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Density and proximity of unconventional oil and gas wells and concentrations of trace elements in urine, hair, nails and tap water samples from pregnant women living in Northeastern British Columbia

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Density and proximity of unconventional oil and gas wells and concentrations of trace elements in urine, hair, nails and tap water samples from pregnant women living in Northeastern British Columbia

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Abstract

The Peace River Valley (British Columbia, Canada) is an area of intensive unconventional oil and gas (UOG) exploitation, an activity that can release contaminants with possible adverse effects on the fetus. My project aimed to estimate the importance of this exposure. For this aim, we 1) measured concentrations of 21 trace elements in tap water and biological (hair, urine, nails) samples from 85 pregnant women in this region; 2) compared them with those from the general population and health-based guidance values; 3) assessed their correlations between matrices; and 4) evaluated their associations with the density and proximity of UOG wells (i.e., wells within radii of 2.5 km, 5 km, 10 km, and with all wells in British Columbia around residences). Spearman's rank correlation and multiple linear regression analyses adjusted covariates were performed. Our results showed higher urinary and hair levels of certain trace elements compared to reference populations (e.g., Co, Ba, Sr, Mn, V, Ga). Concentrations in tap water correlated strongest with concentrations in hair, followed by nails and urine. Positive (e.g., Al, Mn, Cu, Ga, Cd, Ba, Cr, Sr, U) and negative (e.g., Fe) associations were observed between the density and proximity of UOG wells and the concentrations of certain trace elements in tap water, hair, and nails. Our results suggest that pregnant women living in an active area of UOG exploitation are likely to be more exposed to certain trace elements than the general population, but the association with density and proximity to wells remains uncertain.

Keywords : trace elements, unconventional oil and gas exploitation, gestational exposure, tap water, hair, nails, urine, Northeastern British Columbia

Résumé

La vallée de la rivière de la Paix (Colombie-Britannique, Canada) est une zone d'exploitation de pétrole et de gaz naturel par méthodes non-conventionnelles (PGNNC), une activité qui est susceptible de libérer des contaminants avec des effets nocifs possibles sur le fœtus. Mon projet visait à estimer l'importance de cette exposition. À cette fin, nous avons 1) mesuré les concentrations de 21 éléments traces dans des échantillons de cheveux, d'ongles, d'urine et d'eau du robinet de 85 femmes enceintes de cette région, 2) comparé celles-ci avec celles de la population générale et des valeurs-guides, 3) évalué leur corrélation entre matrices et 4) évalué leur association avec la densité et proximité des puits (mesures pour des rayons de 2,5 km, 5 km, 10 km, et sans limite autour des résidences). Des analyses de corrélation de Spearman et de régression linéaire multiple ajustées pour certaines variables ont été effectuées. Les concentrations urinaires et capillaires étaient plus élevées pour certains éléments traces par rapport aux populations de référence (p. ex. Co, Ba, Sr, Mn, V, Ga). Les concentrations dans l'eau du robinet étaient plus fortement corrélées avec les concentrations dans les cheveux, suivis des ongles et de l'urine. Des associations positives (p. ex. Al, Mn, Cu, Ga, Cd, Ba, Cr, Sr, U) et négatives (p. ex. Fe) ont été observées entre la densité/proximité des puits de PGNNC et les concentrations de certains éléments traces dans les échantillons d'eau, de cheveux et d'ongles. Nos résultats portent à croire que les femmes enceintes vivant dans une zone active de PGNNC sont susceptibles d'être plus exposées à certains éléments traces que la population générale, mais le lien avec la densité et la proximité des puits demeure incertain.

Mots-clés : éléments traces**,** exploitation pétrolière et gazière non conventionnelle, exposition gestationnelle, eau du robinet, cheveux, ongles, urine, Nord-Est de la Colombie-Britannique

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Acronyms and abbreviations

EXPERIVA	Exposures in the Peace River Valley
BC	British Columbia
BE	Biomonitoring equivalents
CHMS	Canadian Health Measures Survey
CI	confidence interval
HF	hydraulic fracturing
HBM	human biomonitoring
ICP-MS	Inductively coupled plasma mass spectrometry
LOD	limit of detection
MAC	maximum acceptable concentration
NADA	Non-detects and Data Analysis
NEBC	Northeastern British Columbia
NHANES	National Health and Nutrition Examination Survey
ROS	regression on order statistics
UOG	unconventional oil and gas

Trace Elements

Li	lithium
Be	beryllium
Al	aluminum
V	vanadium
Cr	chromium
Mn	manganese
Fe	iron
Co	cobalt
Ni	nickel
Cu	copper
Zn	zinc
Ga	gallium
As	arsenic
Se	selenium
Sr	strontium
Ag	silver
Cd	cadmium
Ba	barium
Tl	thallium
Pb	lead
U	uranium

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1. Introduction

The Peace River Valley located in Northeastern British Columbia (Canada) is an area of intensive unconventional oil and gas (UOG) exploitation (1). Such industrial activity may release several contaminants, including, trace elements, lead to environmental contamination, to unwanted exposure, and to adverse health outcomes in local people (2–12). Health impacts can be particularly important for vulnerable population groups such as pregnant women, with potentially deleterious effects on reproductive functions and the fetus (13,14).

1.1 Natural gas production in Northeastern British Columbia (B.C.)

In Canada, natural gas has been first discovered in New Brunswick in 1859 as an important energy resource (15). It is used for cooling buildings, heating water, cooking, electricity, etc. (16). Today about one third of Canada's energy needs are met by natural gas (16). In the world, Canada ranks fifth in the resources of natural gas and fourth in its production (17–19). British Columbia is the second largest natural gas producer in Canada (27%) after Alberta (71%) for 2021-2022 (19), and is one of the most productive regions of shale gas in the world (5,20). As natural gas industry is growing very fast with increasing local and global demands, about 10% increase is expected by 2040 in Canada, which is expected to be met with the growing shale gas and tight gas production in Western Canada (18).

Natural gas is extracted from rock reservoirs using conventional and unconventional methods (17,21). Conventional techniques involve traditional simple vertical drilling, pumping and compression techniques used to extract gas from porous geological formation or rock reservoirs such as sandstones. Conventional natural gas activities today are mainly located in Western Canada Sedimentary Basin (i.e., including in British Columbia, Alberta, and in Saskatchewan), and some also in Ontario and New Brunswick (21). On the other hand, unconventional methods involve, typically, vertical (depth) and horizontal (length) drilling and hydraulic fracturing techniques and are used to extract gas from tight or non-porous low permeability rock reservoirs such as coal seams and shale (21–23). Drilling a well involves creating a hole through many rock layers to reach the oil and gas reservoirs. In Canada, conventional natural gas production techniques decline gradually and are replaced with the increasing use of unconventional methods (21). Unconventional natural gas activities are mainly located in Northeastern British Columbia (B.C.) and Northwestern Alberta (21). The northeast of B.C. is the only

region where commercial quantities of gas and oil are being produced in B.C. (21). This region houses major geologic formations with high shale content for gas production including the Liard Basin, the Cordova Embayment, the Horn River Basin and the Montney Play (20).

1.2 Hydraulic Fracturing Activities & Environmental Contamination

Approximately 30,000 wells have been drilled in Northeastern B.C. (1). Hydraulic fracturing (also known as fracking, hydrofracking and hydrofracturing) is an unconventional method used in B.C. since the 1960s to extract natural gas from rock formations (24). Most hydraulic fracturing activities are located in the Montney Play or Montney Formation which covers approximately an area of three million hectares (25). Today, 86% of the annual natural gas production of British Columbia comes from the Montney Play (26).

