# CHARACTERIZING ARSENIC SPECIATION AND METALLOMIC PROFILES IN TOENAIL SAMPLES OF ATLANTIC CANADIANS: A MATCHED CASE-CONTROL STUDY OF BREAST, CERVICAL, PROSTATE, URINARY, AND SKIN CANCERS

by

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## ABSTRACT

Chronic exposure to arsenic and metals has been associated with cancer risk. The research objectives were to 1) characterize the profiles of arsenic speciation and metallomes in the toenails of several cancer groups and healthy participants; 2) determine whether there is an association between the profiles of arsenic speciation and metallomes and cancers; while 3) adjusting for potential confounders. Total concentrations of metals and the levels of arsenic species were measured using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and High Performance Liquid Chromatography (HPLC). The levels and profiles of arsenic speciation and metallomes were compared between cases and controls using MANOVA in adjusted and unadjusted analyses. The profiles of arsenic speciation and metallomes were significantly different between cancer cases and controls. Arsenic methylation capacity may be inversely associated with cancer risk. Toenail arsenic speciation and metallomes (e.g., zinc levels) may be an important biomarker for cancer risk. Further research is needed to use toenails as a prognostic measure of trace metal-induced cancer.

## LIST OF ABBREVIATIONS USED

| | |
|---|---|
| As | Arsenic |
| AsB | Arsenobetaine |
| AsC | Arsenocholine |
| Atlantic PATH | Atlantic Partnership for Tomorrow's Health |
| BMI | Body mass index |
| Ca | Calcium |
| CAE | Cumulative arsenic exposure |
| CanPATH | Canadian Partnership for Tomorrow's Health |
| Cd | Cadmium |
| Co | Cobalt |
| CI | Confidence Interval |
| Cr | Chromium |
| CRM | Certified Reference Materials |
| Cu | Copper |
| DMA | Dimethylarsinic acid |
| DNA | Deoxyribonucleic acid |
| Fe | Iron |
| Ga | Gallium |
| $H_2O_2$ | Hydrogen peroxide |
| HERC | Health and Environments Research Centre |
| $HNO_3$ | Nitric acid |
| HPLC | High Performance Liquid Chromatography |
| HPLC-ICP-MS | HPLC coupled with ICP-MS |
| HR | Hazards Ratio |
| iAs | Inorganic arsenic |
| ICP-MS | Inductively Coupled Plasma-Mass Spectrometer |
| IPAQ | International Physical Activity Questionnaire |
| K | Potassium |
| KED | Kinetic energy discrimination |
| Li | Lithium |
| MANCOVA | Multivariate analysis of covariance |
| MANOVA | Multivariate analysis of variance |
| MDL | Method detection limit |
| MMA | Monomethylarsonic acid |
| Mg | Magnesium |
| Mn | Manganese |
| Na | Sodium |
| Ni | Nickel |
| PMI | Primary methylation index |
| Pb | Lead |
| *r* | Pearson's correlation coefficient |
| Rb | Rubidium |
| RD | Risk Difference |

| | |
|---|---|
| ROS | Reactive oxygen species |
| RR | Risk Ratio |
| SAM | *S* – adenosymlethionine |
| Sb | Antimony |
| Sc | Scandium |
| Se | Selenium |
| SMI | Secondary methylation index |
| Sr | Strontium |
| Th | Thorium |
| Tl | Thallium |
| U | Uranium |
| V | Vanadium |
| WHO | World Health Organization |
| Zn | Zinc |

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# CHAPTER 1 INTRODUCTION

The overarching goal of this thesis was to characterize arsenic speciation, as measured by toenail clippings, in a population of Atlantic Canadian adults, both with and without a history of cancer, and to determine whether the two are associated. As such, this work is highly interdisciplinary, and includes the fields of analytical chemistry, toxicology, and epidemiology. This chapter serves to:

i. Explain relevant terms and mechanisms related to arsenic (As) toxicity and metabolism, metal exposure and toxicity, in addition to the contextual aspects of As and cancer in Atlantic Canada,
ii. Review the literature of other scientific and epidemiologic studies pertaining to As exposure/speciation, metal exposure, and cancer outcomes, and
iii. Describe the rationale for and the objectives of this research.

## 1.1 Arsenic

Arsenic, an infamous chemical element, has been both a toxin and a medicine throughout history. Once known as both the "king of poisons" and "poison of kings," arsenic was an effective lethal agent due to its potency and discreetness, the latter referring to its odor- and taste-less characteristics (1). Its notoriety as a murder agent spurred important advances in As toxicology. Perhaps even before its use as a poison, however, As was used medicinally, dating back to 2000 BCE, and its use was especially prominent in the 18$^{th}$ century (2). Although early treatments are no longer used in practice, the use of As led to a better understanding of its effects on the human body, and the use of As trioxide as an effective chemotherapeutic drug for acute promyelocytic leukemia (3–5). Recall Paracel-

sus, the 'Father of Modern Toxicology', who became famous for the idea that the dose makes the poison (6). Certainly, the implications of As exposure are related to its form and dosage. Nevertheless, As consumption can be lethal in both acute poisonings and long-term exposure (7). To date, the mechanisms of As toxicity have yet to be fully elucidated, and a so-called 'safe-limit' for ingestion has not yet been determined (2,8).

### 1.1.1 Environment

Arsenic is a metalloid[1] ubiquitously found in the earth's crust (10), and is commonly associated with the ores of metals, including copper (Cu) and lead (Pb) (11), which contribute to its presence in local ecosystems (12). Despite its natural, geogenic existence, the presence of arsenic is also due to anthropogenic sources, including metal mining and smelting, pesticide application, coal and wood combustion, and waste incineration (13). Many forms can dissolve in water (14), and it is one of the most common water contaminants due to its mobility capabilities over many redox conditions (15). Arsenic naturally exists in four oxidative states: $As^{-3}$, $As^{0}$, $As^{+3}$, $As^{+5}$ (11). In water, As is commonly found in its inorganic form (iAs), and is stable as both arsenite and arsenate, which respectively, represent the trivalent and pentavalent[2] forms (12). Due to the threat of As to public health, the World Health Organization (WHO) recommends the concentration of As in drinking water not exceed the limit of 10 $\mu$g/L (16).

[1] An intermediate chemical element, with characteristics from both typical metals and non-metals (9).
[2] Referring to the valency, or the affinity the atom. Valency is determined by the number of hydrogen atoms an element could combine with. E.g., for trivalent species, this number would be three.

### 1.1.2 Exposure

The WHO concedes that its recommended limit for As in drinking water (10 $\mu$g/L) is provisional and based on the difficulties of extracting As from water. If possible, they recommend concentrations should be kept below the limit, while acknowledging that some regions are exposed to levels far exceeding the guideline (16). For example, some of the highest As levels globally are found in the South East Asia Region countries, and report As concentrations beyond 1,000 μg/L (17).

Echoing the WHO, Health Canada's guideline for As in drinking water is set at a maximum acceptable concentration of 10 μg/L. Canada, too, provides this recommended limit accompanied with the acknowledgement that it is based on the feasibility of treatment at the municipal- and residential-level, and that it is subject to change as new developments and technologies become available (18). However, in many provinces, including those in the Atlantic region, the concentration of As in drinking water frequently exceeds the recommended limit (18). Private well users may be at an increased risk of As exposure via drinking water because water quality is not systematically monitored and regulated; in fact, this responsibility falls upon the well owner with no government oversight. Many Atlantic Canadians, including 42% of Nova Scotians and 60% of New Brunswickers, rely on private wells for domestic water supply (18). Private wells in these areas have reported As levels that exceed the 10 μg/L guideline, including 20% in Nova Scotia (19) and 6% in New Brunswick (18). On Prince Edward Island, groundwater As concentrations range from 0.1–26 μg/L, but 99% of wells sampled had concentrations below 10 μg/L (18). Clearly, As levels for many residents of this region exceed this guideline, which could lead to the development of various adverse health consequences.

It is widely accepted that the consumption of contaminated drinking water is one of the greatest sources of As exposure (16). Other sources of exposure include tobacco, foods (rice, grains, meats, seafood), soil, and to a lesser extent, air (16). In the case of seafood, the As is commonly found in its organic form, which was once thought to be less toxic[3], but is now considered to have toxicity levels comparable to iAs (21–23). In fact, the organic form of As is the form most commonly found in most foods (24). However, there are foods that do contain some iAs, including, meat, poultry, dairy products, and cereals (24). The source of iAs in plants is the uptake of contaminated water and soil (25). Animals then consume these plants, in addition to contaminated drinking water. This phenomenon of iAs uptake has resulted in As being dubbed a food chain contaminant (25).

Another potential exposure pathway is the inhalation of airborne As. Some common sources of airborne As are tobacco smoke and occupational exposures, where the latter refers to As in aerosol or dust generated from mining, mineral processing, fossil fuels containing As, and other extraction industries (26,27). The amount of As absorbed in the body from inhalation depends on the size and solubility of the particles, but is thought to be between 60–90% (26,27). However, inhalation is not considered a primary source of exposure for the general population (26). Similarly, dermal contact with As-containing products is not considered a major source of exposure (24), and very little is known about its toxicokinetic properties (27). This thesis assumes that the majority of iAs exposure occurs via drinking water, which is supported by the literature (2,16,28).

[3] Arsenobetaine (AsB) and arsenocholine (AsC) are organic arsenic compounds that are commonly found in ocean and estuarine fish and shellfish. Toxicokinetic data showed these As species were not as bioavailable for interaction with biological molecules (20).

### 1.1.3 Absorption, Distribution, Metabolism, and Excretion

#### *1.1.3.1 Absorption & Distribution*

Arsenic is largely distributed throughout the body in the blood (24). Approximately 95% of ingested As is readily absorbed into the gastrointestinal tract before entering the blood (29). Up to 70% of absorbed As is rapidly excreted via urine (29), but the remaining amount is stored in keratin-rich tissues, such as hair, nails, skin, and, to a lesser extent, bones and teeth (30). The absorption of As by a cell depends on physiochemical conditions, including the form of As and pH, as well as the types of proteins present in a given cell or tissue (31). However, before As is excreted or stored, it undergoes a series of biotransformations, a process known as As methylation.

#### *1.1.3.2 Metabolism*

Arsenic metabolism in humans, among other mammals, occurs via successive methylation (32). This process is thought to occur primarily in the liver, especially in cases where ingestion was the route of exposure as As initially passes through the liver. Although, in addition to the liver, As methylation has also been observed in the testes, kidney, and lungs of mice (33). Current hypotheses suggest iAs is methylated via an alternating reduction from pentavalent to trivalent As, with the addition of a methyl group, and results in the metabolites, monomethylarsonic acid (MMA), and subsequently, dimethylarsinic acid (DMA) (32). It is thought that iAs is bonded to a dithiol, with *S*-adenosylmethionine (SAM) serving as the primary methyl donor (32). It is important to note that this biotransformation process has not been fully elucidated, and various pathways have been proposed (34). A simplified pathway of the As methylation process is depicted in Figure 1.1.1.

This process was once considered a detoxification pathway as the biotransformation of iAs ($As^{III}$ + $As^{V}$) to pentavalent MMA and DMA results in metabolites that are less reactive with tissue and more readily excreted in urine. However, an incomplete methylation process may produce residual trivalent MMA and/or DMA, which are more reactive and thus considered more toxic (32). In fact, an *in vitro* study demonstrated that $MMA^{III}$ is the most toxic intermediate produced from As metabolism (35).

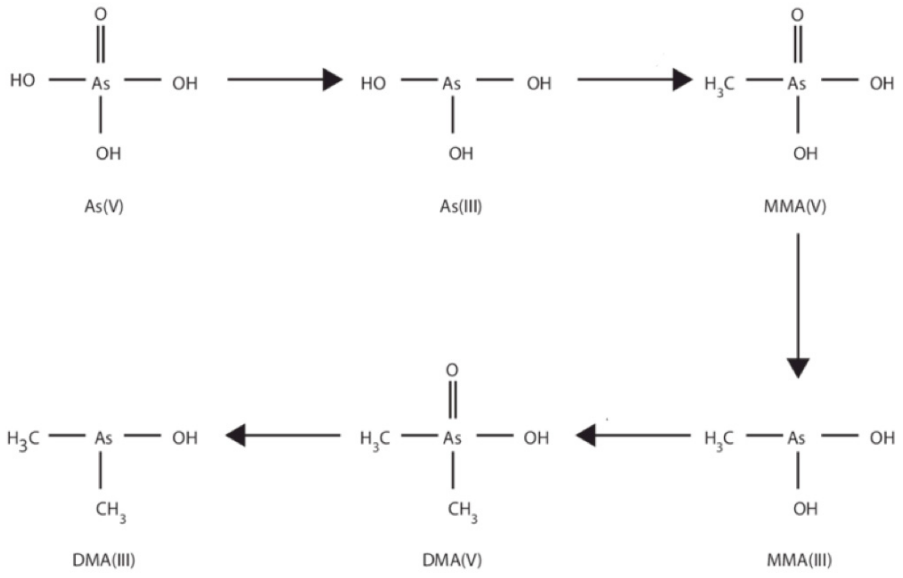


**Figure 1.1.1** Simplified pathway of arsenic methylation
Notes: Modified from Dieter H, Schuhmacher-Wolz, and colleagues (36)

The ability to methylate As is highly varied. In addition to a range of capability displayed across mammalian species, human methylation capacity is varied at both population- and individual-levels (37). The methylation of iAs is thought to be influenced by the form of As one is exposed to, route of exposure, dose, and diet (37,38). Research by Concha and Volger suggest age and pregnancy may play a key role as well (39). While

further research is warranted to better understand the variations, from both molecular and population health perspectives, As methylation capacity is widely considered to be important in understanding As-related toxicity and carcinogenesis.

The extent of methylation capacity is frequently quantified using two measures in toxicological and epidemiological studies: the primary methylation index (PMI) and secondary methylation index (SMI). The former is the ratio of MMA to iAs $\left(\frac{\%MMA}{\%iAs}\right)$ and the latter is the ratio of DMA to MMA $\left(\frac{\%DMA}{\%MMA}\right)$ as measured in the given biomarker.

### 1.1.4 Arsenic Toxicity

Arsenic toxicity can be acute or chronic. In humans, a lethal dose of iAs lies in the range of 1–3 mg/kg of total body weight (40). Symptoms of severe As toxicity include gastrointestinal discomfort, vomiting, diarrhea, bloody urine, anuria, shock, convulsions, coma, and death (41). On the other hand, several potential cellular mechanisms have been proposed to explain how chronic As exposure could cause cancer. These include chromosomal abnormalities, deoxyribonucleic acid (DNA) damage, modified cell proliferation, and oxidative stress (42,43). The metabolites $MMA^{III}$ and $DMA^{III}$ can inhibit certain enzymes, which in turn, can induce cell proliferation and cause DNA damage, and have been linked to reactive oxygen species (ROS) generation (42). Arsenic methylation has also been associated with genotoxic effects. The mechanisms underlying As toxicity based on recent research are described in more detail in the literature review (refer to section 1.4.4 *Mechanisms of arsenic carcinogenicity*).

## 1.2 Metallomics

Trace metals are often categorized as either essential or toxic. For example, elements such as As and Pb, are considered toxic even at low-doses. Conversely, other elements, including magnesium (Mg), iron (Fe), Cu, zinc (Zn), and selenium (Se), among others, are consider important for basic biological processes (44). However, dose-response curves indicate that, even for essential elements there may be a threshold level to maintain physiological benefits, as depicted in Figure 1.2.1 (44). The excess or deficiency of trace metal composition may play a role in cancer development (45,46). There is a need to better understand the inter-relationship between trace metal concentrations and cancer development. This is required for the development of exposure guidelines, as well as a framework for future monitoring and prognosis criteria of cancer and other chronic diseases.

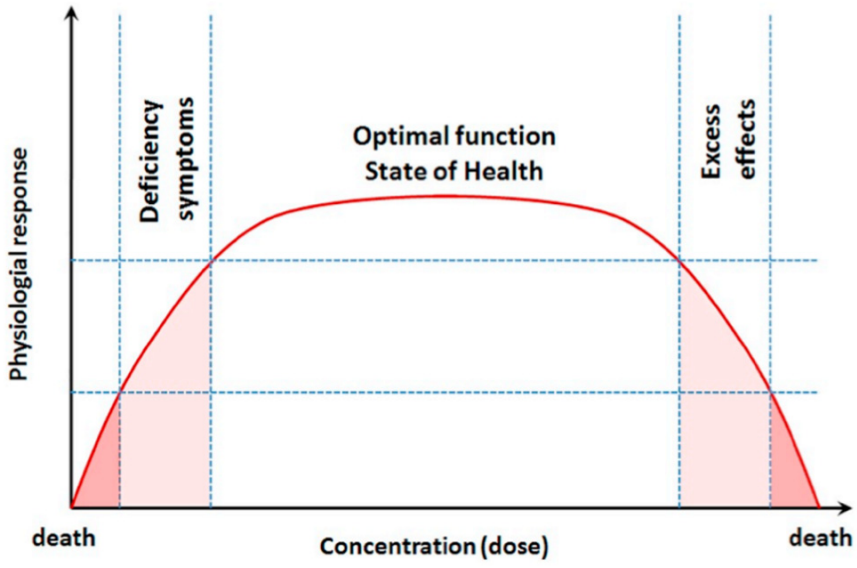


**Figure 1.2.1:** Dose-response visualization for essential elements.
Notes: Source, Zoroddu Aaseth and colleagues (44)

The emerging field of *metallomics* is concerned with research surrounding the metal-dependent biological processes (47). This field is closely related with genomics and proteomics, as metallomes are required to enable the synthesis and metabolic function of genes and proteins (48). The term 'metallome' refers to the entirety of a metal or metalloid species that is present in a cell or tissue, as well as its type, quantity, and localization (47). A study investigating the association between cancer risk and both essential and toxic metals concluded that As, Se, iodine, Zn, sodium (Na), and vanadium (V) were important in determining cancer risk (49). This thesis characterized metallomic profiles in addition to the As speciation profiles to determine how they are associated with cancer.

## 1.3 Cancer

In Canada, approximately 50% of the population will develop cancer, and one quarter will die from the disease. In fact, cancer is currently the leading cause of death in Canada (50). Aging is one of the primary risk factors for cancer. Statistics Canada (2017) found that the incidence rates of cancer increased with each decade of life[4] (Figure 1.3.1) and that the likelihood of being diagnosed and of dying from cancer increase with age (Figure 1.3.1 and 1.3.2).

[4] With the exception of incidence rates over the age of 90-years

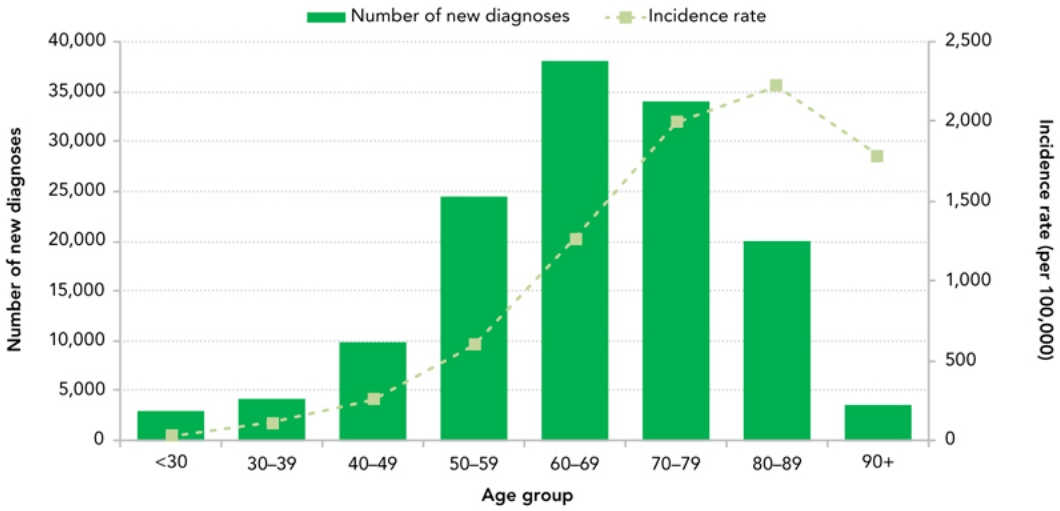


**Figure 1.3.1:** Number of new cancer diagnoses and cancer incidence rates, by age group, Canada (excluding Quebec), 2014

**Notes:** Data Source: The Canadian Cancer Registry database at Statistics Canada. Graphic provided by Public Health Agency of Canada (50).

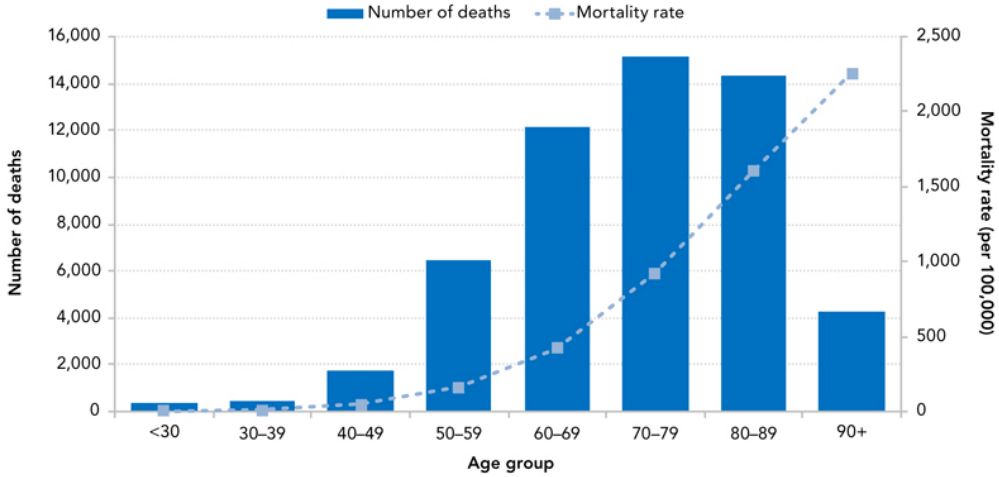


**Figure 1.3.2:** Number of cancer deaths and cancer mortality rates, by age group, Canada (excluding Quebec), 2013

**Notes:** Data from Canadian Vital Statistics – Death Database at Statistics Canada. Data from Quebec are excluded for consistency with incidence. Graphic provided by Public Health Agency of Canada (50).

Incidence rates of cancer differ when stratified by province. In particular, the rates in the Atlantic provinces are higher compared the national average (see Table 1.3.1). Indeed, this region is older than the rest of Canada, but age-standardized incidence rates show that higher rates persist even when age is considered (50). Finally, a recent projection study estimated that cancer incidence rates will continue increase in Canada in 2020, largely due to an aging population. Thus, it is imperative to conduct more research in treatment, as well as screening and prevention to mitigate the burden of disease (51).

