the current knowledge [16,58]. The literature concerning other exposures (e.g., soil, dust) via ingestion or inhalation in non-occupational is comparatively limited. Thus, an expansion of current knowledge is needed.

Another advantage of urine and toenail biomarkers is the application of speciation techniques which may be used to distinguish between the different arsenic species present in the biomarker medium [49,59,60]. Speciation is important because total As concentrations do not always indicate arsenic toxicity. The inclusion of organic arsenic may be confounding and lead to overestimating potential toxicity. For toenails, speciation is possible, but the existing literature is still limited. Its applicability and potential advantages over the use of total toenail arsenic are to be elucidated. In this study, arsenic species in urine and toenail were analyzed and compared to better understand applicability in biomonitoring.

1.3.2 Biomarkers of Effects

Biomarkers of effect reflect the impacts of the chemical in question on a biological system [47]. They may be molecular, reflecting a change in chemical processes, or a health indicator of symptoms, reflecting a pathological response.

Biomarkers of effect for arsenic remain the less defined of all the biomarker groups. Arsenic has been shown to interact with over 200 enzymes involved in many key pathways. Therefore, potential biomarkers of effect are numerous [18]. However, the exact mechanisms remain largely unclear. Hence, there is difficulty in establishing arsenic biomarkers of effect due to these diverse modes of action and subsequent health outcomes. Additionally, many health outcomes are confounded by other contributing factors, such as cigarette smoking for lung cancer and kidney disease. Thus, there is a need to find proteins that can be specific and sensitive candidate biomarkers for arsenic.

The thesis examined two proteins as candidate biomarkers of effect for arsenic-induced impairment of specific organs (i.e., kidney and lung). The candidate biomarkers are club cell secretory protein (CC16) and kidney injury molecule-1(KIM-1), reflecting lung and kidney function, respectively.

CC16 is a lung protein observed to play a role in protecting and repairing the respiratory tract following lung epithelial damage from xenobiotics, such as environmental contaminants [61]. Increased circulating levels of CC16 have been observed following acute environmental exposures (e.g., smoking and firefighting). Conversely, lower overall circulating CC16 concentrations were observed in those chronically exposed (e.g., firefighters and smokers), possibly due to decreased responsive club cells. Lower CC16 levels have also been associated with reduced lung function, lower airflow, and increased risk of lung cancer [62]. Additionally, a longitudinal cohort study observed that lower levels of CC16 in young children might result in decreased lung capacity later in their teenage years [63]. Past studies have observed inverse relationships between CC16 levels and arsenic exposure in different media [61,64–66]. For these

reasons, CC16 has the potential to be a sensitive lung peripheral biomarker to assess lung damage from environmental exposures such as arsenic.

KIM-1 is a transmembrane glycoprotein involved in kidney repair. [67]. It is expressed by proximal tubular epithelial cells in the kidneys early after injury. Following secretion, it is cleaved and then excreted via urine, making it a potential biomarker for kidney function. Chronic KIM-1 secretion is maladaptive and linked to various inflammatory and fibrotic pathogeneses [68,69]. In environmental settings, KIM-1 has been demonstrated to be more sensitive than renal biomarkers N-acetyl-β-D-glucosaminidase and β2 microglobulin for detecting kidney injury induced by chronic cadmium exposure [70,71]. In recent studies, urinary KIM-1 concentrations have been significantly associated with increased urinary As concentrations in children but not in adults [72,73]. Thus, more research is needed to evaluate urinary KIM-1 as a potential biomarker of effect for arsenic-induced kidney damage, particularly in children.

1.3.3. Biomarkers of Susceptibility

Biomarkers of susceptibility reflect the biological, often genetic, differences between individuals that impact their susceptibility toward the effects of chemical exposure [6]. Genetic differences within individuals have been found to result in varying responses to biological and metabolic processes and disease susceptibility. Biomarkers of susceptibility are commonly genetic polymorphisms, which are variations occurring at a specific location in a genome that is observed to an appreciable degree in a population. Patterns of polymorphic variation can serve as genetic markers reflecting one's ancestry. Thus, they are a tool for understanding the heritability of small differential genetic responses to biological processes or diseases. Biomarkers of susceptibility are not evaluated in the scope of this thesis.

1.4. Arsenic in Yellowknife

In Yellowknife, arsenic is predominantly present in bedrock as arsenopyrite, a gold-containing mineral [74]. Due to the natural abundance of arsenopyrite, gold mines were established in the Yellowknife area, with Giant Mine beginning its operations in 1949. The roasting process resulted in the release of arsenic trioxide (As_2O_3), which was emitted freely for the first three years until 1951 when attempts to control emissions were made, where mine by-products were stored in underground chambers. Such uncontrolled emissions have been suggested to have contributed to environmental arsenic levels. Studies have found a temporal increase of arsenic concentrations through dated profiling which corresponded to the duration of Giant Mine operations in lacustrine sediment core samples of two nearby lakes [75,76]. Spatial distribution of arsenic in lake sediments was also observed, in which arsenic concentrations of sediments along a 90 km transect corresponded with historical mining emissions and wind dispersion [77]. Additional studies have observed associations between historical emissions and spatial gradients of environmental arsenic levels in lake waters, sediment, and soil [78–80]. Elevated arsenic levels were also observed in wildlife such as terrestrial birds, hares, and muskrats [81–83]. These findings of elevated environmental levels, therefore, suggest potential human exposure from different contaminated sources such as soil, lake waters, and local foods (e.g., fish, berries, mushrooms, etc.). As such, advisories are provided for local waters, informing residents where it is safe to fish and swim (https://www.hss.gov.nt.ca/en/newsroom/arsenic-lake-water-around-yellowknife). The government of NWT does not recommend drinking water directly from any bodies of water around Yellowknife. Meanwhile, municipal drinking waters are regularly tested for arsenic, and levels are below the national drinking water guideline (10 µg/L). Thus, drinking water is unlikely to be the predominant source of arsenic exposure for the Yellowknife population.

Nevertheless, the extent to of the current human population is exposed and impacted by arsenic is largely unknown, as the last known health study was conducted in the 1970s.

A series of human health studies concerning arsenic were conducted in Yellowknife from 1950-1970s by the government following concerns over the health hazards of arsenic [9]. Overall, arsenic levels in hair and urine were reported to be low with some exceptions in miners. The government studies were criticized for inconsistency and lack of transparency as they were not immediately disclosed to the public. Following an independent study in 1977 in which hair samples of indigenous children were observed to be elevated, a government task force was established in response. However, they concluded that arsenic did not pose a public health threat in Yellowknife. These findings were again criticized as an emphasis on the investigation was on acute arsenic risk, not chronic exposures. No studies have been conducted since then as the technological advancements led to a significant reduction of emissions in the 1980s, and public concern about arsenic subsequently subsided. Thus, there is a 40-year knowledge gap concerning arsenic and human health in Yellowknife. Nevertheless, the toxic legacy of Giant Mine has persisted as the local community has remained concerned about the impact that the mine has had on their past and current health.

1.5. Thesis Rationale

Exposure and risk assessments often involve assessing environmental sources such as water, air, and food. Measuring the body burden in humans by biomonitoring can usually confirm the exposure and estimate the potential health effects. Toxicity is complex, with different factors affecting the toxicokinetics and toxicodynamics of chemicals after they are absorbed into the body. Because of this complexity, assessments warrant a multi-faceted approach involving multiple indicators that may better characterize the risk. Furthermore, most

studied populations in the literature are exposed to arsenic in drinking water. Arsenic exposure and its human health impacts can also be more challenging to assess and interpret in populations with relatively low to moderate chronic exposures from sources other than drinking water. In addition, exposure assessment becomes more complicated when they may be multiple other sources such as diet, ingested dust, soil, etc. Biomarkers can be a more accurate tool to assess the total exposure from different sources, allowing us to better assess the health risks of a population.

1.6. Thesis Outline

This thesis aims to demonstrate how different biomarkers can be used to evaluate arsenic exposure and its impact in a biomonitoring study, with each biomarker contributing to the understanding of arsenic along the continuum of exposure to disease.

First, I established baseline levels by analyzing the contaminant exposure within the Yellowknife population by measuring arsenic concentrations in urine and toenail samples in **Chapters 2** and **3**, respectively.

In **Chapter 2**, I measured arsenic and its species in urine as a biomarker of exposure to establish baseline exposure levels of the population and to assess health risk by using known guideline values and national biomonitoring data. I compared urinary inorganic arsenic concentrations from my study, which is representative of the Yellowknife population, with data from the Canadian Health Measures Survey (CHMS), representative of the general Canadian population. I also assessed health risks using established urinary guideline values to calculate hazard quotients and cancer risk. Additionally, I determined risk factors associated with elevated urinary inorganic arsenic in child and adult study participants. I hypothesized that the Yellowknife population would have higher arsenic concentrations in urine than the general

Canadian population. This chapter is presented in manuscript format and was published in the journal International Journal of Hygiene and Environmental Health in 2020 (DOI: 10.1016/j.ijheh.2020.113623). It is formatted according to the journal's guidelines.

In **Chapter 3**, I assessed arsenic exposure using toenails as another biomarker of exposure. I measured total arsenic and its species in toenail samples to determine the applicability of arsenic speciation. I also compared arsenic species concentrations in urine and toenail to evaluate their different uses as biomarkers of exposure. Finally, I identified risk factors associated with elevated inorganic arsenic exposure in toenails. Compared to urine, toenails reflect a longer exposure of several months rather than a recent exposure of several days. Thus, I hypothesized that arsenic species in toenails would have a different composition than in urine and that risk factors associated with elevated arsenic in toenails would also differ due to the different periods of exposure reflected by the two biomarkers. This chapter was prepared in a manuscript format intended for submission to Environmental Research. It is formatted according to the journal's guidelines and uses the Vancouver citation style.

In **Chapter 4**, I assessed both KIM-1 and CC16 as potential urine biomarkers of effect for detecting early impairment of two target organs of arsenic, kidneys, and lung, from exposure to As and other chemicals of potential concern (COPCs) in children. I measured urinary concentrations of KIM-1 and CC16 in a cohort of children under age 11. I compared concentrations of these candidate biomarkers with urinary concentrations of arsenic species and other metals to evaluate their relationship. I predicted that increasing urinary inorganic arsenic concentrations would be associated with increasing KIM-1 concentrations while increasing urinary inorganic arsenic concentrations would correlate with decreasing CC16 concentrations. This chapter was prepared in a manuscript format intended for submission to the International

Journal of Environmental Research and Public Health. It is formatted according to the journal's guidelines and uses the Vancouver citation style.

In **Chapter 5**, I reviewed available archival literature and data for past urinary, hair, or toenail arsenic measurements. I searched relevant libraries and archives for past reports, data, or documents related to human health and arsenic in Yellowknife. I compiled available archival records of past urine and hair arsenic test results and summarized the findings. I compared the urinary concentrations between past results and current study data to understand arsenic exposure over time. I hypothesized that current arsenic concentrations would be significantly lower than past historical data. This chapter uses the Vancouver citation style.

In **Chapter 6**, I summarize and discuss the findings of all the chapters of my thesis. This chapter uses the Vancouver citation style.

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2. Health risk assessment of arsenic exposure among the residents in Ndilǫ, Dettah, and Yellowknife, Northwest Territories, Canada

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JSJC and HMC designed the study, with assistance from RR and RPP; while AT, AS, and EL provided input. RR and JSJC oversaw sample collection and analysis. JSJC, XFH, RPP, and HMC analyzed and interpreted the data. JSJC and HMC wrote the manuscript, which was reviewed by XFH, RPP, RR, AT, AM, and EL.

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2.1. Abstract

There are concerns in Yellowknife, Northwest Territories, Canada, about arsenic exposure due to past mining operations, particularly the former Giant Mine. The objective of this study was to characterize the risk of arsenic exposure and associated risk factors among the residents. Arsenic (As) and its species were quantified in urine (n=1966) using inductively coupled mass spectrometry. Children in the study were found to have significantly higher ($p<0.05$) urinary inorganic related As (uiAs) concentrations than children in the general Canadian population, as well as adults in the study. Additionally, uiAs concentrations in children, particularly those above the 95th percentile, are above the Biomonitoring Equivalents (BE) levels that are associated with dermal effects, vascular problems and cancer risks. Multiple linear regression results showed that market seafood (fish and shellfish) and rice consumption frequency were significantly positively associated with uiAs. Specific to children, drinking lake water was positively associated with uiAs. Specific to adults, consumption of local mushrooms and berries was significantly positively associated with uiAs while there was a significant negative association with age, smoking and recreational water activities. The risk factors identified in this research can be used for public health education to lower arsenic intake. Overall, these results support the need for an ongoing monitoring program.

2.2. Introduction

Arsenic (As) is a naturally occurring metalloid in the Earth's crust that can be released into the environment through natural processes (e.g., volcanic eruption and weathering) and anthropogenic activities such as mining and smelting (Oremland and Stolz, 2003). Arsenic exists in the environment in both inorganic and organic forms. Inorganic arsenic (e.g., AsIII and AsV) is the more abundant form found in minerals, rock, water, air and food (Gomez-Caminero et al., 2001). While organic forms of arsenic (e.g., arsenobetaine, arsenocholine, arsenosugars, and arsenolipids) are predominantly present in fish, shellfish, and algae. Human exposure to arsenic, through sources such as contaminated drinking water and food, is a global public health concern (Ng et al., 2003). Arsenic, specifically inorganic arsenic and related forms (e.g., methylated arsenic species), is known to be toxic. Chronic exposure to inorganic arsenic has been associated with adverse health outcomes, including dermal effects, cardiovascular disease, and impaired lung function 86–88. Furthermore, inorganic arsenic is a human carcinogen as classified by the International Agency of Research on Cancer (IARC). Moderate to elevated chronic exposures have been observed to lead to increases in cancers of the lung, skin, bladder and kidney in arsenic-endemic areas (Chiang et al., 1993; Ferreccio et al., 2000; Smith et al., 1998; Tseng et al., 1968; Yuan et al., 2010a).

Given its established toxicity and ubiquitous presence in the environment, arsenic has been measured in national biomonitoring programs in countries including France, Germany, South Korea, the United States and Canada (Aylward et al., 2014; Health Canada, 2010; Lee et al., 2012; Saoudi et al., 2012; Schulz et al., 2007). Human biomonitoring is broadly defined as the measurement of a given chemical or product in a biological medium (e.g., arsenic in urine) as a proxy for body burden. The use of biomonitoring data for human health risk assessment can be

limited because of the lack of guideline values for concentrations of contaminants in biological matrices 94. The biomonitoring equivalents (BE), which relates the concentration of a chemical in a biological matrix (e.g., urine) to existing exposure guideline values 95, has become a useful screening tool for biomonitoring data. BEs have been developed for inorganic-related arsenic species (As^{3+} and As^{5+}) as well as its metabolites monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)) in urine (Hays et al., 2010). It should be noted, the consumption of certain seafood can contribute to urine DMA concentrations (Taylor et al., 2017). Thus, seafood consumption needs to be taken into consideration as a potential confounder for risk assessments. Nevertheless, the sum of urinary inorganic-related arsenic (AsIII + AsV + MMA + DMA) is commonly used as a biomarker for arsenic exposure in biomonitoring studies and risk assessment. Additionally, urine is considered the most consistent and reliable biomarker of recent arsenic exposure, reflecting exposure of 4-5 days (Hughes, 2006). However, urinary arsenic values represent exposure for a single point in time and are not necessarily reflective of chronic, long-term exposure.

In Canada, arsenic is generally present in the environment at low levels except for some regions (Wang and Mulligan, 2006). Nonetheless, arsenic is one of the chemicals included in the Canada Health Measures Survey (CHMS), a nationally representative government-run longitudinal study aiming to characterize the environmental health of Canadians (Haines et al., 2017). Based on the results of CHMS, a reference value derived from the 95th percentile (RV95) of 27 µg/L has been calculated for total arsenic in urine, which includes both organic and inorganic forms (Saravanabhavan et al., 2017). The reference value can be used as a screening value indicating the upper margin of background exposure to a chemical for the Canadian population.

Arsenic exposure is of more significant concern in areas of Canada with a higher geological presence of arsenic or anthropogenic sources. Yellowknife, Northwest Territories, has been shown as a hotspot of arsenic due to the geological presence of arsenopyrite and its proximity to past mining operations, particularly Giant Mine (Jamieson, 2014). Giant Mine was a gold mine in operation from 1948 to 2004, located 4 kilometres north of city limits (Keeling and Sandlos, 2012). Gold extraction from arsenopyrite ores consisted of a roasting process that resulted in arsenic trioxide (As_2O_3) as a by-product, which was emitted freely for the first three years until 1951 when attempts to control emissions were made. Such emissions have been linked to contributing to local environmental arsenic levels. In addition, mining operations have left a legacy of 237,000 tonnes of arsenic trioxide in underground chambers (Jamieson, 2014). While local geology is rich in arsenic, spatial gradients in soil, lake water and lake sediment concentrations corresponding to historical mining emissions from Giant Mine have been reported (Galloway et al., 2012; Houben et al., 2016; Jamieson et al., 2017; Palmer et al., 2015). For example, arsenic concentrations in local lake waters have been reported to be as high as 60 times the drinking water guideline of 10 μg/l, with the highest concentration measured in a small lake near the mine (Palmer et al., 2015). Meanwhile, soil concentrations as high as 4700 mg/kg have been linked to historic roaster emissions (Jamieson et al., 2017). As a result, there are public health concerns about environmental contamination of arsenic and other metals due to the initial uncontrolled roaster emissions as well as potential surface runoff and groundwater migration. However, the degree to which the population living in the Yellowknife area is exposed to arsenic is currently unknown.

The first objective of this study was to characterize the risk of arsenic exposure by comparing inorganic-related arsenic (uiAs) concentrations to the established reference values

from the CHMS and the BE values. The second objective was to identify risk factors associated with elevated inorganic arsenic exposure among the residents of the City of Yellowknife, the North Slave Métis Alliance (NSMA) and the Yellowknives Dene First Nation (YKDFN) communities of Dettah and Ndilǫ.

2.3. Materials and Methods

2.3.1. Ethics

The presented research was conducted following the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans and in particular Chapter 9, research involving the First Nations, Inuit, and Métis Peoples of Canada (Canadian Institutes of Health Research et al., 2010), and the document entitled: Indigenous Peoples & Participatory Health Research: Planning & Management, Preparing Research Agreements published by the World Health Organization (Fediuk and Kuhnheim, 2003). The study also follows the First Nations principles of Ownership, Control, Access, and Possession (OCAP®) of data (Schnarch, 2004).

The study is approved by the Health Sciences and Sciences Research Ethics Board of the University of Ottawa (http://research.uottawa.ca/ethics/reb) and the Aurora College Research Ethics Committee. In addition, the study has been granted a Scientific Research License from the Aurora Research Institute in Northwest Territories. Individual participation in the project was voluntary and based on informed written consent following an oral and written explanation of each project component.

2.3.2. Study Population and Data Collection

The Health Effects Monitoring Program is a prospective cohort study. A more detailed description of the study and its design can be found in Chan et al. (2020). A total of 2037 individuals from ages 3 to 86 participated in the baseline study. Recruitment and data collection

of the baseline cohort was conducted in two waves; the first wave occurred from September to December 2017, and the second wave occurred from April to June 2018.

The study consisted of residents living in Yellowknife as well as the First Nation communities of Dettah and Ndilǫ. Under the recommendation of the local community members, different recruitment strategies were developed for data collection from population groups in the area: (1) Yellowknives Dene First Nation (YKDFN), (2) the North Slave Métis Alliance (NSMA), and the general population of Yellowknife, which was further grouped into (3) Random Selection and (4) Volunteer.

For the Yellowknife general population, households were recruited to participate via random selection. A random sampling methodology was developed with the help of Statistics Canada to ensure recruitment and the sample size would be representative of the Yellowknife population. The population of Yellowknife is approximately 20, 000 residents. Thus, the target sample size was 1000 for a confidence level of 5%. Prior to sampling, a sampling frame was developed based on the stratum, district, street name, civic address, and unit number. Then, the sample size was calculated factoring in the following parameters: 20% desired level of precision, 1.00 variability of the variable to be measured, 5% level of confidence, 50% expected response rate, and 90% occupancy rate. A final sampling size of 1,900 households was selected at random from the total of 6,886 addresses to obtain a target sample size of 1000. Participants were selected randomly using a list of residential addresses provided by the city of Yellowknife. Selected residences were sent letters of invitation a week prior to the start of the study. The inclusion criteria were residents of Yellowknife between the ages of 3 to 79 that have lived in Yellowknife for at least one year on the day of the interview. In the first wave of recruitment, the age range was 6 to 79 years, and it was expanded to ages 3 to 79 in the second wave, which was

done to include more child participants. One adult and one child, if applicable, were selected from each consenting household based on the upcoming birth date. In response to the request of the Yellowknife residents during the consultation period, the study also included individuals not selected for random sampling above the age of 3, as a separate sample group labelled as volunteers. All members of the local indigenous communities, the Yellowknives Dene First Nation and North Slave Métis Alliance, above the age of 3, were also invited to participate voluntarily.

All participants were asked to provide a biological sample of urine and to complete a questionnaire. The questionnaire was conducted by a trained research assistant consisting of lifestyle and potential exposure information (e.g., age, gender, smoking status, water sources, hunting, fishing, etc.), as well as a food frequency questionnaire regarding the consumption of local fish. All participants were asked to refrain from seafood consumption three days before urine sampling to control for the potential contribution of organic arsenic and DMA that may come from certain seafood. First-morning urine samples were collected by the participants at their earliest convenience. Once collected, urine samples were kept at 4ºC and shipped to the laboratory at the University of Ottawa within five days.

A total of 2037 individuals, ages 3 to 86, participated in the baseline study of the Health Effects Monitoring Program, as detailed in Figure 1. Of the 2037 participants, a total of 1966 (497 children, 1469 adults) participants in the study provided urine samples for chemical analysis: 870 randomly selected participants (211 children, 659 adults), 856 volunteers (198 children, 658 adults), 194 YKDFN members (75 children, 119 adults) as well as 46 NSMA (13 children, 33 adults).

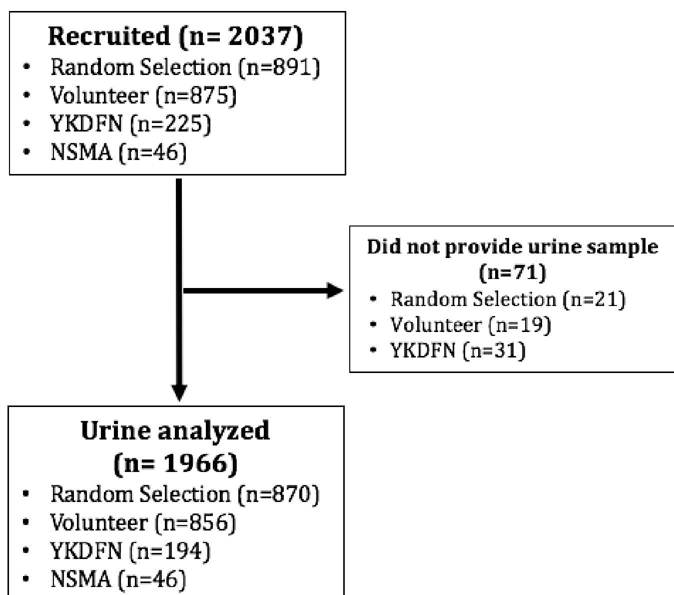

Figure 2.1. Summary of recruitment and urine sample collection.

2.3.3. Laboratory Analysis

Urine samples (n=1966) were shipped on ice from Yellowknife to the University of Ottawa. Within 24 hours of arrival, the urine samples were divided into six aliquots. The aliquots for arsenic quantification were then stored at -20°C until analysis for total arsenic and arsenic speciation.

All chemical analyses were performed at the Laboratory for the Analysis of Natural and Synthetic Environmental Toxicants (LANSET) at the University of Ottawa.

On the day of analysis, urine samples were thawed, then kept on ice. Total arsenic (TAs) was analyzed in urine using inductively coupled plasma mass spectrometry (ICP-MS) (7700x ICP-MS, Agilent Technologies, Mississauga, ON). Urine was diluted 1:10 in 1% nitric acid (Sigma Aldrich (cat. # 84385-2.5l) before total metal analysis. For arsenic speciation, samples were diluted in 10mM ammonium phosphate dibasic, which was prepared by dissolving

ammonium phosphate dibasic (Sigma, cat#379980-100G) in Milli-Q water (Millipore) and pH adjusted to 8.25 with 28% Ammonium hydroxide solution (Sigma, Cat#: 338818-100ML). Measurement of As species (As^{3+}, As^{5+}, MMA, DMA and arsenobetaine) was performed with an Agilent 1200 Infinity Liquid Chromatography (LC) system coupled to an Agilent 7700x ICP-MS (Agilent Technologies, Mississauga, ON). The limit of detection (LOD) of TAs in urine was 0.012 μg/L, and the LOD of arsenic species was 0.005 μg/L. Concentrations below the LOD were replaced with half the LOD.

For quality assurance/quality control (QA/QC), certified reference materials from the National Institute of Standards and Technology, field blanks, and spiked samples were used. The As standards included 1000mg/l of arsenite (Spex Certiprep, Cat#SPEC AS3M), arsenate (Spex Certiprep, Cat#SPEC-AS5M), 10mg/L of dimethylarsonic acid (SpexCertiprep, cat# SPEC-AS-DMA) and methylarsonate (SpexCertiprep, cat# SPEC-AS-MMA). Arsenobetaine stock solution of 1000mg/l was prepared by precisely weighing and dissolving arsenobetaine salt. The recovery rates of all NIST reference materials and spiked samples tested were not significantly different from 100%. The precision expressed as the relative standard deviations of 20 measurements of the spiked standard was 5% for AsIII, 11% for AsV, 3% for MMA, 3% for DMA and 6% for arsenobetaine. Arsenic in the blank samples was not detectable.

An interlaboratory comparison was also conducted to assess laboratory performance. A total of 50 randomly selected urine samples (~2.5% of all urine samples collected) were sent to the Institut Nationale de Santé Public du Québec (INSPQ) for duplicate analysis. There was no statistical difference ($p > 0.05$) between the results obtained from the University of Ottawa and INSPQ laboratories (Appendix 2.A).

2.3.4. Statistical Analysis

Statistical analyses were performed using Stata v14.1 (StataCorp LP, College Station, TX, USA) and R v.3.5.3. Data were organized into three categories based on the recruitment strategy, including random selection, volunteer, and YKDFN. Due to the small sample size (n=46), the NSMA participant group was excluded from group comparisons (Table 2.1, Figure 2.2-2.4). Within each population group, results were subdivided by age into children (ages 3-19) and adults (20-86). Data for the random selection group of our study were weighted to represent the population of Yellowknife and compared to the CHMS data for the Canadian population (Team R Development Core, 2018).

2.3.4.1 Summary of urinary arsenic concentrations

Descriptive statistics were generated for concentrations of urinary inorganic-related arsenic, including geometric mean (GM), minimum, maximum, and percentiles (P5, P25, P50, P75, and P95). Urinary-related inorganic arsenic (uiAs) concentrations were calculated as the sum of inorganic arsenicals and their metabolites: AsIII+ AsV + MMA + DMA. The sum of urinary inorganic-related arsenic rather than total arsenic was used as a focus for this risk assessment as the latter may not accurately reflect potential toxicity as non-toxic forms (i.e., arsenobetaine) can be a major contributor to total arsenic concentrations. However, considerations must be made concerning the contribution of certain organic arsenic species in seafood to DMA (Taylor et al., 2017). To control for this potential confounder, participants were asked to refrain from eating seafood for 3 days before urine collection.

2.3.4.2. Comparison with the Canadian Health Measures Survey (CHMS)

Biomonitoring data from the CHMS was accessed and performed at the Carleton, Ottawa, Outaouais Research Data Center (COOL RDC) at the University of Ottawa, a facility part of the

Canadian Research Data Centre Network (CRDCN). Statistics for uiAs (AsIII+ AsV + MMA + DMA) were calculated from combined data from CHMS cycles 2 (2009-2011), 3 (2012-2013), and 4 (2014-2015). The total sample size was a total of 7640, consisting of 4593 children, and 3047 adults. An RV95 of 27 μg/L for total arsenic has been previously reported by Saravanabhavan et al. (2017). For this risk assessment, a reference value (RV95) of 21 μg/L for uiAs was calculated based on the 95th percentile of CHMS data using a similar methodology. Welch's two-sample *t*-tests were used to assess the difference in As concentrations between the population groups and the CHMS data. These tests were corrected for multiple comparisons using the Bonferroni correction and statistical significance using an $\alpha = 0.05$.

2.3.4.3. Biomonitoring Equivalents

Biomonitoring Equivalents (BE) for uiAs (AsIII+ AsV + MMA + DMA) have been derived from exposure guideline values associated with both non-cancer and cancer endpoints. For non-cancer endpoints, there is the BE_{POD} and the BE. The BE_{POD} is the urine concentration derived from the point of departure (POD) associated with vascular problems, hyperpigmentation, and dermal effects, while BE is the value after an intraspecies uncertainty factor (UF) of 3 was applied to the BE_{POD} (Hays et al., 2010). For uiAs, the BE_{POD} is 19.3 μg/L, and the BE is 6.4 μg/L. For cancer endpoints, there is the BE_{RSD}, which is based on a risk-specific dose (RSD) derived from Health Canada's cancer slope factor. BE_{RSD} is the estimated steady-state concentration of urine associated with a given risk level (Ex: 1 in 10, 000) as a result of lifetime chronic exposure at risk-specific doses (Faure et al., 2020). The BE_{RSD} can be used to calculate cancer risk. For this study, a risk level range of 1 in 10,000 (i.e., 10^{-4}) to 1 in 1, 000, 000 (i.e., 10^{-6}) was used, where BE_{RSD} is 1.4 μg/L, 0.14 μg/L and 0.014 μg/L for risk levels 10^{-4},

10^{-5} and 10^{-6}, respectively. Health Canada considers cancer risk at the risk levels of 10^{-5} to 10^{-6} to be essentially negligible.