In hydraulic fracturing, large quantities of highly pressurized fracking fluid -comprised of water, sand, and chemical additives (e.g., biocides, surfactants)- is injected into a bedrock formation to create fractures (22,23). This allows to extract the natural gas from the shale formations which have otherwise very small pores and low permeability blocking the flow of gas (22,24,25,27). Increasing in this way the permeability of the rocks, it facilitates the movement of gas through the induced factures up a wellbore (24). This activity generates wastewaters emitted to the surface; it includes flowback water from the initial pumping of the fracking fluid and produced water resulting from the production processes (13,22). The content of wastewaters resulting from hydraulic fracturing, determined also by the content of geological formations, can contain various environmental contaminants, including, trace elements, sometimes in considerably high concentrations. (e.g., Ba, Al, Sr, Mn) (13,22). In the Montney Formation, various elements are present: Al, Ca, Fe, K, Mg, Mn, Na, Si in high concentrations as major elements forming the rock, trace elements such are Ag, Ba, Cd, Co, Cr, Mo, Ni, Pb, Sn, Sr, V, etc., in lower concentrations, and other rarer earth elements (28,29). The release of such contaminants through wastewaters leads to environmental contamination of soil and water (13,22). Runoffs, spills and leaks during the handling, the storage and the disposal of these wastewaters may also contribute to this environmental contamination (2–5).

1.3 Trace elements, exposure through drinking water and Toxicity

1.3.1 Essential and non-essential trace elements, and trace metals

Trace elements are minerals present in the body in trace amounts (30,31). Some trace elements are considered essential micronutrients and are required in trace amounts based on their role and participation to metabolic processes in the human body (e.g., Fe, Mn, Zn, Cu, Se), and others are non-essential (e.g., Al, Hg, As, Pb) (32). Trace elements play different roles in the human body. For example, Fe is a component of myoglobin and hemoglobin and is needed for the transport of oxygen in blood, and along with Cu, it is also essential in oxidation-reduction reactions in energy metabolism (32). Zn is a necessary component in over 300 enzymes and plays an important role in the creation of DNA, in growth of cells, in healing damaged tissues, and in supporting a healthy immune system (33). Many trace elements function as catalysts for enzymes (e.g., Fe, Ni, Co, Mo, W, Zn) (32,34). Other trace elements (e.g., Ca, Cu, Mn, Mg, Zn and Se) play roles in the reproductive health of males as components of semen (35). Trace elements also include heavy metals, that is, metallic chemical elements that have relatively high density and are toxic at even very low concentrations (e.g., As , Pb, Cd, Tl, Ni, Cu, Cr) (31,36,37). Their presence in the environment comes from natural geological and anthropogenic sources (36). Trace metals are used widely in industrial, domestic, agricultural, medical, technological, and other settings, leading to increasing presence in the environment, and concerns over their potential effects on human health (36).

1.3.2 Absorption, distribution and excretion

Essential trace elements are mainly absorbed from the gastrointestinal lumen by passive diffusion or by specialized transport systems (38). They are then transported to different organs via blood (38). Their distribution in the body varies depending on the absorption-excretion systems for each trace element. Mainly, essential trace elements are excreted via gastrointestinal and renal pathways (38). For trace elements, homeostasis is maintained through regulated absorption (e.g., Zn, Cu, Mn, Fe) or excretion of trace elements (e.g., Se, I) (39). Trace elements in the body bind to other molecules often to enzymes as cofactors (i.e., as ion components required for the enzyme's roles as a catalyst). If the molecules they are bound to are broken down, they are released and reused by other molecules in the cell or released into the blood stream and used by other cells (40). They can also be released in saliva or in bile (40). Retention

and utilization of trace elements in various tissues are influenced by different factors: the tissue type and functions, pregnancy, lactation, stage of foetal development, genetic factors, etc. (40).

1.3.3 Exposure to trace elements and toxicity

Exposure to trace elements may happen via different pathways (e.g., ingestion of food, inhalation, dermal contact) (32). Although, it has been reported that food serves as the primary source of populational exposure to most of the trace elements, drinking water is also an important exposure pathway for humans to environmental contaminants (32,41,42). Groundwater naturally contains various trace metals (e.g., Cd, Co, Cr, Cu, Mn, Pb, Zn) (43). Drinking water contamination (e.g., surface water, groundwater, private drinking water) from the release of trace elements from hydraulic fracturing activities has been previously observed. For example, increased water levels of Ba have been observed in the Peace River Valley groundwaters, and of As, Ba and Sr in private drinking water in Texas (44–46). Trace element exposure namely through the consumption of contaminated water can yield toxicity and bring about serious health outcomes, and this, when in excessive amounts for essential elements or when even at very low concentrations for non-essential elements (e.g., for highly toxic heavy metals such as As, Pb, Cd) (36). Toxicity may be induced due to short-term and long-term exposure to trace elements (41,47). For example, Ba is a metal present naturally in the environment primarily as barium sulfate or barium carbonate (e.g., may be present in moderate concentrations in drinking water) and is used for example in petroleum industry, steel industry, and in medicine as in medicine as a radiographic contrast agent (48,49). These human activities or anthropogenic sources increase the metal concentrations in our environment. U.S. EPA's oral reference dose (i.e., the maximum acceptable oral dose or the daily oral exposure dose that is likely to be without a risk of adverse effect) for Ba is 0.07 mg Ba/kg/d (50). Ingestion of high amounts of Ba above this level can have acute health effects : gastrointestinal effects (e.g., nausea, vomiting), hypokalemia, muscle weakness, heart rhythm changes (e.g., ventricular tachycardia), hypertension and paralysis (49). Chronic exposure to barium may result in kidney damage (49). Mn is an essential element involved in the synthesis and activation of many enzymes and in the regulation of glucose and lipid metabolism (51). It is found in food -plants being the main source of Mn for humans (52,53). Mn also has natural occurrence in water in areas with Mn-rich bedrock and in areas of high industrial activity (52). Mn toxicity (manganism) often results from contaminated water, or from occupational exposure (from welding, smelting, and mining), and can result in neurological disorders, neurobehavioral impairment, and hyperactivity; other toxic effects include cardiotoxicity and hepatotoxicity as well (12,53). Sr is a non-essential trace element found naturally as a mineral in soil and

seawater, and in seafood and grains; other sources include its release as a by-product in mining operations or industrial usage, including in plastics and paints (54). Main sources of exposure to Sr include food and drinking water (55). Sr, like calcium, concentrates in the bones (e.g., 99% of the Sr in the human body is stored in bones) (54). Stable Sr has been shown to improve bone density in osteoporotic patients (54,56). In addition, Sr concentration in drinking water has been shown to correlate negatively with the occurrence of dental cavities (54). EPA's limit is 4000 Sr µg/L of drinking water. Studies show that exposure to high levels of stable Sr can impair bone growth in children, and exposure to high levels of radioactive Sr may cause cancer (e.g., leukemia) or anemia in adults (54,55). As and Cd are highly toxic elements. The main toxic effects attributable to these metals include different cancers, targeting of cardiovascular, renal, gastrointestinal, neurological, reproductive, and respiratory systems (57,58). Similarly, Pb is also associated with hypertension, kidney damage, and central nervous system injury with behavioural and learning problems especially in children (32). Finally, adverse health outcomes may also result from serious deficiencies of essential elements (32). For people with deficiencies, mineral supplementation, often in form of prescription drugs of some trace elements, may be used (59). For example, both Fe deficiency and Fe excess are risk factors for the development of different cancers (e.g., Fe excess and lung cancer in hematite miners and foundry workers) (32,60).