**Table 1.3.1:** Number and incidence rate of new cases of primary cancer by region in Canada (2017)

| Region | Cancer incidence rate, per 100,000 | Number of new cancer cases |
|---|---|---|
| Canada (excluding Quebec) | 529.1 | 149,435 |
| Newfoundland and Labrador | 670.0 | 3,540 |
| New Brunswick | 631.9 | 4,845 |
| Nova Scotia | 631.8 | 6,005 |
| Prince Edward Island | 614.7 | 925 |

**Notes:** Data provided by Statistics Canada (52)

## 1.4 Literature Review

The link between As exposure and cancer has been explored in depth, and the consensus is that As is an established risk factor for cancer (53). Recently, there has been a shift in focus to better understand the mechanism of effect, including how the metabolism of As (also frequently referred to as As methylation) and its resulting metabolites, are associated with cancer.

This section provides a brief review of the scientific literature concerning iAs exposure, As methylation, and trace metal exposure as they relate to cancer, as well as their potential mechanisms of effect, and the concept of inter-individual variability.

### 1.4.1 Total arsenic exposure and cancer

There is consensus that As exposure can cause several types of human cancer, including liver, lung, urinary bladder, skin, kidney, breast, and prostate cancers (31,54). The majority of the research pertaining to As exposure and cancers has been conducted in regions with very high levels of As, including Taiwan, Argentina, Chile, however, there are also studies in European and North American regions (55). Additionally, the existing research in this field has been predominantly focused on certain cancers, including skin, lung, and bladder cancers, with less attention to breast, cervical, and prostate cancers, and most are limited by small sample sizes.

#### *1.4.1.1 Bladder and kidney cancers*

There is a large body of evidence supporting causal associations between As exposure and bladder cancer incidence and mortality. Dose-response relationships have been observed, although the effects at lower As exposure levels is still unclear (56). There is much less available research concerning kidney cancers.

A case-control study in Finland examined the risk of bladder and kidney cancers among those exposed to As in well water, although total As concentrations in this region were considered low (median As-water concentration = 0.1 $\mu$g/L) (57). Neither As-well water concentrations, daily dose, nor cumulative dose displayed statistically significant associations with kidney cancers. However, the risk of bladder cancer increased with daily dose of As in drinking water: for those exposed to 0.1–0.5 $\mu$g/L and $\geq$ 0.5 $\mu$g/L, the relative risk ratios (RR) were 1.53 (95% confidence interval (CI): 0.75–3.09) and 2.44 (95% CI: 1.11–5.37), respectively, compared to the reference group (<0.1 $\mu$g/L) (57).

A more recent Chilean case-control study assessed bladder cancer risk following a 13-year period of extremely high As exposure in drinking water[5] (58). This study found a dose-response, where the odds of cancer increased with As exposure ($\mu$g/L). An interesting finding of this study was the observed latency period: among those in the high exposure group during the aforementioned 13-year period, the odds ratios (OR) were four to seven times higher compared to those in the low-exposure group. The authors found that an average of 38-years passed from the time of high exposure to their diagnosis, a unique finding that needs further inquiry (58). Nonetheless, these results point to potentially lasting effects of As exposure, which may be explained by epigenetic effects, including select genomic hypo- and hyper-methylation (these mechanisms are explained in greater detail in section 1.4.4).

Evidence for the associations between As exposure and bladder and kidney cancers is less conclusive at low-exposure levels. A cohort study in Denmark examined the effects of low-level As exposure in water (0.05–25.3 $\mu$g/L; mean 1.2 $\mu$g/L) and found no significant associations (59). However, the authors state that only a small proportion of the participants were exposed to As concentrations exceeding 2 $\mu$g/L.

Another study investigated the risk of bladder and kidney cancers from As in drinking water in an ecological study in Nova Scotia, Canada (60). Arsenic concentrations in this region were much lower than those in Taiwan and Bangladesh, but were higher than those in Denmark. This study sought to assess whether there were elevated risk of bladder and kidney cancers even at low levels of exposure in water, categorized as <2 $\mu$g/L; 2–

[5] In the 1950s, river water from the Andes Mountains containing very high levels of naturally occurring As was diverted to the largest city for drinking water. The population of Antofagasta was subsequently exposed to As levels up to 860 $\mu$g/L a day for approximately 13 years, before a treatment plant was installed to lower levels to 10 $\mu$g/L

5 $\mu$g/L; and ≥5 $\mu$g/L. The authors found the risk of developing bladder cancer for the 2–5 $\mu$g/L and ≥5 $\mu$g/L exposure groups to be 16% (1.16 [0.91–1.45]) and 18% (1.18 [0.95–1.44]), respectively for both sexes combined (60). The RRs for both groups were higher among males compared to females, which could be explained by relatively small count of female representation in the sample. Regarding kidney cancer, the respective RRs in the 2–5 $\mu$g/L and ≥5 $\mu$g/L exposure groups were 5% (1.05 [0.79–1.37]) and 14% (1.14 [0.89–1.44]) for both sexes combined. Similar to bladder cancer, the rates were higher among males, and was non-significant among females.

Finally, a systematic review of As exposure via drinking water and urinary cancers (40 studies) concluded that As exposure is indeed associated with an increased risk of bladder and kidney cancers. However, when As concentrations in water are less than 150 $\mu$g/L there is some uncertainty in the associated risk, but their meta-analysis (17 studies) suggested that even at concentrations as low as 10 $\mu$g/L, the risk of bladder cancer may be 40-50% higher compared to unexposed populations (56). Thus, there is evidence of a positive association between As exposure and bladder cancer, and there is preliminary evidence to suggest there may be a similar effect for kidney cancers, but more research is required.

#### *1.4.1.2 Breast cancer*

There are fewer studies available that have assessed As exposure and breast cancer. A systematic review found that As exposure was associated with an increased risk of breast cancer (61). Of the seven studies included, three were ecological and found no evidence of an association (59,61–63). However, of the remaining studies, three were able to measure As species (using urine and blood/plasma biomarkers), and one measured total As

(using toenail biomarkers); all four studies found significant associations between As and breast cancer (61,64–67). This supports growing evidence that As metabolism may play a key role in cancer development. These associations are discussed further in section 1.4.2 *Arsenic methylation and cancer*.

#### *1.4.1.3 Cervical Cancer*

Despite its relatively high prevalence as a cancer type, there is very little existing evidence regarding cervical cancer and As exposure. One ecological study in Bangladesh used administrative health records, and exposure data was determined from the nearest thana[6]. Arsenic concentrations in drinking water were categorized as <10 $\mu$g/L, 10–50 $\mu$g/L, 50–100 $\mu$g/L, and >100 $\mu$g/L; non-detected levels were set to 0.05 $\mu$g/L. Several types of cervical cancers were assessed, none of which had statistically significant associations with As exposure level (68). This study had a number of limitations, including that it was unknown whether the women were, in fact, drinking well water, as well as a high variability of As concentration between wells in the same thana. Additionally, there was no adjustment for confounders, and the reference group was selected from another region. Despite the null association in this one study, the lack of high-quality evidence warrants further investigation regarding cervical cancers and As exposure.

#### *1.4.1.4 Prostate Cancer*

Several studies have reported a positive association between As exposure and prostate cancer. In addition to ecological studies and those measuring As via drinking water samples, some studies have measured As concentrations in biomarkers, as well as *in vitro.*

[6] A thana is a administrative region in Bangladesh, which function as a sub-units of districts.

The Singapore Prostate Cancer Study, a hospital-based case-control study, assessed the association between 10 heavy metals[7] and prostate cancer (69). This study measured metal concentrations in serum and found that As was statistically significantly associated with prostate cancer, while controlling for the concentrations of the other metals[8]. In univariate analysis, As was associated with a prostate cancer risk difference (RD) of 0.29 (95% CI: 0.12, 0.41). Using a Bayesian kernel machine regression analysis, holding all other metals at fixed values, the RD increased (0.15 to 0.45) as As concentrations increased from the 25$^{th}$ percentile to the 75$^{th}$ (69). Similarly, a case-control study in Taiwan measured As in urine ($\mu$g of As per L of urine) of 318 prostate cancer cases and 318 controls (70). The results found that those in the highest quartile of As concentrations (> 29.28 $\mu$g/L) had increased odds of prostate cancer (OR=1.75, 95% CI: 1.06–2.89) compared those with As concentrations <29.28 $\mu$g/L (70).

An ecological study in the United States provides evidence for prostate cancer carcinogenesis at low-exposure levels of As in drinking water (10 $\mu$g/L) (71). Arsenic exposure was measured via drinking water and was categorized as follows: low exposure (1.08–2.06 $\mu$g/L), medium exposure (2.07–2.98 $\mu$g/L), and high exposure (2.99–18.6 $\mu$g/L). This study found that the RR of prostate cancer increased with As exposure; for those in the medium exposure group, RR=1.23, and for high exposure, RR=1.28. A sensitivity analysis was conducted for aggressive cancers, which found that the RR for the high exposure group increased to 1.36 (95% CI: 1.28–1.45) (71).

[7] This study assessed the following heavy metals: Manganese (Mn), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), nickel (Ni), antimony (Sb), cobalt (Co), cadmium (Cd), and lead (Pb).
[8] Zinc was also statistically significant. Refer to section 1.4.3

#### *1.4.1.5 Skin Cancer*

Skin lesions and malignancies are one of the most commonly researched effects of chronic As exposure. A recent systematic review investigated the link between chronic As exposure via drinking water and skin lesions (15 studies) and cancer (10 studies). The authors found evidence of a dose response relationships with certain skin cancers, where risk of cancer increased with As-water concentrations (72). Finally, the review highlights that carcinogenetic effects may occur at lower levels than previously thought (72).

### 1.4.2 Arsenic methylation and cancer

Despite the growing recognition of the importance of As metabolism in cancer development, there is limited evidence available concerning As methylation and cancer. In fact, there is no *in vivo* evidence on As speciation in kidney, prostate, and cervical cancers. Moreover, very few studies have used toenails as a biomarker – urine and blood have been more frequently used. As such, many of the studies presented below used biomarkers that are different from those used in this thesis. Nevertheless, the results are important in understanding the link between As methylation and several cancers, and it is likely that these biomarkers present different, and perhaps complementary, As speciation profiles.

#### *1.4.2.1 Bladder Cancer*

A case-control study of bladder cancer (n=49 cases, n=224 controls) in Taiwan examined total As and As species in urine, and found that higher levels of cumulative As exposure (CAE) was associated with increased odds of cancer when SMI was low (73). In addition, this work found that both smoking and non-smoking men had higher odds of bladder cancer than women; this pattern was also observed given the same levels of PMI and SMI levels (73). Another case-control study in both Argentina (n=114 cases, n=114 controls) and the

United States (n=23 cases, n=49 controls) (74). The authors found that among ever-smokers, high levels of urinary %MMA was associated with an increased odds of cancer (adjusted-OR=2.17, 95% CI: 1.02–4.13)[9]; however among never-smokers in Argentina, and both groups in the United States, the results were insignificant (74). This lack of association observed in the United States may be attributed to small sample sizes, a different exposure level, or population differences in methylation capacity. Finally, a cohort study (n=1563) in Taiwan (2013) found urinary %iAs, %DMA, and SMI to be associated with higher hazards ratios (HR) of bladder cancer (75). Specifically, the proportions of iAs (> 7.86%) was associated with an adjusted-HR = 3.53 (95% CI: 1.16, 10.77), the proportions of DMA (< 76.13%) was associated with an adjusted-HR = 3.05 (95% CI: 1.11, 8.37), and SMI (< 4.9) was associated with and adjusted-HR = 2.85 (95% CI: 1.04, 7.83)[10]. Moreover, significant dose-dependent associations were observed for increasing urinary %iAs, and decreasing urinary %DMA and SMI, and bladder cancer.

#### *1.4.2.2 Breast Cancer*

A large case-control study (n=1016 cases, n=1028 controls) of female breast cancer in Northern Mexico and analyzed As metabolites in urine (65). The authors found that women with higher urinary %MMA and/or PMI had a significantly increased odds of breast cancer. Specifically, the odds of breast cancer for women in the highest quintile of %MMA in urine were 2.63 times that of those in the lowest quintile, and similarly, for those with PMI in the

[9] Analysis adjusted for age, sex, bombilla use, and smoking.
[10] Analysis adjusted for age, sex, education, and smoking.

highest quintile, the odds were 1.9 times that of those in the lowest quintile of %MMA in urine[11] (65).

#### *1.4.2.3 Skin Cancer*

A matched case-control study in Taiwan found that skin cancer cases (n=26) had higher proportions of iAs and MMA and lower proportions of DMA compared to controls (n=26), as measured in urine samples (76). This study found that neither As exposure levels in water nor total urinary As metabolites were statistically significantly different between the two groups (76). By contrast, a larger case-control study (n=76 cases, n=224 controls) found that none of the As speciation variables measured in urine (%MMA, %DMA, %iAs, PMI, SMI) were associated with skin cancer (77). However, cases had statistically significantly higher CAE (mg/L·year) compared to controls (77). This research also found that women had higher mean PMI and SMI compared to men, but the difference was only statistically significant for PMI (77).

#### *1.4.2.4 Prostate Cancer*

A recent study measured As speciation in toenails of Atlantic Canadians, and found significant associations between toenail As speciation profiles and prostate cancer. These findings are discussed in more detail in section 1.5 *Pilot study and preliminary findings.*

### 1.4.3 Trace Metals and Cancer

Trace metal concentrations were measured in the plasma of metastatic cancer patients and controls in Pakistan. The study found significant differences in metal distributions and

[11] The odds of breast cancer for a 1% increase in, %MMA was 2.63 (95%CI: 1.89, 3.66), and for a 1-unit increase in PMI was 1.90 (95%CI: 1.39, 2.59).

patterns between the cases (n=112) and controls (n=118) (78). Specifically, significantly higher[12] concentrations of Cu, Pb, Zn, cadmium (Cd), chromium (Cr), manganese (Mn), nickel (Ni), antimony (Sb), and strontium (Sr), and significantly lower concentrations of Fe, Mg, and calcium (Ca) were observed in cancer patients (78). In addition, cancer patients displayed very strong and statistically significant correlations (Pearson's correlation coefficient, $r \geq 0.7$; $p < 0.01$) between several metals, which were different from those observed in controls[13]. Sex differences were also observed; the average concentrations of Ca, Mg, Mn, Ni, and Pb were higher among male cases compared to female cases, and mean concentrations of Cr, Cu, Na, potassium (K), and lithium (Li), were higher among female cases compared to male cases (78). These results indicate that the profiles of metallomes differ between cases and controls, and there may be differences by sex.

Select metals have also been linked to specific cancer types in clinical and observational studies. A case-control study (n=50 cases, n=50 controls) of Malaysian men with prostate cancer found statistically significantly lower hair and nail concentrations of Se and Zn, and higher concentrations of Cu, Fe, and Mn among cases compared to controls (79). Moreover, lower Se concentrations, and higher Mn and Fe concentrations were significantly associated with increased odds of prostate cancer[14] (79). Similarly, a case control study (n=318 cases, n=318 controls) in Taiwan found that Se levels in plasma were inversely associated with prostate cancer (70). In another study, As, Zn, Mn, and Sb levels

[12] The results found that the heavy metals, Cd, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sr and Zn were 1.6 to 7.4 times higher among cases compared to controls (78).
[13] Metals with strong positive correlations ($r \geq 0.7$) among cancer cases: Fe–Mn, Ca–Mn, Ca–Ni, Ca–Co, Cd–Pb, Co–Ni, Mn–Ni, Mn–Zn, Cr–Li, Ca–Zn. Metals with positive correlations ($r \geq 0.5$) among controls: Ca–Mn, Ca–Mg, Fe–Zn, Ca–Zn, Mg–Mn, Mg–Zn, Cd–Sb, Cd–Co, Cd–Zn, Co–Sb and Sb–Zn (78).
[14] Odds ratios for each metal (measured in nail) and prostate cancer – Se: OR = 5.91, $p < 0.001$; Mn: OR = 3.78, $p < 0.007$; Fe: OR = 5.47, $p < 0.001$

measured in serum were significantly and positively associated with prostate cancer, and that the association persisted for As and Zn in an analysis controlling for 10 other metals (69). Previously, trace metals measured were in breast tissue, and researchers found that Mn and Zn were significantly higher in cancerous tissue compared to non-cancerous tissue (80). While Zn is considered an essential trace metal, over-exposure to Zn may be associated with increased risk of cancer (81).

Taken together, the current evidence indicates there is an inverse association between Se exposure and prostate cancer risk (70,79,81–84), whereas the association with other cancers, including breast and bladder, is still uncertain (81,85,86). In addition, Zn levels may play a role in cancer risk, where both a deficiency and an excess of Zn may increase cancer risk (87,88). On the other hand, some metals may not be safe at any exposure levels, such as Cd exposure, which is considered a risk factor for cancer (89,90). Specifically, studies have shown that Cd is associated with lung cancer, prostate cancer, renal cancer, and there is some evidence to suggest Cd is also associated with bladder cancer and breast cancer (89,90). Finally, some observational studies have found Cd-nail concentrations to be higher in prostate cancer cases than in healthy controls (91,92).

### 1.4.4 Mechanisms of arsenic carcinogenicity

At the present time, the etiology of As-induced carcinogenicity at the molecular level are still unknown. There are, however, several hypotheses that describe the mechanisms of effect. Findings from *in vitro* studies indicate that the response is dependent on the dose: at lower doses, there is an adaptive response, with an upregulation of genes related oxidative stress, inflammation, and proteotoxicity responses, whereas higher doses may result in changes to apoptotic genes (93,94). These observed responses at the cellular level provide

clues that point to the molecular mechanisms that may result from As exposure, including oxidative stress, genotoxic damage, epigenetic regulation of gene expression, and activation of critical proliferative, and anti-apoptotic signaling pathways (31).

Arsenic induces oxidative DNA damage which, in turn, affects the normal expression of cancer related genes (31). This occurs because As increases the production of ROS that cause DNA lesions (31,95). Such lesions have been found in 8-hydroxyl-2'-deoxyguanosine (8-OHdG) adducts of breast cancer cell lines (95). The formation of ROS, including superoxide anion ($O_2^-$), hydroxyl radical ($OH^-$), and hydrogen peroxide ($H_2O_2$), are associated with DNA damage, and inhibit the activity of an enzyme (PARP-1) that is critical for DNA damage repair (31,96,97). Additionally, in some *in vitro* studies, arsenite has been observed to inhibit base excision repair, and reduce DNA damage recognition proteins resulting in nucleotide excision repair, and mismatch repair (98–100). These impairments of DNA repair could provide more favourable conditions for cancer development.

Another pathway of As-induced carcinogenicity is by altered gene expression. In particular, As can induce DNA hypomethylation in human keratinocytes (31). Interestingly, this effect can persist in further cell generations, even in the absence of As, suggesting long-lasting effects related to As exposure (31). In fact, iAs has been found to promote the hypomethylation of oncogenes in human liver cells (101). At the same time, iAs has been shown to cause the hypermethylation of tumour suppressor genes in a skin cancer cases (102). These aberrant gene modifications may yield uncontrolled cell proliferation and apoptosis resistance.

### 1.4.5 Mechanisms of trace metal-induced carcinogenicity

The hypothesized mechanisms of trace metal carcinogenicity are similar to those proposed for As, and largely depend on dosage, oxidation states, and chemical structures. Some metals will, in the presence of DNA, catalyze redox reactions, and thus, oxidize DNA, which can lead to DNA damage (103). In the case of Cd carcinogenicity, proposed mechanisms include, activation of proto-oncogenes, inactivation of tumour suppressor genes, cell proliferation, inhibition of DNA repair, and generation of ROS (89). On the other hand, the deficiency of an essential metal can lead to the loss of protective factors. For example, a nutritional deficiency of Zn may increase cancer risk; Zn is important in the synthesis of metallothionine, which thought to inhibit free radical production (87,88). The mechanisms of effect are better understood for some metals than others, but the inter-relationship between metals may play an important role. Lead is a key example of this, where in addition to inhibited DNA repair and chromosomal aberrations, Pb has been reported to creates synergistic effects with other metals and carcinogens (104).

### 1.4.6 Inter-individual variability

There is sufficient evidence to support the link between As exposure and cancer, and there is mounting evidence to suggest that As methylation plays a crucial role in mediating this association. As such, important research has been conducted to help explain why As methylation capacity may vary between individuals and population groups.

A case-control study in northern Chile examined the As methylation patterns of 73 exposed individuals after changing from high to low concentrations of As in drinking water (105). After intervention, the %iAs in urine and the SMI decreased. Factors that were associated with the change in SMI included smoking, sex, age, years of residence, and

ethnicity, but only accounted for 20% of the variation observed. The authors suggested that some of the unexplained variation could be attributed to genetic polymorphisms in enzymes and other co-factors important to As methylation (105,106). Evidence of this has been observed more recently in a 2011 study with two populations of interest, one in Argentina and one in Bangladesh (107). The results showed that AS3MT polymorphisms were significantly associated with the As metabolite patterns measured in urine of both populations. Given the differences in these populations, this finding may be generalizable to populations around the globe.

In addition to genetic differences, sex and age have been shown to be significant predictors of As metabolism in a exposed population in Bangladesh (108). The same study found that women methylated As more efficiently than men, but only during their childbearing years, which suggests hormone levels may play a role (108). Finally, Se levels have also been positively associated with As methylation capacity, but the evidence is limited (109). It seems that As methylation capacity may be influenced by genetic, biological, and environmental factors.

### 1.5 Pilot study and preliminary findings

The Atlantic Partnership for Tomorrow's Health (PATH) cohort has been engaged in a series of research projects pertaining to arsenic exposure. Some early work, conducted at Acadia University, used drinking water and toenail samples (n=960) to evaluate the association between the consumption of drinking water containing As, and As body burden (110). In addition, private drilled wells were found to have greater amounts of As compared to municipal sources ($p < 0.001$), and there were associations observed between drinking

water and toenail total-As concentrations among the participants with drinking water levels exceeding 1 $\mu$g/L ($p < 0.001$) (110).