2.3.4.4. Calculation of Hazard Quotients and Cancer Risk

Hazard quotients (HQ) were calculated for the non-cancerous endpoint as a ratio of biomarker concentration to the BE (Eq.1). Here, [Biomarker] is the concentration of uiAs (e.g., GM and P95), while the Biomonitoring Equivalent is one of the BEs of interest (BE: 6.4 µg/L; BE_{POD}: 19.3 µg/L). HQ values were calculated at both the GM and P95 levels for both BEs. HQs with values exceeding 1 suggest that the population in question may be exceeding guidance values from which the BE was derived.

$$HQ = \frac{[\text{Biomarker}]}{Biomonitoring\ Equivalent} \qquad \text{Eq.1}$$

Cancer risks were calculated using BE_{RSD} derived from risk-specific doses corresponding to the risk level range of 10^{-4} to 10^{-6} (BE_{RSD} for 10^{-4}: 1.4 µg/L; BE_{RSD} for 10^{-5}: 0.14 µg/L; BE_{RSD} for 10^{-6}: 0.014 µg/L) and biomarker concentrations at the 5th, 25th, 50th, 75th and 95th percentiles (Eq.2).

$$\text{Cancer Risk} = \frac{[\text{Biomarker}]}{BE_{RSD}} \qquad \text{Eq.2}$$

2.3.4.5. Analysis of Risk Factors

Factors associated with uiAs concentrations were identified using bivariate analyses and multiple linear regression. For bivariate analyses, Welch's t-tests and ANOVA were used to test for statistical differences in GMs for various potential risk factors associated with arsenic exposure. For all statistical tests, significance was set at $\alpha=0.05$ and Bonferroni corrections were made. For multiple linear regression analysis, a forward stepwise selection was used, starting

with age and sex as the base model. Age and sex were included in the final model, regardless of significance. Variables were included in the final model if the newly entered variable was significant, or if the addition of the variable contributed to an increase in variance explained by the model (i.e., R^2). Age and BMI were continuous variables, while all other variables were categorical. Separate regression models were created for children (ages 3-19) and adults (ages 20 and over) due to the difference in potential risk factors.

2.4. Results

2.4.1. Urinary Arsenic Concentrations

There was no significant difference in uiAs concentrations of adult and child participants between the two waves of data collection. All results of both waves were combined. A summary of uiAs concentrations by population groups and age group is reported in Table 2.1. uiAs concentrations ranged from 0.1 µg/L to 152 µg/L. The P95 overall was 19.4 µg/L, which was lower than the CHMS RV95 of 21 µg/L. However, P95 for children (23.9 µg/L) exceeded the RV95, particularly for volunteer children (31.2 µg/L). Overall, 7.9% of child participants exceeded the RV95 of 21 µg/L. Exceedance was highest in volunteer children (11.1%). The GM of uiAs for all study participants was 5.6 µg/L. The GM for CHMS was 5.4 µg/L for both age groups and overall. Overall, no statistical difference ($p<0.05$) was observed between the GM representative of the Yellowknife population and that of the CHMS data representative of the Canadian population (5.6 µg/L and 5.4 µg/L for Yellowknife and CHMS, respectively). However, uiAs concentrations in children from Yellowknife (GM: 6.6 ug/L) had significantly higher ($p<0.05$) concentrations than the general Canadian population of the same age group (GM: 5.4 µg/L). For the other population groups, uiAs concentrations were also significantly higher ($p<0.05$) in children of the Volunteer (GM: 7.3 µg/L) and YKDFN groups (GM: 6.4 µg/L)

than the CHMS children (GM: 5.4 μg/L). Among adults, uiAs concentrations in YKDFN (GM: 4.5 μg/L) were significantly lower ($p<0.05$) than the CHMS participants (5.4 μg/L). Within the study groups, uiAs concentrations were significantly higher in children than adults for the random selection, volunteer and YKDFN groups. When comparing the results of adults across the study groups, uiAs concentrations were significantly higher ($p<0.05$) in the volunteer group. Among children, no significant difference was observed between the Random Selection, Volunteer and YKDFN study groups.

Table 2.1. Descriptive statistics of inorganic arsenic (As3+ + As5+ + MMA + DMA) in urine (ug/L) among study participants by population (random selection, volunteer, YKDFN and total participants) and age group (Child: 3-19 years old; Adult: 20-86 years old). Starred (*) geometric means indicate a significant difference ($p < 0.05$) in the geometric mean of study participants and CHMS participants of the same age group.

Population	Age	n	Weighted n	GM (95% CI)	P95[a]
Random Selection	Child	211	3794	6.6 (6.0, 7.3) *	22.9
	Adult	659	14290	5.3 (5.0, 5.6)	19.7
	Total	870	18084	5.6 (5.3, 5.9)	20.8
Volunteer	Child	198		7.3 (6.5, 8.1) *	31.2
	Adult	658		5.7 (5.4, 6.0)	18.2
	Total	856		6.0 (5.7, 6.3)	21.7
YKDFN	Child	75		6.5 (5.7, 7.3) *	15.2
	Adult	119		4.5 (4.1, 5.0) *	11.0
	Total	194		5.2 (4.8, 5.7)	14.2
Total Participants[b]	Child	497		6.746 (6.3, 7.2)	23.8
	Adult	1469		5.3 (5.10 5.5)	18.0
	Total	1966		5.6 (5.5, 5.8)	19.3
CHMS	**Child**	4593	7111445	5.4 (5.1,5.7)	19.3
	Adult	3047	21996414	5.4 (5.1,5.7)	22.3
	Total	7640	29809443	5.4 (5.1,5.7)	21.0

[a] P95: 95th Percentile
[b] Total population includes the NSMA population group (n=46), which was excluded from group comparisons due to the small sample size.

2.4.2. Biomonitoring Equivalents

Figure 2.2 summarizes the distribution of iAs concentrations for each population group by age for the selected BEs (BE: 6.4 µg/L; BE_{POD}: 19.3 µg/L). The GMs for child participants in all population groups (6.6, 7.3, 6.5 µg/L) are above the BE but below the BE_{POD}. Meanwhile, the GMs for adults in all groups were below both BEs. Comparatively, the GM from CHMS data (5.4 µg/L) was below both the BE and BE_{POD}. The P95 for both child and adult participants of the Random Selection group (22.9 and 19.7 µg/L), as well as child Volunteers (31.2 µg/L), are

above the BE_{POD} while the other groups are above the BE but below the BE_{POD}. Comparatively, the P95 for CHMS participants (21.0 µg/L) is also above the BE_{POD}.

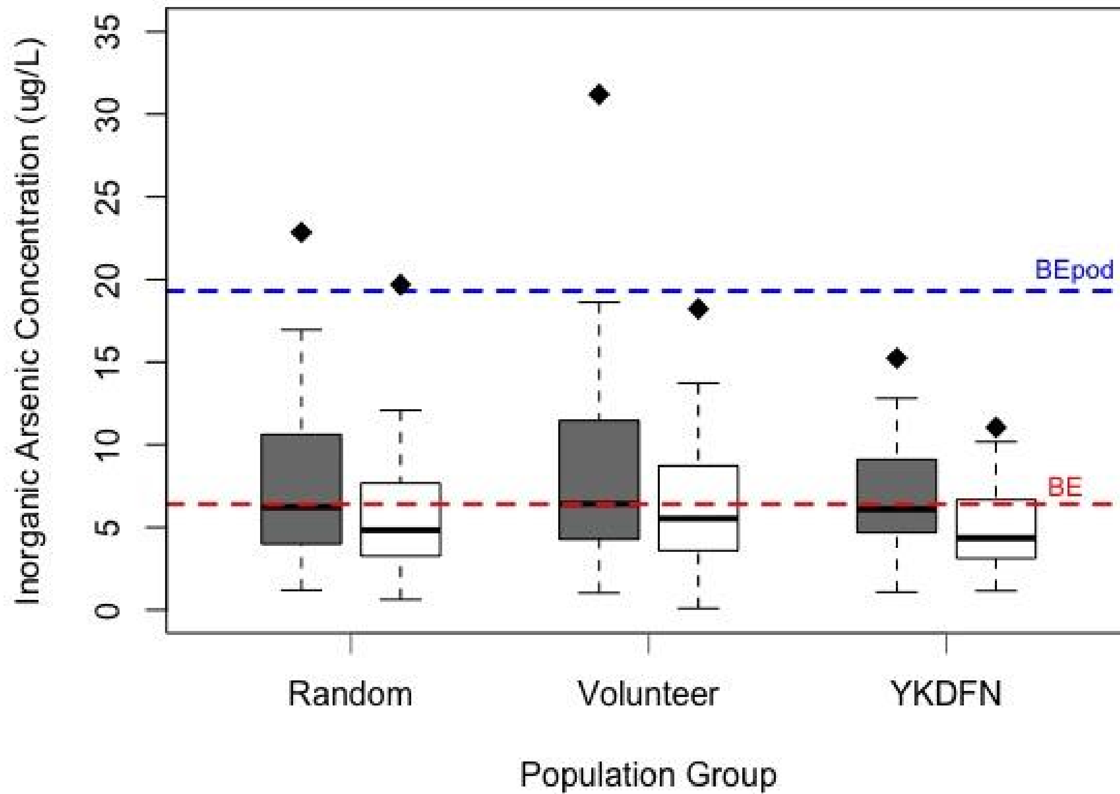

Figure 2.2. Boxplot of inorganic arsenic concentrations by population (Random Selection, Volunteer and YKDFN) and age group (Child, 3- 19 years old: dark grey; Adult, 20-86 years old: white. The line corresponds to the median, and box corresponds to the interquartile range (IQR), and the whiskers represent 1.5xIQR. For each group, the 95th percentile is depicted as ♦. The blue and red dotted lines represent the BE (6.4 µg/L) and the BE for points of departure (19.3 µg/L), respectively.

2.4.3. Calculation of Hazard Quotients and Cancer Risk

The HQs values calculated for the GM and P95 are shown in Figure 2.3. For the BE, all HQ values were similar using the GM concentrations, while all calculated HQ values were above 1 for the P95 concentrations, ranging from 2.8 to 4.9. (Fig. 2.3A). Comparatively, results for HQ values based on the BE_{POD} were below 1 for the GM concentrations, and HQ values approached 1 or exceeded 1 at P95 concentrations, except for the YKDFN population group (Fig. 2.3B).

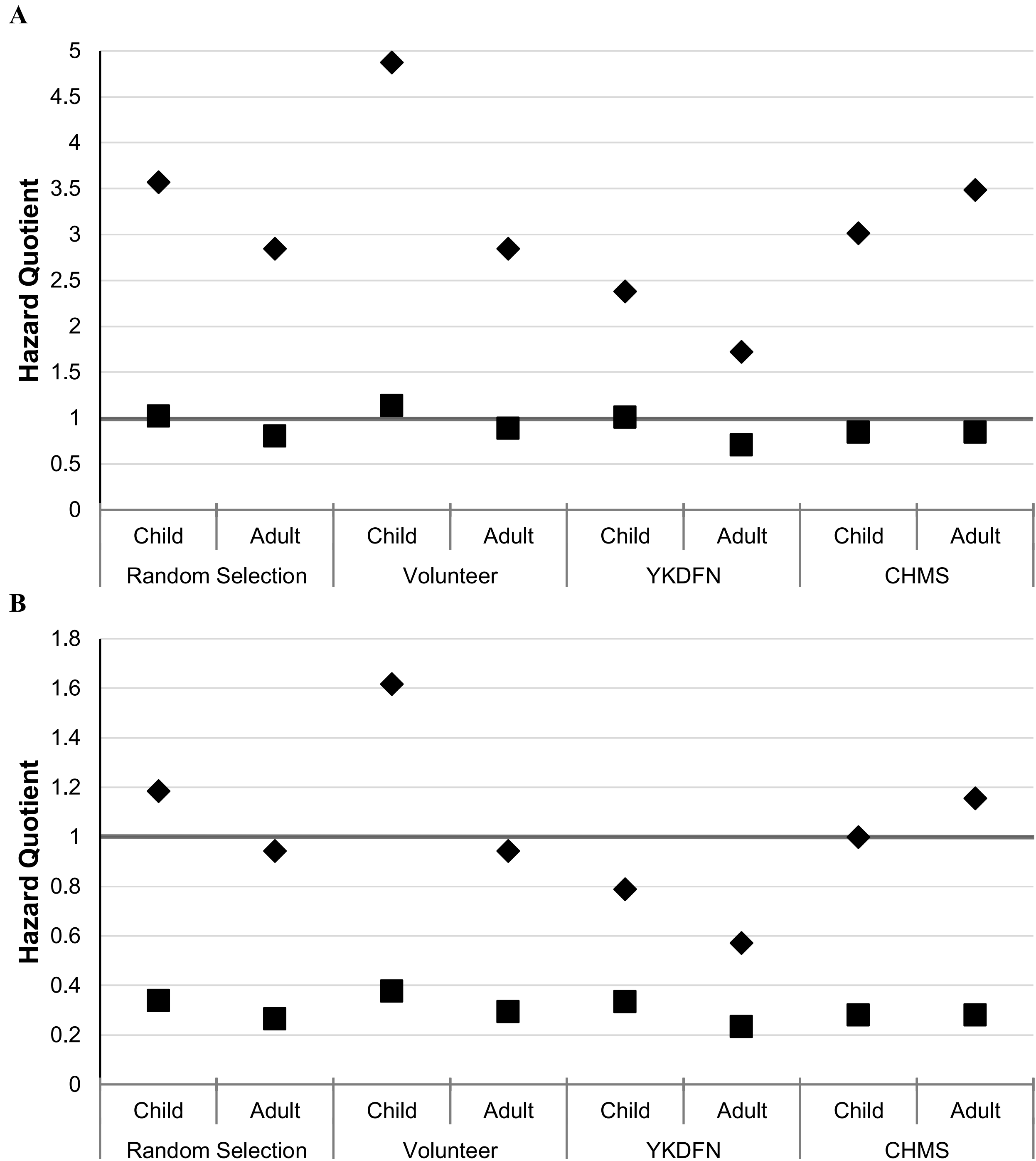

Figure 2.3. Hazard quotients (HQ) for inorganic arsenic by population and age group. Participants ages 3-19 are classified as children; participants ages 20-86 are grouped as adults. Squares (■) represent HQ at the geometric mean (GM), and diamonds (♦) represent HQ at the 95th percentile (P95). (A) depicts HQs calculated from the BE (6.4 ug/L), and (B) depicts HQs calculation from the BE_{POD} (19.3 ug/L). The line represents an HQ of 1, in which the [biomarker] is equal to the corresponding BE.

The cancer risk levels for all three groups of participants (both child and adult) ranged from 10^{-3} to 10^{-4}, with the highest risk observed for Volunteer children at the 95th percentile (Figure 2.4). These values are above the 10^{-5} to 10^{-6} range defined by Health Canada as essentially negligible.

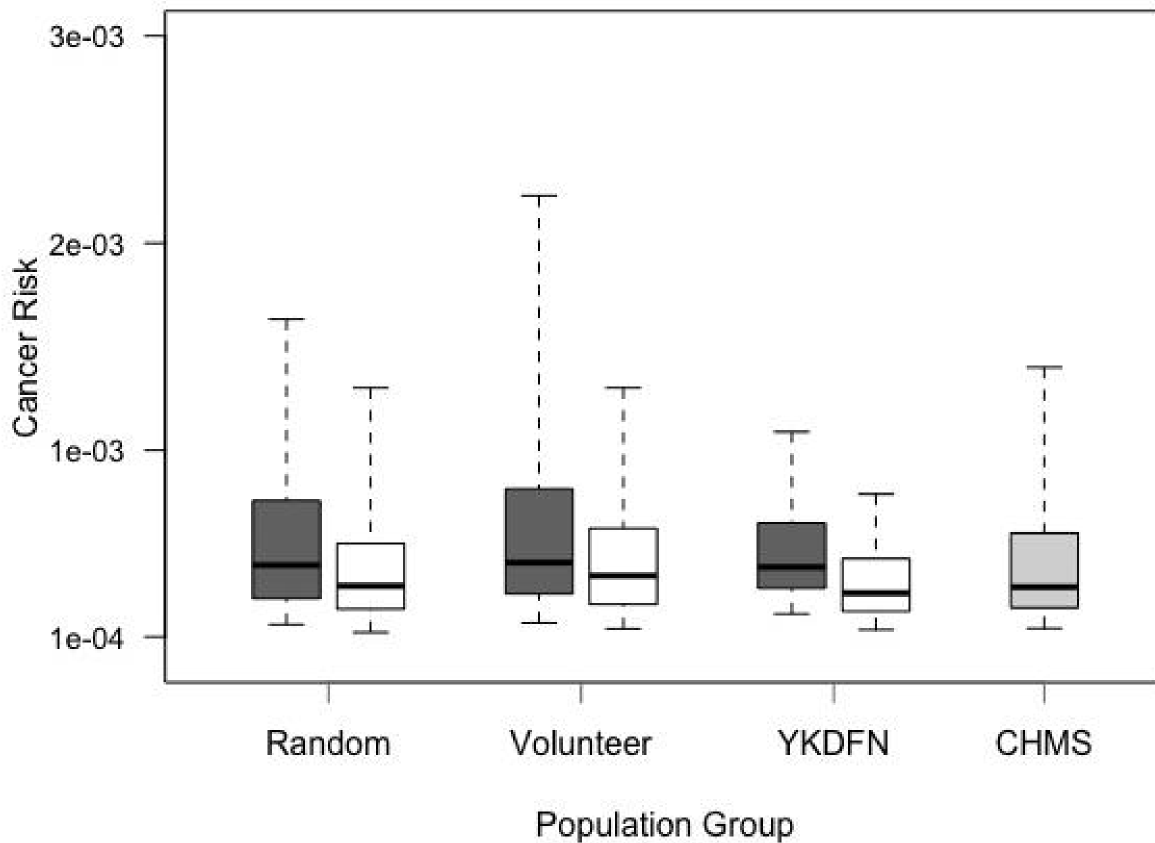

Figure 2.4. Cancer risk for inorganic arsenic by population and age based on cancer exposure guideline values (BE_{RSD}) from Health Canada and US EPA. Age is divided into children (ages 3-19) in dark grey and adult (ages 20-86) in white. The CHMS data was adopted from Faure et al. (2020) and represented in light grey. The medians are represented by the horizontal line in the box plot. The 25th and 75th percentiles are represented by the ends of the box. The 5th and 95th percentile are represented by the ends of the whiskers.

2.4.4. Analysis of risk factors

The results for uiAs concentrations of participants separated into groups with different potential risk factors are summarized in Table 2.2. UiAs concentrations were significantly higher in younger participants, those with lower BMI and non-smokers. Additionally, uiAs concentrations were higher in those who consumed market seafood (fish and shellfish) or rice at least once a week or more.

Table 2.2. Summary of urinary inorganic arsenic (μg/ L) by different risk factors.

Variable		**n(%)**	**GM**	**p-value**
Gender	F	1073 (54.6)	5.6	1.0
	M	892 (45.4)	5.6	
	Other	1 (0.1)	17.6	
Age	3-5	81 (4.1)	7.4	**<0.001**
	6-11	226 (11.5)	7.0	
	12-19	190 (9.7)	6.2	
	20-39	576 (29.3)	6.0	
	40-59	617 (31.4)	5.0	
	60+	276 (14.0)	4.6	
BMI	**<18.5**	260 (13.4)	7.5	**<0.001**
	18.5-25	667 (34.5)	6.0	
	25-30	555 (28.7)	5.4	
	>30	454 (23.5)	4.6	
Smoking status (18+ only)	Non-Smoker	739 (50.0)	5.8	**0.02**
	Smoker	304 (20.6)	4.7	
	Former Smoker	435 (29.4)	5.0	
Drink from lake	No	1377 (70.4)	5.6	1.0
	Yes	580 (29.6)	5.7	
Water activities	No	631 (32.3)	5.7	1.0
	Yes	1325 (67.7)	5.5	

Market seafood (Fish and Seafood) intake frequency	None	298 (15.3)	5.4	**<0.001**
	Less than once per month	485 (24.8)	5.5	
	At least once per month	737 (37.7)	5.3	
	At least once per week	424 (21.7)	6.6	
	At least once per day	10 (0.5)	6.7	
Rice Frequency Intake	None	43 (2.2)	5.0	**<0.001**
	Less than once per month	83 (4.2)	4.7	
	At least once per month	394 (20.2)	4.7	
	At least once per week	1218 (62.3)	5.5	
	At least once per day	216 (11.1)	9.4	
Eat local berries	No	890 (45.5)	5.8	0.2
	Yes	1064 (54.5)	5.4	
Eat local mushrooms	No	1687 (86.3)	6.5	0.4
	Yes	267 (13.7)	5.5	
Wave of sampling	Fall/Winter 2017	877 (44.6)	5.7	1.0
	Spring/Summer 2018	1089 (55.4)	5.5	

[a] After Bonferroni correction (Significance: $p<0.05$)

Multiple regression models for children and adult participants are summarized in Table 2.3. Market seafood (fish and shellfish) intake frequency and rice intake frequency were positively associated with both children and adults after adjusting for age and sex. Drinking lake water was positively associated with uiAs in children only. Meanwhile, in adults, age, smoking status and recreational water activities were negatively associated with uiAs exposure, and consumption of local mushrooms and berries were positively associated with uiAs exposure.

Table 2.3. Multiple linear regression model of factors associated with uiAs (μg/ L) for children and adults.

	Child		Adult	
Variable	**β coefficients**	***p* value**	**β coefficients**	***p* value**
Intercept	6.79	0.09	11.57	**<0.001**
Sex				
Male	Reference		Reference	
Female	0.01	0.99	0.63	0.11
Age (years)	-0.18	0.10	-0.05	**<0.001**
Market Seafood (Fish & shellfish) intake frequency				
None	Reference		Reference	
Less than once per month	-0.45	0.74	0.75	0.27
At least once per month	0.41	0.75	0.03	0.97
At least once per week	4.64	**0.003**	1.83	**0.01**
At least once per day	N/A		3.04	0.21
Rice intake frequency				
None	Reference		Reference	
Less than once per month	-0.90	0.85	-0.53	0.73
At least once per month	2.83	0.50	-1.46	0.27
At least once per week	2.00	0.62	-0.39	0.76
At least once per day	8.80	**0.03**	3.61	**0.01**
Drink lake water				
No	Reference			
Yes	2.87	**0.02**		
Cigarette smoking status				
Non-Smoker			Reference	
Smoker			-1.03	**0.05**
Former Smoker			-0.65	0.16
Local berry consumption				
No			Reference	
Yes			1.14	**0.008**
Local mushroom consumption				

No		Reference	
Yes		1.33	**0.01**
Recreational water activities			
No		Reference	
Yes		-1.61	**<0.001**
BMI		-0.08	**<0.001**
Adjusted R-Squared	0.075	0.081	

2.5. Discussion

2.5.1. Urinary arsenic concentrations

Results from our biomonitoring program showed that the urinary arsenic concentrations of the participants were within a comparable range reported in other national biomonitoring programs (Table 4), except for South Korea, which has much higher concentrations. This has been attributed to diet, particularly seaweed (Lee et al., 2012). However, when compared to a Canadian cohort in rural Québec, our results are comparable to reported uiAs concentrations in children (GM= 7.5 μg/L; n=43) but lower than concentrations in adults (GM= 8.1 μg/L; n= 261) (Gagnon et al., 2016). Additionally, results from our study were lower than studies in areas of known As contamination such as in Nevada, USA where the uiAs GM in adults (n=904) was 31 μg/L 51 or in the HEALS study in Bangladesh where the means for total arsenic were 140 μg/L and 136 μg/L in adult males and females (n=11,746), respectively (Ahsan et al., 2006).

Table 2.4. Arsenic concentrations (GM: Geometric Mean, P95: 95th percentile) in urine reported in national biomonitoring programs in Canada, France, Germany, South Korea and the United States.

Country	Arsenic Species	Study	Year	Age	n	GM (µg/L)	P95 (µg/L)
Canada	As^{3+} + As^{5+} + MMA + DMA	Health Effects Monitoring Program	2017-2018	3-79	1966	5.6	19.4
Canada	As^{3+} + As^{5+} + MMA + DMA	Canadian Health Measures Survey (CHMS)	2009-2011, 2012-2013, 2014-2015	3-79	7640	5.4	21
France	As^{3+} + As^{5+} + MMA + DMA	French National Nutrition and Health Study (ENNS) (Saoudi et al., 2012)	2006-2007	18-74	1500	3.8	10.7
Germany	Total	German Environmental Survey (GerES) (Schulz et al., 2007, 2009)	1990-1992	25-69	4001	6.3	30.2
			1998	25-69	4052	3.9	19.3
			2003-2006	3-14	1734	4.4	14
South Korea	As^{3+} + As^{5+} + MMA + DMA	Korea National Survey for Environmental Pollutants in the Human Body (KorSEP) (Lee et al., 2012)	2008	≥20	4702	43.5	119.7
United States	As^{3+} + As^{5+} + MMA + DMA	National Health and Nutrition Examination Survey (NHANES) (CDC, 2019)	2003-2004	≥6	2572	6.52	19.1
			2005-2006	≥6	2588	6.84	18.5
			2007-2008	≥6	2576	6.47	16.8
			2009-2010	≥6	2852	6.56	20.8
			2011-2012	≥6	2517	5.59	17.2
			2013-2014	≥6	2654	4.8	14.7
			2015-2016	≥6	3094	4.41	14.5

Children participants in our study had higher uiAs than adults, and their levels were also higher than children from the general Canadian population (CHMS). For example, uiAs concentrations in children at the P95, particularly volunteer children, were higher than the inorganic As RV95 value of 21 µg/L for CHMS, indicating a higher proportion of child

participants were at the upper margin of exposure of the Canadian population level (Table 1 and Figure 2). Our findings were similar to other cohorts with children and adults where reported urinary concentrations were higher in children compared to adults in studies conducted in Argentina, Bangladesh, and Denmark (Chowdhury et al., 2003; Concha et al., 1998; Jensen et al., 1991). This could be due to two possible reasons. Children have lower body weights than adults and, as a result, have higher intakes per body weight (Tsuji et al., 2004). Also, children may be exposed to higher intake via hand-to-mouth contact as they play and touch various objects (Cohen Hubal et al., 2000). For example, Canadian children are estimated to have much higher intakes of soil and indoor dust compared to teenagers and adults (Wilson et al., 2013). To assess this possible pathway, we are in the process of gathering additional information on possible soil and dust exposures of child participants with elevated arsenic.

2.5.2.Biomonitoring Equivalents, Hazard Quotients and Cancer Risk

Our screening results using BEs were similar to the findings reported for the CHMS (Faure et al., 2020). For both studies, urinary arsenic levels were close to or exceeded the BE for non-cancer endpoints, which are associated with increased risk of hyperpigmentation, dermal effects, and vascular complications (Hays et al., 2010). It is important to note that biomonitoring equivalents are not diagnostic but may be used to interpret potential population risk and prioritize follow-up assessments, which is appropriate for a longitudinal study. Concentrations are considered a low priority for any intervention efforts if they are below BE, a medium priority if they fall in between the BE and BE_{POD}, and a high priority if they are above the BE_{POD} value. For the present study, child participants are of medium priority, while the adults are of low priority with regard to the risk of non-cancer endpoints (i.e., hyperpigmentation, dermal effects, and vascular complications).

Inorganic arsenic is classified by the International Agency for Research on Cancer (IARC) as a Class-I human carcinogen (Straif et al., 2009). Cancer risk levels calculated based on BE_{RSD} ranged from 10^{-4} and increased to 10^{-3} at the P95 level (Figure 4). These results are similar to those reported by the CHMS, where the cancer risk estimated for uiAs was also higher than the BE_{RSD}; the median risk was in the 10^{-4} range, and the P95 was in the 10^{-3} range. Risk levels from both CHMS and our study were higher than the 10^{-5} to 10^{-6} range that Health Canada defines as negligible (St-Amand et al., 2014). The elevated cancer risk from arsenic exposure observed both in our study and the CHMS may be a result of over-estimation due to the contribution of the metabolite DMA to the concentration of iAs as DMA derives from both sources of organic and inorganic arsenic (Faure et al., 2020). Arsenic-induced cancers may have delayed occurrence, manifesting years after, even after exposure has ceased. Latencies may range from 10 to 50 years, depending on the type of cancer (Martinez et al., 2011; Yuan et al., 2010b). Therefore, continuous efforts to study the long-term relationship between arsenic exposure and cancer rate among the residents of Yellowknife are needed.

2.5.3. Analysis of risk factors

Our results showed that the frequency of market seafood (fish and shellfish) and rice intake was significantly associated with increased uiAs for both adults and children (Table 3). Fish, seafood, and rice are known to be major dietary sources of both inorganic and organic forms of arsenic (Cubadda et al., 2017). Organic species (e.g., arsenocholine and arsenobetaine), most commonly found in seafood and fish, are generally not associated with adverse health outcomes (Taylor et al., 2017). The concentration of iAs in rice varies widely, depending on the region in which it is grown in as anaerobic growing environmental conditions favour root uptake.

There is no rice production locally. Yellowknife residents may be consuming rice imported from areas with higher arsenic in the soil which will warrant further investigations.

We found that uiAs exposure decreased with age, and overall, children had higher exposure than adults. This may be due to the difference in lifestyle and possible exposure scenarios. For example, drinking lake water was observed to be a significant predictor of uiAs in children only. Drinking water is the primary source of arsenic exposure globally. Several studies have observed associations between arsenic concentrations in drinking water and urinary arsenic. Drinking water arsenic concentrations are generally less than 5 µg/L in Canada and, therefore, not the primary source for the majority of Canadians (Health Canada, 2006). However, drinking water may be a major contributor to arsenic exposure for populations living near a source of arsenic (e.g., naturally elevate geological source or contaminated site). We did not measure arsenic in lake waters in our study. However, previous studies have observed spatial gradients linked to mine emissions for water arsenic concentrations in lakes and sediments in Yellowknife (Houben et al., 2016; Palmer et al., 2015). Lake and drinking water concentrations in Yellowknife are regularly tested by the local government. Municipal drinking water in Yellowknife is below Canadian drinking water guidelines of 10 µg/L (GNWT Health and Social Services, 2019). Public health advisories have been issued for certain lakes that are not safe for swimming and fishing (https://www.hss.gov.nt.ca/en/newsroom/arsenic-lake-water-around-yellowknife). Additionally, the Government of Northwest Territories (GNWT) also does not recommend drinking untreated water from anywhere in the Northwest Territories.