1.3.4 Interconnections

Exposure to one trace element or different trace elements can affect the absorption or excretion of others, altering the trace element body burden and the body balance of trace elements. These trace elements may compete, may cause additive, synergistic, or antagonistic effects, alter trace element homeostasis and even cause new effects (61). For example, exposure to As and high body levels of As may increase the secretion of Cu and Mo in the urine, thus causing deficiencies in these elements (32,43). Studies have also shown that, for example, exposure to As through drinking water inhibited the excretion and increased the accumulation of Ba and Mn in hair, and increased the excretion of Cd, Cu and Mo in urine (43). This exposure can happen namely from the ingestion of trace elements from food or drinking water (62). Inter-element relationships and metabolic cycles of the elements are believed to be important determinants for urinary excretion of trace elements (63). These interactions of trace elements in metabolic pathways can override exposure footprints (63).

1.4 Toxic metal exposures and public health

Essential trace element deficiencies and the excessive exposure to potentially toxic elements (namely, heavy metals) have impacts on public health around the world, including in Canada. The growing research regarding the distribution of trace elements in the environment, their role and interaction in the human body, and the known potential adverse health effect, has driven public health agenda, policy, and populational interventions. The research on essential trace elements has driven nutritional policy and Food Program of Health Canada, including the determination of recommended levels for essential trace elements, the addition of vitamins and minerals to foods during food processing (e.g., fluoridation of drinking water), the establishment of mineral levels for infant formulas, the active collection of national data, surveillance, risk assessment, and determining intervention needs (e.g., Canadian Health Measures Survey) (64,65). The research on exposure to toxic elements and their adverse health impacts in humans has driven the evaluation of natural and anthropogenic sources releasing trace elements into the air, soil, water, and food, and led to populational interventions to address these sources and reduce exposures (66).

1.5 Pregnant women as a vulnerable population group

Exposure to trace elements can be especially important for vulnerable population groups like pregnant women. The intake of essential elements or micronutrients within the recommended levels is particularly important during pregnancy (e.g., copper, selenium, zinc) (33). A healthy diet also is particularly important during pregnancy, and supplements may be taken to fulfill trace element needs. For example, iron is often recommended and taken as part of supplements during pregnancy (67). Deficient intakes of trace elements can result in low birth weight, preterm birth, fetal malformations, developmental delays, and miscarriage (33). Exposure to trace elements in excessive amounts during pregnancy can also have deleterious health effects on the fetal development. Heavy metals can cross placental barrier and harm the fetus (10,68). Maternal exposure to heavy metals even at very low concentrations has been associated with adverse birth outcomes including low birth weight and developmental delays (33). For example, early exposure to Cd, Pb, Hg and As has been associated with low birth weight, and neurological, developmental, and endocrine disorders in infants (10,33). Exposure to Cd has been also linked to decreased head circumference (33). On the other hand, high exposure to Mn, for instance, has been shown to affect early psychomotor development (12). Exposure to trace elements can happen also via UOG activities, which could bring about adverse health outcomes. Many epidemiologic studies in Canada

and the US (in California, in Pennsylvania, in Colorado) have suggested associations between exposure to hydraulic fracturing activities during pregnancy and pregnancy outcomes like preterm birth and low birth weight (7–11).

1.6 Water contamination

In B.C., the water for drinking and other domestic purposes is supplied mostly from groundwater source and surface water sources (69). Consumption of contaminated drinking water can be an important source of human exposure and pose significant health risks (37,41,47). For example, a study investigating trace element contamination of Cambodian groundwater found elevated As, Mn, Ba and Fe in groundwater (e.g., at levels higher than Cambodian permissible limits), which were associated with increased risk of non-carcinogenic effects in residents of Kandal and Kratie (relative to non-exposed residents of Kampong Cham) (47). Another example is the concentrations of the trace elements As, B, Ba, F and U in some potable water aquifers in Manitoba exceeding drinking water quality guidelines (70). Surface water and groundwater contamination has also been associated with industrial activities such as hydraulic fracturing activities in areas of intense natural gas exploitation (44). For example, an increase in the concentrations of Ba in groundwater has been detected in the Peace River Valley with the expansion of hydraulic fracturing activities since 2007 (46). Relatively high concentrations of trace metals like As, Ba and Sr were also detected in private drinking water in hydraulic fracturing areas in Texas (45). The study found increased odds of preterm birth and fetal death associated with UOG activities, suggesting an association between maternal residential proximity to UOG wells and preterm birth and fetal death (45). Drinking water can be also contaminated by pipe components, which is especially important to take into consideration for certain trace elements like Pb and Cd (71,72). Pre-1950 homes and low occupancy sites have been shown to have significantly higher water Pb content (71). For this, flushing tap water has been used as a short-term technique for trace metal content reduction (71).

In the Peace River Valley, surface water and groundwater monitoring lacks baseline levels of trace elements before industrial activities in this region started (prior to 1970s), and general environmental monitoring of water in this region with the expansion of intense oil and gas activities is not routinely performed (46).

1.7 Human biomonitoring, pathways of exposure and bio-indicators of exposure

The environmental contamination resulting from industrial activities like hydraulic fracturing raises concerns for associated potential health impacts, especially on pregnant women and fetuses. In the context of human health risk assessment, human biomonitoring (i.e., the measurement of contaminants or their metabolites in biological samples) has been extensively used to assess human exposure (73–75). In Canada, human biomonitoring activities have been carried out as part of Canadian Health Measures Survey (CHMS), which is the most comprehensive nationally representative data of exposure to various environmental contaminants in the Canadian population, including metals and trace elements (76).

Depending on the trace element and exposure period of interest, urine, blood, hair, and nail sampling have been used to estimate short- and/or long-term exposures (41,77–79). Biological fluids like blood and urine have been used widely in previous studies for reflecting human exposure to trace elements (41,77). Urinary excretion levels reflect current or recent body burden and may be influenced by relatively recent exposures (from days to weeks to months) (62,80). If the body burden reflects bioaccumulation of a given element, even if exposure completely stops, and excretion can continue over days to months (81). In addition, hair and nails have also been used as recognized bioindicators of past long-term exposures (from months to years) to various contaminants, including trace elements (63,78,80). The levels of trace elements in hair and nails may also reflect their levels in other tissues of the body (78). Hair and nails (including toenails) are tissues made primarily from keratin (78,79). Due to keratin protein composition with sulfur-containing amino acids, these matrices offer spots for complexation of metals and metalloids (e.g., As, Pb) (36,82). Trace element composition of hair and nails can thus reflect trace element burden and exposure profile. Namely, hair grows on average about 1 cm per month from scalp, with some variations dependent on age, gender, ethnicity, nutrition, metabolic rate, and other factors (83). Toenails grow on average about 1.62mm per month from nail matrix, with some variations dependent once again on age, gender, metabolic rate, and other factors (84). The distal free edges of the hair and nails are isolated from body's metabolic activities, and reflect the body burden from months to years prior to sample collection (37,84). Hair, that can be collected entirely (e.g., in hair bundles), allows reconstructing the temporal profile of the body burden over multiple months, including recent exposures (i.e., segment closest to scalp). Short hair samples do not allow reconstructing the temporal profile.

These biomaterials (hair and nails) present several advantages for their use in the laboratory : they involve painless and easy collection and transportation, high stability at room temperatures, and often contain higher concentrations of trace elements relative to other body tissues or fluids (75,78). The appearance and the composition of hair and nails can give insight about physiological processes in the body and deficient or excessive presence of different substances, including trace elements (78). Different factors influence the trace element composition of hair and nails in the body. Workers in industries can be exposed to various toxic metals and other trace elements through their work environment (e.g., painting, welding, electroplating, and electronics manufacturing, alloying, mining), which can lead to various health outcomes such as lung disease and cancer (61,85). Abnormal concentrations of metals and metalloids in serum and in urine attributable to occupational exposure has been reported in various studies (e.g., Pb, Cd, As) (61). Nutrition and diet are another major source of trace elements affecting the trace element composition of hair and nails.