Subsequently, a method for As speciation analysis in toenails was developed in a pilot study at the Health and Environments Research Centre (HERC) Laboratory at Dalhousie University (111). This method yielded extraction efficiencies of 97.6% (111), a notable improvement upon those previously reported, 62.7% and 53% (112,113). The pilot study, conducted by Nathan Smith, analyzed 60 toenail samples including, 10 samples from participants with healthy controls, and 50 samples from participants with a history of cancer or diabetes (10 skin cancer cases, 10 lung cancer cases, 10 bladder cancer cases, 10 kidney cancer cases, and 10 participants with diabetes) (111). The findings indicated no significant differences in total toenail-As concentrations between the groups, but statistically significantly higher proportions of MMA in the lung cancer ($p = 0.017$) and the kidney cancer ($p = 0.017$) groups compared to the healthy controls. In addition, the healthy control group displayed statistically significantly higher concentrations of Se compared to cancer groups (111). In a larger, prevalent case-control analysis of prostate cancer (139 cases, 417 controls), we found mean %DMA and SMI to be statistically significantly higher, and mean %MMA and PMI to be statistically significantly lower among prostate cancer cases compared to age- and sex-matched controls (114). No statistically significant effects were observed among the additional 18 metals considered (114).

## 1.6 Study Rationale

This thesis builds upon the previous research of the HERC laboratory, investigating the association between As speciation and metallomic profiles with several cancer types. In particular, the study design has been ameliorated by increasing sample sizes and including

age- and sex-matched controls with a 1:1 ratio. Matching is important in this study because age is an important risk-factor for cancer development, and sex-differences have been observed in As methylation capacity.

The present work aimed to address the gap in the literature regarding As speciation and cancer types with little or no research available to date (cervical, prostate, kidney, and breast cancers). In addition, it provides richer context and a better understanding to the existing knowledge on As speciation by making use of a potentially more advantageous biomarker, toenails, which have not been widely used in previous work, and may capture a longer period of exposure than urine due to their prolonged growth period. This is important considering that cancer development is associated with chronic As exposure. Currently, urine is the gold-standard for measuring As *in vivo*, but this biomarker is limited as it only reflects an exposure-window of three to four days (the residence time of As in urine) (115). As such, urine may be better suited as a short-term indicator of exposure.

Based on available research, current hypotheses suggest that As metabolism plays a key role in carcinogenesis and that adverse effects can occur at lower-levels than previously thought. However, the existing literature on the effects of chronic low-level As exposure is inconsistent and limited. This study sought to address this gap in the literature related to low-level arsenic exposure, and more importantly, the role of As metabolism, by determining As speciation for a large sample of Atlantic Canadians, both with various types of cancer as well as healthy controls. In addition, trace metal profiles were also determined as there is evidence that they may differ between those with and without cancer. There is evidence to suggest inter-relationships between metal concentrations, which highlights the importance of the approach used in this thesis, where a range of metals are characterized *in vivo* for Atlantic Canadians with a history of cancer and matched controls. Furthermore,

As speciation and metallomic profiles may differ by population groups, sex, and cancer type, and as such, this thesis assessed whether these factors are associated with the variation. Finally, this analysis sought to provide a baseline characterization of As speciation and metallomic profiles for these participants in the Atlantic PATH cohort, and as such, will serve as a comparator for future research.

## 1.7 Research Objectives

The objectives of this research were to:

1. characterize the profiles of As speciation and metallomes of several cancer groups (skin, lung, bladder, kidney, prostate, breast, and cervical cancer) and healthy participants with no history of cancer using an advanced analytical method;
2. determine whether there is an association between the profiles of (a) As speciation profiles, and (b) metallomic profiles and cancers; and
3. determine if environmental and lifestyle factors are significantly associated with the profiles of (a) As speciation and (b) metallomes.

# CHAPTER 2 METHODOLOGY

## 2.1 Study Design

This study used a prevalent case-control design. Arsenic speciation and other metallomes are the exposures of interest and were measured via toenail samples to compare between cases of cancer and healthy controls. Biological samples (toenails) and questionnaire data for cases and controls were provided by the Atlantic PATH cohort study from the baseline collection period.

The Atlantic PATH study is part of the Canadian Partnership for Tomorrow's Health (CanPath), formerly the Canadian Partnership for Tomorrow Project, a national longitudinal cohort study examining the contributions of genetic, environmental, lifestyle, and behavioural factors in the development of cancer and chronic disease (116). Baseline data collection for Atlantic PATH occurred between 2009 and 2015, and is the source of data used in this study. The cohort has been described in detail elsewhere (117). In brief, participants completed health-related questionnaires, physical measures, and biosamples including blood, urine, saliva, and toenails. All participants consented for their data and samples to be used for the purposes of health research.

## 2.2 Ethics

The Dalhousie University Health Sciences Research Ethics Board granted approval to this study July 16, 2018 as an amendment to the pilot study conducted by Nathan Smith (REB # 2016-3896).

## 2.3 Study Population

Participants in the Atlantic PATH were recruited via advertising, media coverage, community, and workplace events, incentive programs, and designated community

members who encouraged friends and families to participate. The first harmonization of the baseline involved 31,173 participants aged 35-69.

### 2.3.1 Inclusion/Exclusion Criteria

In the present MSc study, cases must have had a history of urinary (bladder or kidney), breast, cervical, lung, prostate, or skin cancer. Furthermore, cases must have had been diagnosed with only one type of cancer[15]. Healthy controls must have had no history of cancer, diabetes, or cardiovascular disease (defined as stroke or myocardial infarction). Participants must have resided in one of the Atlantic provinces (Newfoundland and Labrador, New Brunswick, Nova Scotia, Prince Edward Island).

### 2.3.2 Sampling

The sampling strategy for cancer cases was limited by the number of cases available for each cancer group. Specifically, the number of eligible participants with toenail samples by cancer group were: bladder (20), breast (343), cervical (146), kidney (20), lung (14) prostate (127), and skin (441). Among cancer groups with larger samples (breast, cervical, prostate, skin), 45 cases were randomly sampled from each group. For the remaining cancer groups (bladder, kidney, lung), all the available samples were used. Healthy controls were age- and sex-matched 1:1 to cancer cases. Due to the small sample sizes in the bladder (14), kidney (15), and lung (4) cancer groups, the lung cancer group was dropped entirely from statistical analysis, and the bladder and kidney cancer cases and controls were combined into one group, urinary cancers, for all statistical analyses[16]. Power

[15] This way defined later in the project development and explains the discrepancy in the number of samples requested from Atlantic PATH and the number of viable samples, and the reason why lung cancer cases (and controls) were dropped from analysis.
[16] This limitation is discussed in more detail in the discussion.

calculations for our sample sizes are provided in Appendix B. In total, 470 samples were requested from Atlantic PATH, of which 418 met our inclusion criteria and were viable for analysis.

## 2.4 Questionnaire data and variables

The questionnaire data were sourced from the Atlantic PATH dataset. The majority of the information for these variables was self-reported in the questionnaire. A summary of the variables and how they were handled in the analysis is provided in Table 2.4.1.

Cases and controls are matched 1:1 based on their year of birth within (+/-) 5-years and sex. Both age and sex have been linked to As methylation capacity; increased age is associated with reduced methylation capacity, and women may methylate more efficiently than men (118). Physical activity was recorded in the Atlantic PATH questionnaire in accordance with the International Physical Activity Questionnaire (IPAQ) short form. Using the IPAQ scoring protocol, observations were categorized by their reported physical activity levels (119). The body mass index (BMI) variable was measured by Atlantic PATH. Height was measured using a Seca stadiometer and a Tanita bioelectrical impedance device was used to measure body weight, which were then used to calculate BMI by dividing weight in kilograms by height in meters squared (117).

**Table 2.4.1** Summary of Questionnaire variables from the Atlantic PATH dataset

| Variable | Variable Name | Variable Type | Description |
|---|---|---|---|
| History of cancer | cancer_ever | Binary<br>0 – no<br>1 – yes | Participants self-reported history of cancer, cancer type, and age at diagnosis. |
| Age | age | Continuous | Age calculated from participants' self-reported date-of-birth. |
| Sex | sex | Categorical<br>1 – male<br>2 – female | Self-reported sex. |
| Family history of cancer | cancer_famhistory | Binary<br>0 – no<br>1 – yes | Lifetime occurrence of cancer in the family. |
| Cigarette smoking frequency | smoke | Categorical<br>0 – never<br>1 – former<br>2 – current | Self-reported cigarette smoking frequency. |
| Physical activity | ipaq_cat | Categorical<br>1 – low<br>2 – moderate<br>3 – high | Self-reported physical activity levels categorized in accordance with the IPAQ scoring protocol (119). |
| Body mass index | bmi_cat | Categorical<br>1 – low/normal<br>2 – overweight<br>3 – obese<br>4 – unknown | The BMI categories are low/underweight (<18.5 $kg/m^2$), normal weight (18.5–24.9 $kg/m^2$), overweight (25.0–29.9 $kg/m^2$), and obese (>30.0 $kg/m^2$). |
| Water Source | water_source | Categorical<br>1 – municipal<br>2 – well<br>3 – other<br>4 – unknown | Self-reported water source. Well water refers to both private drilled and dug wells. |

## 2.5 Analytical Methods for Toenail Analysis

### 2.5.1 Objective 1: Laboratory Toenail Analysis

The first objective of this study was to measure and characterize the As speciation and metallome concentrations for each toenail sample. The following subsections detail the analytical techniques used to digest and analyze the toenail samples for As speciation and metal concentrations. Briefly, the total metal concentrations were measured using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, iCAP Q, Thermo Scientific, MA USA) and, subsequently, the As species were determined using High Performance Liquid Chromatography (HPLC, SpectraSYSTEM, Thermo Scientific, MA USA) coupled with ICP-MS (HPLC-ICP-MS). All processes were performed in the HERC Laboratory unless otherwise specified.

### 2.5.2 Reagents and standards

All reagents used in this analysis were of trace metal grade. Milli-Q Advantage A10 unit (MilliporeSigma, ON Cananda) was used to deionize any water used for the preparation, digestion, or analysis of the toenail samples (resistivity, 18.2 MΩ-cm). Acetone (99.5%, Acros Organics, NJ USA) was used in cleaning process. A solution with concentrated nitric acid ($HNO_3$, Fisher Scientific, NJ USA) and hydrogen peroxide ($H_2O_2$, Sigma-Aldrich, MO USA) was used to dissolved toenail clipping samples during microwave digestion.

The following reagents were used for the tuning of, and analysis with, the ICP-MS. Multi-element tuning solution (P/N THERMO-4AREV-500M, Inorganic Ventures, VA USA) was used to perform ICP-MS tuning. For the total metals analysis, a multi-element standard (P/N IV-ICPMS-71A, Inorganic Ventures, VA USA) and a scandium (Sc) single-

element standard (P/N ICP-MS-IS-SC-10X-1, AccuStandard, CT USA) were used to prepare the calibration standard and internal standard. For As speciation analysis, Arsenite ($As^{3+}$, Sigma-Aldrich, MO USA), arsenate ($As^{5+}$, Sigma-Aldrich, MO USA), MMA (Chem Service, PA USA), and DMA (Chem Service, PA USA) were used to prepare calibration standards for the HPLC-ICP-MS analysis of arsenic speciation; AsB (Sigma Aldrich, MO USA) was used to prepare the internal standard for speciation analysis.

Calibration and internal standards were prepared in 1% (v/v) $HNO_3$ to replicate the concentrations in the digested toenail sample solutions. Ammonium carbonate ($[NH_4]_2CO_3$, 99.999%, Fisher Scientific, ON Canada) was used to prepare mobile phases for the HPLC-ICP-MS analysis.

### 2.5.3 Toenail Sample Preparation

The toenail samples provided by Atlantic PATH were stored in sealed plastic bags and labelled with participant and toenail ID numbers. The toenail preparation process can generally be described by the following steps: measurement of wet weights, cleaning, drying, measurement of the dry weights, digestion, dilution. A detailed description is provided below.

Toenail clippings were extracted from the bag and weighed using an analytical balance (Pinnacle-114, Denver Instruments, CO USA) to an approximate mass of 50 mg and recorded as "wet" weights. A target sample mass of 50 mg was chosen for best digestion results, as determined by the pilot study (111). For samples with insufficient mass, the entirety of the sample was used. After being weighed, samples were transferred to labelled, 10 mL quartz vials (CEM Corporation, NC USA) for cleaning. First, the toenail clippings were submerged in acetone and sonicated for five minutes. Vials were decanted

and the samples were rinsed with acetone once before being submerged in water and sonicated again for five minutes. To complete the cleaning process, samples were rinsed with water three times before being placed in a Heratherm 60 L gravity convection oven (Thermo Scientific, MA USA) at 105 °C overnight. The following morning, dehydrated samples were weighed once again to determine their "dry" weights, which were recorded, and were the weights used in further calculation.

Toenail samples were dissolved using a Discover SP-D microwave digestor (CEM Corporation, NC USA). Then, using a pipette, 100 µL of concentrated $HNO_3$, 500 µL of $H_2O_2$, and 400 µL of water were measured and dispensed into each sample vial. In addition to each sample batch, three blanks were created with the same solution. The microwave digestion method was 300 W power, with five minutes of ramp time to 165 °C, and held for four minutes. The pressure in the digestion vessel was approximately 200 psi under the method conditions.

Following digestion, samples were transferred to 15 mL polypropylene tubes and diluted tenfold with water. After dilution, the final concentration of $HNO_3$ was 1% (v/v). For the speciation analysis, a 995 µL aliquot of each sample and the blanks were transferred to 1.8 mL polypropylene vials (Thermo Scientific, MA USA). Finally, 5 µL of 20-µg/L AsB (Sigma Aldrich, MO USA) in 1% v/v $HNO_3$ was added to each vial as an internal standard.

Samples were vortexed (Maxi Mix I, Thermo Scientific, MA USA) immediately before ICP-MS and HPLC-ICP-MS analysis to ensure homogeneity.

### 2.5.4 Metallome determination

Total concentration of As and other heavy metals in the toenail samples were measured using an ICP-MS with a quartz torch, PFA nebulizer, and quartz spray chamber. The torch position, nebulizer flow rate, and lens voltages were tuned daily according to the instructions of the manufacturer using a multi-element tuning solution. The sample introduction system consisted of an ESI SC-4 DX autosampler and FAST valve (Elemental Scientific, NE USA) to facilitate on-line addition of a 50 µg/L Sc internal standard (AccuStandard, CT USA) in 1% (v/v) $HNO_3$.

Kinetic energy discrimination (KED) mode was used to measure the total concentrations of all metallomes (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Cd, Tl, Pb, Th, U) except Se, which was measured in standard mode. High purity helium (>99.999% He) was used as the collision gas. The multi-element calibration standard was diluted to concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, and 100 µg/L in 1% (v/v) $HNO_3$ to form a nine-point calibration curve. Quality control check standards (1 and 10 µg/L) were measured every 15 samples. Qtegra Intelligent Scientific Data Solution software (version 2.7, Thermo Scientific, MA USA) was used to collect and process data from total arsenic analysis.

### 2.5.5 Arsenic speciation determination

To determine arsenic speciation, a second analysis was performed coupling HPLC with the ICP-MS. The HPLC was fit with a P4000 pump, AS3000 autosampler, and SN4000 interface module. PEEK tubing was used to pair the HPLC with the ICP-MS. Sample injection and measurement was coordinated using the Qtregra Intelligent Scientific Data solution software. Ammonium carbonate was used as the mobile phase using a gradient

solution between 20 mM and 200 mM to achieve adequate separation for the As species measured. A calibration stock solution was made by mixing individual $As^{3+}$, $As^{5+}$, MMA, and DMA standards. The stock solution was then diluted to 0.02, 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/L in 1% (v/v) $HNO_3$ to form a seven-point calibration curve. During sample preparation, AsB (Sigma Aldrich, MO USA) was added as the internal standard. An example chromatogram displaying As species separation is provided in Figure C1 (Appendix C).

### 2.5.6 Method validation and quality control

To date, there is no certified reference material for trace metal concentrations or As speciation in toenails. Validation of the analytical methods was performed instead using certified human hair and frozen urine. NIES No. 13 Human Hair (National Institute for Environmental Studies, Tsukuba, Japan) samples were prepared using the toenail protocol (refer to Table C2, Appendix C). The method was tested for different sample masses (as little as 5 mg), as not all toenail samples had sufficient mass (50 mg). NIST 2669 Arsenic Species in Frozen Urine were used to validate the arsenic speciation method (Table C1, Appendix C).

Method detection limits (MDL) were calculated using United States Environmental Protection Agency procedures (EPA 821-R-16-006) and the method blanks that were analyzed alongside the toenail samples (Table C3, Appendix C). Previous studies (120–123) have found that replacing values below MDL with MDL/√2 does not introduce bias when the percentage of values below MDL is below 25%. As such, values for As species that fell below MDL were replaced with the MDL/√2 for that species before being normalized by sample mass.

## 2.6 Objectives 2 & 3: Statistical Analysis

Stata 15 was used to analyze all data (124). None of the observations had missing data for the primary outcome of interest, cancer. Regarding the primary exposure, observations that failed to pass quality control measures during the lab phase were excluded from the statistical analysis as they were deemed unreliable measures.

To determine if As speciation and/or metallomes were associated with history of lifetime cancer diagnosis (Objective 2), a multivariate analysis of variance (MANOVA) was used to assess the effect of cancer on As speciation (Model A) and metallomic profiles (Model B). This analysis was performed for each cancer group separately. The independent variable was history of cancer (case/control status). In Model A, the dependent variables were the As speciation measures: %MMA, %DMA, %iAs, PMI, and SMI. For Model B, the dependent variables were metals that were statistically significantly associated with the predictors (cancer type/control). This was determined using *a priori* independent Student's t-tests, and relationships with a significance level of $p < 0.1$ were included in the MANOVA and MANCOVA models.

To determine whether any covariates had an effect on As speciation or metallomic profiles among cancer cases and controls (Objective 3), multivariate analysis of covariance (MANCOVA) models were replicated from Objective 2, with adjustment for selected covariates: age, sex, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI.

Assumptions of the *a priori* Student's *t*- and MANOVA tests were conducted and were found tenable. No univariate outliers were found from univariate detection. The assumption of normality and linearity were satisfied, and homogeneity of variance was

assessed using Box's M test ($p > 0.05$). There was no multicollinearity; dependent variables were moderately correlated with one another.

Analyses were performed separately for five cancer groups with sufficient sample size (urinary cancer, breast cancer, cervical cancer, prostate cancer, and skin cancer). The bladder and kidney cancer cases (and their respective controls) were combined into one group, urinary cancer.

The "nearest-neighbor matching" 1:1 was used for matching cases to controls. This is a popular tool to create balance between treatment and control groups in observational studies. In current empirical practice the matching step is often ignored in the calculation of standard errors and confidence intervals. However, this may lead to differences in standard errors had it be a correction for matching (125). Given the matching design of this study, we recognize that this is clustered data. We therefore conducted one analysis (Objectives 2 and 3) that included cluster variance estimators and found that the results were comparable to those obtained from the original analyses. Therefore, the original analyses rather than the cluster variance estimator sample design analyses are presented below.

# CHAPTER 3 RESULTS

The questionnaire data and biological samples of 418 eligible participants were received from Atlantic PATH. Specifically, there were 29 urinary cancer (14 bladder cancer and 15 kidney), 45 breast cancer, 45 cervical cancer, 45 prostate cancer, 45 skin cancer samples, and 209 age- and sex-matched controls. However, not all of the samples provided had enough mass to be properly analyzed, and thus were excluded from further analysis. After the completion of the ICP-MS analysis for total metal concentrations and, HPLC-ICP-MS analysis for As speciation, the breakdown of the available matched cases and controls was as follows: 27 urinary cancer cases, 41 breast cancer cases, 41 cervical cancer cases, 44 prostate cancer cases, 43 skin cancer cases, and 196 total matched controls.

The following sections present the results from each of the five-cancer specific case-control analyses.

## 3.1 Urinary Cancer

### 3.1.1 Participant characteristics

The participant characteristics and demographics of the urinary cancer cases and controls are summarized in Table 3.1.1. Mean age of participants was 60.3 years (*SD* = 5.3); 48% were between the ages of 49-59, and the remaining 52% were between 60 and 69 years. The distribution of men to women was 59% and 41%, respectively. The majority of participants had a family history of cancer, 69% of cases, and 74% of controls. Province of residence was the same between cases and controls: 67% from New Brunswick, 18% from Newfoundland, 11% from Nova Scotia, and 4% from Prince Edward Island. There was no information on source of water for 63% of cases, and the remaining 37% of cases reported having municipal water. Among healthy controls, 37% had an unknown water

source, 37% from municipal water, and 26% sourced their water from a private well. Regarding level of physical activity, 30% of cases and 48% of controls were classified as highly active, 52% of cases and 33% of controls were moderately active, and 18% of both cases and controls had low levels of physical activity. Few participants were current cigarette smokers (11% of cases, 4% of controls), a large portion (59% of cases, and 48% of controls) were former smokers, and 30% of cases and 48% of controls reported never having smoked. BMI data were missing for 19% of cases, and 33% of controls. Regarding BMI categories, 11% (cases and controls) had low or normal BMI, 33% of cases and 30% of controls were overweight, and 37% of cases and 26% of controls were obese. None of these variables were statistically significantly associated with cancer, as assessed by chi-squared tests.

**Table 3.1.1** Participant characteristics and demographics for urinary cancer cases (n=27) and matched controls (n=27) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** | **Urinary Cancer Cases** | **Healthy Controls** |
|---|---|---|---|
| | n=54 | n=27 | n=27 |
| | Count *(%)* | Count (%) | Count (%) |
| **Age,** mean *(SD)* | 60.3 *(5.3)* | 60.3 *(5.5)* | 60.2 *(5.3)* |
| 49-59 | 26 *(48%)* | 13 *(48%)* | 13 *(48%)* |
| 60-69 | 28 *(52%)* | 14 *(52%)* | 14 *(52%)* |
| **Sex** | | | |
| Female | 22 *(41%)* | 11 *(41%)* | 11 *(41%)* |
| Male | *32 (59%)* | 16 *(59%)* | 16 *(59%)* |
| **Family History of Cancer** | 38 *(72%)* | 18 *(69%)* | 20 *(74%)* |
| **Province** [a] | | | |
| NB | 36 *(67%)* | 18 *(67%)* | 18 *(67%)* |
| NL | 10 *(18%)* | 5 *(18%)* | 5 *(18%)* |
| NS | *6 (11%)* | 3 *(11%)* | 3 *(11%)* |
| PEI | 2 *(4%)* | 1 *(4%)* | 1 *(4%)* |
| **Water Source** | | | |
| Municipal | 20 *(37%)* | *10 (37%)* | 10 *(37%)* |
| Well | 7 *(13%)* | 0 *(0%)* | 7 *(26%)* |
| Unknown | 27 *(50%)* | 17 *(63%)* | 10 *(37%)* |
| **Physical Activity** | | | |
| Low | 10 *(18%)* | *5 (18%)* | 15 *(18%)* |
| Moderate | 23 *(43%)* | 14 *(52%)* | 9 *(33%)* |
| High | 21 *(39%)* | 8 *(30%)* | 13 *(48%)* |
| **Smoking** | | | |
| Never | 21 *(39%)* | 8 *(30%)* | 13 *(48%)* |
| Former | 29 *(54%)* | 16 *(59%)* | 13 *(48%)* |
| Current | 4 *(7%)* | 3 *(11%)* | 1 *(4%)* |
| **BMI, mean *(SD)*** | 28.9 *(4.3)* | 29.3 *(4.4)* | 28.4 *(4.2)* |
| **BMI Categories** | | | |
| Low/Normal | 6 *(11%)* | 3 *(11%)* | 3 *(11%)* |
| Overweight | 17 *(31%)* | 9 *(33%)* | 8 *(30%)* |
| Obese | 17 *(31%)* | 10 *(37%)* | 7 *(26%)* |
| Unknown | *14 (26%)* | 5 *(19%)* | 9 *(33%)* |

[a] New Brunswick (NB), Newfoundland and Labrador (NL), Nova Scotia (NS), Prince Edward Island (PEI)

### 3.1.2 Toxicological characteristics (Objective 1)

The first objective was to characterize the As species and total metals measured in the toenail samples using analytical instrumentation. The levels of As species were measured and compared between cases and controls. The mean values and standard deviations of %iAs, %MMA, %DMA, PMI, and SMI are presented in Table 3.1.2 for the overall group, urinary cancer cases, and their healthy matched controls. The proportions of iAs, MMA, DMA, and the ratios, PMI and SMI did not appreciably differ between the cases and controls. Briefly, their composition was predominantly iAs, which accounted for 80% (*SD* = 11.77%) of the total arsenic in the nail. The urinary cancer cases had similar proportions of %MMA (10.05%) compared to their controls (9.30%). The proportion of DMA slightly lower among cases, 8.59%, than in controls, 11.24% (Table 3.1.2). The PMI was slightly higher, and the SMI lower among cases compared to controls, but the difference was not statistically significant.