For adults in our study, smokers and the use of recreational water activities were significantly associated with decreased urinary As. The finding that non-smoking adults were associated with higher urinary concentrations was surprising as cigarette smoke has been

associated with increased arsenic (Chen et al., 2004; Ferreccio et al., 2000; Hays et al., 2006). The observed negative association between uiAs and water recreational activities was also surprising as it was expected that water activities such as swimming might increase the accidental ingestion of water. We cannot explain these results, but they may be attributed to other confounding factors that were not assessed in our study. Adult uiAs concentrations were also significantly associated with the consumption of local berries and mushrooms. Mushrooms and berries were not considered to be substantial contributors to arsenic in the diet. Mushrooms generally have low arsenic content ranging from 0.27-0.51 mg/kg DW (Melgar et al., 2014; Rashid et al., 2018), though one study assessing mushrooms in Hungary reported concentrations >10mg/kg DW but for certain species (Vetter, 2004). One study assessed arsenic in both mushrooms and berries near a smelter complex, but arsenic levels in both foods were not correlated to smelter emissions (Barcan et al., 1998). Another study analyzed country foods in Canada, including different mushrooms and berries, for arsenic species and found that As^{3+}+As^{5+} ranged from 0.06-1.7 mg/kg wet weight for mushrooms and 0.02- 5.0 mg/kg for berries (Koch et al., 2013). Recently, a risk assessment study conducted in Yellowknife showed that arsenic concentrations for both mushrooms and berries foraged closer to the Giant Mine were higher, but the estimated risk at the current consumption rate was considered low (Canada North Environmental Services, 2018). Public health advisories in Yellowknife have been issued for mushroom picking and berry foraging. Local berry consumption poses a low health risk though berry picking near historical and current industrial sites is not recommended. Mushrooms within 10 km of Giant Mine should not be harvested. Meanwhile, consumption of mushrooms within 10-25 km poses a low health risk, except for the Tricholomataceae family, which should not be consumed within 25 km of the Giant mine (GNWT Health and Social Services, 2019). Our

results suggest that the contribution from the consumption of local mushrooms and berries may need to be further characterized.

In conclusion, our study showed that children had higher uiAs compared to adults as well as children in the general Canadian population. Our current results may be used to inform rights and stakeholders about the susceptible groups and risk factors of arsenic exposure. Public engagement through public health messages, education and media should be implemented to inform local communities of how to mitigate exposure to arsenic. Given that children are the vulnerable group to arsenic, children in Yellowknife should be prioritized in follow-up assessments, including a potential further investigation into the association with drinking lake water and characterization of frequency or quantity. Furthermore, as urine is an indicator of recent arsenic exposure, future follow-up could include measurements at multiple time points. The Health Effects Monitoring Program aims to follow-up with child participants every five years, with additional recruitment every cycle.

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2.7. Conflicts of Interest

There are none to declare.

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Appendix 2.A: QA/QC results of the inter-laboratory comparison between the University of Ottawa and INSPQ

Table 2.A. Statistical comparison of urinary arsenic concentrations of randomly selected samples (n=50) analyzed by the University of Ottawa and INSPQ for QA/QC.

	Total As (tAs)	Inorganic As (iAs-met)
Pearson correlation coefficient (r)	0.976	0.972
p value- Pearson correlation	< 0.001	<0.001
p value- Paired t-test	0.318	0.094

3. Arsenic species in toenail as biomarkers for arsenic exposure in a mining legacy population in Yellowknife, Northwest Territories, Canada

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Author contributions:

JSJC and HMC designed the study, with input from AM. JSJC oversaw sample collection and analysis. JSJC, XFH, and HMC analyzed and interpreted the data. JSJC and HMC wrote/revised the manuscript, which was reviewed by XFH and AM.

3.1. Abstract

Toenail arsenic is a biomarker that reflects chronic exposures of several months compared to recent exposures reflected by urinary arsenic. While arsenic species in urine are the most commonly used biomarkers of arsenic exposure, there is comparatively limited information on the application of arsenic speciation in toenail samples. This study aims to assess arsenic and its species in toenails as biomarkers for exposure in a population residing near an abandoned gold mine.

Toenail samples were measured for arsenic via speciation analysis in residents ages 3-79 (n=1872) living in Yellowknife, Dettah and Ndilo, in the Northwest Territories, Canada. Total arsenic (tAs) concentrations in toenails were significantly higher ($p<0.05$) in children (GM: 424 µg/kg) than in adults (GM: 116.2 µg/kg). Geometric means speciated arsenic in toenail were 50.1 µg/kg for AsIII, 451.7 µg/kg for AsV, 0.1 µg/kg for monomethylarsonic acid (MMA), 0.1 µg/kg for dimethylarsinic acid (DMA), and 93.38 µg/kg for organic arsenic species in children. In adults, geometric mean concentrations were 7.7 µg/kg for As III, 105.7 µg/kg for AsV, 0.2 µg/kg for MMA, 0.3 µg/kg for DMA, and 33.59 µg/kg for organic arsenic species. Arsenic species were all significantly higher in children except for MMA and DMA. Children's mean arsenic speciation patterns were 20.4% AsIII, 67.5% AsV, 0.6% MMA, 1.2% DMA, and 10.2 % organic arsenic. For adults, speciation patterns were similar, with 17.5% AsIII, 63.2% AsV, 1.7% MMA, 3.1% DMA, and 14.5 % organic arsenic. The percentage of inorganic-related arsenic (iAs-met), defined as the sum AsIII+AsV+MMA+DMA, was significantly higher in participants with elevated toenail tAs concentrations (i.e., > 80th percentile in children and >95th percentile in adults) both children and adults. The relationship between urine and toenail concentrations in children was significant but weak for iAs-met ($r= 0.14$, $p=0.003$) but not significant for tAs ($r=0.03$, $p=0.59$). While in adults, the relationship was significant but weak for both iAs-met ($r=0.15$, $p<0.001$) and

tAs($r=0.13$, $p<0.001$) in adults. Arsenic speciation patterns differed between toenails and urine, where AsV, AsIII and organic As were the dominant species in toenails. In contrast, urinary arsenic was mainly composed of DMA and organic As. Seasonality (spring/summer), recreational water activities, fishing, eating garden-grown produce and eating local wild plants were identified risk factors for children with elevated tAs. For adults, elevated toenail tAs was associated with seasonality, lake water as a main drinking water source, working at Giant Mine, and eating local meat, mushrooms, and wild plants. The study results of toenail arsenic speciation contribute to understanding arsenic exposure in the human population of Yellowknife and support the use of tAs in toenails as a complement to urinary arsenic in prospective monitoring.

3.2. Introduction

Arsenic is a toxic metalloid naturally found in the Earth's crust as a component of minerals and rocks. Release of arsenic into the environment may occur through natural processes (e.g., volcanic eruption and weathering) and anthropogenic activities, such as mining and smelting [1]. In the environment, arsenic exists in both inorganic and organic forms. Inorganic arsenic species include trivalent arsenite (AsIII) and pentavalent arsenate (AsV), which may be found in minerals, rocks, and water. Organic arsenic species (e.g., arsenosugars, arsenolipids, arsenocholine and arsenobetaine) are typically found in the environment in algae, seafood and fish [2]. Toxicity varies with arsenic species, where toxicity is associated with inorganic arsenic (e.g., AsIII and AsV) and its related methylated forms, such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA).

Meanwhile, organic species (e.g., arsenobetaine) are generally considered to be non-toxic. Human exposure to arsenic, particularly inorganic arsenic, is a global public health concern [3]. Chronic exposure to inorganic arsenic has been associated with adverse health outcomes, which include dermal effects, cardiovascular disease, impaired lung function, and various cancers [4–7].

In Canada, the presence of arsenic in the environment is generally low due to the higher geological presence of arsenic or release via anthropogenic emissions, except for some regions [8]. For instance, arsenic has been a public health concern in Yellowknife in the Northwest Territories (NWT) due to the geological presence of arsenopyrite and proximity to past mining operations, particularly Giant Mine. Giant Mine was a gold mine in operation from 1948 to 2004, located 4 kilometres north of city limits [9]. Despite the mine's closure, mining operations, particularly gold extraction processes, have left a legacy of 237,000 tonnes of arsenic trioxide by-product in underground chambers [10]. Consequently, Giant Mine has been regarded as one of

the most contaminated sites in Canada [11]. Though local geology is abundant in arsenic, there is an observed spatial gradient in soil, lake water and lake sediment concentrations corresponding to historical mining emissions from Giant Mine [12–15]. As a result, there are public health concerns about environmental contamination of arsenic and other metals due to initial uncontrolled roaster emissions, as well as potential surface runoff and groundwater migration. Currently, overall environmental levels of arsenic exposure (e.g., in drinking water, vegetation, etc.) are not considered significant risks to the health of the local population of Yellowknife [16].

We had previously reported that urinary concentrations of inorganic-related arsenic (iAs-met), defined as the sum of AsIII, AsV, MMA and DMA, in children in Yellowknife were significantly higher than that of Yellowknife adults, as well as children in the general Canadian population[17]. Our results also suggest that arsenic exposure in Yellowknife is unlikely to be from drinking water, but other sources (e.g., diet or exposure to soil and dust) may be more significant contributors [18]. Given the public health risks of chronic arsenic exposure, biomarkers are valuable tools in biomonitoring and risk assessment to estimate exposure and toxicity. Several biological matrices can be used to measure the internal dose of arsenic, such as urine, hair, and nail (finger and toenail). Urine is the most frequently used biomarker to assess arsenic body burden in human biomonitoring. However, urinary arsenic represents a relatively recent exposure of up to 5 days, therefore may not always accurately reflect chronic exposure [19]. Furthermore, total urinary arsenic (tAs) concentrations may be influenced by dietary intake of non-toxic organic arsenic species (e.g., arsenobetaine) commonly found in foods like seafood. Thus, the contribution of organic arsenic to tAs may be confounding, leading to an overestimation of arsenic toxicity [2]. Arsenic speciation in urine is therefore important for evaluating inorganic arsenic exposure via ingestion. Alternatively, toenails are a suitable

biological matrix for measuring arsenic body burden because arsenic has an affinity for thiol groups present in keratin-rich tissues such as nails [20]. Nails are exposed to blood vessels as they grow, where contaminants like arsenic with an affinity for keratin may accumulate in nails [21].

The toenail is a useful biomarker for population studies and biomonitoring due to its feasibility in terms of ease of storage, stability, and non-invasiveness [22]. It is also less susceptible to contamination than other biological matrices like hair or fingernail. Compared to urine, toenails reflect a more prolonged exposure of up 12 months rather than an exposure of several days [23]. Toenail has been previously shown to be a reliable biomarker to estimate arsenic exposure via drinking water, particularly for elevated concentrations [24–26]. Toenails have also been used as a biomarker to assess arsenic exposures from diet [27], soil [28,29] and dust [29–31]. Arsenic is often measured as total arsenic in toenails though quantification of arsenic species through speciation analysis is possible. Arsenic species in toenails as a biomarker may be useful in estimating inorganic arsenic exposure and potential toxicity in risk assessment. However, previous reports on arsenic speciation in toenails are limited, consisting of populations with elevated arsenic exposures from drinking water or contaminated sites [32,33]. Thus, the application is yet to be elucidated, particularly for low-to-moderate exposures and populations where drinking water is not the primary exposure source, such as in Yellowknife.

In this study, toenail arsenic was assessed as a biomarker of exposure in the human population where the primary exposure is not drinking water. The main objective of this study was to compare the applicability of toenail arsenic speciation over total metal analysis, as well as compare arsenic in urine and toenail as biomarkers of exposure. We also aimed to identify potential risk factors associated with elevated toenail arsenic concentration among the residents

living in Yellowknife and the Yellowknives Dene First Nation (YKDFN) communities of Dettah and Ndilǫ.

3.3. Materials and Methods

3.3.1. Ethics

The study is approved by the Health Sciences and Sciences Research Ethics Board of the University of Ottawa (http://research.uottawa.ca/ethics/reb) and the Aurora College Research Ethics Committee. In addition, the study has been granted a Scientific Research License from the Aurora Research Institute in Northwest Territories. Individual participation in the project was voluntary and based on informed written consent following an oral and written explanation of the study and each component of participation.

The conducted research followed the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, particularly chapter 9, which concerns research involving the First Nations, Inuit and Métis Peoples of Canada [34], as well as the World Health Organization publication entitled: Indigenous Peoples & Participatory Health Research: Planning & Management, Preparing Research [35]. The study also follows the First Nations principles of Ownership, Control, Access and Possession (OCAP®) of data [36].

3.3.2. Study Population and Data Collection

The presented research is part of the Health Effects Monitoring Program, a prospective cohort study established to monitor levels of arsenic and other chemicals of potential concern (COPCs) in the human population of Yellowknife and the First Nation communities of Dettah, and Ndilo as remediation of Giant Mine progresses. A total of 2037 individuals from ages 3 to 79 participated in the baseline study. Recruitment and data collection of the baseline cohort was conducted in two waves; the first wave occurred from September to December 2017, and the

second wave occurred from April to June 2018. Under the recommendation of the local community members, different recruitment strategies were developed for data collection from population groups in the area: (1) Yellowknives Dene First Nation (YKDFN), (2) the North Slave Métis Alliance (NSMA) and the general population of Yellowknife, which were further grouped into (3) Random Selection and (4) Volunteer. For the general Yellowknife population, households were randomly selected where one adult and one child, if applicable, were invited to participate based on the upcoming birth date. The inclusion criteria were residents of Yellowknife between the ages of 3 to 79 that have lived in Yellowknife for at least 1 year. The study also included individuals not selected for random sampling as a separate sample group labelled as volunteers due to interest from the local community. All members of the local indigenous communities, the Yellowknives Dene First Nation and North Slave Métis Alliance, were invited to participate voluntarily. A detailed description of the Health Effects Monitoring Program and the study's methodology is described previously in the reported cohort profile. [17]

All participants were asked to complete a lifestyle questionnaire and provide biological samples of toenails and urine. The questionnaire was conducted by a trained research assistant consisting of lifestyle and potential exposure information (e.g., age, sex, smoking status, water sources, diet, etc.), as well as a food frequency questionnaire regarding local fish consumption. Biological samples of urine and toenail were also collected for total metal and arsenic speciation analysis. Stainless steel clippers were provided to all participants, and they were recommended to provide clippings from all toes. For urine sampling, participants were instructed to abstain from eating seafood three days before sampling and to provide the first-morning void at their earlier convenience. Once collected, samples were kept at the local research office under appropriate storage conditions (at room temperature for toenails and in the refrigerator at 4°C for

urine) until shipment to the University of Ottawa. There were 1966 urine samples (497 children, 1469 adults) that were provided, and 1872 (447 children, 1425 adults) toenail samples were obtained.

3.3.3. Laboratory Analysis

3.3.3.1 Sample Preparation and Digestion

Biological samples of urine (n=1966), kept on ice, and toenails (n=1872) were shipped from Yellowknife to the University of Ottawa.

Toenail samples (n=1872) were stored in ambient temperatures until analysis. Toenails were washed and digested following methods adapted from previously reported methods [37,38]. Exogenous materials were removed from toenails using tweezers, then cleaned via repeated cycles of sonication in acetone and rinsed with Milli-Q water. Samples were then dried overnight in a 60 °C oven. Before digestion, toenails were mechanically ground via ball bearings to fine dust. Toenail samples were then digested using 0.28 M HNO_3.

Urine samples were thawed overnight and then kept on ice on the day of sample analysis. Before quantification, urine samples were diluted 1:10 in 1% HNO_3 (Sigma Aldrich, cat. # 84385-2.5l).

3.3.3.2. Total Arsenic Analysis

Toenail and urine samples were analyzed for total arsenic via inductively coupled plasma mass spectrometry (ICP-MS). Chemical analyses were performed at the Laboratory for the Analysis of Natural and Synthetic Environmental Toxicants (LANSET) at the University of Ottawa. Inductively coupled plasma mass spectrometry (ICP-MS) (7700x ICP-MS, Agilent Technologies, Japan) was used to quantify total arsenic in urine and toenail. The system is equipped with a low-flow borosilicate glass Micro Mist concentric nebulizer and quartz, Scott-

type double pass spray chamber. The interface consisted of a 1mm diameter Ni-typed sampling and a 0.4 mm diameter Ni-typed skimmer cone. Octopole Reaction System (ORS) was used as interference removal in Helium mode. An Agilent ASX-500 was used as an ICP-MS autosampler.

3.3.3.4. Arsenic Speciation Analysis

For arsenic speciation analysis, ICP-MS coupled with liquid chromatography (LC-ICP-MS) was used to quantify the following arsenic species in urine and toenail: arsenic species arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA), and organic arsenic species (i.e., arsenobetaine). Samples were diluted in 10mM ammonium phosphate dibasic, which was prepared by dissolving ammonium phosphate dibasic (Sigma, cat#379980-100G) in Milli-Q water (Millipore) and pH adjusted to 8.25 with 28% Ammonium hydroxide solution (Sigma, Cat#: 338818-100ML). The chromatographic separation of the arsenic species was performed using a 10mM ammonium phosphate dibasic buffer with pH adjusted to 8.25 on an Agilent 1200 Infinity LC system consisting of a 1260 Isocratic pump and 1260 Autosampler with a Hamilton PRP-X100 anion exchange column (250mm x 4.1mm,10um particle). The LC system was connected to the Agilent 7700x ICP-MS via Peek tubing and equipped with a low-flow Micro Mist Nebulizer and quartz, low-volume Scott-type double-pass spray chamber. The mobile phase was delivered at 1mL/min, and the injection volume was fixed at 100uL.

The detection limit in the urine samples was 0.012 µg/L for total arsenic and 0.005 µg/L for the arsenic species. The detection limit in the toenail samples was 0.05, 0.098, 0.136, 0.78, 0.079, and 0.079 µg/kg for total arsenic, AsIII, AsV, MMA, DMA, and arsenobetaine, respectively.

3.3.3.5. Standards and Quality Control

Stock solutions were diluted in 1% nitric acid and used to provide a working calibration curve of at least five points. For analytical quality controls, an element quality control standard stock (High-Purity Standards, Cat# QCS-19) and urine multi-element stock (High-Purity Standards, Health solution A) were used as check standards after calibration and then every 10 to 20 samples. In addition, different reference materials (NIST reference materials: SRM 2669 level 1 and level 2 arsenic species in frozen human urine, SRM 2668 level 1 and level 2 Toxic elements in frozen human urine, IAEA 407-trace elements and methylmercury in fish tissue, and IAEA 085-human hair references from the International Atomic Energy Agency and Dolt-4-Dogfish liver certified reference material for trace metals and other constituents from the National Research Council Canada), and pooled samples for a spike recovery test were included in the analysis. The results for both check standard, spike recovery and reference materials were within 10-20% of expected values.

For quality control of the study, ~2.5% of the collected urine samples (n=50) were randomly selected and sent to the Institut Nationale de Santé Public du Québec (INSPQ) for interlaboratory comparison. No statistical difference ($p > 0.05$) was observed between the results obtained from the University of Ottawa and INSPQ laboratories (Appendix 3.A). Additionally, a portion of toenail samples was sent to the Geochemistry Laboratories at the University of Ottawa for total metal analysis.

3.3.4 Statistical Analysis

Statistical analyses were performed using R v.4.2.1. [39] Descriptive statistics for arsenic species concentrations in urine and toenail were calculated for the following: Arsenic species (AsIII, AsV, MMA, DMA, and arsenobetaine) as well as the sum of As species from speciation

analysis, total As, iAs (AsIII + AsV), and inorganic related arsenic (iAs-met) which is defined as the sum of AsIII + AsV + MMA + DMA. Additionally, % As for each species was calculated as % concentration of As species/% sum of As species in the toenail.

Spearman correlation was used to assess the relationship between arsenic species and total arsenic in toenails. Additionally, spearman correlation and linear regression models were used to evaluate the relationship between the same arsenic species in urine and toenail. Cook's Distance was used to identify potential influential outliers. Statistical tests and regression models were conducted without potential outliers to assess influence. Identified outliers did not change the results of the analyses.

Arsenic species in toenails were also assessed by comparing concentrations and percentages of different arsenic species between participants with normal and elevated total toenail arsenic concentrations. The 80th percentile for children (1350 μg/kg) and 95th percentile (504 μg/kg) for adults were chosen as cut-off screening levels to identify participants with elevated exposure levels. Those with normal levels were classified as those with concentrations below the respective cut-offs. The cutoff of the 80th percentile was chosen for children due to the overall elevated levels in children. Welch's t-test and Z-test for proportions were used to assess differences in concentrations and proportions, respectively, between normal and elevated groups. Risk factors of elevated toenail arsenic were identified via Chi-Square tests of responses for different variables between elevated and normal groups.

Multilinear regression analyses were used to compare different arsenic species in urine and toenail. Each species in both urine and toenail was evaluated by their adjusted R^2 in regression models with all factors obtained from the lifestyle questionnaire. The variables included were: sex; age; BMI; population group; waves of sampling; district of residence; smoking status (adult

only); primary water source; drinking from lakes (yes/no); filter drinking water (yes/no); daily water intake; recreational water activities (yes/no); hunting (yes/no); eat locally hunted meat (yes/no; fishing (yes/no); eat local fish (yes/no); eat local garden grown vegetable/herbs (yes/no); eat locally harvested berries; eat locally harvested mushrooms (yes/no); consumption frequency of market seafood; consumption frequency of rice; currently work at Giant Mine (adult only); and former mine worker (adult only).

3.4. Results

3.4.1 Summary of arsenic species in toenail

Among the 2037 participants, 1872 toenail samples were collected from 447 children (ages 3-19) and 1425 adults (ages 20-79). Overall, the mean age was 37 years old, and the sex distribution was approximately equal (45.8% male, 54.2 % female). The mean age was 10.3 years old for children, while the mean age was 45.7 years old for adults.

Concentrations of arsenic species and total arsenic in toenails are summarized in Table 3.1. Arsenic speciation analysis showed that most of the arsenic in toenails is AsV, followed by AsIII. The arsenic speciation patterns in children were 20.4% AsIII, 67.5% AsV, 0.6% MMA, 1.2% DMA, and 10.2 % organic arsenic. Arsenic speciation patterns in adults were similar, with 17.5% AsIII, 63.2% AsV, 1.7% MMA, 3.1% DMA, and 14.5 % organic arsenic. Total toenail concentrations were significantly higher in children ($p<0.05$), as were concentrations of AsIII, AsV, and organic arsenic. No significant difference in toenail concentrations was observed between the sexes.

Table 3.1. Summary of concentrations of Arsenic species in toenails (µg/kg) for children (3-19 years old) and adults (>20 years old). Individual arsenic species are presented as well as inorganic arsenic (iAs), defined as the sum of AsIII+V and inorganic related arsenic (iAs-met), defined as the sum of AsIII + AsV + MMA + DMA.

	Child			**Adult**		
As Species	GM (SD)	Range	% As	GM (SD)	Range	% As
As(III) *	50.1 (18.2)	0.03-3093	20.4	7.7 (16.6)	0.03-2365	17.5
As(V) *	451.7 (3.1)	23.81-7826	67.5	105.4 (2.8)	0.07-4976	63.2
MMA	0.1 (7.9)	0.04-66	0.6	0.2 (10.5)	0.04-51	1.7
DMA	0.1 (14.7)	0.01-273	1.2	0.3 (18.1)	0.01-223	3.1
Organic*	51.8 (3.0)	2.55-886	10.2	18.8 (2.9)	0.02-1020	14.5
iAs (AsIII+AsV)*	598.9 (3.0)	29.6-8970	87.9	138.0 (2.6)	0.1-5857	80.7
iAs-met* (AsIII+AsV+MMA+DMA)	612.5(2.9)	41.4-8970	89.8	146.7(2.5)	0.14-5867	85.5
Sum of As species*	685.9 (2.9)	45.75-9474	100	174.6 (2.4)	0.16-6866	100
Total As *	424.0 (3.3)	10.1-7335.2	-	116.2 (2.4)	0.31- 4155.1	-

* Significantly different between adults and children
GM: Geometric mean
SD: Geometric standard deviation
% of total As: % concentration of species in toenail/% sum of total As in toenail

3.4.2 Comparison between arsenic species and total arsenic analysis in toenail

The relationship between arsenic concentrations via speciation and total metal analysis in toenails was explored via Spearman correlation and summarized in Table 3.2. In children, the sum of arsenic species is highly correlated ($r=0.83$, $p<0.05$) with total arsenic measured via the total metal analysis. The correlation between the sum of arsenic species and total arsenic is lower in adults ($r=0.63$, $p<0.05$).

Table 3.2. Spearman correlation coefficients (r) between total arsenic from total metal analysis and arsenic species from speciation analysis.

	Child	Adult
As Species	r	r
Total As	1	1
Sum of As species	0.81	0.63
iAs (AsIII+AsV)	0.83	0.63
iAs-met (AsIII + AsV + MMA + DMA)	0.83	0.66

To understand the difference between speciated arsenic and total arsenic in toenails, As species concentrations and speciation profiles were compared between participants with elevated total arsenic and those with normal levels (Tables 3.3a-b). Elevated was defined as total toenail concentrations above the 80th percentile (i.e., Top 20%) for children and total toenail concentrations above the 95th percentile (i.e., Top 5%) for adults. Species concentrations between the two groups were all significantly different, except for MMA and DMA for children. When comparing the proportions of As species in the toenail, percentages (%) of AsV, organic As, iAs-met (AsIII+AsV+MMA+DMA), and iAs (AsIII+AsV) were significantly higher in participants with elevated total toenail As concentrations for both children and adults.

Table 3.3a. Comparison between toenail concentrations of normal (Children: <80th percentile; Adults: <95th percentile) and elevated (Children: >80th percentile; Adults: >95th percentile) for different arsenic species for children (ages 3-19) and adult (ages 20-79) participants.

	Children (3-19)			Adult (20-79)		
	Normal (n=357)	Elevated (n=90)		Normal (n=1357)	Elevated (n=68)	
Species	mean (ug/kg)	mean (ug/kg)	p-value	mean (ug/kg)	mean (ug/kg)	p-value
AsIII	132.1	609.8	**<0.001**	35.6	175.8	**<0.001**
AsV	435.6	2429.2	**<0.001**	146.3	968.3	**<0.001**
MMA	3.0	4.3	0.356	2.7	8.2	**<0.001**
DMA	5.2	12.8	0.109	4.9	8.4	*0.054*
Organic	70.8	182.9	**<0.001**	34.0	68.0	*0.053*
iAs-met (AsIII+AsV+MMA+DMA)	575.9	3056.1	**<0.001**	189.4	1160.7	**<0.001**
iAs(AsIII+V)	567.7	3039.1	**<0.001**	181.8	1144.1	**<0.001**
Sum of As species	646.8	3239.0	**<0.001**	223.4	1228.7	**<0.001**

Table 3.3b. The proportion of As species (%) in toenail between toenail exceedance (n=158) and non-exceedance (n=1712) groups for different species by age groups.

	Children (3-19)			Adult (20-79)		
	Normal (n=357)	Elevated (n=90)		Normal (n=1357)	Elevated (n=68)	
Species	%	%	p-value	%	%	p-value
AsIII	20.4	18.8	0.37	15.3	14.3	0.78
AsV	67.3	75.0	**<0.001**	62.7	78.8	**<0.001**
MMA	0.4	0.1	0.20	1.2	0.7	0.70
DMA	0.8	0.4	0.28	0.2	0.7	0.87
Organic	10.9	5.6	**<0.001**	14.6	5.5	**<0.001**
iAs	89.0	94.4	**<0.001**	81.1	94.5	**<0.001**
AsIII+V	87.8	83.8	**<0.001**	77.9	93.1	**<0.001**

3.4.3 Comparison between the toenail and urinary arsenic

The relationship between urinary and toenail for iAs-met and total arsenic concentrations is shown in Figure 3.1. For children, the relationship between urine and toenail concentrations was significant but weak for iAs-met (Linear regression: adjusted $R^2 = 0.009$, p=0.02; spearman correlation: r= 0.14, p=0.003) but no correlation was observed for tAs (Linear regression: adjusted $R^2 = -0.002$, p=0.97; Spearman correlation: r=0.03, p=0.59), as shown in panels A and C respectively. For adults, the relationship via linear regression between urine and toenail concentrations was significant but weak for both iAs-met (Linear regression: adjusted $R^2= 0.01$, p<0.001, Spearman correlation: r=0.15, p<0.001) and tAs (Linear regression: adjusted $R^2= 0.009$, p<0.001; spearman correlation: r=0.13, p<0.001), as shown in panels B and D respectively.

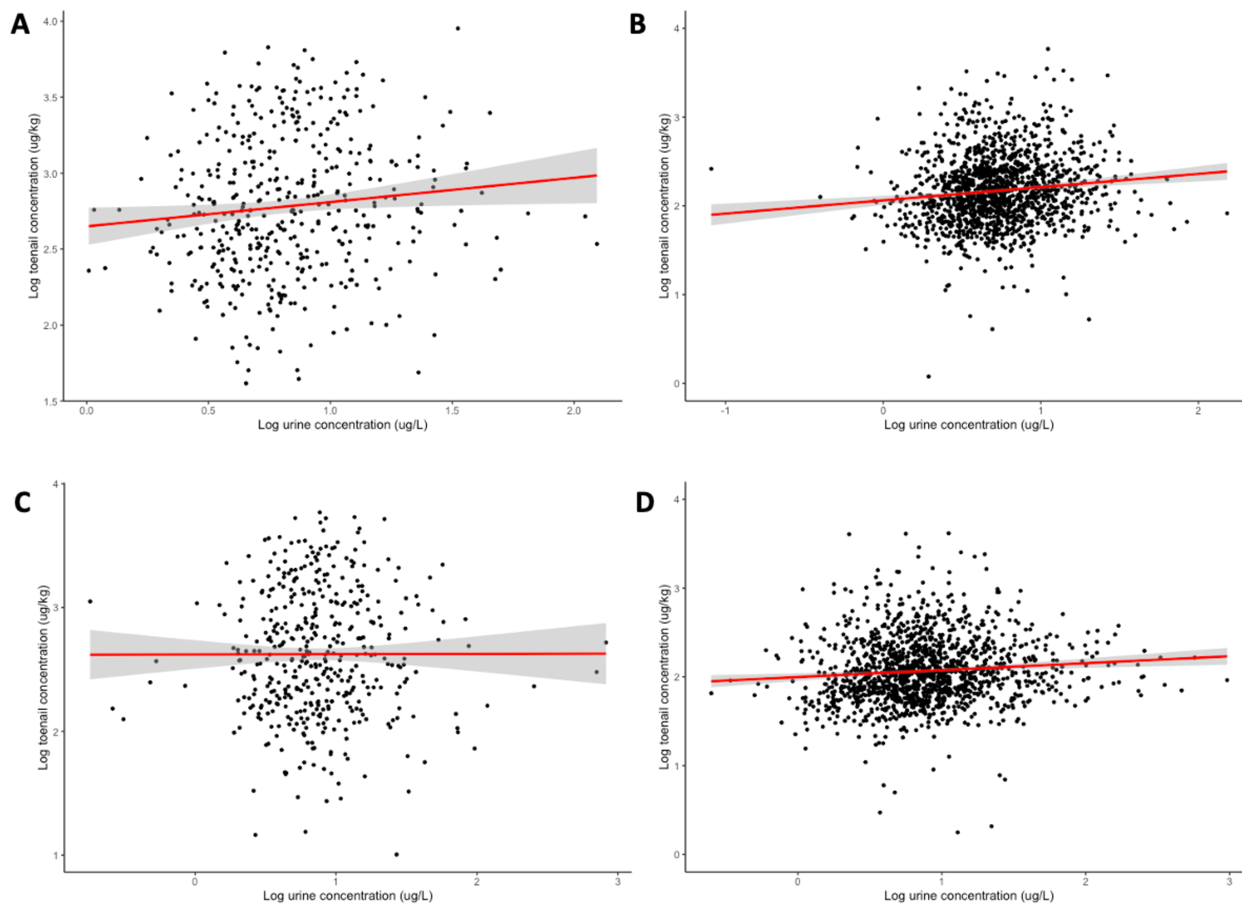

Figure 3.1. Relationship between urine and toenail concentrations of total arsenic and iAs-met(AsIII+AsV+ MMA+ DMA) by age group (children and adults) where the simple linear regression is represented by the solid red line, while the standard error of the regression is represented by the solid red line, while the standard error of the regression is represented by the grey shaded area. The relationships between urine and toenail concentrations are depicted in: A) iAs-met for children (p=0.02), B) iAs-met for adults (p<0.001), C) Total As for children (p=0.97), D) Total As for adults (p<0.001). Concentrations are log-transformed.