The consumption of local food (e.g., wild meat, fish, locally grown berries, fruits and vegetables) and the use of traditional recipes (i.e., home-processed food), can influence the total metal intake from the diet (e.g., for Fe, Cd, Pb, Hg, Al) (86–89). In addition to occupational or environmental exposures, along with nutrition (the intake of food and water), age, sex, gender, body mass index (BMI), habits such as smoking, the presence of underlying diseases and finally educational, racial and ethical factors all play a part in and affect the trace element content of these tissues (62,73,90–92). For example, smoking or smoke exposure is considered an important source for toxic heavy metals (e.g., Cd, Cu, V, Sr) and can alter the body burden and homeostasis of trace elements (93–95). Cigarettes or cigarette smoke contain various trace elements like Cd, Cu, V, Sr, Se, Pb, Al, Cr, Mn, Se. For example, studies have shown increased body burden of Cd (e.g., in blood, urine, organs) of smokers compared to occupationally unexposed non-smokers (95). Urinary concentrations of Cr have also been shown to be influenced by smoking (95). Smokers and former smokers have higher blood Pb levels than non-smokers (95). Education and income can also indirectly influence trace element burden in individuals (influencing habits such as smoking). For example, a study showed decreasing whole blood Cd concentrations with increasing levels of education and income in models adjusting for other covariables (96). The source and the rate of consumption of drinking and cooking water may also affect exposure to trace elements (37,91). For hairs, hair colour, hair care routines, and the source of bathing water also are important factors that affect trace element content of hair tissue (62,73,90,91). In nail and hair sampling, exogenous contamination -due to dust, use of cosmetics such as nail polish, or use of pharmaceutical

products- should be also taken into consideration (75,78,97–99). These factors may be increasing the measure of trace elements in these tissues, thus affecting the trace element relative source contribution and overall exposure (75,78,97,98). For example, Al, Ba, Fe and Mg have been found in nail polish as ingredients, and Al, Pb, Ni, Sr and Sb as heavy metal impurities (98,99).

During pregnancy, the body undergoes different changes, and biomarkers of trace element exposure can be affected. A major documented change includes namely increased urination with trace element mobilization. Studies show higher levels of trace elements in the urine of pregnant women compared to levels in non-pregnant women (33). For example, higher levels have been observed in pregnant women for mean urinary As, Pb, Ba (33). Studies also show that serum Cu levels are significantly higher after pregnancy than before pregnancy, while Ca and Fe levels are decreased (especially during the third trimester of pregnancy) (100). Overall, studies so far indicate that pregnancy can affect the trace element levels for certain trace elements in the body compared to non-pregnant women.

In sum, assessment of trace elements from three biomarkers -urine, hair and nails- allows to draw an image on both past and present exposures, and there are variety of factors that can influence levels of trace elements in these matrices (80). Many of these different factors (e.g., nutrition, smoking status) discussed here need to be considered in exposure assessment as they can override exposure footprints.

1.8 Research problem

Northeastern British Columbia, an area of intensive unconventional oil and gas (UOG) exploitation (namely by hydraulic fracturing), could be contaminated by several contaminants, e.g., trace elements, with potentially deleterious health effects on vulnerable population groups, such as pregnant women and the developing fetus. To date, there is limited data on pregnant women's exposure to contaminants associated with UOG activity. In the past, specifically in Montney, concerns have been raised by local people regarding the health consequences of living near UOG sites. However, biomonitoring data lacked in Northeastern British Columbia. In 2016, in the Peace River Valley, a pilot study was conducted by our research team to assess exposure to 19 trace elements in 29 pregnant women using urine and hair samples (101,102). Relatively high levels of certain trace elements such as Ba, Al, Sr, and Mn were observed in the participants compared to reference populations (101,102). Although these results were suggestive of higher exposure in this region, many questions remained unanswered, namely in terms of

their association with the density and proximity of UOG wells. This led our team to conduct a larger biomonitoring study in the area called the Exposures in the Peace River Valley (EXPERIVA) study.

1.9 Objectives

In the present study, as part of this larger biomonitoring initiative called the EXPERIVA study, we collected and measured trace element concentrations in an extensive set of hair, nails, and repeated urine and tap water samples from 85 pregnant women from the Peace River Valley. Specifically, we aimed to

1) measure concentrations of 21 trace elements in biological (hair, urine, nails) and tap water samples from pregnant women;

2) compare levels of trace elements with those from reference populations and health-based guidance values;

3) assess correlations between concentrations measured in different matrices; and

4) evaluate associations between the density/proximity of UOG wells and trace element concentrations in samples.

2. Materials and methods

2.1 Study area, recruitment, data collection and sampling

The study took place in the Peace River Valley in Northeastern British Columbia, an area of intensive UOG activity. More precisely, the study was conducted in the Treaty 8 territory, the traditional territory of the Cree, Saulteau and Dunne-Za people. Our research team collaborated with the Treaty 8 Tribal Association, the Saulteau First Nations and the West Moberly First Nations for the initiation of this project. The details of the project (e.g., the objectives, the methods, the research problem) were shared with them and their consent and support were received for this project. Their collaboration was especially important for the recruitment of participants from the communities and for having their trust that their best interests were a priority throughout the study. Overall, 92 pregnant women were recruited through four medical and midwifery clinics offering prenatal care. The recruitment of eligible participants -English speaking pregnant women above 18 years old- was completed from May to September 2019. Initially, the health care practitioners in these clinics -that is, the physician, the nurse practitioner, or the midwife- informed their patients about the research project, and those who expressed interest were privately met by a member of the research team. At this first meeting, further information regarding the entirety of the research project and its full implications was shared with them, and they were invited to ask questions before filling out a written consent form.

At recruitment, participants received the instructions on how to collect and freeze urine samples at home for 7 consecutive days, and were provided with all the material needed for this. For toenails, participants received instructions and materials (toenail clipper and minicentrifuge 2 mL tube) to collect polish-free toenail clipping samples. Participants were asked to let their toenails grow before collection. Most also provided hair samples at the time of recruitment, which was sampled by a member of the research team. The frozen urine samples, along with nail, tap water, and hair (if not collected at recruitment) samples, were collected at participants' home approximately 7 days later by a member of the research team. At that time, the team member administered a questionnaire on sociodemographic data, lifestyle habits, food intakes and many other characteristics. Data on housing and other characteristics (including characteristics regarding the potential covariates to be used in the multiple regression models) were also collected.

As a compensation, participants received a 25$ gift card and their results for contaminants with health-based guidance values. Out of the 92 recruited pregnant women, seven withdrew at some point during the study, resulting in a total of 85 participants. Some participants did not provide certain samples, resulting in the availability of hair samples from 79, nail samples from 70, and urine and water samples from 85 pregnant women. The collected samples are considered on loan for the aims of the study and will be ultimately returned to nature. The approach to return samples to nature is to be determined with the communities at the end of the biobanking period. In addition to these samples, as part of the EXPERIVA study, additional samples were collected (e.g., air samples) and other investigations were conducted as well (e.g., on air VOCs and radon). The present study considered only the abovementioned samples.

The study protocol and the consent form for recruiting the participants have been approved by the ethics committee of the University of British Columbia, the "Northern Health Research Review Committee", and by the Ethics Committee for Clinical Research from the University of Montreal (#CERC-18-003-P) (Annex A).

2.2 Urine sampling

Daily spot urine samples of 12 ml were collected by the participants at their home over 7 consecutive days (a sample per day). Participants were asked to collect urine between dinner and bedtime. The collection of urine samples over several days allowed to account for day-to-day variations in the participant's exposure and pharmacokinetics (103). Sample handling involved initial storage in participant's freezer at -20°C, and their pick-up and transportation on ice to the laboratory for analysis.

2.3 Hair sampling

Hair sampling involved collecting one sample per participant in its entire length over an area of 2-3 cm^2. Scissors were used by a member of our research team to cut the hair at the area closest to the scalp. Sample handling included storage in plastic bags at room temperature until analyses.