**Table 3.1.2** Arsenic Speciation Profiles for urinary cancer cases (n=27) and matched controls (n=27) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=54 Mean *(SD)* | **Urinary Cancer Cases** n=27 Mean *(SD)* | **Healthy Controls** n=27 Mean *(SD)* |
|---|---|---|---|
| %iAs | 80.40 *(11.77)* | 81.35 (*11.14)* | 79.45 *(12.50)* |
| %MMA | 9.67 *(5.68)* | 10.05 *(5.78)* | 9.30 *(5.66)* |
| %DMA | 9.92 *(7.36)* | 8.60 *(6.68)* | 11.25 *(7.88)* |
| PMI | 0.13 *(0.11)* | 0.14 *(0.10)* | 0.13 *(0.12)* |
| SMI* | 1.09 *(0.66)* | 0.92 *(0.61)* | 1.27 *(0.67)* |

Notes: Students' t-test comparing cases to controls * $p < 0.1$

Eighteen metals were detected and measured in the toenail samples: As, V, Cr, Mn, Fe, Co, Ni, Co, Cu, Zn, Ga, Se, Rb, Sr, Cd, Tl, Pb, Th, and U. The mean concentrations ($\mu$g/g) for the overall group, the urinary cancer cases, and the healthy controls are presented in Table 3.1.3. The mean concentrations of the cases and controls were compared using the Student's *t*-test, eight of which were flagged to be included Model B, based on the inclusion criteria of $p < 0.1$. In particular, the concentration of Zn was statistically significantly higher among urinary cancer cases compared to their controls ($p = 0.0062$). Similarly, statistically significantly higher concentrations of Co ($p = 0.0372$) and Pb ($p = 0.0192$) were observed among cases compared to controls. The other metals included in subsequent analysis were Mn, Fe, Rb, and Th, all of which were higher among cases compared to controls ($p < 0.1$).

**Table 3.1.3** Metallomic Profiles ($\mu$g/g) for urinary cancer cases (n=27) and matched controls (n=27) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=54 Mean *(SD)* | **Urinary Cancer Cases** n=27 Mean *(SD)* | **Healthy Controls** n=27 Mean *(SD)* |
|---|---|---|---|
| As | 0.069 *(0.028)* | 0.073 *(0.034)* | 0.065 *(0.012)* |
| V | 0.019 *(0.016)* | 0.020 (0.019*)* | 0.019 *(0.012)* |
| Cr | 0.31 *(0.20)* | 0.33 *(0.21)* | 0.29 *(0.19)* |
| Mn * | 0.52 (*0.46)* | 0.62 *(0.59)* | 0.41 *(0.23)* |
| Fe * | 28.42 *(26.61)* | 34.73 *(25.26)* | 22.11 (*10.81)* |
| Co ** | 0.019 *(0.014)* | 0.023 *(0.017)* | 0.015 (0.010*)* |
| Ni | 11.31 *(14.09)* | 11.81 *(14.29)* | 10.81 *(14.13)* |
| Cu | 4.18 *(0.90)* | 4.27 *(0.79)* | 4.08 *(1.01)* |
| Zn *** | 104.45 (*15.01)* | 109.92 *(14.50)* | 98.97 *(13.66)* |
| Ga | 0.006 (0.005*)* | 0.006 (0.005*)* | 0.006 (0.004*)* |
| Se | 0.90 *(0.11)* | 0.90 (*0.13)* | 0.90 (*0.08* |
| Rb * | 0.50 *(0.19)* | 0.55 *(0.18)* | 0.46 *(0.19)* |
| Sr | 0.69 *(0.48)* | 0.77 *(0.52)* | 0.61 *(0.43)* |
| Cd | 0.006 *(0.003)* | 0.006 (0.004*)* | 0.005 (0.003*)* |
| Tl | 0.0005 *(0.0002)* | 0.0005 *(0.0002)* | 0.0004 *(0.0002)* |
| Pb ** | 0.20 *(0.13)* | 0.24 *(0.15)* | 0.16 (*0.09)* |
| Th * | 0.005 *(0.004)* | 0.006 (0.005*)* | 0.004 *(0.003)* |
| U * | 0.006 *(0.007)* | 0.007 *(0.009)* | 0.004 *(0.005)* |

Notes: Students' t-test comparing cases to controls * $p < 0.1$ ** $p < 0.05$ *** $p < 0.01$

### 3.1.3 Arsenic speciation profiles and urinary cancer (Objective 2a)

The second objective was to determine whether there was an association between As species and cancer using statistical methods. To test this, a one-way between subjects MANOVA was performed on five dependent variables: %iAs %MMA, %DMA, PMI, and SMI. The independent variable was history of urinary cancer (yes and no). The combined As speciation variables were not significantly affected by history of urinary cancer, $F(1, 52) = 1.03$, $p = 0.4105$. PMI was marginally significant ($p = 0.065$), with the mean difference of 0.03 ($SE = 0.02$) between cases and controls, however the effect size was small, partial $\eta^2 = 0.06$ (95%CI: 0.01, 0.09).

### 3.1.4 Factors associated with arsenic speciation profiles (Objective 3a)

The third objective was to determine whether lifestyle and/or environmental factors were associated with As speciation. To do this, a MANCOVA was used to replicate the first model while adjusting for age, sex, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI. The combined covariates were significantly associated to the combined dependent variables, $F(16, 36) = 1.66$, $p = 0.0034$. The adjusted mean differences of cases and controls were not significantly different between cases and controls and are presented in Table 3.1.4. However, the statistically significant predictors of this model were family history of cancer ($p = 0.0080$), province of residence ($p = 0.0250$), and BMI ($p = 0.0491$).

**Table 3.1.4** Model A: MANOVA analysis comparing arsenic speciation between urinary cancer cases (n=27) and matched controls (n=27) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | Unadjusted Model<br>Wilks' Lamda p-value: 0.4105 | | | Adjusted Model[b]<br>Wilks' Lambda p-value: 0.0034 | | |
|---|---|---|---|---|---|---|
| | Mean difference *(SE)* | Effect size[a] | p-value | Mean difference *(SE)* | Effect size[a] | p-value |
| %iAs | -0.89 *(1.54)* | 0.01 | 0.565 | -0.58 *(1.58)* | 0.03 | 0.714 |
| %MMA | 1.32 *(0.85)* | 0.04 | 0.126 | 1.00 *(0.89)* | 0.01 | 0.270 |
| %DMA | 0.87 *(1.20)* | 0.01 | 0.473 | 0.59 *(1.32)* | <0.01 | 0.657 |
| PMI | 0.03 *(0.02)* | 0.06 | 0.065 | 0.03 *(0.02)* | 0.04 | 0.245 |
| SMI | -0.14 *(0.15)* | 0.02 | 0.350 | -0.12 *(0.16)* | 0.02 | 0.433 |

[a] Partial $\eta^2$ as a measure of effect size. [b] Model adjusted for age, sex, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI.

### 3.1.5 Metallomic profiles and cancer (Objective 2b)

Part two of the second objective sought to determine whether metallomic profiles were associated with urinary cancer. As such, eight metals that were associated with urinary

cancer[17] were included in the one-way MANOVA analysis (Model B): Zn, Co, Pb, Mn, Fe, Rb, Th, and U. The assumption of homogeneity of variance was assessed using Box's M test and was violated ($p < 0.05$). However, MANOVA is robust against this violation with a sample size >30 (126), therefore no adjustment to the significance criterion was performed. All other assumptions were tenable.

The results of the model indicated that the combined concentrations of these metals were statistically significantly different between cases and controls, $F(1, 52) = 2.45$, $p = 0.0270$. Specifically, mean Zn concentrations were statistically significantly higher among cases (mean difference = 10.94 $\mu$g/g, $SE$ = 3.83 $\mu$g/g, $p = 0.006$), with a large effect size, partial $\eta^2 = 0.13$. The concentration of Pb was statistically significantly higher among cases compared to controls, mean difference = 0.08 $\mu$g/g ($SE$ = 0.03 $\mu$g/g, $p = 0.019$), with a large effect size, partial $\eta^2 = 0.10$. Finally, the mean difference of Co, albeit small, (0.01 $\mu$g/g, $SE$ = 0.003 $\mu$g/g) was statistically significant, $p = 0.037$, with a moderate effect size, $\eta^2 = 0.08$. None of the other variables were statistically significant; mean differences and test statistics can be found in Table 3.1.5, which summarizes the findings of Model B.

### 3.1.6 Factors associated with metallomic profiles (Objective 3b)

To determine whether lifestyle or environmental factors were associated with metallomic profiles, a MANCOVA model was used to replicated Model B, while adjusting for age, sex, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI. The combined dependent variables were not significantly associated to the combined covariates, approximate $F(16, 36) = 0.95$, $p = 0.6108$. However,

[17] Based on the inclusion criteria of $p < 0.1$ from independent t-test between cases and controls (Table 3.1.2).

the mean differences of Zn and U between cases and controls were statistically significant ($p < 0.05$). After controlling for covariates, the mean difference and the effect size of Zn increased: 15.13 $\mu$g/g ($SE$ = 4.66 $\mu$g/g, $p$ = 0.003), $\eta^2$ = 0.23. Similarly, the mean difference and effect size of U increased, 0.005 $\mu$g/g $SE$ = 0.002 $\mu$g/g, $p$ = 0.049, $\eta^2$ = 0.10). The other variables were not statistically significantly different between cases and controls.

**Table 3.1.5** Model B: MANOVA analysis comparing metal concentrations ($\mu$g/g) between urinary cancer cases (n=27) and matched controls (n=27) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | Unadjusted Model Wilks' Lamda p-value: 0.0270 | | | Adjusted Model[b] Wilks' Lambda p-value: 0.6108 | | |
|---|---|---|---|---|---|---|
| | Mean difference *(SE)* | Effect size[a] | p-value | Mean difference *(SE)* | Effect size[a] | p-value |
| Mn | 0.21 *(0.12)* | 0.05 | 0.093 | 0.24 *(0.13)* | 0.08 | 0.083 |
| Fe | 12.61 *(7.10)* | 0.06 | 0.081 | 15.22 *(9.09)* | 0.07 | 0.103 |
| Co | 0.01 *(0.003)* | 0.08 | 0.037 | 0.01 *(0.004)* | 0.08 | 0.081 |
| Zn | 10.94 *(3.83)* | 0.13 | 0.006 | 15.13 *(4.66)* | 0.23 | 0.003 |
| Rb | 0.09 *(0.05)* | 0.06 | 0.073 | 0.10 *(0.06)* | 0.08 | 0.095 |
| Pb | 0.08 *(0.03)* | 0.10 | 0.019 | 0.08 *(0.04)* | 0.10 | 0.053 |
| Th | 0.002 *(0.001)* | 0.06 | 0.070 | 0.003 *(0.001)* | 0.10 | 0.053 |
| U | 0.003 *(0.002)* | 0.06 | 0.078 | 0.005 *(0.002)* | 0.10 | 0.049 |

[a] Partial $\eta^2$ as a measure of effect size. [b] Model adjusted for age, sex, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI.

## 3.2 Breast Cancer

### 3.2.1 Participant characteristics

A total of 90 samples were requested for the breast group (45 cases, 45 controls). Following the laboratory analysis, there were 41 matched cases-controls available for analysis.

Participant demographics are provided in Table 3.2.1. The mean age of the sample was 57.4 ($SD$ = 7.2); 51% of the participants in this group were between the ages of 39 and 59, and 49% were between 60 and 69 years. The majority of participants reported a family history of cancer (71% of cases, 76% of controls). Regarding province of residence, 42%

were from New Brunswick, 34% from Nova Scotia, 15% from Newfoundland, and 10% from Prince Edward Island. Data for water source was missing for many observations (34% of cases, 39% of controls). However, a plurality reported municipal water as their source (37% of cases, 34% of controls), followed by well water (24% for both cases and controls), and the remaining reported 'other' as primary water source (5% of cases, 2% of controls). Both breast cancer cases and healthy controls had large proportions of individuals with high levels of physical activity (39% of cases, and 44% of controls); 34% of cases and 37% of controls were moderately active, and 27% of cases and 19% of controls had low levels of physical activity. Only one breast cancer case (2%) and two healthy controls (5%) reported being current smokers, however 32% of cases and 49% of controls were former smokers; 66% of breast cancer cases and 46% of healthy participants were never smokers. Finally, regarding BMI, data were not available for 44% of cases, and 32% of controls. Among those for whom data were available, mean BMI was 29.8 ($SD$ = 9.4) for cases and 27.3 ($SD$ = 4.9) for controls. Fifteen percent of women with a history of breast cancer were obese compared to 19% of controls; 19% of cases and 27% of controls were overweight, and 22% of both groups were of normal or low BMI.

**Table 3.2.1** Participant Demographics for breast cancer cases (n=41) and matched controls (n=41) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=82 Count *(%)* | **Breast Cancer Cases** n=41 Count (%) | **Healthy Controls** n=41 Count (%) |
|---|---|---|---|
| **Age, mean *(SD)*** | 57.4 *(7.2)* | 57.4 *(7.2)* | 57.4 *(7.2)* |
| 39-59 | 42 *(51%)* | 21 *(51%)* | 21 *(51%)* |
| 60-69 | 40 *(49%)* | 20 *(49%)* | 20 *(49%)* |
| **Family History of Cancer** | 60 *(73%)* | 29 *(71%)* | 31 *(76%)* |
| **Province** | | | |
| NB | 34 *(41%)* | 17 *(42%)* | 17 *(42%)* |
| NL | 12 *(15%)* | 6 *(15%)* | 6 *(15%)* |
| NS | 28 *(34%)* | 14 *(34%)* | 14 *(34%)* |
| PEI | 8 *(10%)* | 4 *(10%)* | 4 *(10%)* |
| **Water Source** | | | |
| Municipal | 29 *(35%)* | 15 *(37%)* | 14 *(34%)* |
| Well | 20 *(20%)* | 10 *(24%)* | 10 *(24%)* |
| Other | 3 *(4%)* | 2 *(5%)* | 1 *(2%)* |
| Missing | 30 *(37%)* | 14 *(34%)* | 16 *(39%)* |
| **Physical Activity** | | | |
| Low | 19 *(23%)* | 11 *(27%)* | 8 *(19%)* |
| Moderate | 29 *(35%)* | 14 *(34%)* | 15 *(37%)* |
| High | 34 *(41%)* | 16 *(39%)* | 18 *(44%)* |
| **Smoking** | | | |
| Never | 46 *(56%)* | 27 *(66%)* | 19 *(46%)* |
| Former | 33 *(40%)* | 13 *(32%)* | 20 *(49%)* |
| Current | 3 *(4%)* | 1 *(2%)* | 2 *(5%)* |
| **BMI, mean *(SD)*** | 28.5 *(7.4)* | 29.8 *(9.4)* | 27.3 *(4.9)* |
| **BMI Categories** | | | |
| Low/Normal | 18 *(22%)* | 9 *(22%)* | 9 *(22%)* |
| Overweight | 19 *(23%)* | 8 *(19%)* | 11 *(27%)* |
| Obese | 14 *(17%)* | 6 *(15%)* | 8 *(19%)* |
| Unknown | 31 *(38%)* | 18 *(44%)* | 13 *(32%)* |

### 3.2.2 Toxicological characteristics (Objective 1)

The first objective was to characterize the As species and total metals measured in the toenail samples. The levels of As species were measured and compared between cases and controls. The mean values and standard deviations of %iAs, %MMA, %DMA, PMI, and SMI are presented in Table 3.2.2 for the overall group, breast cancer cases, and their healthy matched controls. The As speciation profiles were similar between cases and controls, however, those with a history of breast cancer had a greater variance in measured proportions of iAs, MMA, and DMA. As such, none of the As methylation variables were significantly different between groups.

**Table 3.2.2** Arsenic Speciation Profiles for breast cancer cases (n=41) and matched controls (n=41) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=82 Mean *(SD)* | **Breast Cancer Cases** n=41 Mean *(SD)* | **Healthy Controls** n=41 Mean *(SD)* |
|---|---|---|---|
| %iAs | 85.04 *(9.97)* | 84.91 *(*12.08*)* | 85.16 *(7.43)* |
| %MMA | 7.41 *(5.52)* | 7.57 *(6.88)* | 7.26 *(3.78)* |
| %DMA | 7.55 *(6.19)* | 7.51 *(6.69)* | 7.58 *(5.72)* |
| PMI | 0.10 *(0.11)* | 0.11 *(0.15)* | 0.09 *(0.05)* |
| SMI | 1.15 *(0.82)* | 1.14 *(0.79)* | 1.17 *(0.85)* |

The mean concentrations ($\mu$g/g) of the 18 detected metals (As, V, Cr, Mn, Fe, Co, Ni, Co, Cu, Zn, Ga, Se, Rb, Sr, Cd, Tl, Pb, Th, and U) are presented in Table 3.2.3 for the overall group, the breast cancer cases, and the healthy controls. The mean concentrations of the cases and controls were compared using the Student's *t*-test, and four metals were flagged to be included Model B, based on the inclusion criteria of $p < 0.1$. This test indicated that Mn, Zn, Pb, and Th concentrations were associated with breast cancer history. Specifically, higher concentrations of Zn ($p = 0.0412$) and Pb ($p = 0.0385$) were

significantly associated with breast cancer. Additionally, the concentrations of Mn and Th were higher among cases compared to controls ($p < 0.1$). No other metals displayed statistically significantly associations with breast cancer history.

**Table 3.2.3** Metallomic Profiles ($\mu$g/g) for breast cancer cases (n=41) and matched controls (n=41) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=82 Mean *(SD)* | **Breast Cancer Cases** n=41 Mean *(SD)* | **Healthy Controls** n=41 Mean *(SD)* |
|---|---|---|---|
| As | 0.078 *(0.028)* | 0.077 *(0.028)* | 0.079 *(0.028)* |
| V | 0.023 *(0.016)* | 0.024 (0.020) | 0.021 *(0.009)* |
| Cr | 0.34 *(0.22)* | 0.32 *(0.19)* | 0.37 *(0.25)* |
| Mn * | 0.61 (*0.49)* | 0.70 *(0.60)* | 0.52 *(0.34)* |
| Fe | 29.69 *(19.32)* | 31.62 *(24.24)* | 27.76 (*12.66)* |
| Co | 0.019 *(0.011)* | 0.019 *(0.011)* | 0.018 (0.011) |
| Ni | 11.04 *(13.72)* | 11.36 *(15.65)* | 10.72 *(11.67)* |
| Cu | 4.24 *(1.21)* | 4.37 *(1.35)* | 4.12 *(1.06)* |
| Zn ** | 108.50 (*14.91)* | 111.85 *(16.76)* | 105.15 *(12.10)* |
| Ga | 0.006 *(0.004)* | 0.006 *(0.005)* | 0.005 *(0.003)* |
| Se | 0.93 *(0.11)* | 0.94 (*0.12)* | 0.93 (*0.11)* |
| Rb | 0.42 *(0.17)* | 0.43 *(0.16)* | 0.41 *(0.19)* |
| Sr | 0.65 *(0.54)* | 0.69 *(0.52)* | 0.62 *(0.55)* |
| Cd | 0.009 *(0.008)* | 0.010 *(0.010)* | 0.007 *(0.006)* |
| Tl | 0.0004 *(0.0001)* | 0.0004 *(0.0002)* | 0.0005 *(0.0001)* |
| Pb ** | 0.16 *(0.10)* | 0.18 *(0.11)* | 0.13 (*0.07)* |
| Th * | 0.006 *(0.004)* | 0.006 *(0.005)* | 0.005 *(0.003)* |
| U | 0.005 *(0.006)* | 0.007 *(0.008)* | 0.005 *(0.004)* |

Notes: Students' t-test comparing cases to controls * $p < 0.1$ ** $p < 0.05$

### 3.2.3 Arsenic speciation profiles and cancer (Objective 2a)

Objective 2a sought to determine whether As species were statistically associated with cancer. To test this, a one-way between subjects MANOVA was performed on the five As methylation variables: %iAs %MMA, %DMA, PMI, and SMI. The independent variable was history of breast cancer (yes and no). The effect of history of breast cancer on the combined As speciation variables was statistically significant, $F(1, 80) = 2.58$, $p = 0.033$.

In particular, %MMA was statistically significantly lower among breast cancer cases compared to controls (mean difference = -1.17%, *SE* = 0.55%, $p = 0.037$). The effect size was moderate, as evaluated by partial eta-squared ($\eta^2 = 0.05$). None of the other measures of As speciation were statistically significant. However, in general, breast cancer cases had lower %DMA and higher %iAs, which was reflected in the PMI and SMI.