Arsenic speciation patterns in toenails and urine are compared in Fig 3.2. In toenails, arsenic is predominantly present as AsIII and AsV, while in urine, arsenic is mainly present as DMA and organic arsenic.

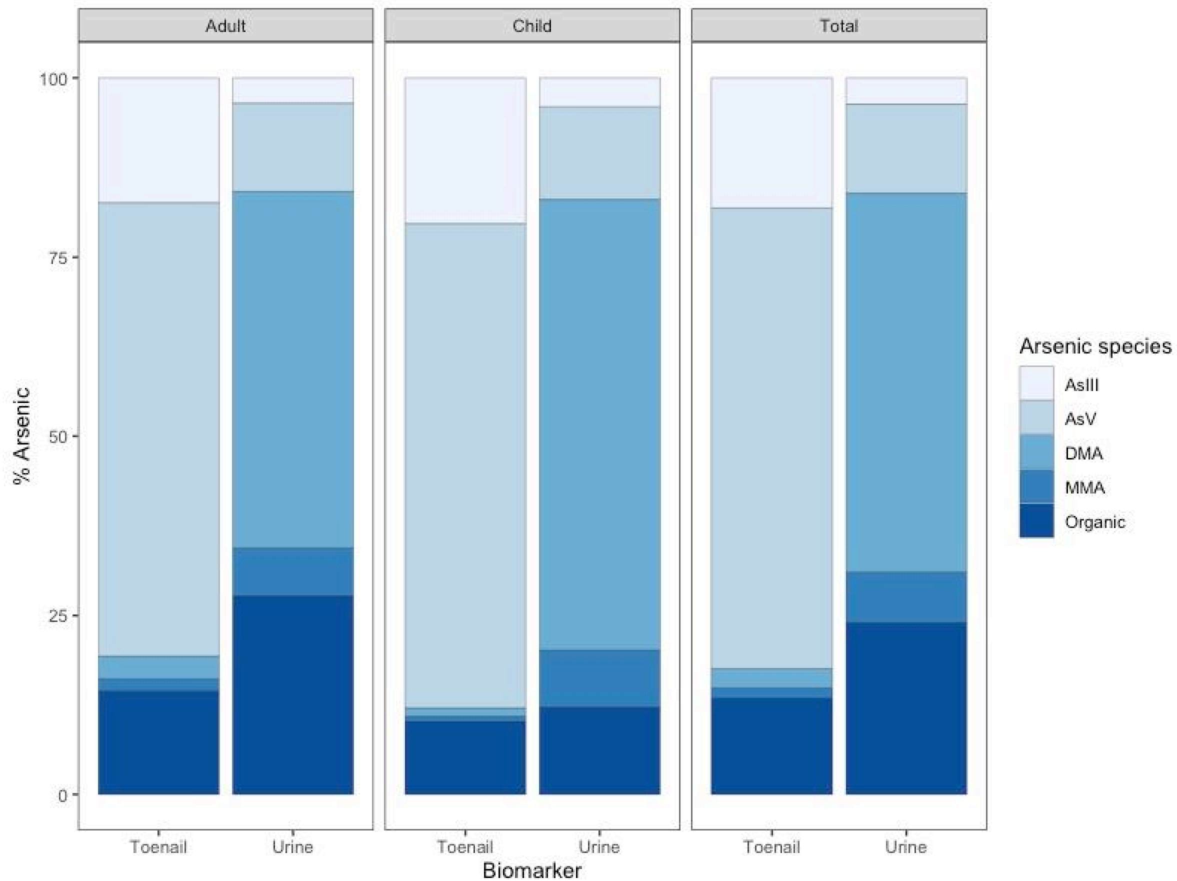

Figure 3.2. Comparison of arsenic in speciation patterns in toenail and urine of children and adults.

To assess the different arsenic species and types of biomarkers (i.e., urine and toenail) for estimating arsenic exposure, multiple linear regression models for each species in toenail and urine were compared (Table 3.5). Overall, the adjusted R^2 was higher for the toenail models compared to the urine models.

Table 3.4. Regression summary arsenic species in urine and toenail accounting for lifestyle factors related to arsenic exposure.

	Toenail				Urine			
	Child		Adult		Child		Adult	
As Species	R^2	p value	R^2	p value	R^2	p value	R^2	p value
Total As	0.160	6.5E-08	0.067	4.1E-11	0.026	0.141	0.027	0.001
Sum of As Species	0.194	2.9E-10	0.064	1.7E-10	0.027	0.133	0.025	0.002
iAs-met (AsIII+AsV+MMA+DMA)	0.198	2.3E-10	0.066	6.6E-11	0.125	1.0E-05	0.083	8.8E-15
iAs (AsIII+V)	0.195	2.6E-10	0.065	9.3E-11	0.025	0.148	0.015	0.029

R^2 values are adjusted R^2.

3.4.4 Analysis of risk factors

To assess risk factors associated with elevated total arsenic in toenails, cross-tabulations of different variables related to arsenic exposure (Table 3.5) were used to compare characteristics of participants with relatively higher toenail arsenic concentrations (i.e., top 20% in children, top 5% in adults) and the other participants with lower concentrations. For children, the responses for wave of sampling, population group, recreational water activities, fishing, eating produce grown from the garden, and eating local wild plants significantly differed between normal and elevated toenail As groups. For adults, the responses between normal and elevated toenail As groups were significantly different for wave of samples, primary drinking water source, working at Giant Mine, and eating local meat, local mushrooms, and local wild plants.

Table 3.5. Cross tabulation (%) of arsenic risk factors between participants with normal and elevated total As concentrations (tAs) in the toenail. For children, the normal/elevated cut-off was the 80th percentile (P80) where normal is defined as tAs<P80 and elevated is tAs>P80. For adults, the normal/elevated cut-off was the 95th percentile (P95) where normal is defined as tAs<P95 and elevated is tAs>P95.

		Child (%)			**Adult (%)**		
Variable		Normal	Elevated	p value	Normal	Elevated	p value
Sex	Male	48.7	58.9	0.31	44	48.5	0.62
	Female	51	41.1		56	51.5	
Wave of sampling	Fall/Winter 2017	42	14.4	**0.001**	49.8	29.4	**<0.001**
	Spring/Summer 2018	58	85.6		50.2	70.6	
Population group	Random Selection	44	36.7	**0.005**	45.5	38.2	0.17
	Volunteer	36.1	54.4		43.8	55.9	
	YKDFN	17.9	6.7		8.3	5.9	
	NSMA	2	2.2		2.3	0	
Main water source	Public Water Supply	93.7	90.9	0.45	93.1	87.9	**0.01**
	Private Well/Spring	0	0				
	Bottled Water	6	8		4.9	4.5	
	Fresh water (e.g., river, lake)	0.3	1.1		2.1	7.6	
Drink from lake	No	81.2	77.5	0.52	67	54.5	0.051
	Yes	18.8	22.5		33	45.5	
Recreational water activities	No	16.2	4.5	**0.007**	38.5	30.3	0.23
	Yes	83.8	95.5		61.5	69.7	
Eat locally hunted meat	No	34.8	42.7	0.2	38.6	25.8	**0.05**
	Yes	65.2	57.3		61.4	74.2	
Fish	No	46.7	33.7	**0.04**	47.2	42.4	0.53
	Yes	53.3	66.3		52.8	57.6	
Eat local fish	No	26.2	31.5	0.38	12.4	12.1	1.00
	Yes	73.8	68.5		87.6	87.9	
Eat locally grown vegetables/herbs	No	43.9	29.2	**0.02**	38.3	25.8	0.054
	Yes	56.1	70.8		61.7	74.2	
Eat local berries	No	51.3	41.6	0.13	12.4	12.1	1

	Yes	48.7	58.4		87.6	87.9	
Eat local mushrooms	No	96.6	92.1	0.12	83.8	71.2	**0.01**
	Yes	3.4	7.9		16.2	28.8	
Eat wild plants	No	84.3	73	**0.02**	79.2	65.2	**0.01**
	Yes	15.7	27		20.8	34.8	
Work at Giant Mine (20+ only)	No	-	-	-	99	93.9	**0.001**
	Yes	-	-	-	1	6.1	

3.5. Discussion

3.5.1 Summary of arsenic species in toenail

Total arsenic toenail concentrations from our study are higher than concentrations observed in unexposed populations in both adults and children [38,40]. Our results are also within range, but generally higher, than toenail arsenic concentrations observed in both children and adults from Canadian populations with low to moderate arsenic exposure concentrations [26,41–43] However, toenail arsenic concentrations in our study are generally lower than populations near mining or contaminated sites [29,44]. Furthermore, toenail concentrations are considerably lower than observed concentrations in known arsenic-endemic areas such as Bangladesh and West Bengal, where mean total arsenic concentrations in toenails were observed to exceed 1000 ug/kg [45–47]. In our studied population, toenail concentrations were significantly higher in children than in adults (Table 3.1), consistent with existing literature with cohorts consisting of both children and adults [28,42]. Children may have higher exposures and subsequently toenail arsenic concentrations due to certain behaviours and activities, such as playing outdoors, barefoot, on the ground, etc. [48].

In this study, arsenic species were quantified in toenails in addition to total arsenic analysis. While there is abundant literature concerning toenails as a biomarker of exposure, there are

limited studies concerning arsenic speciation in toenails. Overall, measured toenail concentrations of speciated arsenic are higher than a population with no known arsenic exposure and a cohort of Canadians [38,49]. However, mean toenail concentrations from our study are lower than cohorts living near mining or contaminated areas [38], as well as populations exposed to elevated arsenic levels in drinking water [21,32].

3.5.2 Comparison between arsenic species and total arsenic analysis in toenail

When comparing toenail arsenic via speciation or total metal analysis, the sum of arsenic species and total arsenic was highly correlated in children (p=0.81) but less so in adult toenails (p=0.63), as observed in Table 3.2. This may be because overall toenail arsenic concentrations in children are higher, thus less variability in concentrations between the analyses. Lower concentrations (i.e., in adults) may lead to higher variability overall, hence the discrepancy between total arsenic and speciated concentrations.

Speciation analysis indicated that arsenic in the toenail of study participants was mainly comprised of AsIII and AsV, representing 87.9% in children and 80.7% in adults (Table 3.1, Figure 1.2). Children have higher percentages of AsIII and AsV, which is likely due to their behaviours (e.g., playing barefoot, outside, etc.). Furthermore, when comparing arsenic profiles between participants with different toenail arsenic concentrations, elevated toenail arsenic groups (i.e., Top 20% in children, top 5% in adults) generally had a lower % of AsIII, MMA and DMA but a significantly higher % of AsV and organic arsenic (Tables 3.3a-b.), indicating a higher presence of inorganic arsenic.

Compared to other Canadian cohorts in Atlantic Canada, our study observed both higher concentrations and % of AsIII+V but significantly lower % of MMA and DMA [49,50]. On the other hand, our results differed from patterns observed in residents exposed to elevated arsenic

concentrations in West Bengal, India, [21,32]. For those studies, AsIII was the species with the highest in toenail species, rather than AsV. In a UK study, AsIII was also observed to be the dominant species for both residents living near a former mine and the control population [38]. The observed difference may be due to the source of arsenic exposure, as those studies were of populations exposed to elevated arsenic levels via drinking water. The difference may also be due to methodology, particularly incubation times and temperatures used during sample digestion [38]. Incubations longer than 30 minutes may result in the gradual oxidation of AsIII to AsV, which can be accelerated by high temperatures (> 90 °C). This is a potential limitation in our study, and inorganic arsenic should be estimated as AsIII+AsV to take that into account. It should also be noted that the compared studies in this discussion measured only AsIII, AsV, MMA and DMA, but not organic arsenic. Thus, further investigations regarding organic arsenic are needed to understand its fate and consequent deposition in toenails.

3.5.3 Comparison between the toenail and urinary arsenic

Toenail arsenic concentrations were significantly associated with adults' urine, though the correlation was weak (Figure 3.1). In children, there was also a significant but weak correlation between toenail and urine inorganic-related arsenic concentrations, but no correlation was observed for total arsenic. Correlations between urine and toenail arsenic concentrations tend to be stronger with increasing levels of environmental arsenic, mainly when drinking water is a significant exposure source [24,51]. In Yellowknife, drinking water is not likely to be a significant source of arsenic exposure and does not pose a significant health risk to the general population [18]. Instead, we posit that arsenic exposure is likely to be from other sources such as diet, ingested dust, soil, etc.

Our results demonstrate that different arsenic species are dominant depending on the biological matrix. Toenails comprised more of AsIII and AsV, while urine was mainly comprised of DMA and organic arsenic. Toenail inorganic arsenic concentrations were significantly associated with urinary concentrations for both adults and children. For total arsenic, toenails are significantly related to urine in adults, but not children. As observed in Figure 3.2, contributions of MMA and DMA to toenail arsenic is minimal, while AsIII and AsV are the dominant species in toenails. Thus, the use of inorganic arsenic may not be a useful indicator for assessing arsenic exposure and toxicity for toenails. In urine, methylated arsenic species (i.e., MMA and DMA) are included in evaluating inorganic arsenic exposure because of arsenic metabolism. Ingested inorganic arsenic is metabolized through a series of methylation reactions generating MMA and DMA as methylated products, excreted via urine [52]. For toenails, the fate of arsenic species is less clear. Organic species such as arsenobetaine are not absorbed by the digestive system when ingested and are excreted via urine relatively unchanged [2]. Thus, ingested arsenobetaine is unlikely to accumulate in toenails.

Further understanding of the source of different arsenic species in the toenail is needed. Despite cleaning methods in preparing samples, surface adsorption is possible. However, it is not always possible to distinguish between arsenic from ingestion and exogenous sources from surface absorption. Advanced techniques, such as laser ablation, can measure arsenic species in the different layers of the toenail. Though costly for human biomonitoring of large populations, the use of laser ablation could elucidate the contribution of arsenic exposure from ingestion and adsorption from exogenous sources.

To evaluate different arsenic indicators and biomarker types, multiple linear regression models for each species in toenails and urine were compared (Table 3.4). Overall, the adjusted

R^2 was consistently higher for the toenail models than the urine models, suggesting that toenails may be a better predictor of arsenic exposure in our cohort compared to urine. Toenails reflect a more prolonged exposure of several months compared to a recent exposure of days for urine and thus may be a better indicator of chronic exposures. In addition, diet is a significant contributor to urinary arsenic concentrations and is susceptible to major fluctuations depending on daily food consumption. Nevertheless, both biomarkers are useful indicators that reflect different types of exposures and sources.

In Canada, urinary arsenic is measured as part of a national longitudinal biomonitoring program called the Canadian Health Measures Survey. Reference values representative of the general Canadian population have been derived [53]. Though toenail is a biomarker that reflects longer exposure periods, there are currently no available reference values or national biomonitoring data like for urine. Thus, interpretation using toenail concentration solely can be difficult in the context of population studies and biomonitoring. The use of urine and toenail offers a more comprehensive understanding of arsenic exposure in a population, with both biomarkers indicating different times of exposure.

3.5.4 Analysis of Risk Factors

When comparing behaviours between participants with elevated total arsenic in toenails (Top 20% for children, 5% for adults) and the remaining participants, certain lifestyle factors, such as water activities for children, drinking lake water for adults and consumption of local foods, were attributed to those with elevated levels of toenail arsenic (Table 3.5). Children with elevated toenail arsenic reported participating in fishing and other recreational water activities. Water activities such as swimming may increase the accidental ingestion of water. Additionally, children have lower body weight than adults and thus may have higher intakes per body weight

via ingestion [54]. The NWT government issues public health advisories indicating which local lakes are safe for swimming and fishing (https://www.hss.gov.nt.ca/en/newsroom/arsenic-lake-water-around-yellowknife). Our results show that children of the volunteer group are represented the most in the top 20% of toenail arsenic concentration. The volunteer sub-group of participants represents a special interest group who were not selected through random selection but wished to participate in the study due to interest in their arsenic levels. Thus, their arsenic concentrations may likely be higher compared to those who were randomly selected. For adults, a higher percentage of those with elevated toenail arsenic reported lake water as their main water source, as well as responded "yes" to drinking lake water. Arsenic in lake waters was not measured in our study. However, spatial gradients of arsenic concentrations in lakes have been linked to historical mine emissions in Yellowknife [12,15]. Lake and drinking waters in Yellowknife are regularly tested for arsenic by the local government. In Yellowknife, municipal drinking is below Canadian drinking water guidelines of 10 μg/L [55]. It should be noted that lake water is not the main water source for most residents. The Government of Northwest Territories (GNWT), however, does not recommend drinking untreated water from any bodies of water in the Northwest Territories.

For all participants, a seasonal difference was observed as toenail arsenic concentrations were significantly higher in the second wave (Spring/Summer 2018) of sampling compared to the first wave (Fall/Winter 2017). In the summer months, there is likely to be greater amounts of adsorption or contamination due to the increased outdoor activities and use of exposed footwear, particularly among children. For both adults and children, there was a significantly higher percentage of participants with elevated toenail arsenic who were reported to eat locally harvested wild plants. Additionally, significantly higher percentages of adult respondents in the

elevated group were reported to eat local meat and mushrooms while a higher percentage of elevated children reported consuming garden-grown produce. Previous risk assessments found that arsenic levels in garden produce may be higher than in market produce, but consumption did not pose a significant health risk. However, imported soil is recommended by local public health authorities due to the variability of As in soil in Yellowknife [56]. An ecological risk assessment observed that arsenic concentrations in local vegetation foraged closer to the Giant Mine were higher than vegetation foraged further away, but consumption of these locally harvested foods generally posed a low health risk [16]. However, the local government does not recommend harvesting mushrooms, berries or other plants near Giant Mine or current industrial sites [55]. Previously, the consumption of local berries and mushrooms was found to be a predictor of elevated urinary inorganic arsenic in adults [18]. Consumption of market rice and seafood was also observed to be a significant predictor of urinary inorganic arsenic for both adults and children, but this was not observed in toenail arsenic. Our results suggest that the contribution from the consumption of local foods may need to be further characterized.

Finally, a significantly higher percentage of Giant Mine workers had toenail arsenic concentrations above the 95th percentile. Occupational exposure generally leads to higher concentrations of arsenic in urine and toenails [44]. Giant Mine workers wear necessary PPE and are regularly tested for urinary arsenic as part of occupational monitoring. However, toenails samples in workers are not tested for arsenic.

3.6. Conclusion

This study quantified total arsenic and arsenic species in residents living in Yellowknife, Dettah, and Ndilo in the Northwest Territories. Arsenic speciation in toenails is a useful tool that may enhance the understanding of chronic arsenic exposures and complement the use of urine

biomarkers, which comparatively reflect shorter exposures. However, a clear standardized methodology is needed for speciation analysis of toenails to ensure that the species analyzed reflect body burden. Method development to ensure minimal oxidation of inorganic arsenic, as well as elucidation of sources of arsenic (i.e., ingestion vs. adsorption), is needed for toenail speciation of arsenic. Currently, these uncertainties in arsenic speciation in toenails may lead to inaccurate estimations of inorganic arsenic exposure. Nevertheless, the use of total arsenic in toenails as a biomarker of exposure is sufficient in estimating body burden from chronic arsenic exposure.

3.7. References

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Appendix 3.A: QA/QC results of the inter-laboratory comparison between the University of Ottawa and INSPQ

Table 3.A. Statistical comparison of urinary arsenic concentrations of randomly selected samples (n=50) analyzed by the University of Ottawa and INSPQ.

	Total As (tAs)	Inorganic As (iAs-met)
Pearson correlation coefficient (r)	0.976	0.972
p value- Pearson correlation	< 0.001	<0.001
p value- Paired t-test	0.318	0.094

4. Associations between urinary biomarkers CC16 and KIM-1 and metal concentrations among children in Yellowknife, Northwest Territories, Canada

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JSJC and HMC conceived and designed the study. JSJC conducted the sample and data analysis. JSJC and HMC interpreted the data. JSJC and HMC wrote/revised the manuscript, which was reviewed by AM.

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4.1. Abstract

Childhood exposures to environmental contaminants, such as arsenic (As), have been associated with an increased risk of disease and mortality later in life. Thus, there is a need for early detection of such exposure in children via biomonitoring. This study aims to assess the potential of two urinary proteins, Kidney Injury Molecule-1 (KIM-1) and Club Cell Secretory Protein (CC16), as biomarkers of potential health effects associated with metal exposure. We investigated the relationship between the KIM-1 and CC16 and the concentrations of arsenic species (AsIII, AsV, MMA, DMA, arsenobetaine), lead (Pb) and manganese (Mn) in urine. Urinary samples were collected from children 3-11 years old (n=244) who lived in Yellowknife, Northwest Territories, Canada, during 2018-2019. Concentrations of KIM-1 and CC16 were measured by enzyme-linked immunosorbent assay (ELISA). Concentrations of As (total and speciated), Pb and Mn were measured by inductively coupled plasma mass spectrometry (ICP-MS). Urinary KIM-1 concentrations were significantly associated ($p<0.001$) with increasing inorganic arsenic (iAs) concentrations, defined as the sum of AsIII+AsV, after adjusting for confounders (i.e., age and sex). Urinary CC16 concentrations were positively associated with iAs, Mn and Pb concentrations ($p<0.001$) after adjusting for confounders (i.e., age, sex, participation in recreational water activities, fishing). These results suggest that KIM-1 may be a candidate biomarker for detecting early iAs effects on kidney function in children. In comparison, urinary CC16 levels may be a potential non-specific biomarker for the impact of metals (As, Pb and Mn) on lung functions in children.

4.2. Introduction

Arsenic, particularly inorganic arsenic, is a natural toxicant of global health concern. Chronic inorganic arsenic exposure is associated with cancer and non-cancer adverse health outcomes impacting many organs, including the lung and kidneys. [1–3]

Biomarkers are standard tools in biomonitoring used to estimate a population's chemical exposures and associated health risks. There are three general types of biomarkers: exposure, effect and susceptibility. [4] Biomarkers of exposure are typically the measured concentrations of a chemical in biological matrices (e.g., blood, urine, or hair) used to indicate its body burden. Biomarkers of effect reflect a biological change induced by a chemical which may result in early biological effects, altered structures, impairment, or disease. Biomarkers of susceptibility reflect the interindividual differences, typically genetic, in biological response to a chemical. Each biomarker type addresses an aspect of the exposure-disease continuum. For instance, biomarkers of effect can be used to detect early effects or adverse health outcomes from chronic environmental exposure to chemicals such as arsenic. Detection of the relationship between exposure and biological effects is important, particularly for sensitive groups like children who are most susceptible to environmental exposure. [5] Childhood exposure to arsenic has been reported to increase the risk of disease and mortality later in life. [6] Although epidemiological literature on arsenic toxicity and human health effects is abundant; there are no established biomarkers for effects from chronic arsenic exposure as the mechanisms of action of arsenic toxicity are diverse and non-specific. Thus, chronic exposure to arsenic is associated with various adverse health outcomes affecting most body systems and major organs, including the lung and kidneys. [7]

Several proteins in literature are potential biomarkers of impairment of specific organs (e.g., kidney and lung) from arsenic exposure, including kidney injury molecule-1 (KIM-1) and club cell secretory protein (CC16). [8–10] KIM-1 is a transmembrane glycoprotein protein secreted by epithelial cells in the proximal tubules following kidney injury, where it is cleaved and subsequently secreted in urine. [11] Normal KIM-1 secretion is an adaptive response following kidney injury. However, prolonged expression of KIM-1 is observed to be maladaptive and is linked to kidney pathogenesis such as fibrosis. [12] KIM-1 may serve as a sensitive biomarker for kidney impairment in human biomonitoring, particularly in early detection of maladaptive responses, as it has been observed to be associated with environmental metal exposure, including arsenic. [13,14] Urinary arsenic concentrations have been observed to be related to the risk of chronic kidney disease (CKD). [15] Furthermore, recent studies have examined the effect of arsenic exposure on urinary KIM-1 concentrations. Urinary KIM-1 concentrations have been significantly associated with As concentrations in children but not adults [10,16] Though more research is needed, urinary KIM-1 may be a candidate biomarker of effect for arsenic-induced kidney damage, particularly in children.

CC16 is a homodimeric lung protein shown to play an anti-inflammatory role in protecting and repairing the respiratory tract following epithelial damage from xenobiotics such as environmental contaminants. [17] Following acute environmental exposures (e.g., tobacco smoke and air pollutants), circulating CC16 serum levels typically increase. Conversely, circulating CC16 levels have been observed to decrease for chronic exposures (e.g., smokers), possibly due to less responsive club cells. CC16 may be an early predictor of lung impairment, and disease as lower circulating CC16 levels have been linked to decreased lung function, lower airflow, and increased lung cancer risk. [18] Furthermore, a longitudinal cohort study observed that

lower levels of CC16 in young children might lead to decreased lung capacity later in their teenage years.[19] Past studies have reported inverse relationships between CC16 levels and arsenic exposure. Thus, CC16 may be used as a sensitive lung peripheral biomarker to assess lung damage from environmental exposures such as arsenic. [8,9,20]

The objective of the present study was to assess both KIM-1 and CC16 as two potential urine biomarkers of effect for detecting impairment of two target organs of arsenic, kidneys and lung, from exposure to As and other chemicals of potential concern (COPCs) in children. Urinary concentrations of KIM-1 and CC16 were measured in a cohort of children under age 11 (n=244) living in Yellowknife, Canada. In the Yellowknife area, there is a legacy of arsenic contamination because of previous mining activities, particularly from the former Giant Mine, where 237 000 tonnes of arsenic by-products were left in the mine's underground chambers despite its closure in 2004.[21] Currently, environmental levels of arsenic exposure (e.g., in drinking water) are not considered to be of significant risk to the health of the local population. [22] However, children in this cohort were previously observed to have elevated urinary inorganic arsenic concentrations compared to adults in the same population, as well as children of the same age group in the general Canadian population. [23] Health effects of chronic arsenic exposure are not always readily observed, particularly in populations with low to moderate arsenic exposures. Using sensitive effect biomarkers such as KIM-1 and CC16 may be a valuable tool for detecting early biological effects of arsenic target organs kidneys and lungs, respectively, for varying levels of arsenic exposure.

4.3. Materials and methods

4.3.1 Ethics

The research was conducted following the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans and, in particular, Chapter 9, research involving the First Nations, Inuit and Métis Peoples of Canada, and the document entitled: Indigenous Peoples & Participatory Health Research: Planning & Management, Preparing Research Agreements published by the World Health Organization (2003). [24,25] The study also follows the First Nations principles of Ownership, Control, Access, and Possession (OCAP®) of data. [26]

The study was approved by the Health Sciences and Sciences Research Ethics Board of the University of Ottawa (http://research.uottawa.ca/ethics/reb) and the Aurora College Research Ethics Committee. In addition, the study has been granted a Scientific Research License from the Aurora Research Institute in Northwest Territories. Individual participation in the project was voluntary and based on informed written consent following an oral and written explanation of each project component.

4.3.2 Study Area and Population

The presented research is part of the Health Effects Monitoring Program, a prospective cohort study established to monitor levels of arsenic and other chemicals of potential concern (COPCs) in the human population of Yellowknife and the First Nation communities of Dettah, and Ndilo as remediation of Giant Mine progresses. [27] A total of 2037 individuals from ages 3 to 79 participated in the baseline study. Recruitment and data collection of the baseline cohort was conducted in two waves; the first wave occurred from September to December 2017, and the second wave occurred from April to June 2018. A detailed description of the Health Effects Monitoring Program and the study's methodology has been reported previously. [27]

The subjects of the present research are child participants ages 3-11 from the Health Effects Monitoring Program (n=244). Child participants were recruited to study randomly or on a parental volunteer basis. Participants were required to have resided in Yellowknife for at least a year. The child and their parent or legal guardian were informed about the details of the project by trained research assistants or registered nurses. Assent and consent were obtained from the child and their parental or legal guardian, respectively, before proceeding with the interview and biological sample collection.

4.3.3 Sample collection

Participants completed a questionnaire on lifestyle and potential exposure factors conducted by a trained research assistant. The child's parent or legal guardian was present for the interview and answered the questions for the child if needed.

At the end of the interview, instructions and supplies were given to provide a urine sample. Participants were instructed to refrain from eating fish and seafood at least 3 days before urine sampling and to provide the first-morning void at their earliest convenience. Once collected, urine samples were kept at 4ºC and shipped on ice to the laboratory at the University of Ottawa within 5 days.