Hair grows in average about 1 cm per month from scalp, with some variations dependent on age, gender, race, ethnicity, nutrition, metabolic rate and other factors (83). Segmental analysis of hair samples allows reconstructing body burden over the past months (104). In this study, the first 2 cm

closest to the scalp were analyzed, which reflects the body burden over the ~2 months prior to hair collection.

2.4 Nail sampling

Nail sampling involved clippings from all toes of the free edge of the nails for each participant. Stainless-steel clippers were used to collect toenail samples. Toenails had to be free of nail polish. Sample handling involved storage in 1.5 mL microtubes at room temperature until analyses.

Toenails grow on average about 1.3-2.10 mm per month from nail matrix, with some variations dependent on toe, age, gender, metabolic rate, and other factors (84,105). The distal free edge of the nail is isolated from the body's metabolic activities and reflects the body burden from months to a year prior to collection (84,105). In this study, all the distal free edges of toenails were analyzed.

2.5 Tap Water sampling

Two water samples were taken from the participant's kitchen tap. The first was collected when turning on the tap and the second after 5 minutes of water running. This was done to capture the possible water contamination from pipes (e.g., lead, cadmium) (71,72). Sample handling involved collection into 15 mL polypropylene tubes, transportation on ice to the laboratory and storage in freezer at -20°C until analyses.

2.6 Chemical determination of trace elements in water, urine, hair, and nails samples

Concentrations of 21 trace elements - lithium (Li), beryllium (Be), aluminum (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), arsenic (As), selenium (Se), strontium (Sr), silver (Ag), cadmium (Cd), barium (Ba), thallium (Tl), lead (Pb) and uranium (U)- were measured in water, urine, hair and toenail samples by ICP-MS 7700x model (Agilent, Mississauga, Canada), using a validated method used for multielement analyses in other studies (101,106–108). Measurements were performed in a trace metal-clean room (ISO 3 146442-1 standard). Essentially, the water and urine samples were diluted five times with HNO_3 at 2%. No other treatment was done on the water prior to analysis. Urine samples were heated for 15 minutes at 45°C to dissolve the residues prior ICP-MS analysis. Hair and toenail samples were sequentially processed as follows:

washed 3 times with a Triton X-100 solution diluted 1/200 in MilliQ water, with 5 minutes sonication at each washing; washed 3 times with acetone, with 3 minutes sonication at each washing; rinsed 3 times with MilliQ water, with 3 minutes sonication between each rinsing; washed again 3 times with acetone, with 3 minutes sonication at each washing. After washing, the samples were dried by evaporation, using a Thermo Scientific Integrated SpeedVac system. Hair and toenail samples were weighed (50 mg minimum for hair and 15 mg for nails) and placed in PFA (perfluoroalkoxy alkanes) digestion tubes prior ICP-MS analysis; 1 mL of HNO_3 at 70% and 0.1 mL of internal standard mixture (rhodium and indium) at 1000 ppb were added to each tube. The tubes were left for 15 minutes at room temperature to dissolve the hair and toenail samples and placed in a block heater at 95°C until completely dissolved; 1.5 mL H_2O_2 was added, left for a few minutes at room temperature and then placed in a block heater at 95°C. H_2O_2 was added again until the solution became colorless, then evaporated to about 0.5 mL. The hair and toenail solutions were placed in 15 mL polypropylene tubes and adjusted to a volume of 2 mL with MilliQ water. They were then diluted 1/5 with HNO_3 at 2 % prior to ICP-MS analysis. For the quantification of metals and trace elements in water, urine, hair and toenails, the ICP-MS was operated under the following conditions: RF power at 1550 W, nebulizer gas flow rate at 0.65 L of Ar/minute, dilution gas flow rate at 0.41 L Ar/ minute, collision gas flow rate in helium mode between 4.3 to 5 mL He/minute for water, urine, hair, and toenails analysis. Limits of detection for the trace elements in this study are provided in Appendix A1. The analytical method has been validated by quality control procedures, which include the analysis of replicate samples and blanks. The mean repeatability from 10 replicate analyses of toenail blanks spiked at two levels (250 and 1000 ng) with reference standards of the analytes was >92% for all elements; the coefficient of variation was less than 7%. The mean repeatability from 10 replicate analyses of urine blanks spiked at two levels (250 and 1000 ng) with reference standards of the analytes was >90%; the coefficient of variation was less than 5%. The mean repeatability from 2 replicate analyses of ClinChek Urine Control QC, Level I (QC1) (Ref: 8847-8849; lot: 122) and Level II (QC2) (Ref: 8847-8849; lot: 122) was >96% for all elements. The mean repeatability from 10 replicate analyses of hair blanks spiked at two levels (1500 and 3000 ng) with reference standards of the analytes was >90% for all elements; the coefficient of variation was less than 10%. The mean repeatability from 20 replicate analyses of external QC hair samples (QM-H-Q1309) from the National Institute of Public Health of Quebec (INSPQ) was >92% (except for arsenic with a value around 85%). The mean repeatability from 10 replicate analyses of toenail blanks spiked at two levels (150 and 300 ng) with reference standards of the analytes was >85% for all elements; the coefficient of variation was less than 12%. No external QC samples were available for toenails. To account for urine dilution, we

also measured creatinine in urine samples using the Jaffé method, an alkaline picric acid method with deproteinization (enzymatic colorimetric test PAP from Boehringer Mannheim, Germany) (109) (Annex C).

2.8 Density and proximity of UNG wells

We calculated UOG well density (number of wells) and proximity (distance between residence and wells) within four buffer sizes: 2.5 km, 5 km, and 10 km around participants residence, and no buffer (i..e, therefore accounting for all wells present in Northeastern British Columbia). Measures of density and proximity were combined using the inverse distance weighting (IDW) method (Fig. 1). Briefly, IDWs for each buffer zone were calculated as follows: $IDWx = \Sigma^{n}_{i=1} * (1/di)$, where "x" is the radius, "i" is the given well inside the buffer, "di" is the distance between the given well and the participant's residence in km (calculated using QGIS) and "n" is the total number of wells inside that buffer. Calculations were carried out using R version 3.5.3. More details about this method can be found in a previous article by our team (27).

2.9 Reference concentrations and reference populations

Descriptive statistics of trace element concentrations in the biological and water samples from EXPERIVA participants were compared to concentrations in reference populations whenever data existed. Although recent reference concentrations and comparable reference populations were preferred (e.g., Canadian, pregnant women, ages 18-40), their availability was limited.

For urine, trace element concentrations (wet-weight urinary concentrations in ng/ml or µg/L) were compared with biomonitoring data of 770 women of 20-39 years of age from the Canadian Health Measure Survey (CHMS 2nd cycle) (74). This applied to V, Mn, Co, Ni, Cu, Zn, As, Se, Cd, Th, and Pb (Appendix A2 Table 1). Urinary trace element concentrations were also compared to data from Canadian First Nations Biomonitoring Initiative (FNBI) (113), where data from 302 first nations women aged 20 years and older are provided for the same elements as for CHMS (Appendix A2 Table 1).

The remaining trace elements, unavailable in CHMS cycle 2, were compared with US populational data that included data on 1537 women above three years of age from the National Health and Nutrition Examination Survey (NHANES 9TH cycle) (110). This applied namely to Sr and Ba (Appendix A2 Table 1). For the remaining urinary trace elements not included in these two biomonitoring reports,

concentrations were compared with reference values from a published study by Goullé et al., that is, with the data of 100 healthy and non-occupationally exposed adults (46 men, 54 women) from France (111,112). This applied to Li, Al, and Ga (Appendix A2 Table 1). Reference urinary values were missing for urinary Cr and Fe.