### 3.2.4 Factors associated with arsenic speciation profiles (Objective 3a)

The third objective was to determine whether lifestyle and environmental factors were associated with As speciation. A MANCOVA was used to control for the effects of age, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI (Table 3.2.4). The combined effect of the covariates was not statistically significantly associated with the dependent variables, $F(16, 65) = 1.17$, $p = 0.1727$. However, the mean difference in %MMA between cases and controls increased to -1.31 (*SE* = 0.56) and was statistically significant ($p = 0.023$). The effect size was moderate, as evaluated by partial eta-squared ($\eta^2 = 0.077$)

**Table 3.2.4** Model A: MANOVA analysis comparing arsenic speciation between breast cancer cases (n=41) and matched controls (n=41) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | Unadjusted Model Wilks' Lamda p-value: 0.033 | | | Adjusted Model[b] Wilks' Lambda p-value: 0.1727 | | |
|---|---|---|---|---|---|---|
| | Mean difference *(SE)* | Effect size[a] | p-value | Mean difference *(SE)* | Effect size[a] | p-value |
| %iAs | 1.41 *(1.36)* | 0.01 | 0.303 | 1.84 *(1.39)* | 0.03 | 0.189 |
| %MMA | -1.17 *(0.55)* | 0.05 | 0.037 | -1.31 *(0.56)* | 0.08 | 0.023 |
| %DMA | -1.23 *(0.78)* | 0.03 | 0.120 | -1.13 *(0.81)* | 0.03 | 0.165 |
| PMI | -0.002 *(0.010)* | >0.01 | 0.814 | -0.01 *(0.01)* | >0.01 | 0.597 |
| SMI | 0.13 *(0.12)* | 0.01 | 0.289 | 0.12 *(0.13)* | 0.01 | 0.372 |

[a] Partial $\eta^2$ as a measure of effect size. [b] Model adjusted for age, history of cancer, province of residence, water source, physical activity, smoking status, and BMI.

### 3.2.5 Metallomic profiles and cancer (Objective 2b)

This objective sought to determine whether there was an association between metallomic profiles and cancer. In Model B, the effect of cancer on select metal concentrations was tested using a MANOVA model (Table 3.2.5). The model showed the combined metal concentrations did not statistically significantly differ between those with and without a history of breast cancer, $F(1, 80) = 2.29$, $p = 0.0671$. However, the mean concentrations of Zn and Pb were higher in cases compared to controls. Specifically, the mean differences for Zn and Pb were 6.70 $\mu$g/g ($SE$ = 3.23 $\mu$g/g, $p = 0.041$) and 0.04 $\mu$g/g ($SE$ = 0.02 $\mu$g/g, $p = 0.038$), respectively.

### 3.2.6 Factors associated with metallomic profiles (Objective 3b)

The third objective was to determine if the covariates were associated with the metallomic profiles. To test this, Model B was replicated using a MANCOVA, with adjustment for age, family history of cancer, province, water source, physical activity, smoking, and BMI. This model indicated the combined covariates were not statistically significantly associated with the combined dependent variables, $F(16, 65) = 0.91$, $p = 0.6720$.

**Table 3.2.5** Model B: MANOVA analysis comparing metal concentrations between breast cancer cases (n=41) and matched controls (n=41) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | Unadjusted Model<br>Wilks' Lamda p-value: 0.0671 | | | Adjusted Model[b]<br>Wilks' Lambda p-value: 0.6720 | | |
|---|---|---|---|---|---|---|
| | Mean difference *(SE)* | Effect size[a] | p-value | Mean difference *(SE)* | Effect size[a] | p-value |
| Mn | 0.18 *(0.11)* | 0.03 | 0.093 | 0.18 *(0.11)* | 0.04 | 0.106 |
| Zn | 6.70 *(3.23)* | 0.05 | 0.041 | 6.90 *(3.54)* | 0.05 | 0.055 |
| Pb | 0.04 *(0.02)* | 0.05 | 0.038 | 0.04 *(0.02)* | 0.05 | 0.059 |
| Th | 0.002 *(0.001)* | 0.03 | 0.090 | 0.002 *(0.001)* | 0.06 | 0.040 |

[a] Partial $\eta^2$ as a measure of effect size. [b] Model adjusted for age, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI.

## 3.3 Cervical Cancer

### 3.3.1 Participant characteristics

A total of 41 matched cervical cancer case-control samples were available for statistical analysis after being measured in the HERC laboratory, totalling 82 samples.

The participant characteristics and demographics for the cervical cancer group are provided in Table 3.3.1. The mean age of participants was 51.8 (*SD* = 8.1); 80% of participants were between 36 and 59 years, and the remaining 20% were between ages 60-68 years. Healthy controls reported a higher proportion of family history of cancer (78%) compared to cervical cancer cases (62.5%). Most of the participants (39% of cases, 37% of controls) were from New Brunswick, 32% of both cases and controls were from Newfoundland, 27% of cases and 32% of controls were from Nova Scotia; only one cervical cancer case (2%) was from Prince Edward Island. A plurality of both cases and controls sourced their water from the municipal system (46% and 44%, respectively), followed by private wells (22% of cases, 24% of controls), and one (2%) case three (7%) healthy controls had other water sources. Water source was unknown for 29% of cases and 24% of controls. Many participants reported high levels of physical activity: 49% of cases and 46% of controls were highly active, 39% of cases and 37% of controls were moderately active, while 12% of cases and 17% of controls were ranked low on the physical activity scale. Smoking was associated with history of cancer ($p < 0.1$). A higher proportion of controls reported having never smoked (63% of controls vs. 39% of cases), 29% of controls were former smoker (vs. 44% of cases), and only three controls (7%) reported being current smokers (vs. 17% of cases). BMI information was not available for 41% of cases and 44% of controls, but among those for whom information was available, mean BMI was comparable between cases and controls. Seventeen percent of both cases and controls were

low/normal weight, 27% of cases and 24% of controls were overweight, and 15% of both cases and controls were obese.

**Table 3.3.1** Participant Demographics for cervical cancer cases (n=41) and matched controls (n=41) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=82 Count *(%)* | **Cervical Cancer Cases** n=41 Count (%) | **Healthy Controls** n=41 Count (%) |
|---|---|---|---|
| **Age, mean *(SD)*** | 51.8 *(8.1)* | 51.8 *(8.2)* | 51.7 *(8.2)* |
| 36-59 | 66 *(80%)* | 33 *(80%)* | 33 *(80%)* |
| 60-68 | 16 *(20%)* | 8 *(20%)* | 8 *(20%)* |
| **Family History of Cancer** | 57 *(70%)* | 25 *(62.5%)* | 32 *(78%)* |
| **Province** | | | |
| NB | 31 *(38%)* | 16 *(39%)* | 15 *(37%)* |
| NL | *26 (32%)* | 13 *(32%)* | 13 *(32%)* |
| NS | *24 (29%)* | 11 *(27%)* | 13 *(32%)* |
| PEI | 1 *(1%)* | 1 *(2%)* | 0 *(0%)* |
| **Water Source** | | | |
| Municipal | 37 *(45%)* | 19 *(46%)* | 18 *(44%)* |
| Well | 19 *(23%)* | 9 *(22%)* | 10 *(24%)* |
| Other | 4 *(5%)* | 1 *(2%)* | 3 *(7%)* |
| Missing | *22 (27%)* | 12 *(29%)* | 10 *(24%)* |
| **Physical Activity** | | | |
| Low | 12 *(15%)* | *5 (12%)* | *7 (17%)* |
| Moderate | 31 *(38%)* | 16 *(39%)* | 15 *(37%)* |
| High | 39 *(47%)* | 20 *(49%)* | 19 *(46%)* |
| **Smoking*** | | | |
| Never | 42 *(51%)* | 16 *(39%)* | 26 *(63%)* |
| Former | 30 *(37%)* | 18 *(44%)* | 12 *(29%)* |
| Current | 10 *(12%)* | *7 (17%)* | 3 *(7%)* |
| **BMI, mean *(SD)*** | 28.7 *(6.2)* | 28.9 *(5.9)* | 28.5 *(6.5)* |
| **BMI Categories** | | | |
| Low/Normal | 14 *(17%)* | *7 (17%)* | *7 (17%)* |
| Overweight | 21 *(26%)* | 11 *(27%)* | 10 *(24%)* |
| Obese | 12 *(15%)* | *6 (15%)* | *6 (15%)* |
| Unknown | *25 (43%)* | 17 *(41%)* | 18 *(44%)* |

### 3.3.2 Toxicological characteristics (Objective 1)

The first objective was to characterize the As species and total metals measured in the toenail samples. Five different As methylation variables were calculated and compared between cases and controls. The mean values and standard deviations of %iAs, %MMA, %DMA, PMI, and SMI are presented in Table 3.3.2 for the overall group, cervical cancer cases, and their healthy matched controls.

The predominant As species measured in the toenail samples of both cases and controls was iAs, followed by smaller proportions of MMA and DMA. However, the speciation profiles differed between cases and controls. There were no statistically significant differences between the As methylation variables between cases and controls.

**Table 3.3.2** Arsenic Speciation Profiles for cervical cancer cases (n=41) and matched controls (n=41) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=82 Mean *(SD)* | **Cervical Cancer Cases** n=41 Mean *(SD)* | **Healthy Controls** n=41 Mean *(SD)* |
|---|---|---|---|
| %iAs | 84.84 *(9.65)* | 83.28 *(9.50)* | 86.40 *(9.67)* |
| %MMA | 8.61 *(7.41)* | 9.40 *(7.40)* | 7.81 *(7.42)* |
| %DMA | 6.56 *(4.74)* | 7.32 *(4.89)* | 5.79 *(4.52)* |
| PMI | 0.11 *(0.15)* | 0.13 *(0.15)* | 0.10 *(0.15)* |
| SMI | 1.00 *(0.96)* | 1.08 *(1.11)* | 0.92 *(0.78)* |

The mean concentrations ($\mu$g/g) of the 18 detected metals (As, V, Cr, Mn, Fe, Co, Ni, Co, Cu, Zn, Ga, Se, Rb, Sr, Cd, Tl, Pb, Th, and U) are presented in Table 3.3.3 for the overall group, the cervical cancer cases, and the healthy controls. The mean metal concentrations of the cases and controls were compared using the Student's *t*-test; three were flagged to be included Model B, based on the inclusion criteria of $p < 0.1$. This test

showed three metals (As, Fe, and Tl) were associated with cervical cancer history. Specifically, the concentration of As was lower ($p$ = 0.0805) among cases compared to controls. Similarly, the concentration of Tl was significantly lower among cases compared to controls ($p$ = 0.0228). On the other hand, higher concentrations of Fe we observed in among cases compared to controls ($p$ = 0.0291).

**Table 3.3.3** Metallomic Profiles ($\mu$g/g) for cervical cancer cases (n=41) and matched controls (n=41) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=82 Mean *(SD)* | **Cervical Cancer Cases** n=41 Mean *(SD)* | **Healthy Controls** n=41 Mean *(SD)* |
|---|---|---|---|
| As * | 0.071 *(0.021)* | 0.066 *(0.018)* | 0.075 *(0.024)* |
| V | 0.020 *(0.013)* | 0.020 *(0.015)* | 0.020 *(0.012)* |
| Cr | 0.35 *(0.22)* | 0.36 *(0.24)* | 0.34 *(0.19)* |
| Mn | 0.54 (*0.36)* | 0.56 *(0.38)* | 0.52 *(0.35)* |
| Fe ** | 29.16 *(18.70)* | 33.64 *(22.34)* | 24.68 (*12.97)* |
| Co | 0.016 *(0.011)* | 0.018 *(0.013)* | 0.015 *(0.008)* |
| Ni | 12.32 *(18.65)* | 13.16 *(21.78)* | 11.48 *(15.11)* |
| Cu | 4.18 *(0.87)* | 4.12 *(0.80)* | 4.24 *(0.94)* |
| Zn | 106.33 (*14.08)* | 108.42 *(14.43)* | 104.24 *(13.57)* |
| Ga | 0.007 (0.005*)* | 0.007 (0.005*)* | 0.006 (0.004*)* |
| Se | 0.94 *(0.11)* | 0.95 (*0.11)* | 0.93 (*0.10)* |
| Rb | 0.44 *(0.17)* | 0.44 *(0.16)* | 0.44 *(0.18)* |
| Sr | 0.59 *(0.49)* | 0.67 *(0.57)* | 0.51 *(0.40)* |
| Cd | 0.008 *(0.006)* | 0.007 (0.006*)* | 0.008 (0.006*)* |
| Tl ** | 0.0005 *(0.0002)* | 0.0004 *(0.0002)* | 0.0005 *(0.0002)* |
| Pb | 0.17 *(0.10)* | 0.18 *(0.14)* | 0.16 (*0.08)* |
| Th | 0.005 *(0.004)* | 0.005 (0.004*)* | 0.004 *(0.003)* |
| U | *0.004 (0.006)* | 0.005 *(0.007)* | 0.004 *(0.003)* |

Notes: Students' t-test comparing cases to controls * $p < 0.1$ ** $p < 0.05$

### 3.3.3 Arsenic speciation profiles and cancer (Objective 2a)

A one-way between subjects MANOVA was performed on five dependent variables: %iAs %MMA, %DMA, PMI, and SMI to determine whether there was an association between As speciation and a history of cancer (yes/no). Model A (Table 3.3.2) indicated that the

combined As speciation variables were statistically significant different between cases and controls, $F(1, 80) = 2.79$, $p = 0.0228$. Statistically significant mean differences were observed for %MMA and %iAs. Specifically, %MMA was higher in cervical cancer cases compared to controls (mean difference = 1.58, $SE = 0.54$, $p = 0.004$) and %iAs was lower in cases than controls (mean difference = -3.04, $SE = 1.07$, $p = 0.006$). Both speciation measures had moderate effect sizes ($\eta^2_{\%MMA} = 0.1$, $\eta^2_{\%iAs} = 0.09$).

### 3.3.4 Factors associated with arsenic speciation profiles (Objective 3a)

The third objective was to determine whether lifestyle and environmental factors were associated with As speciation. To do this, a MANCOVA was used to replicate Model A, while adjusting for several covariates (age, family history of cancer, province of residence, water source, physical activity, smoking status, BMI). The adjusted model indicated that the combined covariates were not statistically significant predictors of the combined As speciation variables, $F(16, 64) = 1.15$, $p = 0.1976$. However, family history of cancer was a significant predictor of the As speciation variables, $p = 0.0276$. In addition, the mean difference in %MMA between cases and controls increased in the adjusted model to 1.80 ($SE = 0.56$, $p = 0.002$) with an increased effect size, $\eta^2 = 0.14$. Furthermore, the mean difference and effect size of %iAs also increased in magnitude to -3.43 ($SE = 1.16$, $p = 0.004$, $\eta^2 = 0.12$). In contrast with the unadjusted model, the mean difference in PMI between the groups was statistically significant (0.02, $SE = 0.01$, $p = 0.029$). The effect size for PMI was moderate, $\eta^2 = 0.07$. %DMA and SMI were not statistically significantly different between the cases and controls.

**Table 3.3.4** Model A: MANOVA comparing arsenic speciation between cervical cancer cases (n=41) and matched controls (n=41) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | Unadjusted Model Wilks' Lamda p-value: 0.0228 | | | Adjusted Model[b] Wilks' Lambda p-value: 0.1976 | | |
|---|---|---|---|---|---|---|
| | Mean difference *(SE)* | Effect size[a] | p-value | Mean difference (SE) | Effect size[a] | p-value |
| %iAs | -3.04 *(1.07)* | 0.09 | 0.006 | -3.43 *(1.16)* | 0.12 | 0.004 |
| %MMA | 1.58 *(0.54)* | 0.10 | 0.004 | 1.80 *(0.56)* | 0.14 | 0.002 |
| %DMA | -0.05 *(0.71)* | >0.01 | 0.948 | -0.03 *(0.73)* | <0.01 | 0.968 |
| PMI | 0.02 *(0.01)* | 0.04 | 0.089 | 0.02 *(0.01)* | 0.07 | 0.029 |
| SMI | -0.16 *(0.12)* | 0.02 | 0.210 | -0.23 *(0.13)* | 0.05 | 0.082 |

[a] Partial $\eta^2$ as a measure of effect size. [b] Model adjusted for age, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI.

### 3.3.5 Metallomic profiles and cancer (Objective 2b)

This objective sought to determine whether there was an association between metallomic profiles and cancer. To test this, a MANOVA was used to compare the dependent variables, the metals, As, Fe, and Tl, between cervical cancer cases and controls. The metals selected were included in Model B as defined by inclusion of variables with $p < 0.1$ in *a priori* independent t-tests. Box's M test was used to assess homogeneity of variance, and was violated ($p < 0.05$). To investigate this violation, Levene's test was used to assess univariate homogeneity of variance for each independent variable. The results of Levene's test indicated that Fe was the source of this violation. However, given the sample size is greater than 30, the MANOVA is robust against this violations to this assumption (126), and as such, the significance criterion was not adjusted. All other test assumptions were found tenable.

The MANOVA showed that the combined metals were statistically significant between those with a history of cervical cancer and those without, $F(1, 80) = 5.00$, $p = 0.0032$. Fe concentrations were statistically significantly higher in cases (mean difference

= 8.96 $\mu$g/g, *SE* = 4.03 $\mu$g/g, $p$ = 0.029) compared to controls, and Tl concentrations were statistically significantly lower (mean difference = -0.0001 $\mu$g/g, *SE* = 0.00004 $\mu$g/g, $p$ = 0.023). Both Fe and Tl had a moderate effect size of $\eta^2$= 0.06. Total As concentrations were lower in cervical cancer cases, but the mean difference was not statistically significant (Table 3.3.5).

### 3.3.6 Factors associated with metallomic profiles (Objective 3b)

To determine whether environmental and lifestyle factors affect the metallomic profiles, a MANCOVA was used to replicate Model B, while adjusting for covariates of interest: family history of cancer, province of residence, water source, physical activity, smoking, and BMI (Table 3.3.5). In this adjusted analysis, the results indicated that the covariates had a statistically significant effect on total metals concentrations, $F(16, 64) = 1.46$, $p$ = 0.0395. In particular, the covariates, province of residence ($p$ = 0.0395) and physical activity ($p$ = 0.0429), were significant predictors of the profile of metallomes. Additionally, the mean difference in Fe between groups decreased and was not longer significantly different (Table 3.3.5). The mean difference for Tl remained unchanged, and was statistically significantly, lower in cases compared to controls.

**Table 3.3.5** Model B: MANOVA comparing metal concentrations between cervical cancer cases (n=41) and matched controls (n=41) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | Unadjusted Model Wilks' Lamda p-value: 0.0032 | | | Adjusted Model[b] Wilks' Lambda p-value: 0.0395 | | |
|---|---|---|---|---|---|---|
| | Mean difference *(SE)* | Effect size[a] | p-value | Mean difference *(SE)* | Effect size[a] | p-value |
| As | -0.01 *(0.005)* | 0.04 | 0.081 | -0.01 *(0.005)* | 0.03 | 0.181 |
| Fe | 8.96 *(4.03)* | 0.06 | 0.029 | 8.10 *(4.27)* | 0.05 | 0.063 |
| Tl | -0.0001 *(0.00004)* | 0.06 | 0.023 | -0.0001 *(0.00004)* | 0.08 | 0.022 |

[a] Partial $\eta^2$ as a measure of effect size. [b] Model adjusted for age, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI.

## 3.4 Prostate Cancer

### 3.4.1 Participant characteristics

The participant demographics for the prostate cancer cases and matched controls (total n=88) are available in Table 3.4.1. The mean age for this group was 60.9 (*SD* = 4.8); 41% of participants were between the ages of 50 and 59 years, and 59% were between 60 and 68 years old. The majority (67% of cases, 69% of controls) had a family history of cancer. The largest proportion of participants were from New Brunswick (43%), followed by Newfoundland (32%), Nova Scotia (23%), and then Prince Edward Island (2%). Municipal water was the most common water source for both cases (27%) and controls (41%), and well water was the second most common with 27% of cases and 18% of controls reporting a private well as their domestic water supply. Two percent (2%) of controls reported other as their water source. There were no water source data available for 45% of cases and 39% of controls. Both cases and controls were physically active: 48% of cases and 57% of controls were highly active, 32% of cases, and 18% of controls were moderately active, and 20% of cases and 25% of controls had low-levels of physical activity. This engagement in healthy behaviours continued in smoking patterns; most participants had never smoked

(48% of cases, 41% of controls), or were former smokers (41% of cases, 52% of controls), while few were current smokers (11% of cases, 7% of controls). Among those for whom BMI data were available (59% of cases, 76% of controls), the mean BMI was 29.4 (*SD* = 4.7). Only 9% of cases, and 5% of controls were of normal or low BMI, while 30% of cases and 36% of controls reported being overweight, and 20% of cases and 25% of controls were obese.

**Table 3.4.1** Participant Demographics for prostate cancer cases (n=44) and matched controls (n=44) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=88 Count *(%)* | **Prostate Cancer Cases** n=44 Count (%) | **Healthy Controls** n=44 Count (%) |
|---|---|---|---|
| **Age, mean *(SD)*** | 60.9 *(4.8)* | 60.9 *(4.8)* | 60.9 *(4.8)* |
| 50-59 | 36 *(41%)* | 18 *(41%)* | 18 *(41%)* |
| 60-68 | 52 *(59%)* | 26 *(59%)* | 26 *(59%)* |
| **Family History of Cancer** | 57 *(68%)* | 28 *(67%)* | 29 *(69%)* |
| **Province** | | | |
| NB | 38 *(43%)* | 19 *(43%)* | 19 *(43%)* |
| NFLD | 28 *(32%)* | 14 *(32%)* | 14 *(32%)* |
| NS | 20 *(23%)* | 10 *(23%)* | 10 *(23%)* |
| PEI | 2 *(2%)* | 2 *(2%)* | 1 *(2%)* |
| **Water Source** | | | |
| Municipal | 30 *(34%)* | 12 *(27%)* | 18 *(41%)* |
| Well | 20 *(23%)* | 12 *(27%)* | 8 *(18%)* |
| Other | 1 *(1%)* | 0 *(0%)* | 1 *(2%)* |
| Missing | *37 (42%)* | 20 *(45%)* | 17 *(39%)* |
| **Physical Activity** | | | |
| Low | 20 *(23%)* | *9 (20%)* | 11 *(25%)* |
| Moderate | 22 *(25%)* | 14 *(32%)* | 8 *(18%)* |
| High | 46 *(52%)* | 21 *(48%)* | 25 *(57%)* |
| **Smoking** | | | |
| Never | 39 *(44%)* | 21 *(48%)* | 18 *(41%)* |
| Former | 21 *(47%)* | 18 *(41%)* | 23 *(52%)* |
| Current | 8 *(9%)* | 5 *(11%)* | 3 *(7%)* |
| **BMI, mean *(SD)*** | 29.4 *(4.7)* | 29.1 *(4.6)* | 29.6 *(4.9)* |
| **BMI Categories** | | | |
| Low/Normal | 6 *(7%)* | 4 *(9%)* | 2 *(5%)* |
| Overweight | *29 (33%)* | 13 *(30%)* | 16 *(36%)* |
| Obese | 20 *(23%)* | *9 (20%)* | 11 *(25%)* |
| Unknown | *33 (37%)* | 18 *(41%)* | 15 *(24%)* |

### 3.4.2 Toxicological characteristics (Objective 1)

The first objective was to characterize the As species and total metals measured in the toenail samples. The proportions of iAs, MMA, and DMA, and the ratio variables, PMI and SMI, were calculated and compared between cases and controls. The mean values and standard deviations of these five variables are presented in Table 3.4.2 for the overall group, prostate cancer cases, and their healthy matched controls. The proportions of iAs and MMA were the same in both cases and controls, while the %DMA and SMI were marginally higher among prostate cancer cases compared to controls.