4.3.4 Laboratory analysis

Within 24 hours of the arrival in the laboratory, the urine samples were divided into aliquots. The aliquots for quantification of chemicals were stored at -20°C and analyzed for total As and COPCs as well as speciated arsenic within five days. The aliquots for KIM-1 and CC16 analysis were stored at -80°C until analysis.

4.3.4.1 Analysis of Arsenic Species and other Chemicals of Potential Concern (COPCs)

All chemical analyses were performed at the Laboratory for the Analysis of Natural and Synthetic Environmental Toxicants (LANSET) at the University of Ottawa. Inductively coupled plasma mass spectrometry (ICP-MS) (7700x ICP-MS, Agilent Technologies, Japan) was used to quantify total arsenic and other chemicals of potential concern (COPCs), which included lead (Pb) and manganese (Mn). The system is equipped with a low-flow borosilicate glass MicroMist concentric nebulizer and quartz, Scott-type double pass spray chamber. The interface consisted of a 1mm diameter Ni-typed sampling and a 0.4 mm diameter Ni-typed skimmer cone. Octopole Reaction System (ORS) was used as interference removal in Helium mode. An Agilent ASX-500 was used as an ICP-MS autosampler.

Arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenobetaine were measured via ICP-MS coupled with liquid chromatography (LC-ICP-MS). The chromatographic separation was performed using a 10mM ammonium phosphate dibasic buffer with pH adjusted to 8.25 on an Agilent 1200 Infinity LC system with a 1260 Isocratic pump and 1260 Autosampler with a Hamilton PRP-X100 anion exchange column (250mm x 4.1mm,10um particle). The LC system was connected to the Agilent 7700x ICP-MS via Peek tubing and equipped with a low-flow Micro Mist Nebulizer and quartz, low-volume Scott-type double-pass spray chamber. The mobile phase was delivered at 1mL/min, and the injection volume was fixed at 100uL.

Stock solutions were diluted in 1% nitric acid and used to provide a working calibration curve of at least five points. For analytical quality controls, an element quality control standard stock (High-Purity Standards, Cat# QCS-19) and urine multi-element stock (High-Purity Standards, Health solution A) were used as check standards after calibration and then every 10 to

20 samples. Analyses also included reference materials (NIST reference materials: SRM 2669 level 1 and 2 arsenic species in frozen human urine, SRM 2668 level 1 and 2 toxic elements in frozen human urine, IAEA 407-trace elements and methylmercury in fish tissue, IAEA 085-human hair references from the International Atomic Energy Agency and Dolt-4- Dogfish liver certified reference material for trace metals and other constituents from the National Research Council Canada), and pooled samples for a spike recovery. The results for both check standard, spike recovery and reference materials were within 10-20% of expected values.

The detection limit (LOD) in the urine samples was 0.01 µg/L for total arsenic, 0.005 µg As /L for the arsenic species, 0.02 µg/L for lead, and 0.10 µg/L for manganese. Out of all the samples received, 5,7%, 3.3% and 6.2% of concentrations were below the LOD for AsIII, AsV, and MMA. For the total metal analysis, 0.4% and 5.3% of concentrations were below LOD for Pb and Mn, respectively. None of the samples had concentrations below LOD for DMA and total As. Concentrations below LOD were replaced with ½ LOD.

For quality control of the study, ~2.5% of all urine samples collected (n=50) were randomly selected and sent to the Institut Nationale de Santé Public du Québec (INSPQ) for interlaboratory comparison. No statistical difference ($p > 0.05$) between the results from the University of Ottawa and INSPQ laboratories (Appendix 4.A).

4.3.4.2 Creatinine Analysis

All urine samples were analyzed for creatinine, a biomarker for kidney function, as well as an indicator of hydration status. Urine samples were diluted ten times in ultrapure water and then analyzed via the Jaffe method using creatinine urinary detection kits (Thermo Fisher Scientific, Cat#: EIACUN). Absorbances were read at 500 nm wavelength using a Biotek Cytation 3 imaging reader (BioTek Instrument, Inc.).

Urinary concentrations of arsenic species (AsIII, AsV, MMA, DMA, arsenobetaine), COPCs (i.e., Pb, Mn), KIM-1, and CC16 were adjusted for creatinine.

4.3.4.3 Biomarker Analysis

Urine samples of child participants under 11 (n=244) were analyzed for KIM-1 and CC16 via commercially available enzyme-linked immunosorbent assay (ELISA) kits (Thermo Scientific Invitrogen Human TIM-1 (HAVCR1) ELISA Kit; Biovendor Club Cell Protein (CC16) Human ELISA). The clonal antibodies used were Uteroglobin Monoclonal Antibody (AY1E6) and ARD5 for CC16 and KIM-1, respectively. Samples were prepared and analyzed according to manufacturer instructions.

The limit of detection (LOD) for KIM-1 and CC16 were 2 pg/mL and 46 pg/mL, respectively. 5.3% and 4.1% of concentrations were below LOD for KIM-1 and CC16, respectively. Concentrations below LOD were replaced with ½ LOD. All biomarker concentrations were converted to μg/L.

4.3.5 Statistical Analysis

Statistical analyses were performed using R v.4.1.1. [28] All urinary measurements (arsenic, other metals and biomarkers) were adjusted for creatinine, a biomarker for kidney function and an indicator of hydration status. Descriptive statistics for arsenic species (AsIII+ AsV, MMA, DMA, arsenobetaine), COPCs (Pb and Mn), and effect biomarkers (CC16 and KIM-1) were calculated. Urinary inorganic As (iAs) concentrations were calculated as the sum of inorganic arsenic species, AsIII and AsV.

Bivariate associations between effect biomarkers (CC16 and KIM-1) and exposure biomarkers (urinary arsenic species and COPCs), as well as potential covariates, which include factors related to lifestyle and arsenic exposure (Table 1), were analyzed using Welch's t-test and

analysis of variance (ANOVA)for categorical variables. Such variables include sex, seafood consumption frequency, rice consumption frequency, participation in recreational water activity within the year (yes/no), fishing within the year(yes/no), and consumption of local fish (yes/no). Continuous variables such as age, BMI and daily water consumption were analyzed via linear regression. For all statistical tests, significance was set at $\alpha=0.05$ and Bonferroni corrections were made to adjust for multivariable testing.

Both simple and multiple linear regression models were used to analyze the relationship between arsenic concentrations and CC16/KIM-1. Prior to regression analyses, assumptions (e.g., normality and heteroscedasticity) were assessed via statistical tests (e.g., Shapiro-Wilk and Breusch-Pagan tests). To address results of the regression assumption analyses, all urinary concentrations were log-transformed. In addition, Cook's Distance was used to identify potential influential outliers. Regression models were conducted without potential outliers to assess influence. Identified outliers did not impact the results of the analyses. For the regression analyses, simple linear regressions were first conducted separately between each potential effect biomarker (CC16 and KIM-1) and urinary metal concentrations, which were arsenic species (iAs, MMA, DMA, and arsenobetaine) and other COPCs. The metals that were significantly associated (iAs, Pb, and Mn) with the effect biomarkers were chosen to be assessed using separate multiple linear regression analyses. Potential confounders were selected by determining the relationship between the effect biomarker (CC16 or KIM-1) and urinary metal concentrations (iAs, Pb and Mn), adjusting for each potential confounding variable individually via linear regression. The possible confounding variables are lifestyle factors and exposure variables related to arsenic-based. If the coefficient for the metal changed more than 10% after adjusting for a variable or if the independent variable was significant in the model, then that

variable was included in the multiple linear regression. Multiple linear regression analyses were conducted to assess the association between urinary metals (iAs, Pb, and Mn) and effect biomarker concentrations.

4.4. Results

4.4.1 Population Characteristics

Urine samples of 244 child participants ages 3-11 were tested for arsenic species, other metals, and the potential biomarkers KIM-1 and CC16. One child was excluded from analyses as creatinine was unable to be detected. The characteristics of the subjects are described in Table 4.1. The mean age was seven years, and the sex distribution was approx. equal (52% male, 48% female).

Table 4.1. General characteristics of subjects. Arithmetic mean (AM) and standard deviations (SD) are presented for continuous variables (age, BMI, water consumption amount). Sample size and percentages are shown for categorical variables. [a] Timeframe of variables is within the last year.

Variable		AM (SD)/ n(%)
Age (years)		7.3 (2.49)
Age group	*3-5*	59 (24.18)
	6-11	185 (75.82)
Sex	*Male*	127 (52.05)
	Female	117 (47.95)
BMI		18.09 (14.49)
Water amount (glass/day)		5.38 (2.60)
Seafood consumption frequency	*None*	64 (26.23)
	< 1/month	55 (22.54)
	1/month	87 (35.66)
	1/week	38 (15.57)
	>1/day	0
Rice consumption frequency	*None*	5 (2.23)
	< 1/month	5 (2.23)
	1/month	40 (17.86)
	1/week	140 (62.50)
	>1/day	34 (15.18)
Participation in recreational water activity[a]	*No*	39 (15.98)
	Yes	205 (84.02)
Fishing[a]	*No*	125 (51.23)
	Yes	119 (48.77)
Eat local fish[a]	*No*	63 (25.82)
	Yes	181 (74.18)

4.4.2 Arsenic and other chemicals of potential concern (COPCs) in urine

Creatinine-adjusted urinary concentrations of arsenic species and other chemicals of potential concern (COPCs) are summarized in Table 4.2. Concentrations of iAs, Pb and Mn were significantly associated with age. No difference was observed between sex ($p>0.05$) for iAs, Pb and Mn concentrations in children.

Table 4.2. Creatinine adjusted biomarker concentrations (µg/g creatinine) in urine (n=243) for arsenic species, chemicals of potential concern (COPCs) lead (Pb) and manganese (Mn), and effect biomarkers (CC16 and KIM-1).

Biomarker	GM (SD)	AM (SD)	Median	Min-Max
Exposure Biomarkers				
Arsenic				
Total	10.2 (2.42)	19.4 (59.0)	9.18	0.78-788
iAs (AsIII+V)	1.27 (2.28)	1.67 (1.31)	1.35	0.019-11.3
MMA	0.54 (3.94)	0.92 (0.85)	0.69	0.001-5.25
DMA	6.44 (2.24)	9.86 (16.60)	5.85	1.21-202
Arsenobetaine	0.40 (8.07)	6.74 (41.90)	0.45	0.002-598
Pb	0.52 (2.12)	0.75 (1.09)	0.48	0.018-11.9
Mn	0.10 (4.04)	0.53 (3.81)	0.09	0.009-58.8
Effect Biomarkers				
KIM-1	0.24 (5.18)	0.42 (0.36)	0.34	0.001-2.16
CC16	0.83 (4.10)	1.79 (4.62)	0.59	0.07-44.1
Creatinine (mg/dL)	85.75 (1.60)	90.90 (41.2)	85.30	12.75-252

GM: Geometric mean
AM: Arithmetic mean
SD: Standard deviation

4.4.3 KIM-1 and metal exposure

As shown in Figure 4.1, there was an overall increase in KIM-1 concentrations when stratified by iAs levels (Low: <25th percentile, medium: 25th-75th percentile, high: >75th percentile). A significant difference was observed in KIM-1 concentrations between the low and medium iAs groups as well as between the low and high iAs groups.

Linear regression analyses were used to assess the relationship between iAs and KIM-1 concentrations (Figure 4.2 and Table 4.3). KIM-1 was positively associated with increasing iAs concentrations after adjusting for confounders (e.g., age, sex) via multivariable linear regression (Table 4.3). There were no significant associations between KIM-1 concentrations and the other arsenic species, Pb and Mn.

4.4.4 CC16 and metal exposure

As shown in Figure 4.1, there was an increase in CC16 when stratified by iAs and Pb exposure. There was a significant difference in CC16 concentrations between the Low iAs and High iAs groups. CC16 concentrations also increased by Pb level, and significant differences were observed between all groups. No significant increase in CC16 concentrations was observed for Mn levels.

Simple linear regressions showed that CC16 was significantly associated with iAs, lead and manganese concentrations (Figure 3.2), and the relationships remained significant after multivariable linear regression (Table 3.4). Additionally, recreational water activities and fishing were significantly negatively associated with iAs, Mn and Pb concentrations in multivariable linear regression models (Table 4.4). There were no significant associations between CC16 concentrations and the other arsenic species.

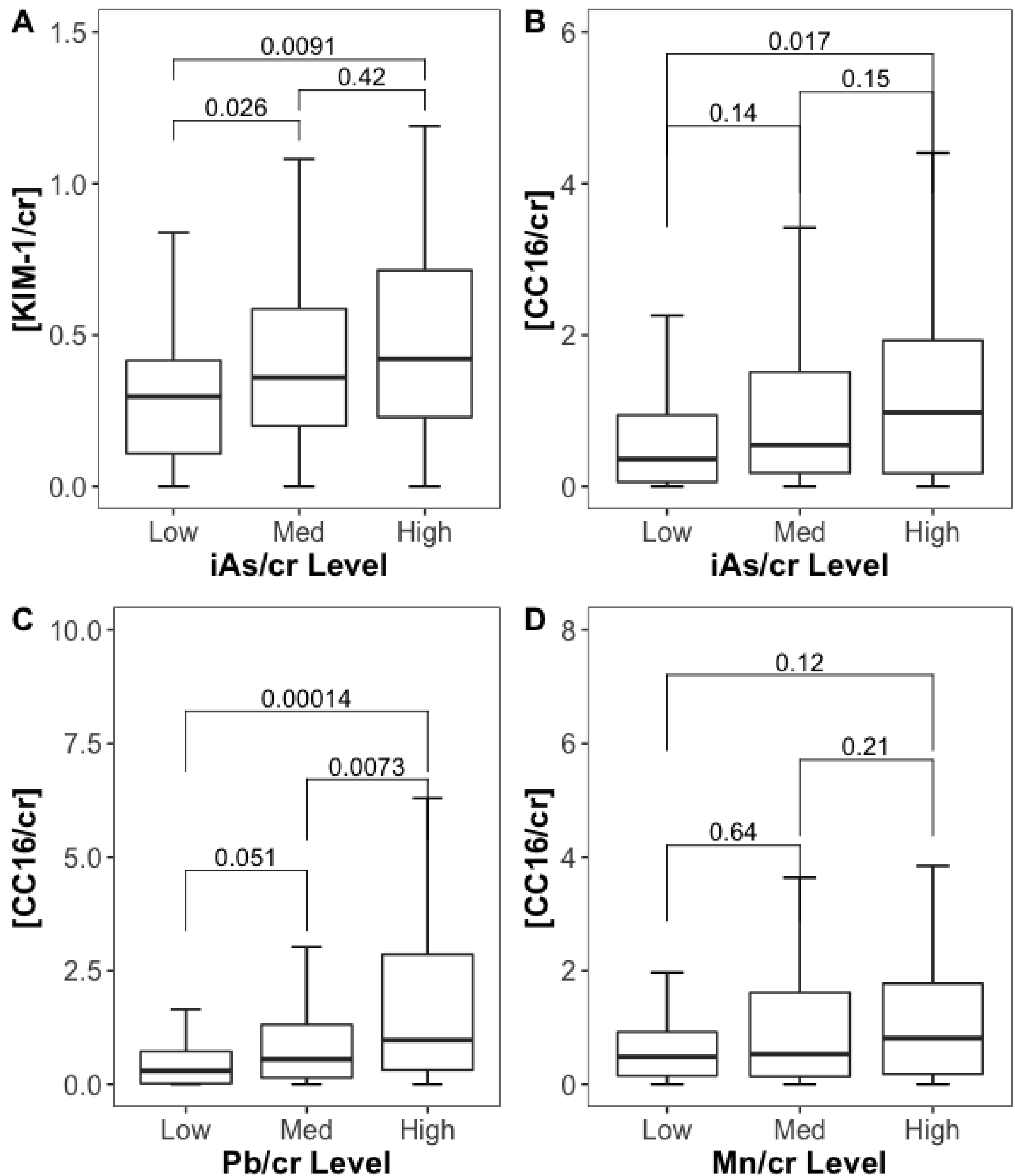

Figure 4.1. Box plot of KIM-1 (A) and CC16 (B-D) by exposure biomarker terciles (Low, Medium, High) in urine where low is defined as <25th percentile, medium is 25-75th percentile, and high is >75th percentile. The line within the box corresponds to the median, the box corresponds to the interquartile range (IQR), and the whiskers represent 1.5xIQR. Differences in KIM-1 and CC16 between the groups were evaluated using Wilcoxon-type tests, p values between groups displayed above the box plots.

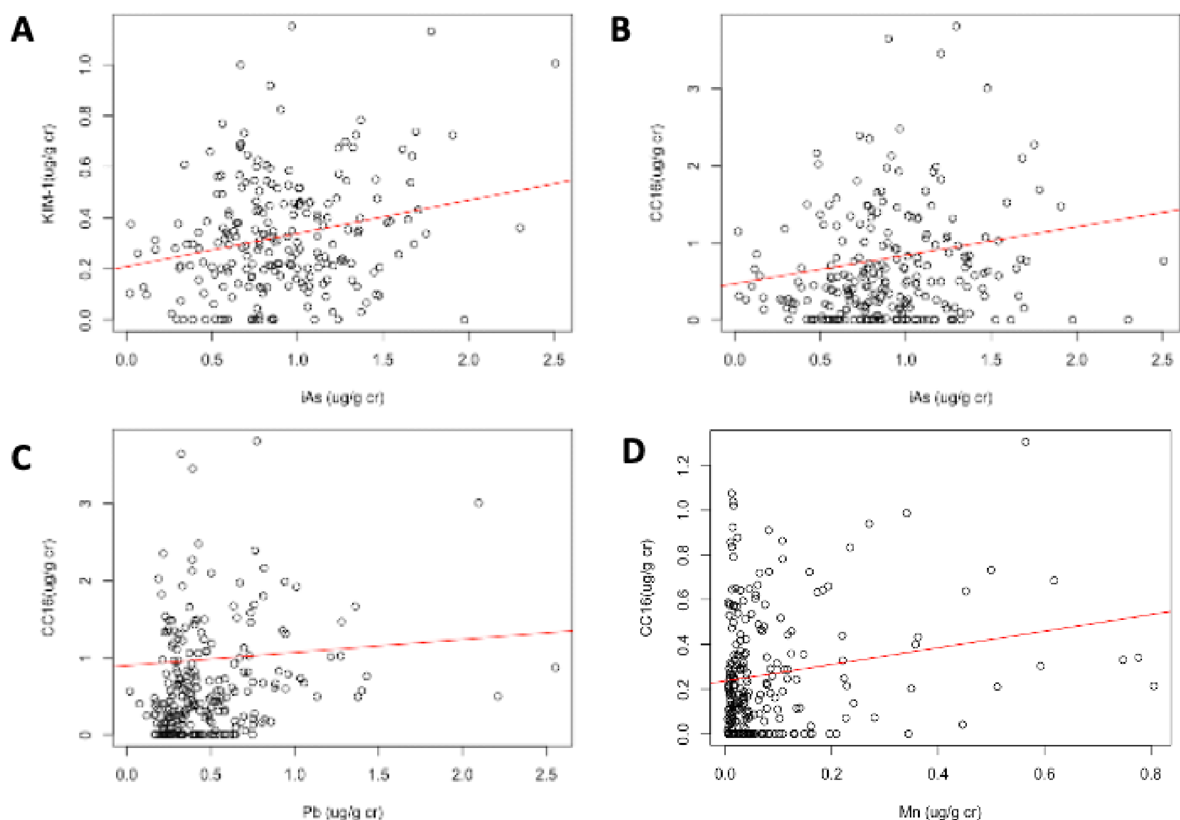

Figure 4.2. Relationship between effect and exposure biomarkers. The relationship between KIM-1 and iAs is depicted in A, while B-D display the relationship between CC16 and iAs (B), Pb (C) and Mn (D). The simple linear regression lines are represented in red ($p<0.05$). Concentrations are log-transformed.

Table 4.3. Multivariable linear regression models between KIM-1 and iAs (AsIII+V) urine concentrations in children under age 11 (n=243).

		Model 1		Model 2	
Variable		β	p value	β	p value
(Intercept)		0.41	**<0.001**	0.34	**<0.001**
[AsIII+AsV]		0.27	**0.016**	0.10	**0.009**
Age				-0.013	**0.032**
Sex	Male			Reference	
	Female			-0.020	0.489
Adjusted R^2		0.050		0.064	
p-value		**<0.001**		**<0.001**	

Table 4.4. Multivariable linear regression models between CC16 and iAs (AsIII+V), CC16 and lead, and CC16 and manganese urine concentrations of children under age 11 (n=243).

		iAs (AsIII+V)						Lead (Pb)						Manganese (Mn)					
		Model 1		**Model 2**		**Model 3**		**Model 1**		**Model 2**		**Model 3**		**Model 1**		**Model 2**		**Model 3**	
Variable		β	p value	β	p value	β	p value	β	p value	β	p value	β	p value	β	p value	β	p value	β	p value
(Intercept)		0.41	**<0.01**	0.03	0.90	0.47	0.04	0.79	**<0.01**	0.51	**<0.01**	0.56	0.00	0.80	**<0.01**	0.59	**<0.01**	0.80	<0.001
Metal		0.27	**0.02**	0.34	**<0.01**	3.10	**<0.01**	0.21	**<0.01**	0.24	**<0.01**	0.54	**<0.01**	0.06	**0.04**	0.09	**<0.01**	0.30	**<0.01**
Age				0.05	**<0.01**	3.06	**<0.01**			0.05	**<0.01**	0.05	**<0.01**			0.05	**0.01**	0.05	**<0.01**
Sex	Male			Reference		Reference				Reference		Reference				Reference		Reference	
	Female			-0.08	0.35	-0.12	0.18			-0.13	0.14	-0.17	0.04			-0.15	0.10	-0.16	0.06
Fishing	No					Reference						Reference						Reference	
	Yes					-0.30	**<0.01**					-0.30	**<0.01**					-0.41	**<0.01**
Water activities	No					Reference						Reference						Reference	
	Yes					-0.40	**<0.01**					-0.39	**<0.01**					-0.30	**<0.01**
Adjusted R^2		0.02		0.04		0.13		0.05		0.08		0.16		0.01		0.04		0.12	
p-value		**0.02**		**0.00**		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**		**0.04**		**0.01**		**<0.001**	

4.5 Discussion

We observed that KIM-1 levels and CC16 levels were associated with environmental metal contaminants in the urine of the child participants. Specifically, KIM-1 levels were significantly associated with inorganic arsenic (iAs) concentrations in urine. CC16 levels are significantly associated with iAs, lead (Pb) and manganese (Mn). As previously reported, child participants had higher urinary inorganic-related arsenic concentrations than adult participants in our study. Child concentrations from our study were also higher than children from the general Canadian population. [23] However, our observed urinary iAs concentrations of children are lower than other cohorts in arsenic-endemic regions such as Bangladesh. Pb and Mn concentrations observed in children in our study are within the normal range of the Canadian population. [29]

4.5.1 KIM-1 and metal exposure

Though limited studies have examined KIM-1 and arsenic, our findings are consistent with reported literature consisting of child cohorts. Our results agree with a study of 83 children in Mexico where elevated urinary KIM-1 levels were significantly associated with high urinary arsenic concentrations. [10] In comparison, results from studies with adults are less consistent. For example, urinary KIM-1 and arsenic in Mexican adults showed no association. [16] Meanwhile, in a Sri Lankan cohort, KIM-1 concentrations in urine were positively correlated with urinary arsenic in patients with CKD of unknown etiology. [30] There are limited studies that have examined the relationship between arsenic exposure and kidney function in children. [31] In chronic arsenic exposures, arsenic has been observed to accumulate in kidneys. [32] Chronic exposure to low arsenic doses has been associated with adverse renal health outcomes in adults, such as CKD and decreased glomerular filtration rate (GFR). [31] Additionally, arsenic exposure

has been associated with kidney cancer. [33] A study in Chile reported an increased relative risk for kidney cancer in adults born before or during high arsenic exposure levels 25 years earlier. [33] Due to the long latency often observed between chronic arsenic exposure and disease, the detection of an increase of urinary KIM-1 with increased As exposure may be valuable as a screening tool and initiate mitigation in lowering As exposure among the high exposure group.

4.5.2 CC16 and metal exposure

Our results for CC16 were less consistent with the existing literature. For chronic exposures to xenobiotics, overall circulating CC16 levels have decreased with increasing exposure concentrations. Conversely, we observed a positive correlation between urinary CC16 and metal concentrations, specifically arsenic, lead and manganese.

Previous studies have observed an inverse relationship between serum CC16 and arsenic exposure. For children, the inverse relationship between CC16 and arsenic varies depending on the environmental source of arsenic and biological matrices considered, as only some of the studies in children observed an association between CC16 and arsenic in biological matrices. Beamer et al. (2016) investigated the associations between CC16 levels in children and arsenic in different media. They found that urinary CC16 was negatively correlated with arsenic concentrations in soil but not in biological matrices such as urine and toenail. In a cohort of Mexican children, there was also no significant association between CC16 and urinary As concentrations. However, CC16 significantly decreased with increasing As in drinking water and was significantly associated with an increased risk of respiratory disease. In a longitudinal cohort of children living in the As-endemic region in Bangladesh, there was a significant association between CC16 and arsenic in urine, where plasma CC16 levels decreased with previously measured urinary arsenic concentrations in early childhood. [8] The study also observed a

significant positive association between urinary CC16 levels and lung function indicators. [34] For studies with adult cohorts, the results are more consistent as inverse correlations between CC16 and arsenic concentrations in biological matrices were observed. [20,35] Decreased respiratory function, and urinary CC16 were also observed in these adult studies.

In comparison, we did not observe an inverse relationship. Instead, urinary CC16 concentrations were significantly associated with increased urinary iAs. In the aforementioned studies, inverse associations between CC16 and arsenic concentrations in biological matrices (i.e., urine and hair) were only observed in adult cohorts. For the child studies, only one study found an inverse relationship between CC16 and arsenic in urine which was a longitudinal cohort that measured urinary arsenic over several years. [8] It is possible that decreased levels of CC16 may not be readily observed in our child cohort due to the relatively short duration of chronic exposure compared to adults, hence the observed significant positive relationship. Furthermore, CC16 was quantified in urine rather than plasma. Additional CC16, as well as urinary and environmental As measurements, may be needed for our cohort to establish temporal trends and further elucidate the observed relationship. Moreover, as the relationship was not specific to As, the value of CC16 as a biomarker for As exposure may be limited.

This is the first study to report an association between CC16 and Pb in human biological matrices in children. Children are particularly susceptible to lead exposure, as lead toxicity is known to affect the brain and lead to neurological issues. Though limited, studies have observed associations between Pb exposure and lung function in children and adults. [36–41] Though the impact of lead exposure on the lungs is not well elucidated, inhalation is one of the routes of exposure for lead. [42] CC16 plays a protective role in the respiratory tract against xenobiotic

substances. Thus, the observed association with Pb could be a protective response against the inhalation of Pb-containing particles (e.g., dust).

There are very few studies investigating the relationship between CC16 and Mn exposure. Of note, a cohort of manganese-exposed shipyard workers was measured for serum CC16. Though no direct association between environmental manganese and CC16 was reported, they reported a link between low serum CC16 and neurological abnormalities. [43] Though Mn is an essential micronutrient and cofactor in many biological processes; Mn toxicity may occur due to environmental and occupational exposures. Mn exposure, most notably from air and inhalation, has been associated with adverse cognitive and neurological outcomes most often related to motor functions. [44–46] Like Pb, CC16 may indicate a protective response against Mn exposure.

In our study, we observed an increase in CC16 levels with increasing urinary metal concentrations rather than a predicted inverse relationship that is observed for chronic exposures. One consideration could be that the decrease in circulatory CC16 levels for chronic exposure is not readily observed in young children. Another consideration is that we measured CC16 in urine rather than plasma or serum. Proposed mechanisms for change in circulating CC16 levels include a change in barrier permeability and renal clearance. [17] CC16 in the airway epithelium enters the blood through the bronchoalveolar-vascular barrier. The permeability of this barrier may be increased due to airway damage, thereby increasing the transport of CC16 to the blood. Circulating blood CC16 is then eliminated into the urine via glomerular filtration. Studies have observed an inverse correlation between GFR and serum CC16 levels in patients undergoing dialysis. [17] Decreased GFR, and renal clearance indicates loss of kidney function and later stages of chronic kidney disease progression, which is more likely observed in clinical settings. For

earlier stages of the disease continuum, kidney damage is marked by increased protein in the urine. [47] In this study, we observed increased urinary CC16 and urinary KIM-1. Thus, increased urinary CC16 observed in our study may be linked to increased epithelial permeability and early biological effects of impaired kidney function. Further studies are needed to understand urinary CC16 concentrations in chronic exposures. The relationship between serum and urinary CC16 concentration in chronic exposures should also be further elucidated.

Arsenic, lead, and manganese as a group have often been associated together as mixtures in occupational environments such as mining. [48,49] Co-exposures of these metals have been examined though there is limited literature concerning the health impacts of all three metals together. [50,51] However, several studies assessing combinations of two of the metals have observed adverse neurological outcomes. For instance, several studies have observed coexposure of Pb and Mn to be associated with decreased neurodevelopmental and cognitive function in children. [52–55] The impact of these coexposures on the respiratory system has yet to be elucidated. Further work is needed to understand the interactions of these metals and the modes of action of such coexposures.

Multivariable linear regression results showed that recreational water activities and fishing were also significantly associated with decreased urinary CC16 in the iAs, Pb and Mn models, which was an unexpected finding as these activities increase the likelihood of environmental exposure to these metals. We do not have plausible reasons for these results, but they may be attributed to other confounding lifestyle and arsenic exposure factors not assessed in our study. Additionally, the inclusion of a more in-depth assessment of these variables (e.g., frequency, duration, seasonality) should be considered for future follow-ups for this longitudinal study.

4.5.3 Limitations

A limitation of the presented research is that we did not measure any clinical indicators of organ health, such as FEV1/FVC for lung and glomerular filtration rate (GFR) for the kidneys to validate the biological significance of the biomarkers. GFR is typically an indicator of kidney function but has been inversely correlated to CC16 concentrations. [17] Thus, GFR may be an indicator to measure in future work to evaluate lung and kidney function in conjunction with CC16 and KIM-1.