Urinary concentration of trace elements (wet-weight in µg/L and creatinine-adjusted urinary concentrations in µg/g creatinine) were also compared with available health-based guidance values using the Biomonitoring Guidance Value Database and Comparison Tool (114). Biomonitoring equivalents (BE) were available for Cd, Zn, and Se, and Human Biomonitoring (HBM) values were available for Cd and Tl from the German Human Biomonitoring Commission (HBM commission 2011) (Appendix A2 Table 2).

For hair, trace element concentrations were preferentially compared to biomonitoring data of 916 women of 20-59 years of age from the Canadian Health Measure Survey (CHMS cycle 5) (115). This applied to Li, Be, Al, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Ba, Tl, Pb, and U (Appendix A2 Table 3). The remaining trace elements, unavailable in CHMS cycle 5, were compared with the published reference values from the Goullé et al. (111,112). This applied namely to Ga and Sr (Appendix A2 Table 3). Reference hair values were missing for Fe.

There were no reference values identified for trace element concentrations in nails.

For water, trace element concentrations were compared with maximum acceptable concentrations available from the Guidelines for Canadian Drinking Water Quality (116) and from Source Drinking Water Quality Guidelines of British Columbia (117) (Appendix A2 Table 4). Reference values were missing for Li, Be, V, Fe, Ga, Ag, and Tl.

Measured concentrations of trace elements were displayed in a table and contrasted to the reference values using gray highlight -the density of the gray highlight being used to give a visual for how many times in a relative and approximated scale the measured values exceeded the reference values (from 1-5 times for our data).

2.10 Statistical Analyses

Concentrations below the LOD were imputed using regression on order statistics (ROS function and NADA imputation packages in R). Descriptive statistics (medians and 95th percentiles) were calculated

for each trace element in the different matrices. For urine, arithmetic mean concentration values for each participant were calculated from the repeated samples (equivalent to pooling). For subsequent analyses, only trace elements with a detection frequency equal or greater than 60% were included. Spearman's rank correlations were used to evaluate the correlation between the trace element concentrations in the different samples. Given the exploratory nature of this analysis, we did not put emphasis on p values, and generated heatmaps (i.e., a visual representation of data as a map in which data are represented as colors) to evaluate patterns of association. Next, associations were assessed between the density/proximity of UOG wells (IDW metrics) and concentrations of trace elements in the biological and water samples in multiple linear regression models controlling for covariates specific to each matrix. We first identified variables potentially associated with increased/reduced trace element concentrations or UOG density/proximity using published literature (37,39,62,63,94,118–121). Because of the large number of potential covariates identified, we selected a subset of covariates by performing bivariate analyse(Wilcoxon and Kruskal-Wallis). If a covariate showed an association with a *p* value of <0.2 with at least one of the trace elements and with at least one of the IDW density/proximity metrics, it was included in the multiple linear regression model (27). For water, multiple linear regression models were adjusted for sources of kitchen tap water (municipal, cistern, private well, or other sources). For biological samples, multiple regression models were adjusted for cooking water (bottled vs. others), drinking water (bottled vs. others), source of tap water (municipal, private well, cistern, or others), work in industry, smoking status at recruitment (active smoking vs. no active smoking) and consumption of local food (Table 1). All statistical analyses in this study were carried out using R version 4.0.4 and Excel.

3. Article

Author Contributions

Lilit Gasparyan

- worked in collaboration with her research supervisors, Marc-André Verner and Delphine Bosson-Rieutort, to develop a research protocol and the resources necessary for its implementation,
- worked in collaboration with her research supervisors to determine the statistical analyses to be implemented to tackle the research problem and to develop data presentation strategy to reach the objectives of the study,
- managed the dataset and realized all the steps following the data collection,
- carried out the statistical analyses in RStudio in close collaboration with Juliette Duc and Delphine Bosson-Rieutort;
- produced the datasets, the figures and the tables, and carried out the interpretation of the results in close collaboration with her research supervisors,
- wrote the manuscript and made corrections according to the comments of her research supervisors and co-authors.

Marc-André Verner

- developed and implemented the research project in collaboration with Élyse Caron-Beaudoin,
- worked in collaboration with Lilit Gasparyan to develop the research protocol, and to mobilize the resources needed to conduct the study,
- worked in collaboration with Lilit Gasparyan to determine the statistical analyses to be implemented,
- supervised Lilit Gasparyan during her entire involvement in the master's program, followed her progress closely and was involved in all stages of her academic journey,
- has participated in obtaining ethics approval for this study,
- contributed to the interpretation of data in collaboration with Lilit Gasparyan,
- contributed to the development of the manuscript by revising it,

Juliette Duc

- worked closely with Lilit Gasparyan in carrying out the statistical analyses using R software,
- helped manage the datasets,
- helped with the layout of datasets with the R software.

Delphine Bosson-Rieutort

- as co-director, collaborated with Lilit Gasparyan and Marc-André Verner, and her student Juliette Duc, in the key stages of the implementation of the statistical tests using R software,
- advised Lilit Gasparyan and Juliette Duc on best practices in data analysis using R software.
- provided advice on the selection and feasibility of statistical analyses.

Michèle Bouchard

- developed analytical protocols and conducted the sample analyses in her laboratory and shared the data necessary to carry out the study.
-

Maryse Bouchard

- involved in advising statistical approaches and interpretation of data.

Élyse Caron-Beaudoin

- developed and implemented the research project in collaboration with Marc-André Verner,
- led the recruitment and sampling phases,
- provided comments and corrections to the scientific article,
- advised on the best types and approaches for data analysis in this study.

Lucie Claustre dit Barbahére

- Collaborated and helped in formatting of the raw data.

Density and proximity of unconventional oil and gas wells and concentrations of trace elements in urine, hair, nails and tap water samples from pregnant women living in Northeastern British Columbia

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Abstract

Background. Northeastern British Columbia (Canada) is an area of unconventional oil and gas (UOG) exploitation, which can release several contaminants, namely, trace elements, with potentially deleterious effects on fetal development. To date, there is limited data on pregnant women's exposure to contaminants associated with UOG activity. **Objectives.** We aimed to 1) measure levels of trace elements in biological and tap water samples collected from pregnant women participating in the EXPERIVA study; 2) compare the concentrations to those from reference populations and to those from health-based guidance values 3) assess correlations between levels measured across matrices; and 4) evaluate associations between the density/proximity of UOG wells and trace element levels. **Methods.** We collected tap water, hair, nails, and repeated urine samples from 85 pregnant women, and measured concentrations of 21 trace elements. We calculated UOG well density/proximity (Inverse Distance Weighting [IDW]) for 4 buffer sizes (2.5 km, 5 km, 10 km, no limit). Trace element concentrations were compared to those from reference populations. Correlations were assessed using Spearman's correlation test; and multiple linear regression models were used to evaluate the associations between IDWs and trace elements concentrations. **Results.** We found higher urinary and hair concentrations of certain trace elements in study participants compared to reference populations (e.g., Mn, Sr, Co, Ba). Correlation coefficients ranged from -0.23 to 0.65 across matrices; correlations with tap water concentrations were strongest for hair, followed by nails, and urine. Positive (e.g., Cu, Ga, Ba) and negative (e.g., Fe) associations were observed between IDW metrics and the concentrations of certain trace elements in water, hair, and nails. **Conclusions.** Our results suggest that pregnant women living in an area of UOG activity may be more exposed to certain trace elements (e.g., Mn, Sr, Co, Ba) than the general population, but the association with density/proximity of wells remains unclear.