**Table 3.4.2** Arsenic Speciation Profiles for prostate cancer cases (n=44) and matched controls (n=44) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=88 Mean *(SD)* | **Prostate Cancer Cases** n=44 Mean *(SD)* | **Healthy Controls** n=44 Mean *(SD)* |
|---|---|---|---|
| %iAs | 85.14 *(7.54)* | 84.85 *(7.53)* | 85.42 *(7.64)* |
| %MMA | 7.46 *(3.96)* | 7.09 *(3.16)* | 7.83 *(4.62)* |
| %DMA | 7.40 *(5.17)* | 8.06 *(6.01)* | 6.75 *(4.13)* |
| PMI | 0.09 *(0.05)* | 0.09 *(0.04)* | 0.10 *(0.07)* |
| SMI | 1.17 *(1.05)* | 1.34 *(1.32)* | 0.99 (*0.63)* |

The mean concentrations ($\mu$g/g) of the 18 detected metals (As, V, Cr, Mn, Fe, Co, Ni, Co, Cu, Zn, Ga, Se, Rb, Sr, Cd, Tl, Pb, Th, and U) are presented in Table 3.4.3 for the overall group, the prostate cancer cases, and the healthy controls. The mean concentrations of the cases and controls were compared using the Student's *t*-test and six metals (V, Mn, Fe, Zn, Se, Pb) were flagged to be included Model B, based on the inclusion criteria of $p < 0.1$. In particular, prostate cancer cases had statistically significantly higher concentrations of Zn ($p = 0.0116$) and Se ($p = 0.0116$). The concentrations of V, Fe, and Pb were also higher among cases compared to controls ($p < 0.1$).

**Table 3.4.3** Metallomic Profiles ($\mu$g/g) for prostate cancer cases (n=44) and matched controls (n=44) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=88 Mean *(SD)* | **Prostate Cancer Cases** n=44 Mean *(SD)* | **Healthy Controls** n=44 Mean *(SD)* |
|---|---|---|---|
| As | 0.069 *(0.027)* | 0.069 *(0.028)* | 0.069 *(0.026)* |
| V * | 0.018 *(0.012)* | 0.020 *(0.015)* | 0.016 *(0.009)* |
| Cr | 0.26 *(0.15)* | 0.25 *(0.16)* | 0.27 *(0.13)* |
| Mn * | 0.55 (*0.48)* | 0.65 *(0.58)* | 0.46 *(0.33)* |
| Fe * | 28.34 *(21.71)* | 32.60 *(28.24)* | 24.08 (*10.90)* |
| Co | 0.015 *(0.009)* | 0.0015 *(0.010)* | 0.0014 *(0.009)* |
| Ni | 12.21 *(17.33)* | 13.46 *(20.17)* | 10.97 *(14.05)* |
| Cu | 4.27 *(1.09)* | 4.27 *(1.15)* | 4.16 *(1.03)* |
| Zn ** | 107.14 (*14.42)* | 110.99 *(14.56)* | 103.30 *(13.57)* |
| Ga | 0.006 (0.003*)* | 0.005 (0.003*)* | 0.006 (0.003*)* |
| Se ** | 0.93 *(0.11)* | 0.96 (*0.12)* | 0.90 (*0.10)* |
| Rb | 0.49 *(0.21)* | 0.47 *(0.20)* | 0.50 *(0.22)* |
| Sr | 0.63 *(0.48)* | 0.67 *(0.47)* | 0.60 *(0.49)* |
| Cd | 0.009 *(0.008)* | 0.010 (0.009*)* | 0.008 (0.006*)* |
| Tl | 0.0004 *(0.0002)* | 0.0004 *(0.0002)* | 0.0005 *(0.0002)* |
| Pb * | 0.18 *(0.13)* | 0.21 *(0.17)* | 0.16 (*0.08)* |
| Th | 0.004 *(0.003)* | 0.004 (0.002*)* | 0.005 *(0.004)* |
| U | 0.004 *(0.004)* | 0.004 *(0.005)* | 0.003 *(0.003)* |

Notes: Students' t-test comparing cases to controls * $p < 0.1$ ** $p < 0.05$

### 3.4.3 Arsenic speciation profiles and cancer (Objective 2a)

The second objective was to determine if As speciation profiles differed between those with a history of cancer and those without. To test this, a MANOVA was used to assess the effect of history of prostate cancer on five As speciation variables, %iAs, %MMA, %DMA, PMI, and SMI (Table 3.4.2). A violation to the homogeneity of variance assumption was noted as Box's M, $p < 0.05$. However, given to the sample size (>30), the recommendation is that MANOVA is robust against this violation (126), therefore no adjustment was made to the significance criterion. All other assumptions were tenable.

Model A indicated there was not a statistically significant effect of prostate cancer history on As speciation profile variables, $F(1, 86) = 1.29$, $p = 0.2750$. However, it is worth

noting that the SMI was statistically significantly higher among cases (mean difference = 0.23, $SE = 0.10$, $p = 0.031$) compared to controls. The effect size was moderate, $\eta^2 = 0.05$. None of the other measures were statistically significant, but in general, %MMA and PMI were lower, and %DMA and SMI were higher among cases compared to controls.

### 3.4.4 Factors associated with metallomic profiles (Objective 3a)

To test the third objective, whether lifestyle and environmental factors were associated with As speciation, Model A was replicated using a MANCOVA. This model adjusted for age, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI. The results showed these covariates had a statistically significant effect on the combined As speciation measures, $F(16, 67) = 1.35$, $p = 0.0373$ (Table 3.4.4). The statistically significant predictors of this model were province of residence ($p = 0.0004$) and smoking status ($p = 0.0095$). However, after adjustment, none of the As speciation variables were statistically significantly different between prostate cancer cases and controls.

**Table 3.4.4** Model A: MANOVA comparing arsenic speciation between prostate cancer cases (n=44) and matched controls (n=44) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | Unadjusted Model Wilks' Lamda p-value: 0.2750 | | | Adjusted Model Wilks' Lambda p-value: 0.0373 | | |
|---|---|---|---|---|---|---|
| | Mean difference *(SE)* | Effect size[a] | p-value | Mean difference *(SE)* | Effect size[a] | p-value |
| %iAs | -0.02 *(1.02)* | >0.01 | 0.986 | 0.08 *(1.17)* | <0.001 | 0.944 |
| %MMA | -0.17 *(0.52)* | >0.01 | 0.746 | -0.52 *(0.59)* | 0.01 | 0.384 |
| %DMA | 0.53 *(0.70)* | 0.01 | 0.450 | 0.64 *(0.77)* | 0.01 | 0.406 |
| PMI | -0.02 *(0.01)* | 0.03 | 0.094 | -0.01 *(0.01)* | 0.03 | 0.187 |
| SMI | 0.23 *(0.10)* | 0.05 | 0.031 | 0.23 *(0.12)* | 0.05 | 0.058 |

[a] Partial $\eta^2$ as a measure of effect size. [b] Model adjusted for age, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI.

### 3.4.5 Metallomic profiles and cancer (Objective 2b)

The second component of Objective 2 was to determine whether metallomic profiles differed between cancer cases and controls. This was tested using a MANOVA (Model B), which assessed the effect of prostate cancer history on select metal concentrations, V, Mn, Fe, Zn, Se, and Pb (Table 3.4.5). The homogeneity of variance assumption was violated, as assessed using Box's M test ($p < 0.05$)[18]. However, MANOVA is said to be robust against this violation when the sample size is >30 (126), thus, no adjustment to the criterion for significance of the test was made to account for this violation. All other assumptions were satisfied.

The unadjusted MANOVA showed there was statistically significant difference in the combine total metals concentrations between those with a prostate cancer history and those without, $F(1, 81) = 3.22$, $p = 0.0069$. Specifically, the mean differences in Zn and Se

[18] Further, univariate investigation of this violation was performed using Levene's test for equal variances; the variables in violation were V, Mn, Fe, and Pb. The null hypothesis (that the variances between the cases and controls are equal) could not be rejected for Zn and Se.

concentrations were statistically significant ($p$ = 0.012 for both Zn and Se). In particular, prostate cancer cases had higher Zn concentrations compared to controls (mean difference = 7.68 $\mu$g/g, $SE$ = 2.98 $\mu$g/g). Similarly, Se concentrations were also higher among cases, albeit the mean difference was smaller, 0.06 $\mu$g/g ($SE$ = 0.02 $\mu$g/g). Both Zn and Se had an effect size of $\eta^2$ = 0.072. The concentrations of Mn, Fe, Pb, and V were higher among cases compared to controls, but the mean differences were not statistically significant (Table 3.4.5).

### 3.4.6 Factors associated with metallomic profiles (Objective 3b)

To determine whether lifestyle and environmental factors are associated with metallomic profiles, a MANCOVA was used to replicate Model B. This model compared metal concentrations between prostate cancer cases and controls, while adjusting for age, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI. The adjusted model showed these covariates did not statistically significantly affect the combined total metal concentrations, $F(16, 67) = 1.20$, $p$ = 0.1163. However, the metallomic profile patterns among cases and controls remained the same (Table 3.4.5).

**Table 3.4.5** Model B: MANOVA comparing metal concentrations between prostate cancer cases (n=44) and matched controls (n=44) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | Unadjusted Model Wilks' Lamda p-value: 0.0069 | | | Adjusted Model[b] Wilks' Lambda p-value: 0.1163 | | |
|---|---|---|---|---|---|---|
| | Mean difference *(SE)* | Effect size[a] | p-value | Mean difference *(SE)* | Effect size[a] | p-value |
| V | 0.004 *(0.003)* | 0.03 | 0.092 | 0.004 *(0.003)* | 0.027 | 0.179 |
| Mn | 0.19 *(0.10)* | 0.04 | 0.062 | 0.18 *(0.11)* | 0.041 | 0.109 |
| Fe | 8.52 *(4.56)* | 0.04 | 0.065 | 7.52 *(5.20)* | 0.034 | 0.153 |
| Zn | 7.68 *(2.98)* | 0.07 | 0.012 | 7.41 *(3.02)* | 0.089 | 0.013 |
| Se | 0.06 *(0.02)* | 0.07 | 0.012 | 0.06 *(0.02)* | 0.092 | 0.014 |
| Pb | 0.05 *(0.03)* | 0.04 | 0.062 | 0.05 *(0.03)* | 0.042 | 0.108 |

[a] Partial $\eta^2$ as a measure of effect size. [b] Model adjusted for age, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI.

## 3.5 Skin Cancer

### 3.5.1 Participant characteristics

There were 43 matched skin cancer case-controls available for analysis (total n = 86). Participant demographics and characteristics are provided in Table 3.5.1. The mean age was 55.7 (*SD* = 8.7); 53% were between the ages of 37 and 59, and the remaining 47% were between the ages of 60 and 69 years. There were more women (60.5%) than men (39.5%). The majority of participants (71% of cases, 67% of controls) had a family history of cancer. Most participants were either from New Brunswick (33%) or Newfoundland (33%), 26% were from Nova Scotia and 9% were from Prince Edward Island. Municipal water was the most common reported source (51% of cases, 39% of controls), followed by well (12% of cases, 26% of controls), while 5% reported 'other'; water source was unknown for about one third of the participants. Physical activity levels were high among both cases and controls: 49% of cases and 40% of controls were in the high physical activity category, 44% of both cases and controls were in the moderate category, and 7% of cases,

and 16% of controls were among those in the low physical activity category. Many participants reported never smoking (56% cases, 44% controls), or were former smokers (27% cases, 47% controls, while only 7% of cases and 9% of controls were current smokers. BMI information was available for approximately two thirds of the participants (70% of cases, and 63% of controls); mean BMI was 28.5 (*SD* = 5.6). Twenty six percent (26%) of cases and 16% of controls had low/normal BMI, 16% of cases, and 30% of controls were overweight, and 28% of cases and 16% of controls were obese.

**Table 3.5.1** Participant Demographics for skin cancer cases (n=43) and matched controls (n=43) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=86 Count *(%)* | **Skin Cancer Cases** n=43 Count (%) | **Healthy Controls** n=43 Count (%) |
|---|---|---|---|
| **Age, mean *(SD)*** | 55.7 *(8.7)* | 55.7 *(8.7)* | 55.7 *(8.7)* |
| 37-59 | 46 *(53%)* | 23 *(53%)* | 23 *(53%)* |
| 60-69 | 40 *(47%)* | 20 *(47%)* | 20 *(47%)* |
| **Sex** | | | |
| Female | 52 *(60.5%)* | 26 *(60.5%)* | 26 *(60.5%)* |
| Male | 34 *(39.5%)* | 17 *(39.5%)* | 17 *(39.5%)* |
| **Family History of Cancer** | 59 *(69%)* | 30 *(71%)* | 29 *(67%)* |
| **Province** | | | |
| NB | 28 *(33%)* | 14 *(33%)* | 14 *(33%)* |
| NFLD | 28 *(33%)* | 14 *(33%)* | 14 *(33%)* |
| NS | 22 *(26%)* | 11 *(26%)* | 11 *(26%)* |
| PEI | 8 *(9%)* | 4 *(9%)* | 4 *(9%)* |
| **Water Source** | | | |
| Municipal | 39 *(45%)* | 22 *(51%)* | 17 *(39%)* |
| Well | 16 *(19%)* | 5 *(12%)* | 11 *(26%)* |
| Other | 4 *(5%)* | 2 *(5%)* | 2 *(5%)* |
| Missing | *27 (31%)* | 14 *(33%)* | 13 *(30%)* |
| **Physical Activity** | | | |
| Low | 10 *(12%)* | *3 (7%)* | 7 *(16%)* |
| Moderate | 38 *(44%)* | 19 *(44%)* | 19 *(44%)* |
| High | 38 *(44%)* | 21 *(49%)* | 17 *(40%)* |
| **Smoking** | | | |
| Never | 43 *(50%)* | 24 *(56%)* | 19 *(44%)* |
| Former | 36 *(42%)* | 16 *(37%)* | 20 *(47%)* |
| Current | 7 *(8%)* | 3 *(7%)* | 4 *(9%)* |
| **BMI, mean *(SD)*** | 28.5 *(5.6)* | 28.2 *(5.1)* | 28.8 *(6.2)* |
| **BMI Categories** | | | |
| Low/Normal | 18 *(21%)* | 11 *(26%)* | 7 *(16%)* |
| Overweight | 20 *(23%)* | 7 *(16%)* | 13 *(30%)* |
| Obese | 19 *(22%)* | 12 *(28%)* | *7 (16%)* |
| Unknown | *29 (34%)* | 13 *(30%)* | 16 *(37%)* |

### 3.5.2 Toxicological characteristics (Objective 1)

The first objective was to characterize As speciation and metallomic profiles of the cancer cases and their matched as measured in toenail samples. Five measures of As speciation were measured and compared between cases and controls. The mean values and standard deviations of %iAs, %MMA, %DMA, PMI, and SMI are presented in Table 3.5.2 for the overall group, skin cancer cases, and their healthy matched controls. A significant difference was observed in the proportion of iAs between cases and their matched controls, where the skin cancer cases had significantly lower %iAs ($p = 0.0427$).

**Table 3.5.2** Arsenic Speciation Profiles for skin cancer cases (n=43) and matched controls (n=43) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=86 Mean *(SD)* | **Skin Cancer Cases** n=43 Mean *(SD)* | **Healthy Controls** n=43 Mean *(SD)* |
|---|---|---|---|
| %iAs** | 84.08 *(10.30)* | 81.84 *(11.10)* | 86.32 *(9.01)* |
| %MMA* | 8.45 *(5.56)* | 9.61 *(6.23)* | 7.29 *(4.57)* |
| %DMA* | 7.47 *(6.10)* | 8.55 *(6.20)* | 6.39 *(5.86)* |
| PMI* | 0.11 *(0.10)* | 0.13 *(0.11)* | 0.09 *(0.08)* |
| SMI | 1.16 *(1.94)* | 1.35 *(2.64)* | 0.97 *(0.76)* |

Students' t-test comparing cases to controls * $p < 0.1$ ** $p < 0.05$

The mean concentrations ($\mu$g/g) of the 18 detected metals (As, V, Cr, Mn, Fe, Co, Ni, Co, Cu, Zn, Ga, Se, Rb, Sr, Cd, Tl, Pb, Th, and U) are presented in Table 3.5.3 for the overall group, the skin cancer cases, and the healthy controls. The mean concentrations of the cases and controls were compared using the Student's *t*-test. Three metals (As, Zn, Ga) were flagged to be included Model B, based on the inclusion criteria of $p < 0.1$. The total concentration of As was lower in cases compared to controls, and the concentration of Ga was higher in cases ($p < 0.1$). Zinc concentration were significantly higher in cases compared to controls ($p = 0.0011$).

**Table 3.5.3** Metallomic Profiles ($\mu$g/g) for skin cancer cases (n=43) and matched controls (n=43) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | **Overall** n=86 Mean *(SD)* | **Skin Cancer Cases** n=43 Mean *(SD)* | **Healthy Controls** n=43 Mean *(SD)* |
|---|---|---|---|
| As * | 0.070 *(0.025)* | 0.065 *(0.022)* | 0.075 *(0.026)* |
| V | 0.017 *(0.011)* | 0.018 *(0.012)* | 0.016 *(0.009)* |
| Cr | 0.28 *(0.19)* | 0.28 *(0.19)* | 0.29 *(0.20)* |
| Mn | 0.54 (*0.40)* | 0.54 *(0.46)* | 0.54 *(0.34)* |
| Fe | 27.56 *(19.63)* | 27.40 *(20.48)* | 27.75 (*18.99)* |
| Co | 0.015 *(0.010)* | 0.014 *(0.009)* | 0.017 *(0.011)* |
| Ni | 11.39 *(17.23)* | 10.26 *(16.62)* | 12.52 *(17.95)* |
| Cu | 4.02 *(0.92)* | 4.02 *(0.93)* | 4.01 *(0.92)* |
| Zn *** | 105.80 (*14.33)* | 110.71 *(12.76)* | 100.88 *(14.27)* |
| Ga * | 0.005 (0.003*)* | 0.006 (0.004*)* | 0.005 (0.002*)* |
| Se | 0.93 *(0.11)* | 0.93 (*0.11)* | 0.93 (*0.12)* |
| Rb | 0.45 *(0.17)* | 0.44 *(0.17)* | 0.45 *(0.17)* |
| Sr | 0.55 *(0.37)* | 0.53 *(0.37)* | 0.57 *(0.37)* |
| Cd | 0.008 *(0.007)* | 0.008 *(0.007)* | 0.008 *(0.006)* |
| Tl | 0.0004 *(0.0002)* | 0.0004 *(0.0001)* | 0.0005 *(0.0002)* |
| Pb | 0.16 *(0.11)* | 0.15 *(0.12)* | 0.16 (*0.09)* |
| Th | 0.004 *(0.003)* | 0.004 *(0.003)* | 0.005 *(0.003)* |
| U | 0.004 *(0.005)* | 0.005 *(0.006)* | 0.004 *(0.003)* |

Notes: All metal concentrations are reported in ug/g, with the exception of As, V, Co, Ga, Cd, Tl, Th, and U, which are reported in ng/g. Students' t-test comparing cases to controls * $p < 0.1$ ** $p < 0.05$ *** $p < 0.01$

### 3.5.3 Arsenic speciation profiles and cancer (Objective 2a)

Objective 2a was to determine if As speciation profiles differed between skin cancer cases and controls. To test this, a MANOVA was used to assess the effect of history of prostate cancer on five As speciation variables, %iAs, %MMA, %DMA, PMI, and SMI (Table 3.5.4). The assumption of homogeneity of variance was assessed using Box's M test and was violated ($p < 0.05$). MANOVA is robust against this violation with a sample size >30, hence no adjustment to the significance criterion was performed (126). All other assumptions were tenable.

The results of Model A indicated that the combined As speciation variables were statistically significantly different between those with and without a history of skin cancer, $F(1, 84) = 3.35$, $p = 0.0085$. In addition, the variables, %MMA, %DMA, and PMI were statistically significantly higher among cases compared to controls (Table 3.5.4). The mean difference in %MMA between cases and controls was 2.27 ($SE = 0.65$, $p = 0.001$). The effect size of %MMA was large, $\eta^2 = 0.13$. The mean difference in %DMA was 2.03 ($SE = 0.80$, $p = 0.013$), with a moderate effect size, $\eta^2 = 0.07$. Skin cancer cases had less %iAs (mean difference = -4.55, $SE = 1.27$, $p = 0.001$) compared to controls. The effect size for %iAs was large, $\eta^2 = 0.13$. Finally, the mean difference in PMI between cases and controls was 0.03 ($SE = 0.01$, $p = 0.001$), with a large effect size, $\eta^2 = 0.11$. The SMI was not statistically significantly different between cases and controls.

### 3.5.4 Factors associated with arsenic speciation profiles (Objective 3a)

To determine if other factors were associated with As speciation profiles, Model A was replicated using a MANCOVA. This adjusted model assessed the effect of cancer history on arsenic speciation while controlling for age, sex, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI. The combined effect of the covariates on the combined arsenic speciation variables was not statistically significant, $F(17, 67) = 1.26$, $p = 0.0799$. However, the dependent variables, %MMA, %DMA, %iAs, and the PMI remained significantly different between cases and controls, and the SMI remained non-significant (Table 3.5.4). The mean difference in %MMA decreased (1.89, $SE = 0.65$), and the mean differences in %DMA (2.83, $SE = 0.84$) and %iAs (-4.78, $SE = 1.38$) increased.