Another limitation of this study is that we did not measure environmental exposures to arsenic, such as arsenic in drinking water, local food (e.g., fish, berries, etc.), dust, etc. A separate risk assessment was conducted in Yellowknife, where environmental exposure factors were tested for arsenic. [22] Additionally, lake waters and drinking waters are regularly tested for arsenic. While municipal drinking water is below the Canadian Maximum Allowable Concentration (MAC) value of 10 μg/L, public health advisories have been issued for certain lakes that are not safe for swimming and fishing (https://www.hss.gov.nt.ca/en/newsroom/arsenic-lake-water-around-yellowknife). [56] The Government of Northwest Territories (GNWT) does not recommend drinking untreated water from anywhere in the Northwest Territories. Most arsenic studies are conducted on populations where the primary source of arsenic exposure is drinking water. Our previous work suggests that the risk of arsenic exposure from drinking water is not likely to significantly contribute to the general Yellowknife population.[23] Instead, we posit that elevated levels of inorganic arsenic in children may be from exposure to dust or soil, which were not measured in the present study. Future work is anticipated where environmental exposures (e.g., house tap water and household dust) are tested on the same cohort.

4.6. Conclusion

This study aimed to measure and evaluate KIM-1 and CC16 as two candidate biomarkers of effect for arsenic in children. KIM-1 levels were significantly associated with inorganic arsenic (iAs) concentrations in urine. CC16 levels were significantly associated with iAs, lead (Pb) and manganese (Mn). Our results suggest that urinary KIM-1 could be used as a potential biomarker for the early effects of iAs on kidney impairment in children, while CC16 may be a potential biomarker of lung impairment from environmental contaminants such as iAs, Pb and Mn.

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Appendix 4.A: QA/QC results of the inter-laboratory comparison between the University of Ottawa and INSPQ

Table 4.A. Statistical comparison of urinary arsenic concentrations of randomly selected samples (n=50) analyzed by the University of Ottawa and INSPQ.

	Total As (tAs)	Inorganic As (iAs-met)
Pearson correlation coefficient (r)	0.976	0.972
p value- Pearson correlation	< 0.001	<0.001
p value- Paired t-test	0.318	0.094

5. Historical Exposure to Arsenic among miners and Indigenous children in Yellowknife from 1950s to 1980s- A review

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JSJC conceived the research question. JSJC conducted the search, acquisition, review, analysis, and interpretation of relevant literature. JC wrote the manuscript. LC oversaw the research and provided feedback and guidance on the study.

5.1. Abstract

In Yellowknife in the Northwest Territories, Canada, arsenic has been a public health concern for decades due to its history of gold mining. While a biomonitoring study, the Health Effects Monitoring Program (YKHEMP), was conducted in 2017-2018, there is an interest in the community to understand how arsenic exposure has impacted residents over time, notably while Giant Mine was in operation from 1948-2004. The objective of this study was to compare the current exposure levels to those measured in a series of biomonitoring studies conducted in the initial decades of mine operations between the 1950s to 1980s.

A search for historical arsenic human data was conducted in relevant libraries and archives. Archival arsenic concentrations in urine (n=554) and hair (n=301) were found for 72 Yellowknives Dene First Nation (YKDFN) children and 172 Yellowknife miners. Arsenic concentrations in urine (n=34) and hair (n=108) from 72 YKDFN children were mainly collected from a 1977 independent arsenic study and a government follow-up study of children retested for hair and urinary arsenic. Follow-up hair arsenic concentrations in YKDFN children (n=34; AM= 2.52 μg/g, GM= 3.37 μg/g) were significantly lower ($p<0.05$) than hair arsenic concentration from the initial independent study (n= 47; AM= 4.81 μg/g, GM= 3.37 μg/g). For urinary arsenic, archival total arsenic concentrations (n= 34; AM= 7.28 μg/L, GM= 6.65 μg/L) were comparable to current levels in YKDFN children (n= 75; AM= 8.20 μg/L, GM= 6.66 μg/L). Archival urinary (n=520) and hair arsenic (n=193) concentrations were also obtained from 172 Yellowknife miners from past occupational monitoring between 1975-1987. When accounting for individuals with multiple measurements, historical urinary total arsenic concentrations of miners (n= 138; AM= 77.5 μg/L, GM= 31.5 μg/L) were significantly higher ($p<0.05$) than recent

levels from YKHEMP adults (n= 1469; AM= 15 μg/L, GM= 7.6 μg/L). More than half of the obtained archival arsenic records belonged to three miners who each provided ~100 samples for analysis of urinary As concentrations. These samples showed very high arsenic exposure (AM= 111, 148 and 408 μg/L), which were at least ten times the baseline level observed among the general population, as reported in the current YKHEMP study.

Available historical data suggests that arsenic levels among YKDFN children in the 1970s were similar to current levels in Yellowknives Dene children. In contrast, historical concentrations in Yellowknife miners were higher in the past compared to the current background levels observed in adult YKHEMP participants.

5.2. Introduction

Arsenic is a naturally occurring, but toxic metalloid found in the Earth's crust. Its release into the environment occurs through natural processes (e.g., volcanic eruption and weathering) and anthropogenic activities (e.g., mining and smelting), leading to the presence of arsenic in air, water, soil, etc. [1]. Contamination of arsenic in the environment (e.g., in soil and drinking water) can result in elevated chronic arsenic exposure, impacting communities for many decades. Historically, arsenic contamination has been observed in Bangladesh, Chile, India, and Taiwan, resulting in a legacy of adverse health outcomes [2–5].

In Canada, environmental levels of arsenic are generally low but the presence of arsenic may be more elevated in certain regions due to geology or anthropogenic activities, as is the case of Yellowknife, Northwest Territories [6]. Arsenic is predominantly present in Yellowknife as gold-containing arsenopyrite in the local bedrock, thus, gold mines were established in the area, the largest of which was Giant Mine [7]. Giant Mine, located 4 km north of the city, was in operation from 1949 to 2004 [8]. The mine's roasting process resulted in the release of arsenic trioxide (As_2O_3), which was emitted freely for the first three years of operations. Efforts were made to control and reduce emissions in the 1950s [9]. However, the initial uncontrolled emissions have been suggested to have contributed to elevated environmental arsenic levels in the area. Previous studies had observed associations between historical emissions and environmental arsenic levels in water, sediment, and soil [10,11]. These findings, therefore, suggest potentially elevated arsenic exposures for the human population during Giant Mine operations.

Since the beginning of mining in Yellowknife, there have been concerns about arsenic exposure and its health impact on the local communities. Residents reported incidences of

arsenic poisoning in people and animals, which included the death of a Yellowknives Dene First Nation (YKDFN) child who had ingested contaminated snow in 1951[8]. The federal government conducted a set of human health studies concerning arsenic in Yellowknife from 1950-1970s (1951, 1966, 1975) (Appendix 5.A). Overall, arsenic levels in hair and urine were reported to be low, with some exceptions in miners. These studies were criticized for inconsistency and lack of transparency, as they were not immediately disclosed to the public. An independent study was conducted in 1977, which observed elevated hair arsenic levels in Indigenous children. A federal government task force was established in response, but it was concluded that arsenic did not pose a public health threat in Yellowknife [12]. However, the findings were criticized for emphasizing acute arsenic risk rather than the risk of chronic exposure. In the 1980s, public concern about arsenic subsided as technological advancements led to reduced mining emissions. No further arsenic health studies were conducted until recently, leaving a 40-year knowledge gap about arsenic and human health in Yellowknife.

Despite the mine's closure in 2004, 237 000 tonnes of arsenic trioxide remained stored in the mine's underground chambers. Consequently, Giant Mine has been regarded as one of the most contaminated sites in Canada [13]. The legacy of the mine has resulted in public health concerns of arsenic exposure from the initial uncontrolled roaster emissions as well as the potential surface runoff and groundwater migration from the former site [14]. Remediation activities of the former mine began in 2021. Moreover, the Health Effects Monitoring Program (YKHEMP), a human biomonitoring study, started in 2017 to establish baseline levels of arsenic and other chemicals of potential concern in the population of Yellowknife and the First Nations communities of Ndılǫ and Dettah.

During the planning of the study, the local community in Yellowknife expressed concerns over the health impact of the mine over the years and an interest to understand their past internal arsenic levels. There are currently no feasible methods in biomonitoring to measure the bioaccumulation of arsenic over a lifespan. Arsenic body burden may be estimated using biomarkers such as urine and toenail, which reflect recent exposures of only 3-4 days and up to 12 months, respectively. Longitudinal exposure may be better understood if an individual has been tested and those data are available for comparison with current measurements. Furthermore, analysis of historical data and comparison with recent results may allow for a better understanding of the health impacts of arsenic over the last 70 years. The objectives of this research were: (1) Summarize and analyze available archival records of urinary and hair concentrations, and (2) Compare the historical data with the current findings from YKHEMP to assess arsenic exposure levels in Yellowknife residents over different periods.

5.3. Materials and methods

5.3.1. Literary search

A literature search was conducted in the databases of the University of Ottawa, the Health Library of the Government of Canada, the Northwest Territories Archives (NWT Archives), the Library and Archives Canada (LAC), and the Canadian Circumpolar Collection at the University of Alberta. The keywords used to search for relevant documents included "Arsenic," "Yellowknife", "Human Health," "Toxicity" "Giant Mine", "Con Mine," etc. The libraries were also searched for relevant documents about previous federal arsenic health studies, Giant Mine, or arsenic in Yellowknife. The NWT Archives, LAC, and the University of Alberta held copies of relevant literature. Documents of interest included past reports, drafts, media reports, letters, and communications of previous federal arsenic health studies in Yellowknife.

Additionally, archival records of arsenic concentrations in hair and urine were also found to be stored in the NWT Archives. Relevant documents were requested for the loan. For the archival records, digital copies of suitable NWT Archives files with arsenic results were acquired, where all names or identifiers (e.g., date of birth, address) were redacted following the Access to Information and Protection of Privacy (ATIPP) agreement with the NWT Archives.

In addition, participants of the Health Effects Monitoring Program were asked during data collection whether they were tested for arsenic in the past. If so, they were asked permission by the study to search for their past results in the NWT Archives. The participants signed a consent form and agreement, and a list of consenting names was sent to the NWT Archives. The names were then searched for in the relevant files at the NWT Archives, and if records of their past results were found, these results were sent to the research team. The participants with available records were sent a letter with their past and current arsenic levels.

5.3.2. Compilation and literary review of historical data

All received documents were reviewed and archival arsenic measurements in hair and/or urine, as well as relevant information (e.g., date of sampling), were recorded and compiled into an anonymized database. Context of the measurements and basic information of the tested individuals (e.g., age, occupation) were identified through the archival documents (e.g., attached reports, memos, and communications) and with the aid of local stakeholders from the Giant Mine Oversight Board (GMOB). The compiled database of archived arsenic measurements contained 855 records of urine and/or hair (554 urinary and 301 hair concentrations) collected from 244 individuals from 1975 to 1987.

5.3.3. Statistical analysis

Descriptive statistics and statistical tests of archival concentrations were calculated using R v.4.1.2. (Team R Development Core, 2022). Welch's *t*-test was used to test the means of the historical data and the data from YKHEMP.

5.3.4. Ethics

The presented research and design were approved by the NWT Archives, the Health Sciences and Sciences Research Ethics Board of the University of Ottawa (http://research.uottawa.ca/ethics/reb) and the Aurora College Research Ethics Committee. In addition, the study has been granted a Scientific Research License from the Aurora Research Institute in Northwest Territories. The presented research was conducted as per ATIPP. Additionally, The Health Effects Monitoring Program was conducted following the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans and, in particular Chapter 9, research involving the First Nations, Inuit and Métis Peoples of Canada, and the document entitled: Indigenous Peoples & Participatory Health Research: Planning & Management, Preparing Research Agreements published by the World Health Organization (2003). [16,17] The study also follows the First Nations principles of Ownership, Control, Access, and Possession (OCAP®) of data. [18]

5.4. Results and Discussion

5.4.1. Summary of Archival Records

Following the review of relevant archival documents, 885 available records of arsenic test results in urine and/or hair (554 urinary and 301 hair concentrations) were found in archival files located in the NWT Archives. These samples were collected from 244 individuals between 1975-1987

From the obtained historical data, archival records of arsenic concentrations belonged to two groups: (1) Yellowknives Dene First Nation (YKDFN) children; and (2) Yellowknife miners. A total of 72 individuals were identified as YKDFN children, and 172 individuals were identified as miners, with one miner identified as also YKDFN. A summary detailing the obtained records for each group is presented in Table 5.1.

Table 5.1. Amount of individuals and urinary concentrations in archival data by group.

Group	Individuals n	Measured Urine Concentrations n	Measured Hair Concentrations n
YKDFN-Child	72	34	108
Miner	172	520	193
Total	**244**	**554**	**301**

A total of 86 individuals (34 YKDFN children and 52 Yellowknife miners) had multiple arsenic test results. Most of these individuals had 2-7 measurements that were generally several months apart, except for three miners, who each had ~100 arsenic tests conducted between 1977-1987 (Fig 5.8a-c and 5.9).

After reviewing the files and documents, most archival arsenic concentrations were found to belong to three main sources. The first source was from an independent arsenic hair study of YKDFN children and mine workers conducted by the National Indian Brotherhood (NIB) and the United Steelworkers of America (USoA) published in 1977 [19]. The second source was from an unpublished follow-up study, conducted by the Northwest Territories (NWT) government in 1977, on the children who participated in the aforementioned independent hair arsenic study. The third source is from the occupational monitoring of mine workers in Yellowknife.

5.4.2. Historical Records of Yellowknives Dene First Nation (YKDFN) Children

Historical arsenic test results of 72 YKDFN children were found. Overall, 34 urine and 108 hair results of YKDFN children from 1976-1978 were obtained. Most of the archival

records were traced back to the 1977 independent hair arsenic study and the NWT government follow-up of the child cohort.

A total of 47 archival measurements of hair arsenic matched the reported sampling dates of the independent hair arsenic study conducted by NIB and USoA, where the children were tested in December 1976. The results were cross-referenced via the median, where the study had reported a median of 3 ppm (µg/g), which matched the calculations from our collected archival data. Additionally, all concentrations were reported in the Final Report of Public Task Force on Arsenic published by the Canadian Public Health Association (CPHA) in 1977. Therefore, all the historical data collected from YKDFN children in the independent study have been obtained.

Archival documents and communications indicate that the Northwest Territories Region (NWT) attempted to follow up with the children who participated in the independent hair arsenic study. The follow-up consisted of a physical examination as well as testing of arsenic in urine and hair. Urine and hair arsenic test results from the NWT follow-up study were found for 34 children, all of whom had one hair and one urine result each.

An additional 27 archival hair concentrations were from neither of the studies above. The exact source of these results could not be determined from the available archival documents. Eight of these results could be from a health study conducted by the Medical Services Branch of the federal government in 1975, as the results are preceded by a government memo that indicates the results are a list of all YKDFN children sampled in 1975 with hair arsenic concentrations above 5 µg/g. There were also 18 hair arsenic measurements found in the final report of the Arsenic Task Force in Table 11, titled Arsenic Levels in Native Children by Residence, January 1977. The report states that the NIB categorized results presented in the table by where the tested children resided. However, there are no further details regarding the origin of the results

presented in the table. It does not clearly state where these additional measurements are from, as they are not from the independent hair arsenic study.

Furthermore, the presented concentrations do not correspond to any data found in the archival documents. Lastly, one arsenic test result, sampled in 1978, could not be accounted for. This child was previously tested, but no information was provided as to why they were tested in 1978. In summary, out of the 72 YKDFN children, 34 had multiple hair arsenic test results, all of whom had 2 results each except for 2 of the children with 3 and 4 measurements, respectively. There were no children who had multiple urinary measurements.

A summary of the descriptive statistics of the available historical data of YKDFN children, compared to YKHEMP results from YKDFN children, is presented in Table 5.2. All obtained historical urine concentrations were from the NWT follow-up study. The distribution of archival urinary arsenic concentrations for historical data of YKDFN children is displayed in Figure 5.1. The concentrations are right skewed as the median is 7 µg/L and the P95 is 13 µg/L. Most obtained urinary concentrations (79.4%) were below 10 µg/L.

Table 5.2. Summary of total arsenic concentrations in urine (µg/L) and hair (µg/g) of historical data and YKHEMP results of YKDFN children. Hair concentrations are summarized by all obtained measurements and by individuals in which a mean concentration was calculated for individuals with multiple concentrations.

		N	A.M.	G.M.	Median	Min	Max	P95
Urine								
Historical Data		34	7.28	6.65	7	0	17	13
YKHEMP		75	8.2	6.66	6.73	0.53	30.8	20.2
Hair								
Historical Data	All measurements	108	4.49	3.33	3.1	0	28	12.7
	By Individual	72	4.78	3.56	3.65	0	22.5	13.0

A.M.: Arithmetic Mean, G.M.: Geometric Mean, P95: 95th Percentile

Compared with current urinary arsenic levels, the historical total urinary concentrations of the 34 YKDFN children (AM= 7.28 µg/L, GM= 6.65 µg/L) were not significantly different

from observed concentrations from YKDFN child participants of the Health Effects Monitoring Program baseline results (AM= 8.20 µg/L, GM= 6.66 µg/L). The maximum concentration of the historical studies was 17 ug/L compared to 30.8 ug/L reported by the YKHEMP.

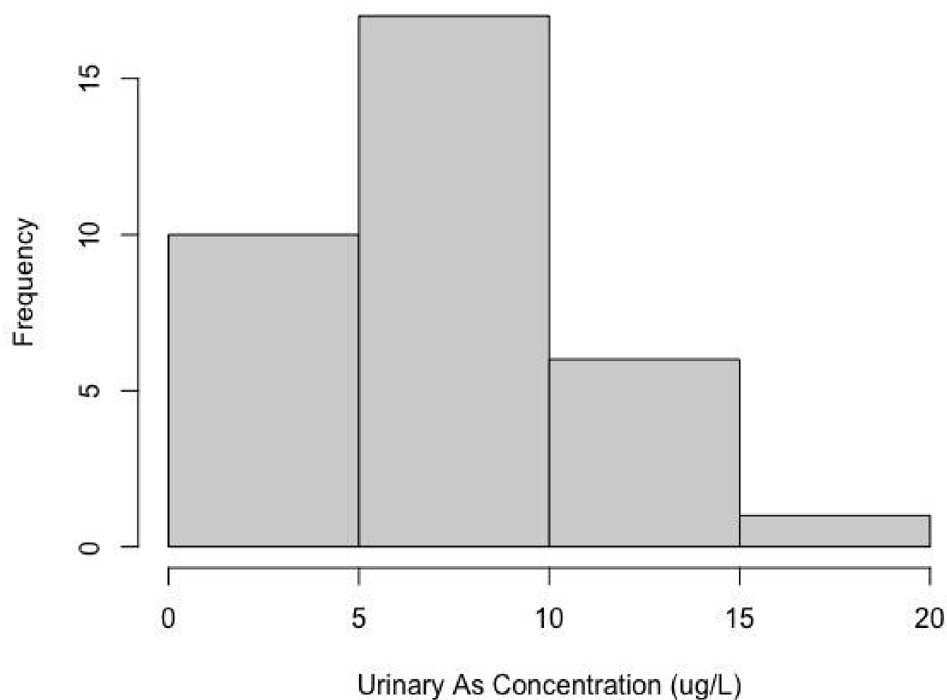

Figure 5.1. Frequency distribution of YKDFN urinary concentration (µg/) of historical measurement from YKDFN children in 1977 (n=34).

For hair, the arithmetic (AM) and geometric (GM) means for all obtained archival hair arsenic concentrations were 4.49 µg/g and 3.33 µg/g, respectively (Table 5.2). Regarding children with multiple hair arsenic measurements, the AM and GM were 4.78 µg/g and 3.56 µg/g, respectively.

A comparison of hair arsenic results from the independent NIB study and the follow-up is presented in Table 5.3 and Figure 5.2. Hair concentrations from the independent study were significantly higher than in the follow-up ($p<0.05$). For the individual child with the highest observed urinary arsenic in the independent study (29.4 µg/g), their levels were comparatively lower when tested again (12 µg/g). Figure 5.3a shows the distribution of total arsenic

concentrations in hair for historical data of YKDFN children. The concentrations are right skewed as the median is 3.1 µg/g and the P95 is 12.7 µg/g. Overall, most acquired arsenic concentrations (77.7%) are below 5 µg/g. At the time of testing, the government of Northwest Territories considered concentrations above 5 µg/g to be elevated, as indicated by the archival documents. In the independent study, 34 % exceeded the 5 µg/g cut-off (Figure 5,3b), which is higher than the follow-up study with the exceedance at 5.9% (Figure 5.3c).

Table 5.3. Summary of total arsenic concentrations (µg/g) in the hair of historical records and NWT follow-up results of YKDFN children.

	# of Children (n)	A.M.	G.M.	Median	Min	Max	P95
Independent Study	47	4.81*	3.37	3	0.65	28	12.76
NWT Follow-Up	34	2.52*	2.57	2.7	0	6.3	5

* Hair concentrations from the independent NIB were significantly higher than in the NWT follow-up study.

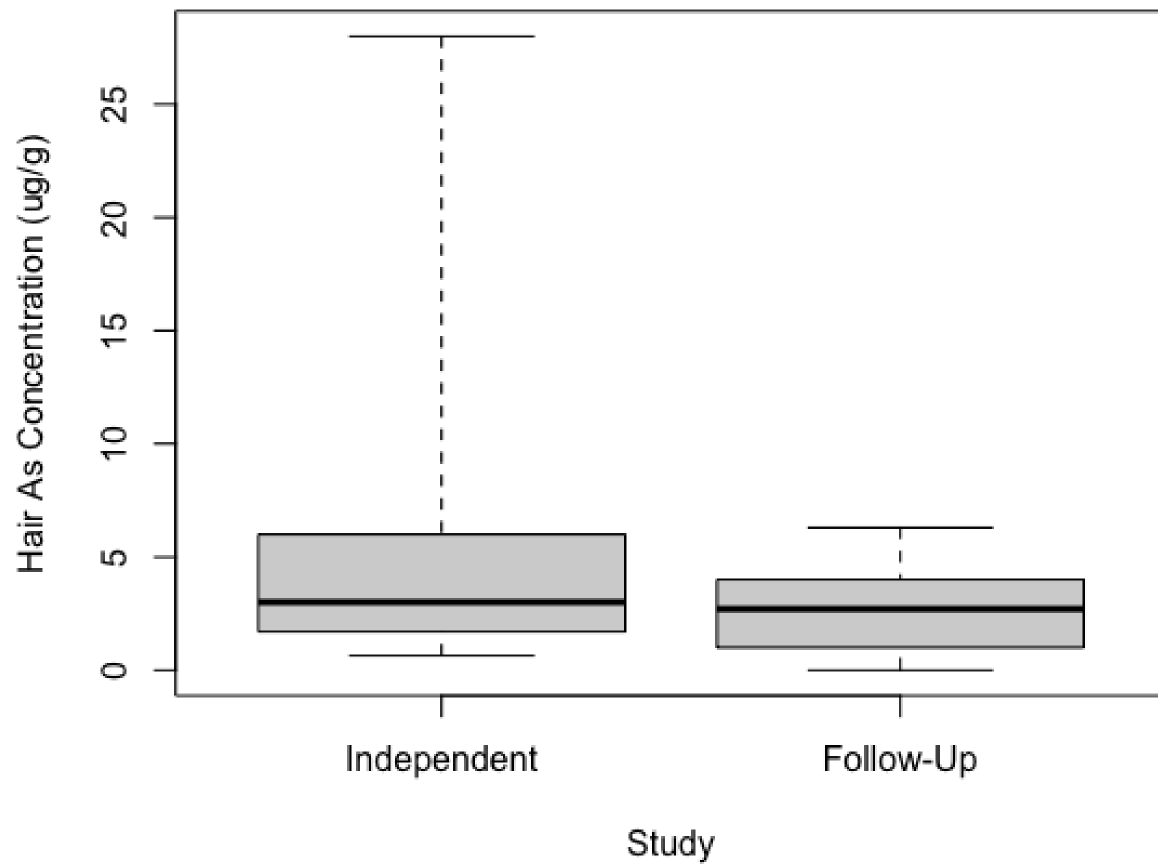

Figure 5.2. Boxplot comparison of hair arsenic concentrations in YKDFN between independent study and government follow-up. The ends of the whiskers represent the minimum and maximum.

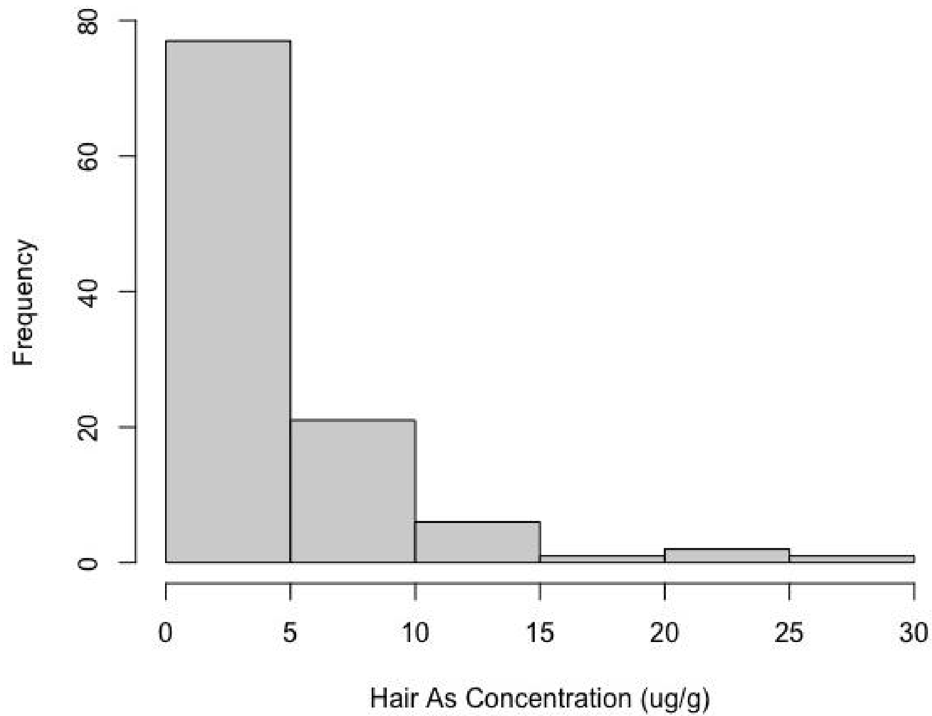

Figure 5.3a. Frequency distribution of YKDFN hair concentrations (µg/g) of historical measurements from YKDFN children (n=108).

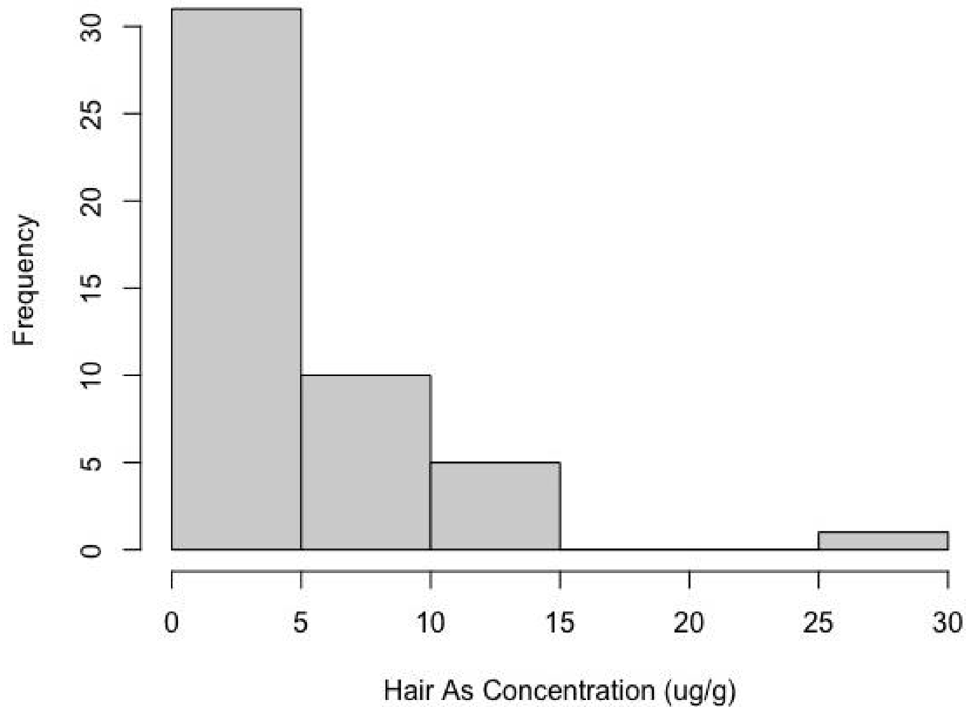

Figure 5.3b. Frequency distribution of YKDFN hair concentrations (µg/g) of historical measurements from YKDFN children from the independent arsenic hair study (n=47).

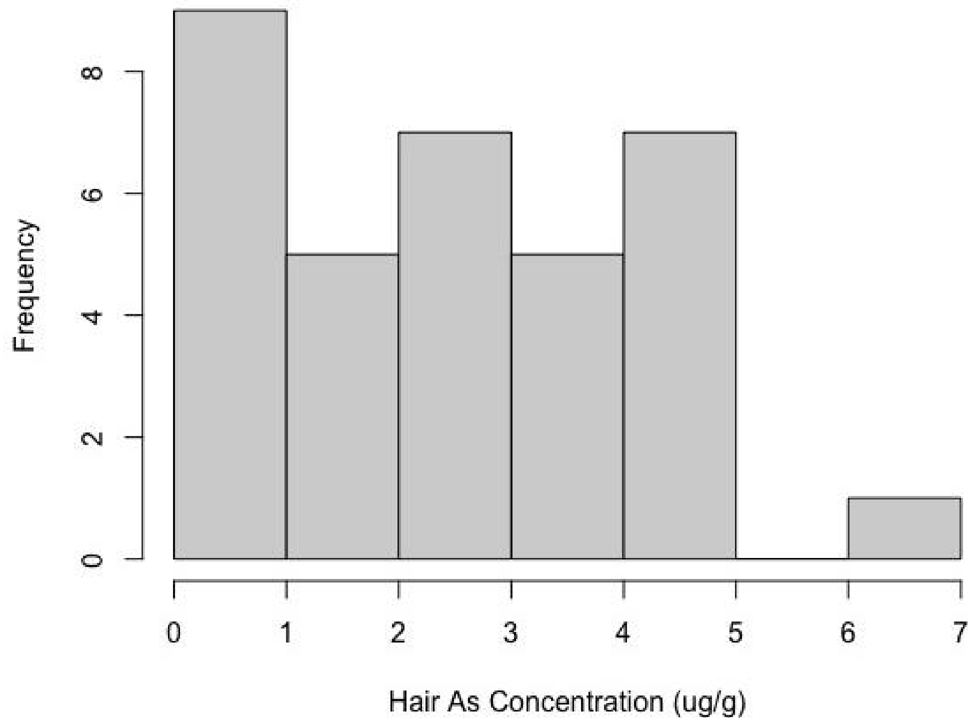

Figure 5.3c. Frequency distribution of YKDFN hair concentrations (μg/g) of historical measurements from YKDFN children from the NWT follow-up study (n=47).