Keywords : trace elements, unconventional oil and gas exploitation, gestational exposure, tap water, hair, nails, urine, Northeastern British Columbia

3.1. Introduction

The Peace River Valley located in Northeastern British Columbia (Canada) is an area of intensive unconventional oil and gas (UOG) exploitation (1). Such industrial activity may lead to environmental contamination, to unwanted exposure and adverse health outcomes in local people (2–12). For example, the content of wastewaters resulting from hydraulic fracturing can contain various environmental contaminants, namely, trace elements, sometimes in considerably high concentrations. (e.g., barium, aluminium, strontium, manganese) (13,22). Water contamination (e.g., surface water, groundwater, private drinking water) from the release of trace elements from UOG activities has been previously observed. For example, increased groundwater levels of barium have been observed in Peace River Valley, and arsenic, barium and strontium in Texas private drinking water (44–46). Drinking water can be an important source of human exposure and pose significant health risks (37,41,47). Some trace elements are considered essential micronutrients and are required in trace amounts based on their role and participation to metabolic processes in the human body (e.g., iron, manganese, zinc, copper, selenium, boron) (30). On the other hand, others are non-essential (e.g., arsenic, lead, cadmium) (32). Exposure to trace elements can occur via different pathways (e.g., ingestion of food, inhalation, dermal contact) (30). Both deficiencies (specifically for essential trace elements) and excess levels of trace elements have been associated with adverse health effects, namely in the developing fetus (32,36). In the context of human health risk assessment, exposure to trace elements is often estimated through biomonitoring (73–75). Depending on the trace element and exposure period of interest, urine, blood, hair, and nail sampling have been widely used to estimate short- and/or long-term exposures (41,77–79).

Many epidemiologic studies in North America have suggested associations between exposure to UOG activity during pregnancy and health outcomes like preterm birth and low birth weight (6–12). The etiologic factors underlying these associations have yet to be determined but may include gestational exposure to contaminants like trace elements. To date, there is limited data on pregnant women's exposure to contaminants associated with UOG activity (13,14). In 2016, in the Peace River Valley, a pilot study was conducted by our research team to assess exposure to 19 trace elements in 29 pregnant women using urine and hair samples (101,102). Relatively high levels of certain trace elements including aluminum, barium, strontium, and manganese were observed in the participants compared to reference populations (101,102). Although these results were suggestive of higher exposure in this region, many questions remained unanswered, namely in terms of their association with the density and proximity of

UOG wells. This led our team to conduct a larger biomonitoring study: the Exposures in the Peace River Valley (EXPERIVA) study.

In the context of the EXPERIVA study, we collected and measured trace element concentrations in an extensive set of hair, nails, and repeated urine and tap water samples from 85 pregnant women from the Peace River Valley. In this study, we aimed to 1) measure concentrations of 21 trace elements in biological (hair, urine, nails) and environmental (tap water) samples from pregnant women; 2) compare levels with those from reference populations and health-based guidance values; 3) assess correlations between concentrations measured in different matrices; and 4) evaluate associations between the density/proximity of UOG wells and trace element concentrations in the samples.

3.2. Materials and methods

3.2.1 Study area and recruitment

In the Peace River Valley in Northeastern British Columbia (Canada), 92 pregnant women were recruited through four medical and midwifery clinics offering prenatal care. The recruitment of eligible participants -English speaking pregnant women above 18 years old- was completed from May to September 2019. Initially, the health care practitioners in these clinics -that is, the physician, the nurse practitioner, or the midwife- informed their patients about the research project, and those who expressed interest were privately met by a member of the research team. At this first meeting, further information regarding the entirety of the research project and its full implications was shared with them, and they were invited to ask questions before filling out a written consent form.

At recruitment, participants received the instructions on how to collect and freeze urine samples at home for 7 consecutive days and were provided with all the material needed for this. For toenails, participants received instructions and materials (toenail clipper and minicentrifuge 2 mL tube) to collect polish-free toenail clipping samples. Participants were asked to let their toenails grow before collection. Most also provided a hair sample at the time of recruitment, which was sampled by a member of the research team. The frozen urine samples, along with nail, tap water, and hair (if not collected at recruitment) samples, were collected at participants' home approximately 7 days later by a member of the research team. At that time, the team member administered a questionnaire on sociodemographic data, lifestyle habits, food intakes and many other characteristics. Data on housing and other characteristics were also collected.

Out of the 92 recruited pregnant women, seven withdrew at some point in the study, resulting in a total of 85 participants. Some participants did not provide certain samples, resulting in the availability of hair samples from 79, nail samples from 70, and urine and water samples from 85 pregnant women.

3.2.2 Urine sampling

Daily spot urine samples of 12 ml were collected by the participants at their home over 7 consecutive days (one sample per day). Participants were asked to collect urine between dinner and bedtime. The collection of urine samples over several days allowed to account for day-to-day variations in the

participant's exposure and pharmacokinetics (103). Sample handling involved initial storage in participant's freezer at -20°C, and their pick-up and transportation on ice to the laboratory for analysis.

3.2.3 Hair sampling

Hair sampling involved collecting hair in its entire length over an area of 2-3 cm^2 in participants whose hair was longer than 2cmScissors were used by a member of our research team to cut the hair at the area closest to the scalp. Sample handling included storage in plastic bags at room temperature until analyses.

Hair grows in average about 1 cm per month from scalp, with some variations dependent on age, gender, racial, ethnical, nutritional, metabolic and other factors (83). Segmental analysis of hair samples allows reconstructing body burden over the past months (104). In this study, the first 2 cm closest to the scalp were analyzed, which reflects the body burden over the 2 months prior to hair collection.

3.2.4 Nail sampling

Nail sampling involved clippings from all toes of the free edge of the nails for each participant. Stainless-steel clippers were used to collect these toenail samples. Toenails had to be free of nail polish. Sample handling involved storage in 1.5 mL microtubes at room temperature until analyses.

Toenails grow is in average about 1.3-2.10 mm per month from nail matrix, with some variations dependent on toe, age, gender, metabolic rate, and other factors (84,105). The distal free edge of the nail is isolated from body's metabolic activities and reflects the body burden from months to a year prior to collection (84,105). In this study, all the distal free edges of toenails were analyzed.

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3.2.6 Chemical determination of trace elements in water, urine, hair, and nails samples

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elements; the coefficient of variation was less than 7%. The mean repeatability from 10 replicate analyses of urine blanks spiked at two levels (250 and 1000 ng) with reference standards of the analytes was >90%; the coefficient of variation was less than 5%. The mean repeatability from 2 replicate analyses of ClinChek Urine Control QC, Level I (QC1) (Ref: 8847-8849; lot: 122) and Level II (QC2) (Ref: 8847-8849; lot: 122) was >96% for all elements. The mean repeatability from 10 replicate analyses of hair blanks spiked at two levels (1500 and 3000 ng) with reference standards of the analytes was >90% for all elements; the coefficient of variation was less than 10%. The mean repeatability from 20 replicate analyses of external QC hair samples (QM-H-Q1309) from the National Institute of Public Health of Quebec (INSPQ) was >92% (except for arsenic with a value around 85%). The mean repeatability from 10 replicate analyses of toenail blanks spiked at two levels (150 and 300 ng) with reference standards of the analytes was >85% for all elements; the coefficient of variation was less than 12%. No external QC samples were available for toenails. To account for urine dilution, we also measured creatinine in urine samples using Jaffe's reaction (picric acid method).

3.2.8 Density and proximity of UNG wells

We calculated UOG well density (number of wells) and proximity (distance between residence and wells) within four buffer sizes: 2.5 km, 5 km, and 10 km around participants residence, and no buffer. Measures of density and proximity were combined using the Inverse Distance Weighting (IDW) method (Figure 1). Briefly, IDWs for each buffer zone were calculated as follows: $IDWx = \Sigma^{n}_{i=1} * (1/di)$, where "x" is the radius, "i" is the given well inside the buffer, "di" is the distance between the given well and the participant's residence in km (calculated using QGIS) and "n" is the total number of wells inside that buffer. Calculations were carried out using R version 3.5.3. More details about this method can be found in a previous article by our team (27).