**Table 3.5.4** Model A: MANOVA comparing arsenic speciation between skin cancer cases (n=43) and matched controls (n=43) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | Unadjusted Model Wilks' Lamda p-value: 0.0085 | | | Adjusted Model[b] Wilks' Lambda p-value: 0.0799 | | |
|---|---|---|---|---|---|---|
| | Mean difference *(SE)* | Effect size[a] | p-value | Mean difference *(SE)* | Effect size[a] | p-value |
| %iAs | -4.55 *(1.27)* | 0.13 | 0.001 | -4.76 *(1.39)* | 0.15 | 0.001 |
| %MMA | 2.27 *(0.65)* | 0.13 | 0.001 | 1.89 *(0.65)* | 0.11 | 0.005 |
| %DMA | 2.03 *(0.80)* | 0.07 | 0.013 | 2.84 *(0.85)* | 0.14 | 0.001 |
| PMI | 0.03 *(0.01)* | 0.11 | 0.001 | 0.03 *(0.01)* | 0.14 | 0.002 |
| SMI | 0.03 *(0.12)* | >0.01 | 0.781 | 0.15 *(0.12)* | 0.02 | 0.222 |

[a] Partial $\eta^2$ as a measure of effect size. [b] Model adjusted for age, sex, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI.

### 3.5.5 Metallomic profile and cancer (Objective 2b)

Objective 3 aimed to determine whether metallomic profiles differed between those with a history of cancer and those without. This was assessed with a MANOVA (Model B), which included three metals (As, Zn, Ga) as dependent variables. History of skin cancer (yes/no) was the grouping variable. The model found the effect of skin cancer history on the combine metals concentrations to be statistically significant, $F(1, 84) = 6.56$, $p = 0.0005$. Zn was statistically significantly higher among skin cancer cases compare to controls; the mean difference was 9.83 ($SE = 2.92$, $p = 0.001$, $\eta^2 = 0.119$). The mean difference in the concentrations of As and Ga were not statistically significantly different between cases and controls.

### 3.5.6 Factors associated with metallomic profiles (Objective 3b)

To determine whether other lifestyle and environmental factors were associated with the metallomic profiles a MANCOVA was used to replicate Model B (Table 3.5.5). This model adjusted for the covariates, age, sex, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI, and found their combined effect

on the combined metals to be statistically significant, $F(17, 67) = 1.53$, $p = 0.0220$. Again, Zn was the only metal that was statistically significantly different between skin cancer cases and controls. The mean difference was 11.62 ($SE = 3.20$, $p = 0.001$). The effect size for Zn was large ($\eta^2 = 0.16$). Despite the model being significant, none of the covariates included in the model were statistically significant predictors of the profiles of the metals, As, Zn, and Ga.

**Table 3.5.5** Model B: MANOVA analysis comparing metal concentrations between skin cancer cases (n=43) and matched controls (n=43) sampled from the Atlantic PATH cohort study (2009-2015), N=392.

| | Unadjusted Model Wilks' Lamda *p*-value: 0.0005 | | | Adjusted Model[b] Wilks' Lambda *p*-value: 0.0220 | | |
|---|---|---|---|---|---|---|
| | Mean difference (SE) | Effect size[a] | p-value | Mean difference *(SE)* | Effect size[a] | p-value |
| As | -0.01 *(0.005)* | 0.04 | 0.067 | -0.01 *(0.005)* | 0.04 | 0.088 |
| Zn | 9.83 *(2.92)* | 0.12 | 0.001 | 11.62 *(3.20)* | 0.16 | 0.001 |
| Ga | 0.001 *(0.001)* | 0.04 | 0.068 | 0.001 *(0.001)* | 0.05 | 0.071 |

[a] Partial $\eta^2$ as a measure of effect size. [b] Model adjusted for age, sex, family history of cancer, province of residence, water source, physical activity, smoking status, and BMI.

# CHAPTER 4 DISCUSSION

The overarching goal of this research was to measure, characterize, and compare the As speciation and metallomic profiles of Atlantic Canadians with and without a history of cancer. The objectives of this work can be categorized as a two-phase process: i) the toxicological measurement of the As species and metals in the biological samples (Objective 1), and ii) the statistical analysis of the combined As speciation and metallomic profiles between the cancer cases and controls for each of the five cancer types considered: urinary (bladder/kidney) cancers, breast cancer, cervical cancer, prostate cancer, and skin cancer (Objectives 2 and 3).

## 4.1 Toxicological Profiles

The toenail As species was measured for each participant included in this study using a combined HPLC-ICP-MS. The proportions of As species measured in toenail samples are markedly different than those previously measured urine. For all of the groups in the present study, the composition of toenail As speciation was approximately 80–90% iAs, 5–10% MMA, and 5–10% DMA. Previous studies have also found iAs to be the predominant (>80%) species found in toenails (113,127). By comparison, the profiles of As species (as measured in urine) of those with bladder cancer have measured a lower proportion of iAs (4.22%), and a higher proportion of DMA (85.80%); the proportion of MMA was 8.34% (75). Similar As species profiles measured in urine samples have been observed in case-control studies of breast cancer and skin cancer (65,76). The compositional differences of As species in these biomarkers may point to differences in the properties of the metabolites and capacity to methylate As. For example, $iAs^{III}$ has been shown to have a strong affinity

to keratin (112). It is likely that each these biomarkers present unique and complementary ways to examine and better understanding As metabolism.

## 4.2 Metallomic profiles and cancer

To determine whether metallomic profiles are associated with a history of urinary, breast, cervical, prostate or skin cancers, a MANOVA was performed for each cancer group. The dependent variables were heavy metals that were associated with history of cancer. While the selected metals were not the same for each cancer type, some metals, such as Zn, were frequently included in the model.

The results showed statistically significant differences between the metallomic profiles of cases and controls in the urinary cancer group ($p$ = 0.0270), cervical cancer group ($p$ = 0.0032), prostate cancer group ($p$ = 0.0069), and skin cancer group ($p$ = 0.0005). The adjusted models were statistically significant in only the cervical cancer and the skin cancer groups. In the cervical cancer group, province of residence ($p$ = 0.0395) and physical activity ($p$ = 0.0429), were significant predictors of the profile of metallomes. By contrast, none of the covariates in the skin cancer group were statistically significant. Due to the limited evidence, no conclusive statements regarding these associations can be made, as further research is required.

### 4.2.1 Zinc

The concentration of Zn was statistically significantly higher in the cases from the urinary cancer group, prostate cancer group, and the skin cancer groups. Despite the breast cancer model not being significant ($p$ = 0.0671), the concentration of Zn in the breast cancer cases was statistically significantly higher compared to the controls ($p$ = 0.041). Currently, the findings regarding the association between Zn and cancer are inconsistent. For example,

one study measuring Zn in the nails of prostate cancer cases found levels consistent with those measured in the present study, but found no association between the concentration of Zn and odds of prostate cancer (128). By contrast, a study of Malaysian men found that those with prostate cancer had lower Zn-nail concentrations compared to controls, however, the authors observed much lower concentrations than those observed in the present study (79). In another study measuring toenail Zn concentrations in breast cancer cases, a non-significant positive association was found between Zn concentrations in toenails and breast cancer risk (64). Moreover, findings from a study in Pakistan found metastatic cancer patients displayed higher Zn concentrations compared to the controls (78). These inconsistencies warrant further investigation to better understand the relationship between Zn and these cancers, but also to determine optimal Zn levels. Previous research has indicated that the degree of Zn exposure may play a key role in determining its association with cancer. Specifically, the effect of Zn deficiency is different from overexposure (81). For example, Zn is considered an to be an antioxidant, thus nutritional Zn deficiencies may be a risk factor for cancer because it is thought to play a key role in metallothionine synthesis, which may inhibit free radical production (87,88). On the other hand, over-exposure to zinc, including occupational exposure and inhalation may be associated with increased risk of cancer (81).

### 4.2.2 Selenium

Toenail Se concentrations did not appreciably differ between the cases and controls in many of the cancer groups. There was, however, one notable exception: the prostate cancer cases had a slightly, albeit statistically significantly, higher mean Se concentration compared to their controls ($p$ = 0.014). This result is inconsistent with previous findings; current

evidence have shown there may be an inverse association between Se exposure and prostate cancer risk (81–84). On the other hand, however, the evidence does not support an association between Se exposure and breast cancer (81,85,86), and the evidence regarding Se and bladder cancer risk is inconclusive (81). The findings of the present study would indicate there is no significant association between toenail-Se levels and urinary, breast, cervical, or skin cancer risk, although prostate cancer cases may have slightly higher selenium levels than controls. Despite the null and conflicting results, further research is necessary to ascertain any associations between Se and cancer risk, particularly prostate cancer risk.

Current hypotheses have explained how Se may mitigate cancer risk. One such hypothesis states that Se is an essential trace element that protects against oxidative stress and can regulate the redox of other molecules, which may provide protective effects against cancer (81). There is also some evidence to suggest that Se can provide protective benefits against arsenic toxicity by forming an As-Se compound that is less toxic and easily excreted (129). To this end, future research should determine how As- and Se-toenail concentrations are linked to cancer risk.

### 4.2.3 Cadmium

The present study found no association between toenail Cd concentration and any cancer type. However, Cd is considered causally associated with lung cancer, and potentially prostate and renal cancers (89,90). There is some evidence to suggest Cd is associated with other cancers, including bladder and breast cancers, but the evidence is limited and requires further investigation (89,90). Some observational studies using nail samples have found Cd-nail concentrations to be higher in prostate cancer cases than in healthy controls (91,92).

The mechanisms of cadmium induced carcinogenicity include activation of proto-oncogenes, inactivation of tumor suppressor genes, cell proliferation, inhibition of DNA repair, and generation of ROS (89).

Nail samples are considered a reliable biomarker for Cd exposure, especially as they capture Cd accumulation over a long period of time. The reported amount of Cd in nail samples is 1.11 ± 0.83 µg/g (89). Whereas in the present study, toenail Cd concentrations were in the range of 0.005–0.01 µg/g. Considered together, the lack of observed association between Cd and the cancer types in this study may be the result of low exposure, and thus, the risk of Cd toxicity and carcinogenicity may be low for residents in Atlantic Canada. However, further research is required to confirm or deny these statements.

### 4.2.4 Lead

The findings of this research showed higher toenail-Pb concentrations among urinary cancer cases ($p = 0.019$) and breast cancer cases ($p = 0.038$) compared to controls. Lead concentrations were higher among cervical and prostate cancer cases compared to their respective controls, but the difference was not statistically significant. Lead has been classified as a possible carcinogen by the International Agency for Research on Cancer, despite limited evidence in both human and animal studies (130,131). At the present time, the human evidence linking lead and cancer is weak, but there may be an association with lung, stomach, and brain cancers (104). In addition, little research has been conducted on the use of nails as a biomarker for Pb-exposure. Some animal and *in vitro* studies have proposed mechanisms of Pb-induced carcinogenicity, including inhibited DNA repair and chromosomal aberrations, in addition to creating synergistic effects with other metals and

carcinogens (104). Our findings suggest there may be an association between Pb concentrations and urinary and breast cancers. However, further research is required to ascertain the ability of nails to both measure Pb body burden, and to assess potential interactions between Pb and other metals, such as As.

### 4.3 Arsenic speciation and cancer

The relative proportions of MMA, DMA, iAs, and the ratio measures, PMI and SMI (as measured by toenail clippings) were compared between cases and controls using MANOVA for each of the five cancer groups from participants in the Atlantic PATH cohort study. Arsenic speciation profiles appeared to differ between groups and in their association with the outcome; a visual depiction of this is provided in Appendix A.

#### 4.3.1 Urinary cancers

No significant associations were found between As speciation profiles and urinary cancers. In fact, none of the five measures of As speciation differed significantly between the cases and controls of this group. This result differs from previous findings, which have assessed As speciation and bladder cancer using urine samples. Previously, a higher %iAs and lower %DMA in urine have been shown to be significantly associated with increased bladder cancer mortality (75). Similarly, another study found a lower SMI (a lower ratio of DMA to MMA) in urine samples was associated with increased odds of bladder cancer (73). This difference in findings could arise from using a different biomarker (urine vs. toenails), or a different study population. The human body metabolizes As by successive methylation in the liver, and the metabolites are ultimately excreted in urine (~80% of internal As doses, which serves as a short-term indicator) or deposited in keratin rich tissues such as hair or nails (~20% of internal As doses, which serve as longer-term indicators) (132).

To the best of the author's knowledge, the present study is the first to measure As speciation in kidney cancer cases and to compare profiles to healthy controls. There is limited and conflicting evidence linking total As exposure in drinking water with kidney cancer risk. A study in Nova Scotia found that both bladder and kidney cancer risk increased with increased total As exposure (60). However, the risk was higher for bladder cancers than kidney cancers (60). On the other hand, a study in Finland found bladder cancer risk increased with total As exposure, but found no significant association between As exposure and kidney cancer (57).

Taken together, the lack of association observed for the urinary cancer group is likely not due to the use of toenail biomarkers, but rather the sample selected for this study. In fact, the null association may be explained by the combination of the bladder and kidney cancer groups. It is possible that arsenic speciation profiles between the two cancer groups are different, thus increasing the variance for As speciation variables. This could have biased the result toward the null. Future research should seek adequate sample size for both the kidney and bladder cancer groups so they can be analyzed independently.

### 4.3.2 Breast Cancer

There was a statistically significant difference in the As speciation profiles of those with a history of breast cancer and those without ($p = 0.033$). In particular, those with breast cancer history had a statistically significantly lower %MMA compared to controls ($p = 0.037$). To date, there are no studies that have assessed As speciation among those with a history of breast cancer using toenails. However, some previous research has found that increased urinary %MMA was significantly associated with an increased risk of breast cancer (65,67). These results provide further evidence that As speciation profiles differ

between urine and toenails. In addition, the proportion of MMA in the breast cancer groups was lower than the proportions observed in all the other groups, suggesting that speciation and methylation also differ by cancer type. Finally, despite differences in As speciation profiles, the total As concentrations between breast cancer cases and controls was not significantly different. Compared to the As speciation profiles of cases versus controls in the other cancer groups, breast cancer cases appear to display a unique speciation profile. Based on the findings from this study, the author concluded that breast cancer cases have a different As methylation capacity when compared to other cancer types. This research should be replicated with larger sample sizes to confirm the results.

Experimental evidence has generated several proposed mechanisms of effect of As induced breast cancer, which include $As^{III}$ induced ROS generation, DNA oxidative damage, metallothioneine and c-Myc proteins, NF- B activation, and cell proliferation in human breast cancer MCF-7 cells, among others (95). There is also evidence of genetic polymorphisms affecting both methylation ability and breast cancer risk. In particular, some polymorphisms may provide protective benefits against arsenic related breast cancers (133).

### 4.3.3 Cervical Cancer

Cervical cancer cases had significantly lower %iAs compared to controls ($p = 0.0058$), as well as significantly higher %MMA compared to controls ($p = 0.0042$). To the best of the author's knowledge, this is the first study to measure As speciation in among those with a history of cervical cancer. The Human Papilloma Virus is considered the primary cause of cervical cancer (134), however there is preliminary evidence to suggest that oxidative stress may play a role (135). Arsenic is known to induce oxidative stress through the production

of ROS (31,95). While not the primary cause, arsenic exposure and methylation capacity may contribute to the development of cervical cancer. Further research is necessary to better understand this association, and to confirm the results found in the present study.

### 4.3.4 Prostate Cancer

In the prostate cancer group, the SMI was statistically significantly higher among cases compared to controls ($p$ = 0.0307). The present study is among the first research determining the link between As speciation and prostate cancer. In fact, this thesis is connected to recent work from the HERC lab, where in collaboration with Atlantic PATH, arsenic speciation and metallomic profiles were compared between prostate cancer cases and matched controls (114). This study found that the %MMA and PMI were lower among prostate cancer cases compared to controls, and the SMI was higher (114). This research employed a similar design to the present study, although the sample size was much larger. Given that the target population is the same, the similarity in findings was expected. However, the smaller sample size in the present study may explain the lack of association in the present study for %MMA and PMI. This highlights the need for larger sample sizes in all groups in future work.

### 4.3.5 Skin Cancer

In the skin cancer group, cases had significantly lower %iAs ($p$ = 0.0006), and significantly higher %MMA ($p$ = 0.0008) and %DMA ($p$ = 0.0127) compared to controls. The PMI was also significantly higher among skin cancer cases compared to controls ($p$ = 0.0015). The existing evidence regarding skin cancer and As speciation is conflicting. One study found that when compared to controls, skin cancer cases had higher urinary %MMA, as well as higher urinary %iAs and lower urinary %DMA (76). However, another study found no

significant associations between %iAs, %MMA, %DMA, PMI and SMI and skin cancer; instead, given a low SMI, a higher cumulative As exposure was associated with increased risk of skin cancer (77).

While the proportions of As species (particularly %iAs and %DMA) differ between toenail and urine biomarkers, the results of this study and previous research indicates that higher %MMA is linked to increased skin cancer risk (76). Higher stored or excreted %MMA may be indicative of a lower methylation capacity or ability, as the methylation process is incomplete, which has been associated with higher risk of As-induced skin malignancies (136). Despite some inconsistencies in the literature, the evidence linking As speciation and skin cancer is quite strong. Based on the findings of this study, the author concludes that As speciation among those with skin cancer is different from those without. In addition, these findings indicate that toenail biomarkers are a viable option for monitoring As speciation, and in the future, with additional evidence, could potentially be used as a prognostic tool.

### 4.3.6 Factors associated with arsenic speciation

To determine if family history of cancer, province of residence, water source, physical activity, smoking, or BMI were associated with As speciation profiles, our MANOVA model was replicated, while adjusting for these covariates. Of the five cancer groups analysed, the adjusted models were statistically significant for only the urinary cancer group ($p = 0.003$) and the skin cancer group ($p = 0.0458$). In the skin cancer group, despite the model being significant, none of the covariates included were statistically significant predictors. This suggests that cancer history is a better predictor of As speciation than family history of cancer, province of residence, water source, physical activity, smoking,

or BMI. On the other hand, in the bladder cancer group, family history of cancer ($p$ = 0.0203), province of residence ($p$ = 0.0430), physical activity level ($p$ = 0.0239), and BMI ($p$ = 0.0184) were significant predictors of As speciation profiles.

There is some evidence that biological, lifestyle, and environmental factors are linked with As methylation capacity, but it is limited and often inconsistent. Those with higher BMI methylate As more efficiently and have a lower As body burden (127,137). The role of excess body weight in As methylation is not fully understood, but the variation is not thought to be the result of adipose tissue, which has been shown to have very low methylation capacity (137). However, body weight has been linked to AS3MT expression, which is important in methylation (137). This study found some evidence that BMI was associated with methylation, but further investigation is needed to ascertain the direction of the association. Smoking may be associated with decreased As methylation capacity, but the results are not conclusive (138). The present study found no significant associations between cigarette smoking status and As speciation, thus the results support the findings that there is no association between smoking and methylation capacity. Other factors that have been related to As methylation in humans include ethnicity, As exposure dosage, age, sex, pregnancy, and breastfeeding (137). The present study found weak evidence linking some of these factors to As speciation profiles, and as such, cannot make conclusive statements regarding these associations without further investigation.

### 4.4 Strengths and Limitations

The strengths of this study include the use of a novel biomarker to measure As speciation and trace metal exposure, analytical methodology, study design, and collaboration with the Atlantic PATH database. Currently, urine is the gold-standard biomarker to measure trace

metals, however the use of urine is limited as it only captures a short exposure window. For the purposes of monitoring chronic exposure, as is the case for cancer risk factors, this is a critical shortcoming. There are drawbacks to other potential biomarkers as well, including blood, whose exposure window is even shorter than that of urine, and hair, which is subject to external contamination. The advantages of using toenails include non-invasive sample collection, and the ease and duration of sample storage. In addition, this research has used a newly developed methodology to measure As speciation in toenails, which yields higher extraction efficiencies than previously observed. Moreover, the approach undergone method validation using two certified reference materials.

To date, research on As exposure and As speciation have been primarily focused on select cancer types (e.g., skin, lung, bladder). Furthermore, very few studies have investigated total As using toenail biomarkers, and even fewer have measured As speciation using toenails. This thesis addressed this gap in the literature by providing data on As speciation for several cancer types, including those often overlooked. In addition, this work provides total metal concentrations for 18 trace metals, and the use of a multivariate analysis provided a more comprehensive look at the inter-relationship between these metals. This thesis employs a prevalent case-control design with 1:1 matching on age and sex, both of which are associated with arsenic speciation. Finally, all of the data generated from this study will be re-integrated into the Atlantic PATH database, which will allow other researchers consider arsenic speciation and trace metal exposure across a range of studies related to the health of Atlantic Canadians.

This study is subject to a number of limitations. The data in this study are cross-sectional, and without temporal order, there is no way to ascertain causality between As exposure/speciation and cancer. Moreover, the cases included in this study were those with

a history of cancer. While some of the participants may have had cancer at the time of data collection, it is likely that some had cancer and are in remission, or have recovered. Future studies should account time of survivorship since diagnosis to address this limitation. The author acknowledges that the status of the cancer cases may affect As methylation ability, or the observed profile of As speciation and metal deposition in the toenails. Given the nature of the Atlantic PATH cohort, these findings may not be generalizable to the entire population of Atlantic Canada. Participation in the Atlantic PATH cohort was voluntary, and as such, there are some notable differences between the cohort sample and the target sample, however these are expected differences in a cohort of this nature. The observed differences in the Atlantic PATH participants compared to the general population are: higher socioeconomic status, higher representations of women, people born outside Canada, people with university degrees. These differences are important as more affluent populations (greater income and education) may reside in healthier communities, and may be less likely to be exposed to environmental contaminants. This thesis assumes that the main source of As is drinking water. However, the amount of As in drinking water at the time of the data collection, and the duration of time they were subject to this exposure is unknown. If participants move from a location of low-exposure to high-exposure or vice-versa, this may influence the profiles measured in the nails.

The questionnaire data used in this study to determine history of cancer, family history of cancer, smoking status, and physical activity levels, among others were self-report. While it is likely that the participants would remember a significant life event, such as a personal or immediate family cancer diagnosis, these data are still subject to recall bias. Additionally, some of the data may be vulnerable to social-desirability bias, as some

of the participants may have over-represented some characteristics, such as physical activity levels, and under-reported others, such as cigarette smoking frequency.

Finally, despite the novel approach used to measure As species in toenails, the present method does not allow the trivalent and pentavalent species to be measured individually. The ability to differentiate between these forms would provide better insight into methylation capacity and efficiency, as well its role in toxicity as trivalent species are thought to be more toxic. In fact, the ability to distinguish between these forms may reveal stronger association between As species and the associated cancer risk.

## 4.5 Implications and Outlook

### Implications

This thesis is the first large study to investigate the association between As speciation and several cancer types using toenail biomarkers. In addition, this work is the first to assess As speciation and cervical and kidney cancers. These findings show that there is an association between As methylation and the history of breast, cervical, prostate, and skin cancers, and that toenails are a viable biomarker for monitoring As exposure. Arsenic speciation profiles appear to differ by cancer type, and between urine and toenail biomarkers. However, the latter may be a more suitable biomarker given that it captures a greater exposure window and As-related cancer are the result of long-term exposure (115).