5.4.3. Historical Records of Miners

Historical data from 1975-1987 of 172 miners were obtained, with a total of 520 urine concentrations and 192 hair concentrations. All of the miners were from Yellowknife, with 56 miners reported to work at Giant Mine and one miner from Con Mine, while no information on the work location was available for 115 miners. Information regarding their job position was also unavailable for nearly all miners. A total of 52 miners had multiple arsenic measurements. Forty-five miners had only hair results. It should be noted that out of the 520 urine records, 297 measurements came from 3 miners who had elevated arsenic exposure.

The majority of obtained measurements for these miners were likely for occupational monitoring as copies of these test results were from the Occupational Health Unit of the Laboratory Medical Service Branch of Health and Welfare Canada. Documents indicate that the tests of Yellowknife miners were for "arsenic surveillance." The Arsenic Task Force report

suggests that Giant Mine surveys were conducted in March-April/October-November of 1976 and 1977, as well as Con Mine surveys in 1972, 1975, and 1977 (Appendix 5.A). Based on the dates and sample size described in the report, urinary and hair arsenic concentrations from 38 individuals in the archival records are likely to be from the 1977 Giant Mine survey. Additionally, 19 hair arsenic concentrations were results from the independent arsenic hair study that included YKDFN children. There were also 16 hair arsenic concentrations from 1975 that may be from the 1975 study conducted by the Medical Services Branch. In an attached correspondence, it is noted that these workers were also previously tested in the 1966 health study of mine workers. However, the results from that study were not disclosed.

A summary of the descriptive statistics of the available historical data of Yellowknife miners compared to YKHEMP results is presented in Table 5.4. Concentrations are summarized by all obtained measurements, as well as by individual where for individuals with multiple measurements, their arithmetic mean was first calculated. The arithmetic means of all the obtained historical urine As concentrations were 159.30 µg/L, while the geometric mean was 76.51 µg/L. For hair, the arithmetic mean for all archival hair concentrations was 46.37 µg/g, and the geometric mean was 17.45 µg/g. When accounting for individuals with multiple measurements, the arithmetic mean for archival urinary arsenic in Yellowknife miners was 77.51 µg/L and the geometric mean was 31.45 µg/L. For hair, the arithmetic mean was 41.6 µg/g, and the geometric mean was 16.2 µg/g.

Table 5.4. Summary of total urinary arsenic concentrations (µg/L) of miners. For miners with multiple measurements, the arithmetic mean of their measurements was used and summarized by the individual.

Group			n	A.M.	G.M.	Median	Min	Max	P95
Miners	All Measurements	Urine	520	159.3*	76.51	90.5	0.25	2345	521.1
		Hair	193	46.3	17.45	17.8	0	350	198.8
	By Individual	Urine	138	77.5*	31.45	33	0.25	970	402.97
		Hair	134	41.7	16.2	16.5	0	350	172.2
YKHEMP	Adults	Urine	1469	15.1	7.62	6.96	0.25	960.27	44.54
YKHEMP	Adult Males	Urine	644	14.4	7.64	7.22	0.34	571.25	40.75
YKHEMP	Current Giant Workers	Urine	17	16.7	11.64	9.59	4.4	59.08	41.33
YKHEMP	Former Miners	Urine	164	14.0	7.01	6.86	0.92	245.06	37.83

* Significantly different than YKDFN adults in YKHEMP (p<0.001)

The mean urinary concentrations of the miners (by individual) were compared with current arsenic concentrations in adult participants of the YKHEMP. Historical urinary concentrations in miners (AM: 77.51 µg/L, GM: 31.45 µg/L) were found to be significantly higher ($p<0.001$) than that of adult participants in the YKHEMP (AM: 15.13 µg/L, GM: 7.62 µg/L). Additionally, historical arsenic concentrations were significantly higher ($p<0.001$) than both current Giant Mine workers (AM: 16.73 µg/L, GM: 11.64 µg/L) and former Giant/Con mine workers (AM: 13.99 µg/L, GM: 7.01 µg/L).

The data were collected in three main time periods: 1975-1978, 1979, and 1980-1987. Total arsenic concentrations in urine and hair by time period are summarized in Table 5.5. Figures 5.4 and 5.5 display arsenic concentrations in urine and hair, respectively, in mine workers from 1975-1987. Overall concentrations from 1979 were highest. The concentrations reported in 1975-1978 were overall lower than those collected in the other two periods. However, it should be noted that the results are skewed by the three miners who were tested extensively, particularly in 1979.

Table 5.5. Summary of total urinary arsenic concentration in urine (μg/L) and hair (μg/g) of miners by year (All data points).

Year		n	A.M.	G.M.	Median	Min	Max	P95
1975-1978	Urine	137	32.2	19.1	21	0.25	253	93.2
	Hair	142	37.4	13.6	12.9	0	313	174.65
1979	Urine	336	210	135	142	2.5	2345	603
	Hair	37	67.3	29.1	25	1.7	350	296.8
1980-1987	Urine	47	166	73.1	76	4	970	541
	Hair	14	81.7	52.3	60	10	219	196.25
Total	Urine	520	159	76.5	90.5	0.25	2345	521
	Hair	193	46.4	17.4	17.8	0	350	198.8

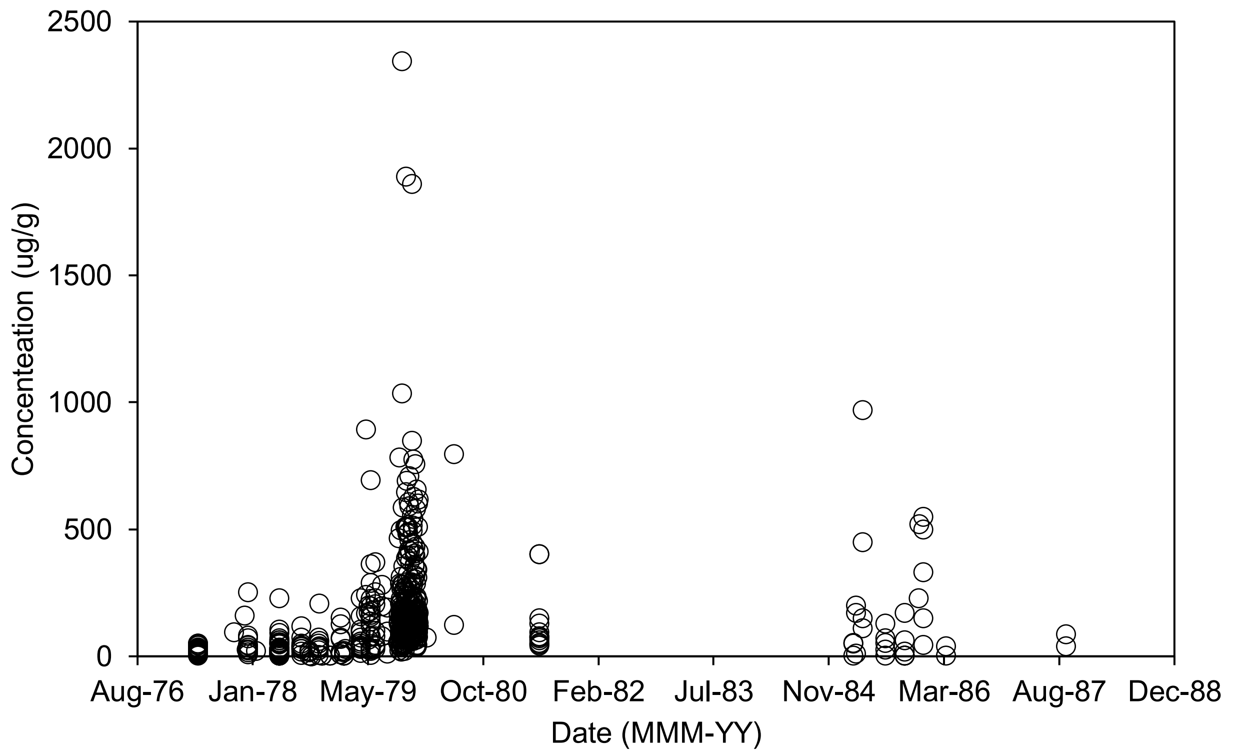

Figure 5.4. Archival urinary total arsenic concentrations(μg/L) of miners (n=520) from 1975-1987.

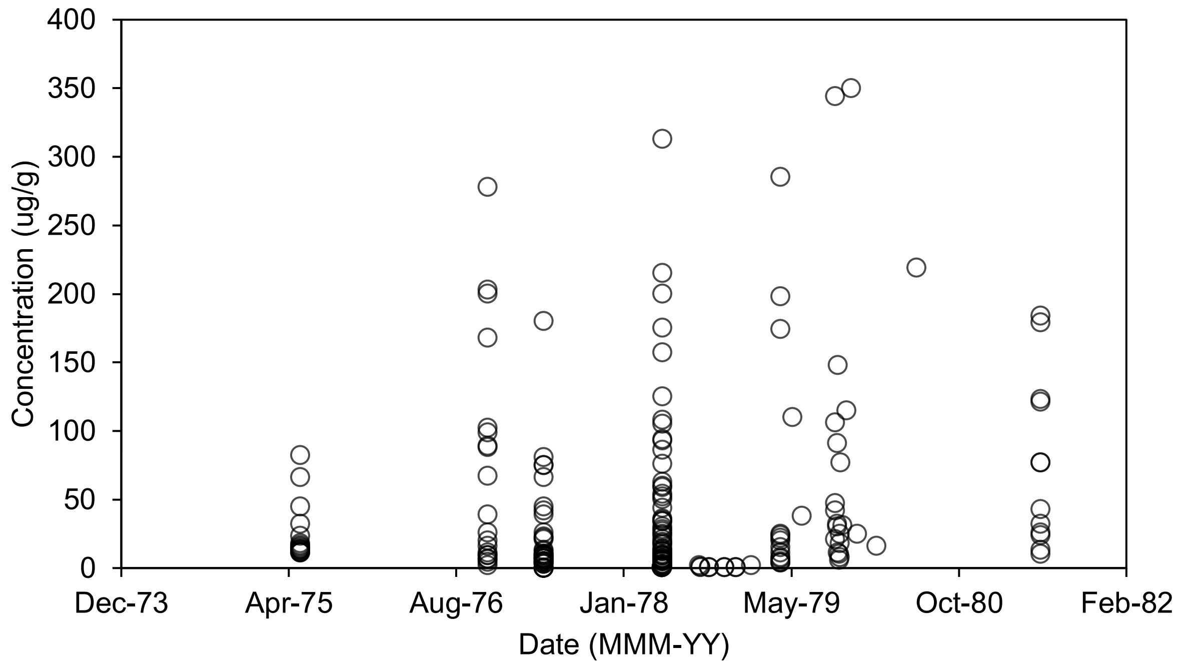

Figure 5.5. Archival hair total arsenic concentrations(µg/L) of miners (n=183) from 1975-1987.

Figure 5.6 shows the distribution of urinary total arsenic concentrations for historical data of Yellowknife miners.

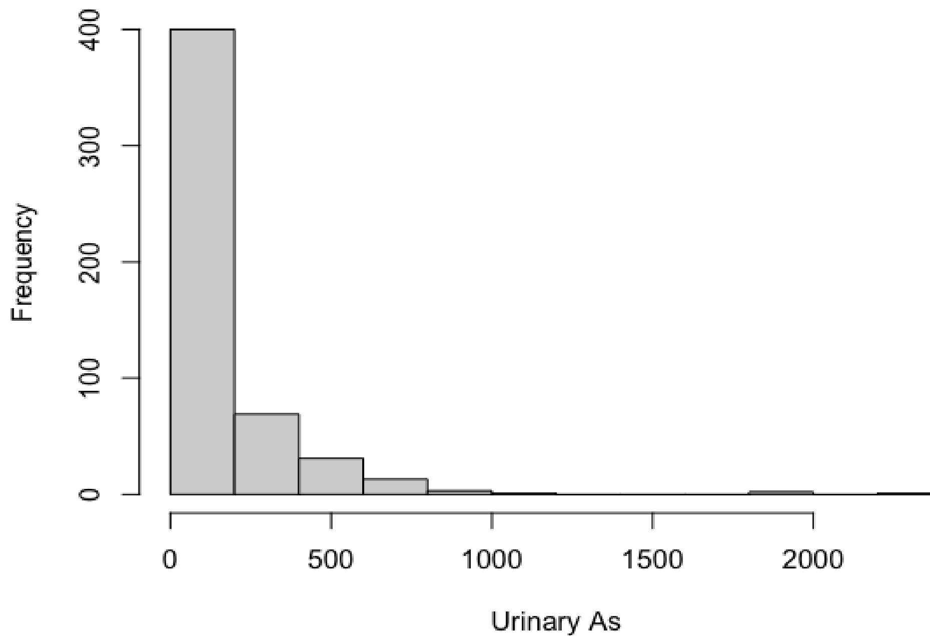

Figure 5.6. Frequency distribution of urinary concentration (µg/L) of historical measurement from Yellowknife miners in 1977 (n=520).

Figure 5.7 shows the distribution of all obtained arsenic concentrations in hair for historical data of Yellowknife miners. The concentrations are right skewed as the median is 17.8 μg/g and the P95 is 198.8 μg/g.

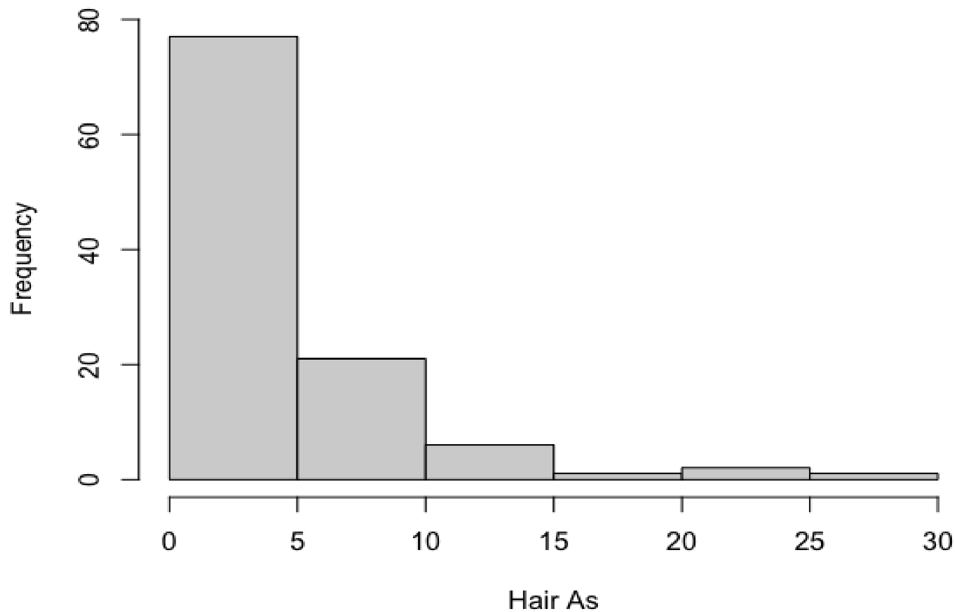

Figure 5.7. Frequency distribution of hair concentrations (μg/g) of historical measurement from Yellowknife miners (n=193).

5.4.3.1 Summary of Historical Data of Three Miners

As previously noted, three miners from Giant Mine were tested extensively, with 101, 99, and 97 obtained records for each. These miners were likely tested multiple times due to their elevated levels from 1977 to 1987. A summary of their concentrations is presented in Table 6, and their concentrations over time are shown in Figures 5.8a-c and 5.9. In 1979, records indicate that they were tested nearly every day from October-December (Figure 5.9). Based on archival communications in 1980 (Chief of Safety Division at the Department of Justice and Public Services), the three miners were part of a 90-day Arsenic survey conducted at Giant Mine. However, we have been unable thus far to locate more information about this survey. These results suggest that these three miners, especially Miner B, had very high occupational arsenic

exposure in 1979, which was at least ten times the baseline level observed among the general population as reported in the current YKHEMP study.

Table 5.6. Summary of total urinary arsenic concentration (µg/L) of miners A-C.

ID	n	AM	GM	Median	Min	Max	P95
A	101	148	119	136	2.5	466	319
B	99	408	303	307	9	2345	853
C	97	111	97	90	21	508	192

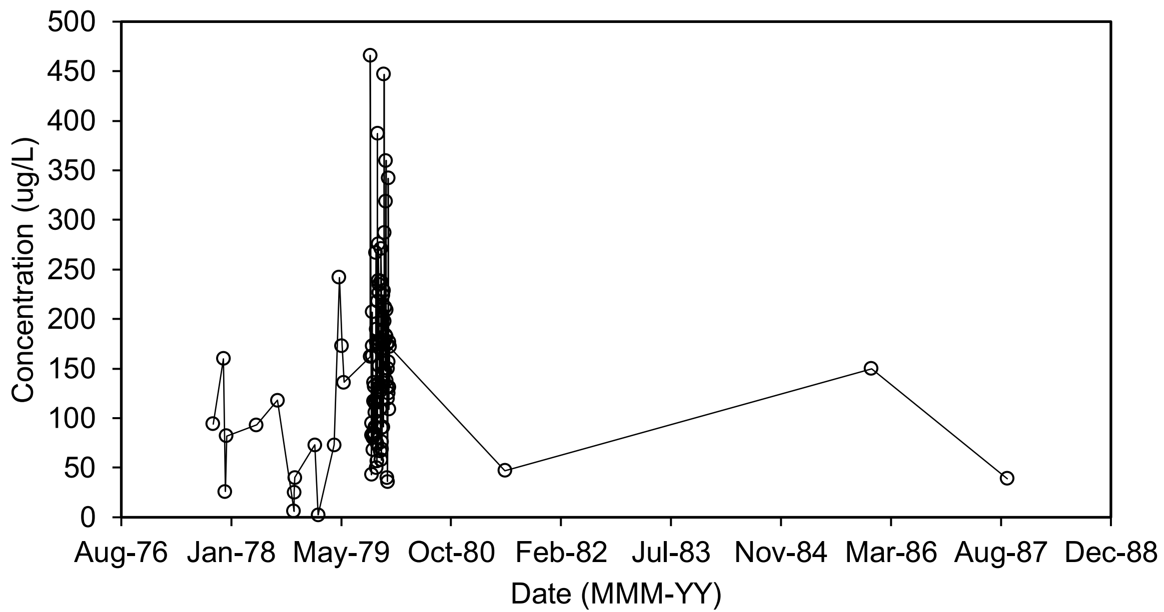

Figure 5.8a. Total urinary arsenic concentrations (µg/L) of miner A (n=101) from October 1977 to September 1987.

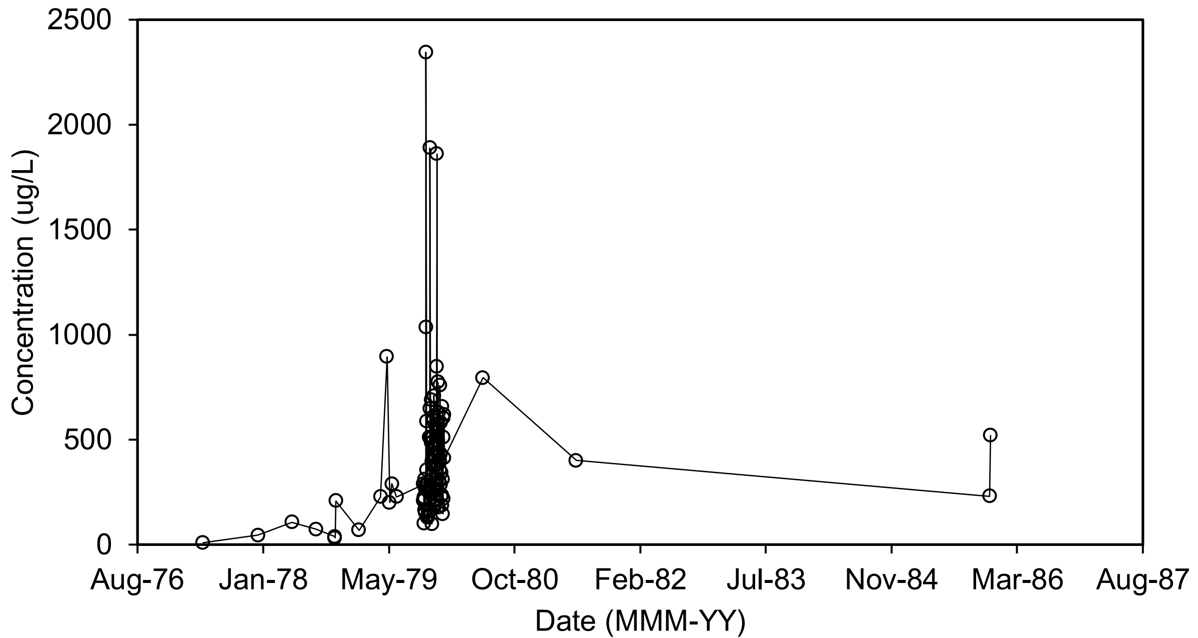

Figure 5.8b. Total urinary arsenic concentrations (µg/L) of miner B (n=99) from May 1977 to December 1985.

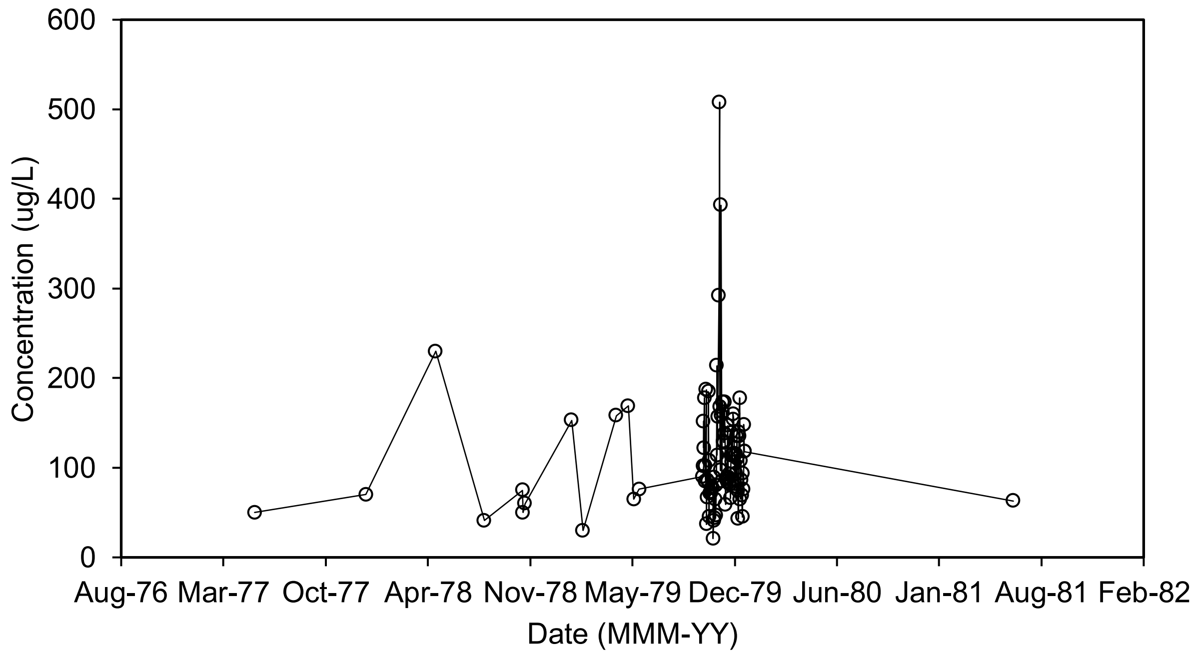

Figure 5.8c. Total urinary arsenic concentrations (µg/L) of miner C (n=97) from May 1977 to June 1981.

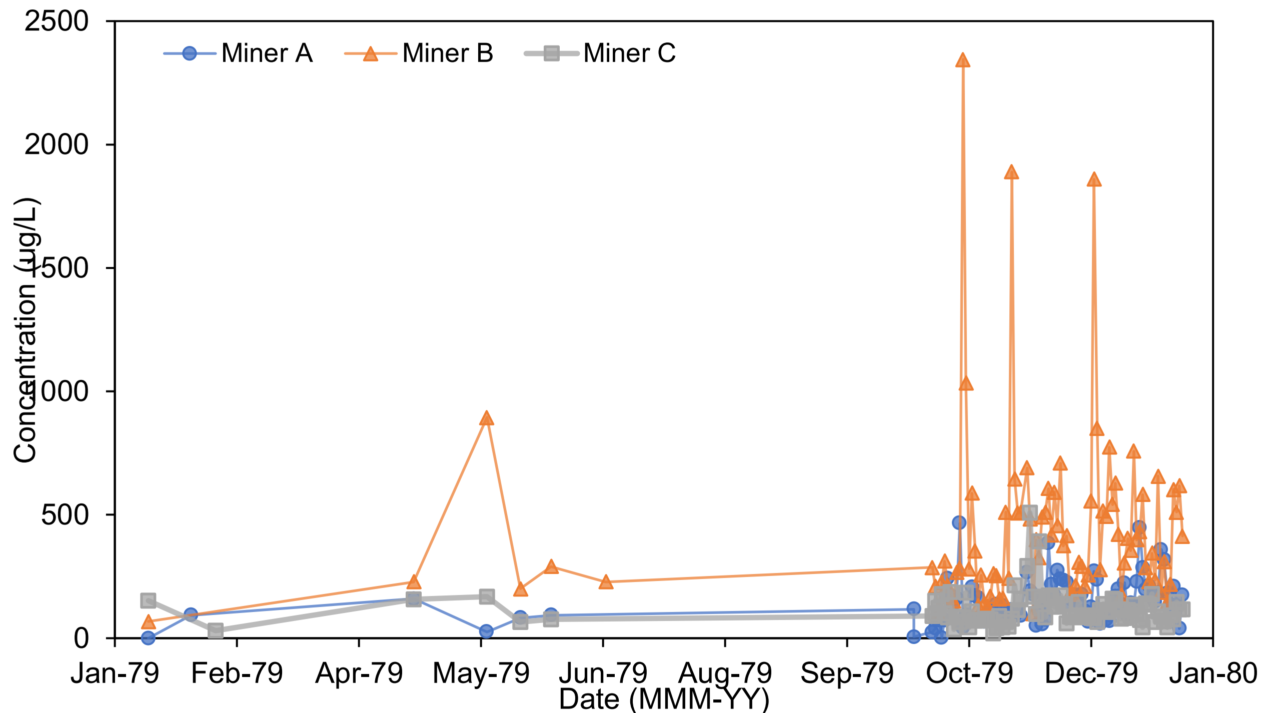

Figure 5.9. Total urinary arsenic concentrations (µg/L) of Miner A, B and C in the year 1979.

5.4.4. Historical Records of YKHEMP Participants

During community meetings before the start of YKHEMP, numerous community members informed the team that they were tested for arsenic in the past. Thus, as part of YKHEMP baseline sample collection, participants were asked whether they were tested in the past and for their consent to look for past results in the NWT Archives. Seventy-two participants signed a consent, and there were only 4 participants whose past results were available. Two were YKDFN (then children), and 2 were miners. A summary of past and current total arsenic concentrations in the urine of each participant is presented in Table 5.7. Two YKDFN participants were tested for arsenic in hair in the past, but no urinary arsenic measurements were found. For the two miners, particularly Participant 3, their previous archival concentrations were higher than their current levels.

Table 5.7. Summary of past and current total urinary arsenic concentrations of four YKHEMP participants. Records of past urinary concentrations were found in archival files at NWT Archives, while YKHEMP arsenic levels were sampled in the Fall of 2017.

		Archival			YKHEMP
Participant	Group	# of results	Mean hair (µg/g)	Mean urine (µg/L)	Mean urine (µg/L)
1	YKDFN	2	3.3	-	5.8
2	YKDFN	4	4.0	-	3.4
3	Miner	4	-	397	11
4	Miner	1	13	-	2.8

5.5. Discussion

Overall, 885 archival arsenic records (554 urinary and 301 hair concentrations) of 244 Yellowknife residents tested from 1975 to 1987 were found in the NWT Archives. These individuals were either YKDFN children (n= 72) or Yellowknife miners (n=172). The obtained records are a partial collection of historical human arsenic data. The available archival arsenic results are very limited, considering historical data for only two demographics were found, though it is known that more Yellowknife residents were tested in the past (Appendix 5.1). The major arsenic human health studies were conducted in 1951, 1966, 1975, and 1977.; the federal government conducted the first three while the latter was an independent study. The complete dataset was only found for the 1977 independent study. Therefore, a critical analysis and review of all the studies could not be made.

The first major study was conducted in 1951 with 230 schoolchildren, a subset tested for urinary arsenic. The study was briefly described in minimal detail in the Final Report of the Arsenic Task Force and reported that urinary arsenic was observed in "small amounts" [12]. After an archival search, neither the original report nor the study results could be found. Thus, the actual levels of the "small amounts of arsenic" in urine are unclear.

In 1966, a study of 361 male residents was conducted in Yellowknife [20]. Overall, the mean urinary arsenic concentration was 12.37 μg/L and urinary arsenic was more elevated in mill mine workers compared to non-mill mine workers. A copy of the study report was available, but the complete dataset could not be located.

In 1975, the Medical Services Branch conducted a study testing arsenic in hair of 700 Yellowknife residents. This was the most extensive study in scope, as it included general Yellowknife residents. Participants included 575 Yellowknife residents and 135 mill workers from the mine. Approximately 33% of the mill workers (n=45) had hair arsenic levels above 10 μg/g, while concentrations for 3% of residents were above 10 μg/g [21]. A report of the study was located in the Canadian Circumpolar Collection at the University of Alberta. Some participants' results are possibly described in memos and correspondence in archival documents at the NWT Archives. However, the hair arsenic results could not be located.

5.5.1. Archival concentrations of arsenic in Yellowknives Dene First Nations (YKDFN) children

5.5.1.1. Urine

Overall, archival urinary arsenic test results for 34 YKDFN children were obtained. All obtained urine results are from an NWT follow-up of 47 children previously tested in the independent arsenic study. Archival memos and correspondences indicated that only some of the children participated in the follow-up study. However, the number of children who participated in the follow-up was not stated. Therefore, some results may be missing. The results of this follow-up were never disclosed to the public as no reports of this data could be found. The study was only described in memos and letters; thus, the follow-up study remained an internal investigation. To our knowledge, the present research is the first to report results for this NWT follow-up study.

The archival urinary arsenic concentrations were similar to the current YKHEMP baseline total urinary arsenic levels of YKDFN children and similar to the total arsenic levels found in the general Canadian population [22]. Compared to other child cohorts living near mining and industrial areas during the same decade, the urinary concentrations of the 34 children are also within range, but generally lower, than the observed concentrations [23–25]. Furthermore, archival urinary arsenic concentrations were lower compared to children living in arsenic-endemic areas such as Bangladesh, where one study observed total urinary arsenic concentrations ranging from 140-1250 μg/L [26].

The limited amount of available urinary data limits the ability to critically assess historical arsenic levels in YKDFN children during active mining periods in Yellowknife. Numerous factors make the direct comparison between the historical and YKHEMP results difficult, such as differences in laboratory analysis and quality control measures. Moreover, missing details, such as study methodology and lifestyle information, make comparisons more difficult. The detailed sampling methodology of the sampled cohort was also not available; thus, the obtained historical concentrations may not be from a representative cohort, and the results may not necessarily reflect the arsenic levels of all YKDFN children at that time.

Compared to hair, there were fewer available archival urine test results in children and urinary data was limited. There was no explanation as to why studies used hair rather than urine to assess arsenic. Hair is susceptible to environmental contaminants (e.g., dust, particulate matter), which may interfere with measuring the internal dose. Ingested arsenic is mainly excreted via urine. Thus, urinary arsenic is the human biomarker most frequently used for measuring arsenic exposure and is more preferred over hair as an indicator of internal dose.

5.5.1.2. Hair

Most archival hair concentrations were from either an independent hair study conducted by the NIB and the UsoA or an NWT follow-up study of child participants from the independent research. Concentrations from the follow-up study were significantly lower than the independent study conducted within a year apart. The differences could therefore be due to seasonality. Differences in methods could also be possible, though a detailed methodology was unavailable for either study.

Background, arsenic hair levels, are typically <1 ug/g in non-exposed populations, thus obtained archival hair arsenic concentrations in YKDFN children indicated arsenic exposure [27]. Furthermore, archival hair concentrations were within the range of study results of child cohort living near mining areas during the 1970s. However, concentrations were higher than the observed means of some of the studies [23–25]. This may suggest that while children were exposed to arsenic, as indicated by their hair concentrations, their arsenic exposure via ingestion was not as elevated, as characterized by their urinary arsenic concentrations.

Arsenic has been shown to have an affinity for thiol (-SH) groups in cysteine residues and thus tends to deposit in keratin-rich tissues such as hair and nails [28]. Therefore, hair may be used as a biomarker for arsenic exposure, though it is currently less preferable than urine for estimating internal dose due to exogenous contamination. All reported archival arsenic concentrations are from washed hair samples. However, the sample preparation and washing protocols were unavailable. Furthermore, cross-sectional analysis of hair showed that arsenic from exogenous contamination could not be distinguished from arsenic from ingestion [27]. Thus, possible external contamination should be considered when assessing hair arsenic. The methods used to analyze hair samples were also not disclosed. Methods used around the same period

included colorimetric tests using chemicals such as silver diethyldithiocarbamine [23]. Higher throughput technologies have developed since then, and current methods of arsenic quantification include mass spectrometry techniques such as inductively coupled plasma mass spectrometry (ICP-MS). Despite these advancements, urinary inorganic arsenic remains the preferred indicator of arsenic body burden.

5.5.2 Yellowknife Miners

5.5.2.1 Urine

Overall, 520 historical urinary concentrations from 138 Yellowknife miners during 1975-1987 were found in archival documents. Archival concentrations were significantly higher than current YKHEMP baseline urinary arsenic levels in adults as well as former and current Giant Mine workers. Such findings are expected as individuals who are mine workers are likely to have elevated arsenic concentrations due to occupational exposure. Obtained archival concentrations of miners are within the range of reported literature of occupationally exposed cohorts [29–31]. YKHEMP participants should have lower exposures than past workers as mining operations have ceased. Urinary concentrations of current workers on the Giant Mine Remediation Project are also likely to be lower as technological advancements have been made to minimize occupational exposure compared to when the mine was open. However, presented archival concentrations may be slightly more elevated than concentrations of all past Giant Mine workers. The available records of some miners were likely tested because their levels were elevated, as is the case with the three serially tested miners. Details regarding the selection of tested miners were not available.

5.5.2.2 Hair

Overall, 192 hair concentrations were obtained from archival records. Compared to the obtained hair concentrations from the YKDFN children, hair arsenic concentrations in Yellowknife miners were higher. Elevated hair arsenic concentrations were expected due to occupational exposure, particularly for mine workers who worked in the mill as they were more likely to be exposed to arsenic dust released during the roasting process. Due to the gold mining process, external contamination of arsenic in hair is possible. Despite the washing and treatment of hair samples, it can be challenging to distinguish endogenous hair concentrations and exogenous contaminations [32]. Thus, hair arsenic may not be an accurate indicator of internal arsenic exposure when external contamination may be a significant contributor, such as for occupational exposures.

5.5.3 Limitations

The presented research is of collected historical data from archival records. The obtained archival data are an incomplete collection of past human arsenic results in Yellowknife as most arsenic results from the early human health studies could not be located. Therefore, critical analysis and review of those studies could not be made. In addition to incomplete data, numerous factors make the assessment of the historical data difficult, such as a lack of information about study participants, details about the methodology used, etc. Furthermore, the collected historical data are of only two population groups. They do not reflect exposure of the general population of Yellowknife at the time nor do the available results necessarily reflect the respective cohort (i.e., YKDFN and miners) as a whole.

Another limitation is that the obtained archival urinary concentrations were of total arsenic only. Total arsenic may not accurately reflect potential toxicity as some organic arsenic

species (i.e., arsenobetaine) can be a significant contributor to total arsenic concentrations but are generally considered to be non-toxic [33].

5. 6. Conclusion

The findings of this study provide an idea of the level of historical exposure to arsenic among YKDFN children and miners. While the data may not be directly comparable to the YKHEMP data, nor does it necessarily reflect the levels of all YKDFN members at the time, results suggest that the arsenic exposure among the tested YKDFN children in the 1970s was similar to the 2017-18 results of YKDFN children. In contrast, the miners were exposed to very high concentrations of arsenic compared to the background level observed by the adult YKHEMP participants. The results of the 4 YKHEMP participants (2 YKDFN and 2 former miners) for whom we could find archival test results also support these observations.

5. 7. References

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Appendix 5.A: Results summary of past Arsenic studies conducted in Yellowknife.

Study	Year	Participants	n	Biomarker	Min	Max	Mean	Other Information	Reference
Medical survey in association with environmental survey	1951	School-aged children	230	Urine	Not reported	Not reported	Not reported	Study consisted of physical examination and measurement of urinary arsenic. **Reported no evidence of ill effects and small amounts of arsenic.**	Canadian Public Health Association(1977)
Investigation of the Health Status of Inhabitants of Yellowknife, NWT	1965-1966	Male residents of Yellowknife	Total: 361 308 Non-Mill Workers 53 Mill Workers	Urine	3 µg/L	>150 µg/L	Total Mean: 12.37 µg/L Non-Mill Workers: 11 µg/L Mill Workers: 20.3 µg/L		De Villiers & Baker (1969)
Con Mine Survey conducted by Cominco	1972	Con Mine employees	Total: 41 Employees 7 Mill Workers 4 Shift Bosses 7 Mechanical & Trade Personnel 13 Staff	Urine	Not reported	300 µg/L	Total mean: 64.73 µg/L Mill Workers: 186 µg/L Shift Bosses: 88 µg/L Mechanical & Trade: 50 µg/L Staff: 50 µg/L		Canadian Public Health Association(1977)
Medical Services Branch	1975	Yellowknife Residents (~20% mine mill workers)	Total: 700 565 YK residents 135 Mine mill workers	Hair	<1 ppm (µg/g)	>10 ppm (µg/g)	Not reported	1/3 of mill workers(n=45) had hair As >10 ppm while 3.4% of other residents(n=19) had >10 ppm 74 mill workers and 49 residents had levels >5 ppm (United Steelworkers Brief, 1977)	Brown, Krishnamoorthy and Scharfer (1970?s)
Con Mine Survey conducted by Cominco	1975	Con Mine employees	55 employees	Urine	Not reported	>100 µg/L	Not reported		Canadian Public Health Association(1977)

March-April 1976 Giant Mine Survey	1976 Mar-Apr	Giant Mine employees	16 employees	Urine	Not reported	>100 µg/L	Not reported	4 out of 16 employees had uAs >100 µg/L	Canadian Public Health Association(1977)
October-November 1976 Giant Mine Survey	1976 Oct-Nov	Giant Mine employees	24 employees	Urine	Not reported	75 µg/L	Not reported		Canadian Public Health Association(1977)
1977 Giant Mine Survey	1977	Giant Mine employees	38 employees	Urine & hair	Not reported	Urine: 51 µg/L Hair: 275 ppm	Hair: 34.4 ppm		Canadian Public Health Association(1977)
Con Mine Survey conducted by Cominco	1977	Con Mine employees	213 employees	Urine	Not reported	235 µg/L	Not reported		Canadian Public Health Association(1977)
Independent Study by the National Indian Brotherhood and United Steel Workers	1977	All mill workers at Giant Mine and Yellowknife FN children	Total: 67 20 Mill workers 47 FN children	Hair	FN children: 0.8 ppm. Mill worker: 1.8 ppm	FN Children: 28 ppm. Mill worker: 278 ppm	Total: 24.9 ppm FN children: 4.8 ppm Mill worker: 72.2 ppm (CPHA, 1977)		CPHA (1977), Jervis, R. (1977)National Indian Brotherhood, United Steel Workers of America & University of Toronto(1977)

6. Conclusion

6.1 Study Outcomes

The research chapters (Chapters **2-5**) of this thesis demonstrate the use of different biomarkers to evaluate arsenic exposure and associated health effects in the human population of Yellowknife. In **Chapters 2 and 3**, I first established a baseline of arsenic exposure in the human population of Yellowknife by analyzing arsenic in urine and toenail as two biomarkers of exposure. Then in **Chapter 4**, I assessed two proteins, KIM-1 and CC16, as candidate biomarkers of effect to detect arsenic-induced kidney and lung impairment in children. Finally, in **Chapter 5**, I reviewed historical records of past arsenic measurements in urine. I compared this data with current study results to understand arsenic exposure in Yellowknife over the past several decades. Overall, this thesis research indicates that arsenic exposure is higher in children in Yellowknife, with urinary and toenail concentrations being higher than that of adults.

In **Chapter 2**, arsenic and its different species were quantified in urine to establish a baseline level of exposure. From this chapter, urinary inorganic-related arsenic (AsIII+AsV+MMA+DMA) concentrations in this study were generally comparable to the Canadian population, except for children. Urinary inorganic-related arsenic concentrations of children, mainly those ages 6-11, were significantly higher than adults of the Yellowknife and children in the general Canadian population. Furthermore, inorganic-related arsenic concentrations in children, particularly those above the 95th percentile, were above the guidance values (i.e., biomonitoring equivalents) associated with increased risk of dermal effects, vascular problems, and cancer. [1,2] As such, children in Yellowknife should be prioritized in future follow-up assessments as biomonitoring continues.

Additionally, various risk factors associated with elevated arsenic in children and adults were identified. Drinking lake water was associated with elevated inorganic-related arsenic concentrations in adults. For adults, consumption of locally harvested mushrooms and berries was significantly positively associated with inorganic-related arsenic concentrations. At the same time, a significant negative association was observed with age, smoking and recreational water activities. These identified risk factors were used in public health communication to bring awareness to potential arsenic exposures. Overall, these results support the need for an ongoing arsenic biomonitoring program. Furthermore, the findings from this chapter have helped inform decision-making for municipal, provincial, and federal stakeholders.

In **Chapter 3**, arsenic and its different species were measured in toenails to build upon the baseline of arsenic exposure established in Chapter 2, which used urine as a biomarker of exposure. Overall, total toenail arsenic and inorganic-related arsenic concentrations were higher in children than adults, which is consistent with the urinary arsenic results from Chapter 2. Arsenic species patterns in toenails, mainly composed of AsV, differed from that of urine, in which DMA was the dominant species. For urinary arsenic, speciation is important for distinguishing between inorganic and organic forms of arsenic to assess toxicity due to the contribution of organic arsenic in dietary sources. For toenails, inorganic arsenic has a high affinity for keratin-rich tissue. Thus, toenails are primarily comprised of these species[3]. As AsIII and AsV largely represent arsenic species in the toenail, total arsenic may be sufficient as a biomarker of exposure.

Further research is needed to distinguish between arsenic sources of potential surface adsorption and internal dose to understand the utility of toenail arsenic speciation better. Furthermore, different factors were associated with elevated toenail arsenic compared to

identified risk factors associated with elevated urinary inorganic arsenic. For instance, identified risk factors in urine were overall related to general diet (e.g., market seafood and rice consumption frequency for both adults and children; consumption of local mushrooms and berries in adults). Whereas the lifestyle factors in toenails are generally related to local environmental exposures, such as outdoor exposures (e.g., fishing, and recreational water activities for children, working at Giant Mine in adults, seasonality for both) and local diet (e.g., eating local meat, mushrooms, and wild plants for adults). Toenails reflect a longer exposure period of months compared to the recent exposure of days for urine. Thus, urine is likely to be more susceptible to the consumption frequency of certain foods (e.g., rice and seafood). At the same time, toenails represent a longer period and thus may be reflective of different lifestyle behaviours related to environmental exposures. Toenails as a biomarker of exposure can therefore be a useful biomarker for understanding chronic arsenic exposure and can complement urinary arsenic, which comparatively reflects shorter exposures. The results from this chapter contribute to understanding arsenic exposure in the human population of Yellowknife and support the use of toenail arsenic in prospective monitoring.

A baseline of arsenic exposure was established using urine and toenails in chapters 2 and 3, where children were observed to have comparatively higher levels of exposure in both urine and toenail. As such, I posited that health effects may be more readily observed in child participants. In **Chapter 4**, KIM-1 and CC16 were evaluated as two candidate biomarkers of effect for arsenic and other contaminants in children [4,5]. KIM-1 and CC16 in urine samples of study participants under age 11 were quantified via ELISA. Urinary KIM-1 concentrations were significantly associated with urinary inorganic arsenic (AsIII+AsV) concentrations.

Meanwhile, urinary CC16 concentrations were positively significantly associated with inorganic arsenic (iAs), as well as lead(Pb) and manganese (Mn)concentrations in urine. This finding differed from existing research in which chronic exposures were linked to overall lower circulating levels of CC16. I posit that the inverse relationship between CC16 and contaminants in chronic exposures may not be readily observed in young children. More research is needed on CC16 and chronic contaminant exposures of various levels in children to better understand the relationship. The results of this chapter suggest that KIM-1 may be a candidate biomarker for detecting early iAs effects on kidney function in children. Meanwhile, urinary CC16 levels could be a potential non-specific biomarker for the effect of metals (As, Pb and Mn) on lung functions in children.

The presented research in this thesis is from the first human health arsenic study conducted in Yellowknife since the late 1970s, filling a large knowledge gap regarding human arsenic exposure in Yellowknife [6]. In **Chapter 5**, past archival hair and urine arsenic data was reviewed and compared to current baseline concentrations, determined in chapter 2, to better understand past arsenic exposures. A literary search was conducted in various libraries and archives for relevant data. Archival arsenic concentrations were found for two groups, Yellowknives Dene First Nations (YKDFN) children and Yellowknife miners. Though numerous health studies were conducted in the 1950-1970s, limited records were available, consisting of one study in the 1970s, an unpublished government follow-up, and occupational monitoring data. Available historical data suggest that arsenic levels among YKDFN children in the 1970s were similar to current levels in YKDFN children.

Meanwhile, historical concentrations in Yellowknife miners were higher compared to the current background levels observed in adult YKHEMP participants and current mine remediation

workers. Due to the limited available data, the findings of this chapter are not necessarily reflective of past exposures of the Yellowknife population. Thus, comparisons to baseline data are complex. Still, the review of archival documents does provide insight into the level of past exposures of two cohorts in Yellowknife, as well as a historical perspective of past attempts at arsenic biomonitoring.

This thesis is part of the Health Effects Monitoring Program (YKHEMP), an ongoing longitudinal biomonitoring study aimed at assessing arsenic and other contaminants in the human population to ensure that exposure does not impact the health of the people living in Yellowknife, Dettah and Ndilo. Throughout the project, the core team has consisted of Dr. Laurie Chan, the principal investigator, as well as a project manager, local project coordinators, graduate students, and post-doctorate fellows. Additionally, a team of research assistants and nurses from Yellowknife were responsible for baseline data collection. Furthermore, YKHEMP is a study under the Giant Mine Remediation Project. The project was subjected to 26 measures upon approval, one of which was implementing a broad health effects biomonitoring program (i.e., YKEMP) of combined cohort and longitudinal design. In addition to YKHEMP, other studies and initiatives have been established under the Giant Mine Remediation Project. For instance, a Human Health and Ecological Risk Assessment (HHERA) was conducted in which environmental exposures (e.g., country foods, soil, sediment, and water) in Yellowknife were measured to assess health risk [7]. Overall, arsenic concentrations in environmental exposures were similar to background concentrations and posed a low risk to the human population except for certain mushroom species found within 10 km of Giant Mine. This assessment was conducted separately from YKHEMP but informs project results. As such, the research

presented in this thesis is one component of a larger environmental project to mitigate arsenic exposure in Yellowknife.

6.2. Community Engagement and Communication

Community engagement has been a vital part of the Health Effects Monitoring Program from the beginning. A biomonitoring program of this scale and matter requires constant communication and transparency. It is important to project that we work closely with the community and explain clearly the choices being made. In turn, feedback from the community allows the project to gain community perspective, expertise and advice in the development, implementation, and communication of the Health Effects Monitoring Program. The project is also conducted under the guidance of an advisory committee (HEMPAC) of local, provincial, and federal stakeholders. The committee meets regularly and has provided advice and expertise, which has informed each component of the study. My contribution to community engagement includes meeting with community members during the planning phase, canvassing and attending monthly advisory committee meetings. I also provided technical support and prepared various briefing materials, such as presentations, plain language summaries, and reports (communication materials of the study are available at: https://ykhemp.ca/resources/study-documents).

During the planning stages of the study, both the local community and advisory committee were informed of the proposed project plan through a series of meetings and community outreach in Yellowknife. Local input informed method development and study design. This included recruitment strategies for the different population groups, a lifestyle questionnaire that is reflective of the local area, the addition of food frequency and medical history questionnaires for the Yellowknife Dene First Nations, etc. Specific to this thesis, the research presented in Chapter 5 was added after residents expressed their interest in

understanding their past exposures. Each research chapter of this thesis has been reviewed by the advisory committee and communicated to the Yellowknife community in short plain language and visual summaries.

Though this research was conducted by the University of Ottawa, the ownership of these results ultimately belongs to the people of Yellowknife, and the Yellowknife Dene First Nations in Dettah and Ndilo. Thus, consistent community engagement is vital to ensure that the operates in an open, inclusive, and transparent manner.

6.3 Recommendations and Future Directions

As this thesis is part of an ongoing biomonitoring study in Yellowknife, the presented results help inform future directions of the long-term program and follow-up assessments. [8]

As noted in **Chapter 2**, the results of this thesis indicate that children should be prioritized in the follow-up phases of the project. This supports the planned follow-ups in the biomonitoring project every 5 years for children and 10 years for adults. The first follow-up of child participants is scheduled for next year in 2023. The baseline results of urinary and toenail arsenic from Chapters 1 and 2 have suggested that exposures are not mainly from drinking water. The local public authorities do not advise drinking from lake water. Additionally, arsenic in municipal drinking water, which is the primary drinking water source of the population, is below Canadian drinking water guideline levels [9]. While drinking lake waters was observed to be a risk factor for urinary arsenic in children and toenail arsenic in adults, this is a relatively small subset of the population and not the primary drinking water source for the overall population. Arsenic exposure is, therefore, likely from other exposures such as food, soil dust, or other water-related exposures (e.g., swimming, fishing). We also posit that children may be exposed to dust or soil by playing on the ground or ingestion via hand-to-mouth contact [10]. A significant

limitation of this research is that we did not measure environmental exposures (e.g., drinking water, soil, dust, food, etc.). A separate risk assessment was conducted in Yellowknife in which environmental exposure factors and local food sources (e.g., plants, fish, soil, dust, and water) were tested for arsenic in relation to distance from Giant Mine. As biomonitoring progresses, environmental exposures should be integrated with biomonitoring data to confirm the sources of elevated arsenic exposures in the human population. Household exposures (e.g., indoor dust, outdoor soil and tap water) of participants should be measured in the upcoming follow-up assessments. Further, lifestyle factors and behaviours in children (e.g., playtime activities and recreational activities) should be investigated to understand their exposures better.

In Chapter 2, urinary arsenic levels in Yellowknife were compared to results from the national biomonitoring data to understand the relative exposure levels of the Yellowknife population. Furthermore, urinary guidance values (e.g., biomonitoring equivalents and reference values) were used to assess the health risk of the population [1,11]. In contrast, there are currently no national biomonitoring data and guidance values for toenails. This lack of comparative data makes it difficult to interpret results and assess health risks in biomonitoring, as noted in **Chapter 3**. Thus, we recommend the development of guidance values for arsenic in toenails as well as the inclusion of toenail analysis in biomonitoring programs. The results from chapter 3 can inform federal stakeholders (i.e., Health Canada) in the development of these guidelines and the use of toenail biomarkers in future national research,

In **Chapter 3**, speciation analysis was used to assess different arsenic species in the toenail. Though toenail arsenic is a frequently used biomarker, there is limited literature regarding arsenic speciation in the toenail. Further research and method standardization is needed to address uncertainties in the quantification of arsenic species to optimize the use of

toenail speciation in population studies and biomonitoring. One limitation in using toenail arsenic is the difficulty in distinguishing between sources of arsenic in toenails (e.g., internal via ingestion and exogenous via adsorption) despite cleaning during sample preparation. Novel analytical techniques can further the understanding of arsenic in toenails. Outside the scope of this thesis, we are measuring arsenic in different layers of the toenail via laser ablation. From this research, we aim to distinguish the contribution of internal sources of arsenic from dietary sources and adsorption from exogenous sources. This future work will further the understanding of the distribution of arsenic in toenails and the utility of speciated arsenic in toenails as a biomarker.

In **Chapter 4**, KIM-1 and CC16 were evaluated as two candidate biomarkers of effect. As biomonitoring in Yellowknife continues, the two proteins should be measured in urine again in participants in the upcoming follow-up. KIM-1 was shown to be a potential specific biomarker for arsenic-induced kidney impairment. Meanwhile, we observed an increasing relationship between CC16 and contaminant concentrations, which differs from the inverse relationships expected in chronic exposures [12,13]. Thus, a follow-up assessment will allow for a better evaluation of the longitudinal relationship between CC16 levels and chronic contaminant exposures.

In this thesis, only two candidate biomarkers of effect were assessed in **Chapter 4**. Arsenic is associated with a wide range of mechanisms thus numerous proteins and molecules could be candidate biomarkers. The next step is to explore candidate biomarkers through biomarker discovery further using high-throughput omics techniques such as metabolomics and exosomics. Metabolomics allows for the quantitative analysis and identification of a mass range of metabolites in a biological sample, such as blood and urine. It is a sensitive analytical tool that

can evaluate the effects of arsenic exposure and its disruption on metabolic processes, resulting in changed excretion of distinct metabolites in urine [14,15]. Urine and other biological fluids are also rich in small extracellular vesicles, known as exosomes, which get released by cells as a mode of intercellular communication[16]. These exosomes are highly stable in urine and indicate the biological state of their parent cells[17]. In recent years, the study of exosome proteins has shown promising applications for identifying novel biomarkers of disease and chemical exposure[18,19]. We plan to identify potential biomarkers using metabolomic and exosomic approaches. We posit that increased exposure to arsenic interferes with many cellular processes in a person's body resulting in changes in metabolites and exosome proteins excreted in the urine. The objective is to identify potential arsenic biomarkers of effect by assessing observed differences in metabolic and urinary exosome protein profiles between different arsenic exposure groups.

Biomarkers of susceptibility were not assessed in this thesis due to scope. For arsenic, these biomarkers are often single nucleotide polymorphisms (SNPs) associated with arsenic metabolism, as well as cellular repair and arsenic transport [20–23]. Biomarkers of susceptibility reflect inter-variabilities of biological responses to arsenic; thus, they can be a modifying factor for biomarkers of exposure and effect. The next step would be to analyze SNPs to assess genetic determinants of biological arsenic response via genomic analysis. Collected saliva samples will be analyzed for known SNPs to determine their effect on metabolic methylation efficiency, arsenic concentrations in urine and toenail samples, and other related outcomes. The results of this future work will complement current thesis findings, addressing the inter-variabilities between population groups that modify arsenic body burden and biological responses.

6.4. Research Contributions

The research of this thesis contributes to the knowledge of arsenic and associated biomarkers, providing value to both the scientific and local Yellowknife communities.

6.4.1. Scientific Value

Biomonitoring, through the use of biomarkers, is a necessary tool in environmental health. Arsenic exposure and its subsequent health impact can be complex. In my thesis, I used a suite of biomarkers to assess arsenic in the population of Yellowknife, demonstrating how various biomarkers can address different aspects of arsenic exposure and aid in addressing this complexity.

As arsenic is a major public health concern worldwide, there is a need for continued research and biomonitoring to better understand global exposure and subsequent health impacts on populations. Existing research generally focuses on exposure via drinking water, as it is the predominant source of elevated exposure globally, thus shaping much of the current knowledge on arsenic and subsequent health outcomes. There is comparatively less understanding regarding other environmental exposures such as dust, soil, etc. The results of this thesis add to the current knowledge of arsenic biomonitoring due to the characteristics of the study population and area. Yellowknife is a unique study population in Canada as it is in proximity to a large legacy contaminated site. The results of this thesis suggest that human exposure in Yellowknife is likely to be from multiple sources of exposure, with drinking water as a less significant exposure source. Despite observed environmental concentrations linked to historical mine emissions, urinary arsenic concentrations in the Yellowknife population are similar to the general Canadian population though concentrations in children are significantly higher. In Canada, arsenic concentrations in the environment are generally low, except for certain regions like Quebec or

Nova Scotia, where exposure via well water is a concern. Arsenic concentrations from our results are within range of these populations [24–27]. In comparison, toenail and urinary arsenic concentrations in our child cohort are lower than in children living near a legacy contaminated smelting site in Arizona, where exposure via tap water and soil is a concern [28]. In comparison, both urinary and toenail concentrations of the Yellowknife population are well below levels observed in areas with known elevated arsenic exposures, such as Bangladesh, where groundwater contamination is a major public health concern [29–32]. Arsenic is a global contaminant by which populations may be exposed in varying degrees and via different sources. As such, the research of this thesis contributes to the overall understanding of arsenic exposure by demonstrating biomarker utility in a population residing near a legacy contaminated site with multiple exposure sources that are not mainly drinking water. The results of this research can aid public health agencies and future biomonitoring studies in assessing populations with similar exposures.

Complex issues often require multi-faceted approaches. The use of only one biomarker cannot adequately assess arsenic exposure. The results of chapters 2 and 3 demonstrated how urine and toenail could be used together as biomarkers of exposure for evaluating chronic arsenic exposure in a population. Both biomarkers reflect different periods of exposure, thus, the use of both allows for a more comprehensive understanding. Additional biomarkers, such as biomarkers of effect assessed in chapter 4, also add to the knowledge of the risk of arsenic exposure and its health impacts. The more that is understood about how arsenic impacts surrounding environments and populations, the better-equipped researchers and public health authorities are to mitigate risk and ensure the health of a population.

6.4.2. Social Value

This research provides a better understanding of arsenic exposure in the human population of Yellowknife. Our research has established a baseline of arsenic exposure in the Yellowknife population. The results have informed Yellowknife residents about their exposure levels and how these levels compare to the rest of Canada. We have also determined contributing factors associated with elevated exposures, which inform residents of their potential risks. Additionally, this research has informed decision-making and policy for local and federal stakeholders.

As one of the most contaminated sites in Canada, Giant Mine and its toxic legacy has a long-withstanding history in Yellowknife resulting in public health concerns for decades, persisting throughout the mine's operation and long after its closure. However, there has been no understanding of human exposure levels in the general population since the 1970s. Our research is the first health study on arsenic in over 50 years, as well as the largest in scope and size in the area, filling a knowledge gap in this region. This research provides insight into human arsenic exposure, contributing to the bigger picture of arsenic in the Yellowknife area.

6.4. Closing Statement

Arsenic is a small chemical with a giant global impact. It is important to understand how we can monitor its exposure and impact to ensure healthy populations. This thesis demonstrates the utility of human biomarkers to evaluate arsenic exposures and its associated health outcomes in the human population of Yellowknife and the First Nations communities of Dettah and Ndilo, in the Northwest Territories. The results of this research indicate that children have higher exposure levels of inorganic arsenic compared to study adults and other children in the general Canadian population. In the past, Yellowknives Dene First Nations (YKDFN) children were

monitored for arsenic in hair and urine, and past available urinary levels are comparable to current baseline concentrations in YKDFN children.

Furthermore, there is increased excretion of potential biomarkers of effect indicative of lung and kidney impairment. As such, children should be prioritized in future monitoring. The results of this research inform local and federal stakeholders in policy and decision-making.

In addition to the scientific contribution of this thesis, the research has significance for the people of Yellowknife, Dettah, and Ndilo. The toxic legacy of Giant Mine has left not only lasting impacts on the surrounding environment but has also been a concern for the local communities, particularly the Yellowknife Dene First Nations, impacting their way of life. We hope this research can be part of a new legacy of remediation and reconciliation.

6.5. References

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