In the case of heavy metals, more research is needed to make conclusive statements about the use of toenail biomarkers for heavy metal exposure. The persistent association between Zn and the various cancers suggest that toenails may be a method of monitoring Zn exposure. On the other hand, the lack of association observed between known carcinogens (e.g., cadmium, lead) might suggest that their deposition in toenails does not

reflect their effect on the body. However, it is possible that this lack of association is due to low exposure levels in the sample analyzed for this thesis.

To date, evidence linking lower-level As exposure and cancer has been inconsistent, and much of the available work has focused on areas where exposure levels are extremely high. These results, coupled with other recent findings suggest that the provisional WHO and Health Canada guidelines are not sufficient, and that low-level arsenic exposure may induce carcinogenic effects. This builds upon the increasingly loud calls-to-action for improved water quality across Canada, but especially for historically Black and Indigenous communities, who have been disproportionately exposed them to environmental pollutants, including As and other toxic heavy metals (139).

**Outlook**

Further research in this area is required to better understand the association between As speciation and metallomic profiles and cancer. Future research requires more advanced approaches from both the analytical chemistry/toxicology perspective and the population health/epidemiology perspective. Regarding the former, the next steps include more experimental studies to determine the cellular and molecular mechanisms of As toxicity and carcinogenicity, and further method development for As speciation analysis. In particular, the ability to differentiate between trivalent and pentavalent arsenicals in biomarkers would provide a more comprehensive understanding of methylation and its role in toxicity. From a population health and epidemiological perspective, the next steps include incident case analysis, an intersectional sex- and gender-based analysis (SGBA+), accounting for the time elapsed since the first cancer diagnosis, and a larger sample. The use of incident cases would allow the researchers to make more definitive inferences about potentially causal mechanisms. Another future direction is to use SGBA to better

understand why there are sex differences in methylation capacities and how this may affect cancer risk. Evidence suggests that hormone levels may be an important factor influencing arsenic methylation (108), future research should investigate whether these are also associated with risk of certain cancers. This may provide a better understanding why breast cancer cases displayed different As speciation profiles compared to the other groups analyzed. Finally, the literature shows that trace metals interact with one another, and may generate synergistic or protective effects. For example, Se has been shown to be positively associated with arsenic methylation capacity (109). Future research should analyze arsenic speciation and metallomic profiles together to determine any potential associations or interactions.

## 4.6 Conclusion

Exposure to arsenic and other heavy metals through contaminated drinking water remains a problem experienced around the world and in Atlantic Canada. While the mechanisms of arsenic toxicity and carcinogenicity are yet to be fully elucidated, this research provides further evidence of the potential mediating role that As speciation may play in cancer development. Moreover, it improves upon previous research by measuring As speciation in addition to total As and other metallomes. Using toenail samples may be a more advantageous biomarker for As speciation related to chronic exposure in drinking water. On the other hand, the suitability of toenails as a method for biomonitoring other heavy metals, and as a potential prognostic tool, has yet to be determined.

The findings of this work provide further evidence that As speciation, rather than total As, is critical in understanding cancer risk. In particular, our findings support the hypothesis that a reduced capacity to methylate As is associated with cancer. In addition,

this research suggests that As speciation profiles may differ slightly by cancer type. Although, further research is required to confirm these findings, to understand the mechanisms underlying this variation, and to develop upstream population health monitoring tools. Finally, this research indicates that toenails can be used as a biomarker for As speciation and breast, cervical, prostate, and skin cancers; more research needs to be conducted to make statements regarding their use as a biomarker for bladder and/or kidney cancers.

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## APPENDIX A ADDITIONAL RESULTS

**Table A1** Arsenic speciation profiles patterns by cancer type. Comparison of mean differences from MANOVA models between cases and controls from the Atlantic PATH cohort study (2009-2015), N=392.

| Speciation | Bladder/Kidney (n=27) | Breast (n=82) | Cervical (n=82) | Prostate (n=88) | Skin (n=86) |
|---|---|---|---|---|---|
| **%MMA** | NS | ↓ ** | ↑ *** | NS | ↑ *** |
| **%DMA** | NS | NS | NS | NS | ↑ ** |
| **%iAs** | NS | NS | ↓ *** | NS | ↓ *** |
| **PMI** | NS | NS | ↑ * | ↓ * | ↑ *** |
| **SMI** | NS | NS | NS | ↑ ** | NS |

*Note: Arrows indicate the direction of the mean difference between cases and controls Not significant (NS), * p < 0.1, ** p <0.05, *** p< 0.01*

**Table A2** Arsenic speciation profiles patterns by cancer type. Comparison of mean differences from MANCOVA models between cases and controls from the Atlantic PATH cohort study (2009-2015), N=392.

| Speciation | Bladder/Kidney (n=27) | Breast (n=82) | Cervical (n=82) | Prostate (n=88) | Skin (n=86) |
|---|---|---|---|---|---|
| **%MMA** | NS | ↓ ** | ↑ *** | NS | ↑ *** |
| **%DMA** | NS | NS | NS | NS | ↑ *** |
| **%iAs** | NS | NS | ↓*** | NS | ↓ *** |
| **PMI** | NS | NS | ↑ ** | NS | ↑ *** |
| **SMI** | NS | NS | NS | ↑ ** | NS |

*Note: Arrows indicate the direction of the mean difference between cases and controls Not significant (NS), * p < 0.1, ** p <0.05, *** p< 0.01*

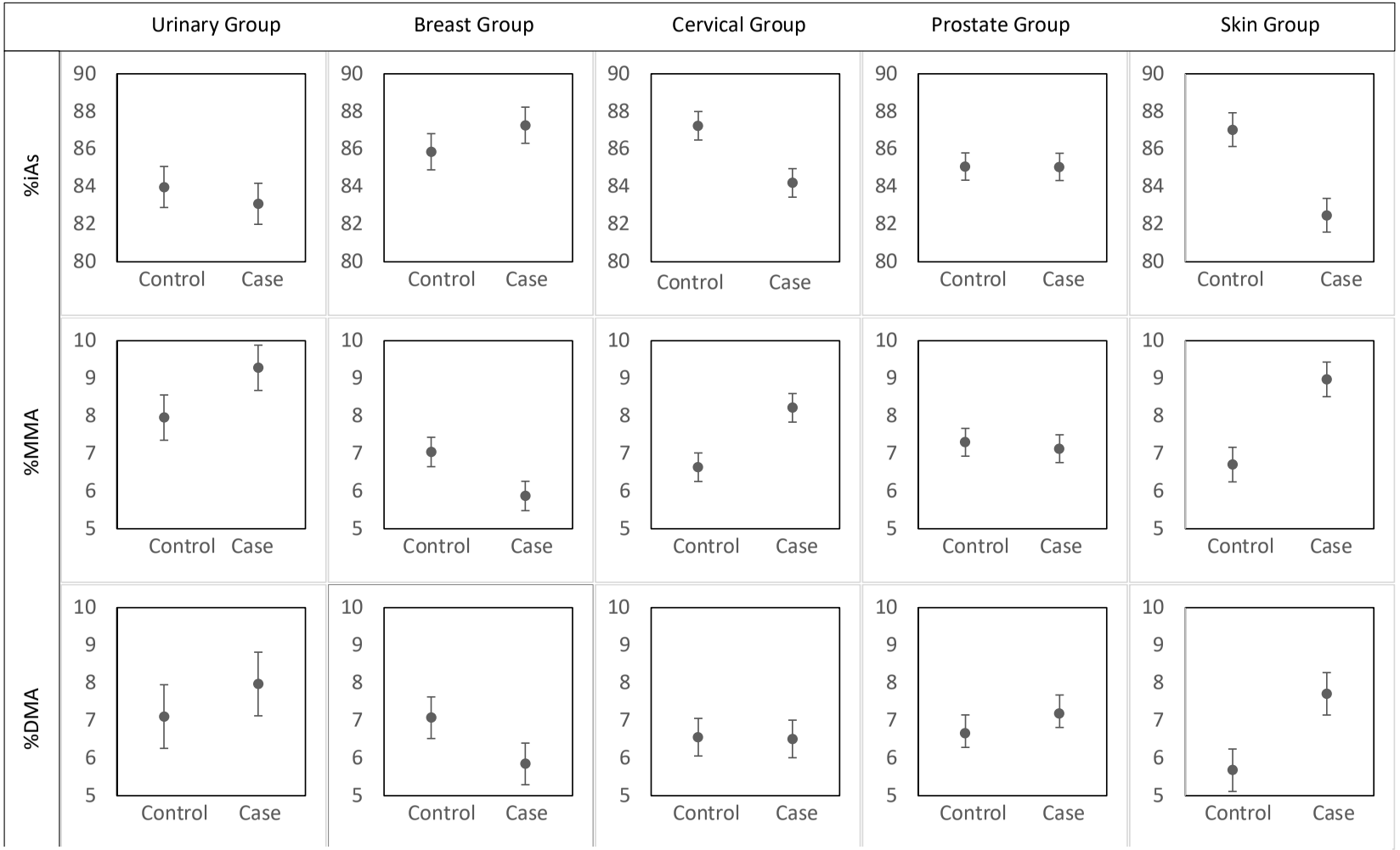


**Figure A1** Comparison of arsenic species (%iAs, %MMA, %DMA) between cases and controls, by disease group from the Atlantic PATH cohort (2009–2015), N=392. Mean values and standard errors from unadjusted MANOVA analyses.

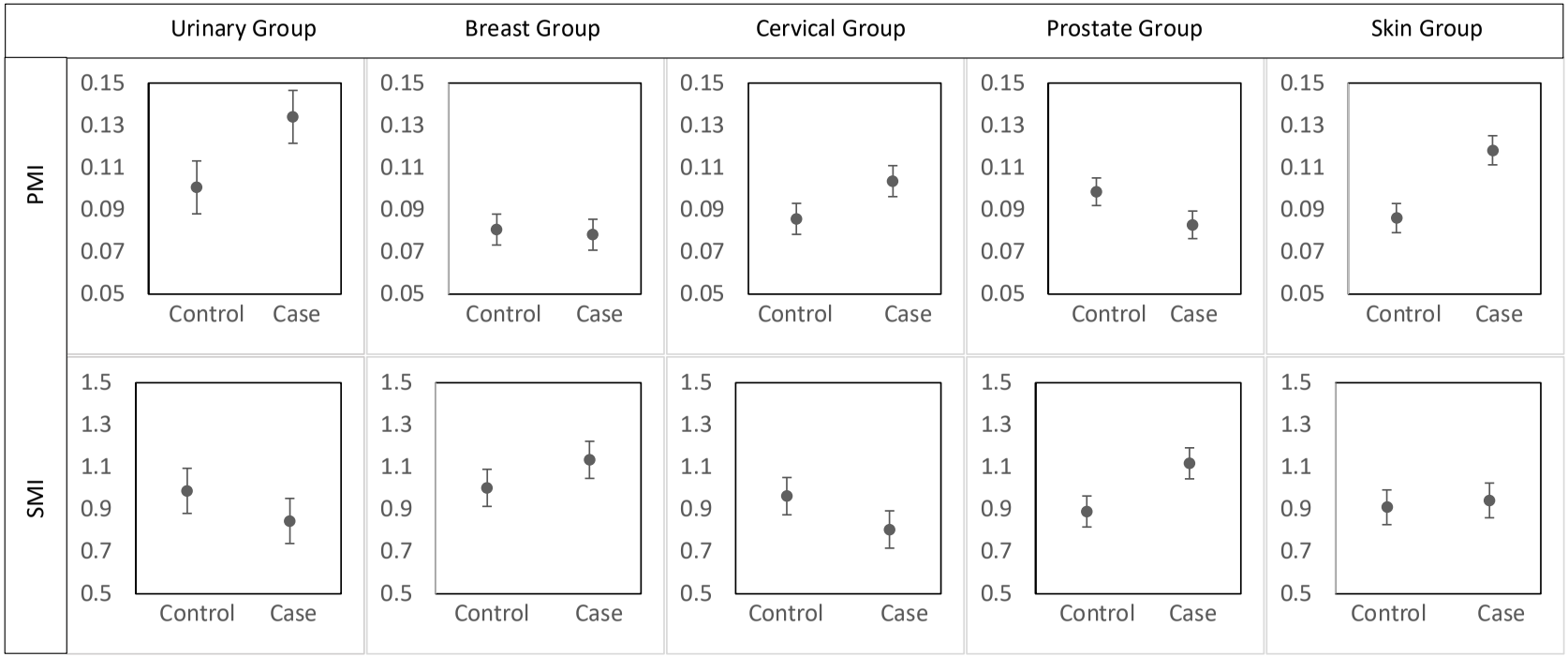


**Figure A2** Comparison of arsenic methylation ratios (PMI, SMI) between cases and controls, by disease group from the Atlantic PATH cohort (2009–2015), N=392. Mean values and standard errors from unadjusted MANOVA analyses.

## APPENDIX B POWER CALCULATIONS

**Table B1** Power calculations by cancer group for each arsenic speciation variable

| | Bladder/Kidney | Breast | Cervical | Prostate | Skin |
|---|---|---|---|---|---|
| **%iAs** | 0.08 | 0.18 | 0.78 | 0.03 | 0.92 |
| **%MMA** | 0.54 | 0.55 | 0.80 | 0.05 | 0.91 |
| **%DMA** | 0.11 | 0.34 | 0.03 | 0.12 | 0.69 |
| **PMI** | 0.45 | 0.04 | 0.40 | 0.39 | 0.88 |
| **SMI** | 0.16 | 0.19 | 0.24 | 0.58 | 0.05 |

Notes: All power calculations were performed for a two-sided test using the sample sizes, means of the given same group and that of their matched controls, and their standard deviations; the $\alpha$ was set to 0.05.

## APPENDIX C SUPPLEMENTARY ANALYTICAL METHODS TABLES

**Table C1** Certified reference materials, arsenic species measured in NIST 2669.

| CRM | Analyte | Method | Measured Concentration (ug/g) | Reference concentration (ug/g) |
|---|---|---|---|---|
| NIST 2669 Level I (Human urine) | Total As | ICP-MS | 22.26 ± 0.42 | 22.2 ± 4.8[a] |
| NIST 2669 Level I (Human urine) | As5+ | HPLC-ICP-MS | 2.35 ± 0.20 | 2.41 ± 0.3[b] |
| NIST 2669 Level I (Human urine) | MMA | HPLC-ICP-MS | 1.84 ± 0.19 | 1.87 ± 0.39[b] |
| NIST 2669 Level I (Human urine) | DMA | HPLC-ICP-MS | 3.46 ± 0.07 | 3.47 ± 0.41[b] |
| NIST 2669 Level II (Human urine) | Total As | ICP-MS | 47.96 ± 0.34 | 50.7 ± 6.3[a] |
| NIST 2669 Level II (Human urine) | As5+ | HPLC-ICP-MS | 6.06 ± 0.30 | 6.16 ± 0.95[b] |
| NIST 2669 Level II (Human urine) | MMA | HPLC-ICP-MS | 6.77 ± 0.29 | 7.18 ± 0.56[b] |
| NIST 2669 Level II (Human urine) | DMA | HPLC-ICP-MS | 24.89 ± 0.61 | 25.3 ± 0.7[b] |

*Notes:* [a] Reference concentration as given by the CRM provider for information only; does not meet the criteria to be a certified reference value. [b] Certified reference concentration given by the CRM provider that has been independently verified using four analytical methods

**Table C2** Certified reference materials, metal concentrations measured in NIES No. 13

| CRM | Analyte | Method | Measured Concentration (ug/g) | Reference concentration (ug/g) |
|---|---|---|---|---|
| NIES No. 13 (Human hair) | Cd | ICP-MS | 0.23 ± 0.01 | 0.23 ± 0.03 [b] |
| NIES No. 13 (Human hair) | Cu | ICP-MS | 14.59 ± 0.12 | 15.3 ± 1.3 [b] |
| NIES No. 13 (Human hair) | Pb | ICP-MS | 5.47 ± 0.45 | 4.6 ± 0.4 [b] |
| NIES No. 13 (Human hair) | Se | ICP-MS | 2.30 ± 0.08 | 1.79 ± 0.17 [b] |
| NIES No. 13 (Human hair) | Zn | ICP-MS | 161.93 ± 2.11 | 172 ± 11 [b] |
| NIES No. 13 (Human hair) | As | ICP-MS | 0.10 ± 0.002 | 0.1 ± 0.002 [a] |
| NIES No. 13 (Human hair) | Co | ICP-MS | 0.06 ± 0.004 | 0.07 ± 0.0014 [a] |
| NIES No. 13 (Human hair) | Fe | ICP-MS | 138.60 ± 17.72 | 140 ± 2.8 [a] |
| NIES No. 13 (Human hair) | Mn | ICP-MS | 3.59 ± 0.22 | 3.9 ± 0.078 [a] |
| NIES No. 13 (Human hair) | V | ICP-MS | 0.20 ± 0.02 | 0.27 ± 0.0054 [a] |

*Notes:* [a] Reference concentration as given by the CRM provider for information only; does not meet the criteria to be a certified reference value. [b] Certified reference concentration given by the CRM provider that has been independently verified using four analytical methods

**Table C3** Method detection limits for metals and arsenic species.

| Analyte | MDL (µg/L) | MDL (µg)[a] | MDL (µg/g)[b] |
|---|---|---|---|
| V | 0.009 | 0.00009 | 0.003 |
| Cr | 0.33 | 0.0033 | 0.10 |
| Mn | 0.081 | 0.0008 | 0.025 |
| Fe | 5.8 | 0.058 | 1.83 |
| Co | 0.027 | 0.0003 | 0.009 |
| Ni | 0.35 | 0.0035 | 0.11 |
| Cu | 0.79 | 0.0079 | 0.25 |
| Zn | 3.7 | 0.037 | 1.16 |
| Ga | 0.0047 | 0.00005 | 0.0015 |
| As | 0.011 | 0.00011 | 0.0035 |
| Se | 0.019 | 0.0002 | 0.0060 |
| Rb | 0.033 | 0.00033 | 0.010 |
| Sr | 0.10 | 0.0010 | 0.032 |
| Cd | 0.016 | 0.00016 | 0.0051 |
| Tl | 0.0003 | 0.000003 | 0.0001 |
| Pb | 0.15 | 0.0015 | 0.047 |
| Th | 0.0007 | 0.000007 | 0.00022 |
| U | 0.0007 | 0.000007 | 0.00022 |
| MMA | 0.0049 | 0.000049 | 0.0015 |
| DMA | 0.0045 | 0.000045 | 0.0014 |
| iAs | 0.017 | 0.00017 | 0.0052 |

*Notes:* Metal and iAs MDLs were calculated according to EPA procedure using method blanks. MMA and DMA MDLs were calculated using 7 replicates of the lowest level calibration standard (0.02 µg/L). [a] Calculated by multiplying the MDL in µg/L by the total sample volume (0.010 L for total metal analysis; 0.01005 L for arsenic speciation analysis). [b] Calculated by multiplying the MDL in µg by average sample mass (0.0391 g).

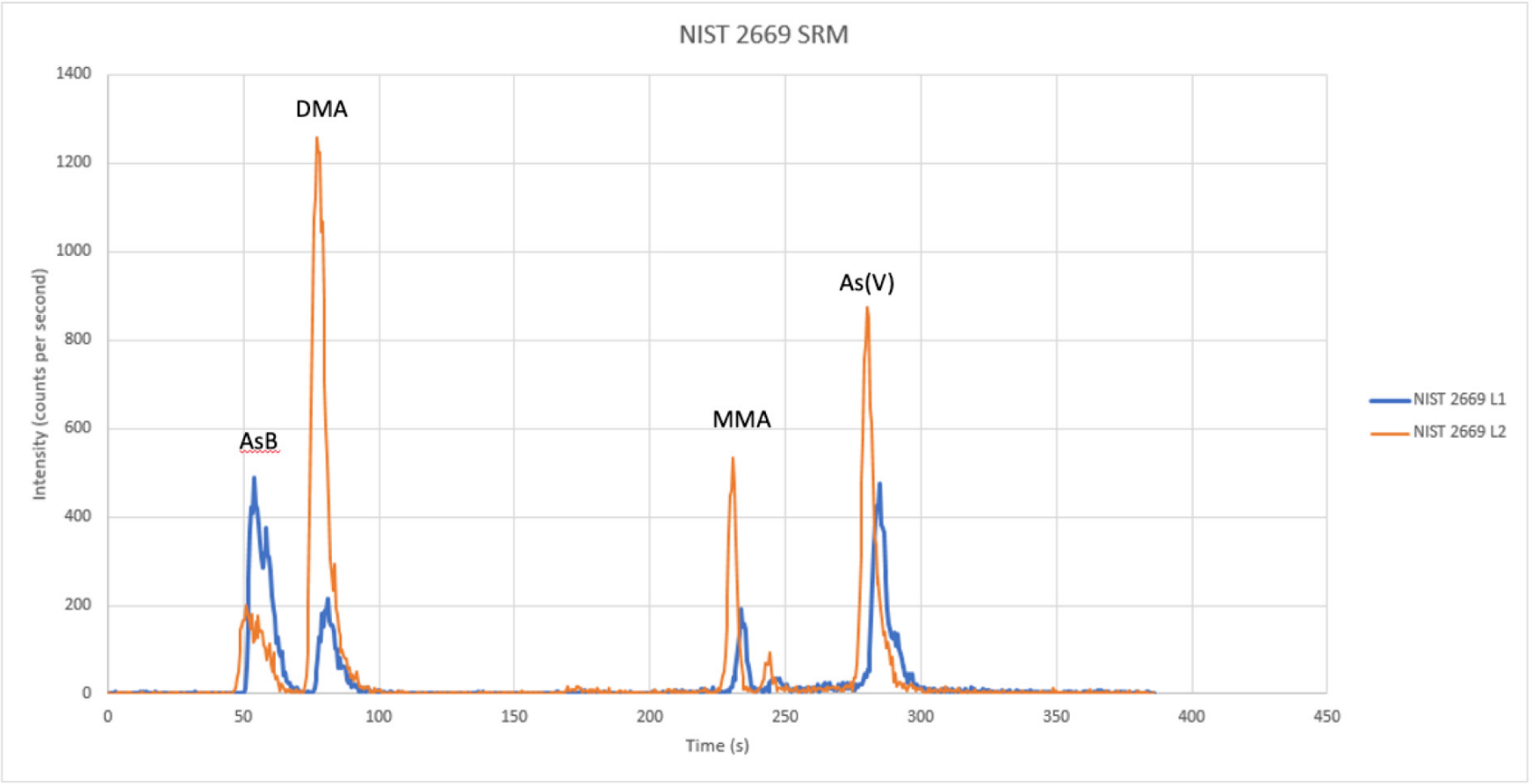


**Figure C1** Example chromatogram displaying arsenic speciation separation from NIST 2669 CRM.