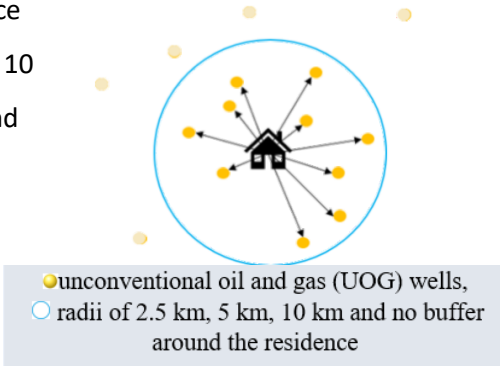


Figure 1 – Density and proximity of UOG wells.

3.2.9 Reference concentrations and reference populations

Descriptive statistics of trace element concentrations in the biological and water samples from EXPERIVA participants were compared to concentrations in reference populations whenever data existed.

Although recent reference concentrations and comparable reference populations were preferred (e.g., Canadian, pregnant women, ages 18-40), their availability was limited.

For urine, trace element concentrations (wet-weight urinary concentrations in ng/ml or µg/L) were preferentially compared with biomonitoring data of 770 women of 20-39 years of age from the Canadian Health Measure Survey (CHMS 2nd cycle) (74). This applied to V, Mn, Co, Ni, Cu, Zn, As, Se, Cd, Th, and Pb (Appendix A2 Table 1). Urinary trace element concentrations were also compared to data from Canadian First Nations Biomonitoring Initiative (FNBI 2011) (113), where data from 302 First Nations women aged 20 years and older are provided for the same elements as for CHMS (Appendix A2 Table 1). For Sr and Ba, unavailable in CHMS cycle 2, concentrations were compared with US populational data that included data on 1537 women above three years of age from the National Health and Nutrition Examination Survey (NHANES 9TH cycle) (110) (Appendix A2 Table 1). For the remaining urinary trace elements not included in these three biomonitoring reports, concentrations were compared with reference values from a published study by Goullé et al., that is, with the data of 100 healthy and non-occupationally exposed adults (46 men, 54 women) from France (111,112). This applied to Li, Al, and Ga (Appendix A2 Table 1). Reference urinary values were missing for urinary Cr and Fe. Urinary concentration of trace elements (wet-weight in µg/L and creatinine-adjusted urinary concentrations in µg/g creatinine) were also compared with available health-based guidance values included in the Biomonitoring Guidance Value Database and Comparison Tool (114). Biomonitoring equivalents (BE) were available for Cd, Zn, and Se, and Human Biomonitoring (HBM) values were available for Cd and Tl from the German Human Biomonitoring Commission (HBM commission 2011) (Appendix A2, Table 2).

For hair, trace element concentrations were preferentially compared to biomonitoring data of 916 women of 20-59 years of age from the Canadian Health Measure Survey (CHMS cycle 5) (115). This applied to Li, Be, Al, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Ba, Tl, Pb, and U (Appendix A2 Table 3). The remaining trace elements, unavailable in CHMS cycle 5, were compared with the published reference values from the Goullé et al. (111,112). This applied to Ga and Sr (Appendix A2, Table 3). Reference hair values were missing for Fe.

There were no reference values identified for trace element concentrations in nails.

For water, trace element concentrations were compared with maximum acceptable concentrations available from the Guidelines for Canadian Drinking Water Quality (116) and from Source Drinking

Water Quality Guidelines of British Columbia (117) (Appendix A2 Table 4). Reference values were missing for Li, Be, V, Fe, Ga, Ag, and Tl.

Measured concentrations of trace elements were displayed in a table and contrasted with the reference values using gray highlight -the density of the gray highlight being used to give a visual for how many times in a relative scale the measured values exceeded the reference values (from 1 to 5 times for our data).

3.2.10 Statistical analyses

Concentrations below the limit of detection (LOD) were imputed using regression on order statistics (ROS function and NADA imputation packages in R). Descriptive statistics (medians and 95th percentiles) were calculated for each trace element in the different matrices. For urine, arithmetic mean concentration values for each participant were calculated from the repeated samples (equivalent to pooling). For subsequent analyses, only trace elements with a detection frequency equal or greater than 60% were included. Spearman's rank correlations were used to evaluate the correlation between the trace element concentrations in the different matrices. Next, associations were assessed between the density/proximity of UOG wells (IDW metrics) and concentrations of trace elements in the biological and water samples in multiple linear regression models controlling for covariates specific to each matrix. We first identified variables potentially associated with increased/reduced trace element concentrations or UOG density/proximity based on published literature (37,39,62,63,94,118–121). Because of the large number of potential covariates identified, we selected a subset of covariates by performing bivariate analyses (Wilcoxon and Kruskal-Wallis). If a covariate showed an association with a *p* value of <0.2 with the concentration of at least one of the trace elements and with at least one of the IDW density/proximity metrics, it was included in the multiple linear regression model (27). For water, multiple linear regression models were adjusted for sources of kitchen tap water (municipal, cistern, private well, or other sources). For biological samples, multiple linear regression models were adjusted for cooking water (bottled vs. others), drinking water (bottled vs. others), source of tap water (municipal, private well, cistern, or others), work in industry, smoking status at recruitment (active smoking vs. no active smoking) and consumption of local food (Table 1). Given the exploratory nature of this analysis, we did not put emphasis on p values, and instead generated heatmaps to evaluate patterns of associations. All statistical analyses in this study were carried out using R version 4.0.4 and Excel.

3.3. Results

3.3.1 Characteristics of the EXPERIVA study participants

The median age of EXPERIVA participants was 29 years (Table 1). Most of the participants at the time of recruitment were identified as non-smokers (92%) and unexposed to second-hand smoke during their pregnancy (76%). Most also had tap water from the municipality (60%), drank bottled or filtered tap water (69%), and consumed local food (69%). For drinking water, 37 (44%) of the participants drank bottled water, 22 (26%) filtered tap water, and only 23 (27%), drank tap water directly; the other 3 (4%) participants used water for drinking from more than one source. In addition, 35% of the participants had at least one UOG well within 2.5 km from their home, and 51% of the participants who did not have a well within 2.5-kms had at least one well from between 2.5 and 5 km from their home.

Table 1. Characteristics of pregnant women from the EXPERIVA study (n=85)

CHARACTERISTIC	N (%)	MEDIAN (MIN ; MAX)
AGE (YEARS)		29 (18 ; 40)
BMI AT PREGNANCY (KG/M^2)		26.4 (19.3 ; 48.7)
SELF-IDENTIFIED AS INDIGENOUS		
Yes	15 (18)	
No	70 (82)	
MATERNAL EDUCATION		
12th grade or less	23 (27)	
Certificate	41 (48)	
Bachelor, Graduate degree, or Master	21 (25)	
SMOKED AT RECRUITMENT		
Yes	7 (8)	
No	78 (92)	
EXPOSED TO SECOND-HAND SMOKE DURING PREGNANCY		
Ever	20 (24)	
Never	65 (76)	
USE OF INDOOR FIREPLACE DURING URINE SAMPLING PERIOD		
Yes	3 (3)	
No	82 (97)	
SOURCE OF DRINKING WATER		
Only tap water	23 (27)	
Only filtered tap water	22 (26)	
Only bottled water	37 (43)	
More than one source [a]	3 (4)	
SOURCE OF COOKING WATER		
Only tap water	72 (84)	
Only filtered tap water	4 (5)	
Only bottled water	5 (6)	
More than one source [b]	4 (5)	
SOURCE OF TAP WATER AT HOME		
Only municipal	51 (60)	
Only private well	5 (6)	
Only cistern	22 (26)	
Only surface source	1 (1)	
Other (e.g., dug out)	5 (6)	
More than one source [c]	1 (1)	
CONSUMPTION OF LOCAL FOOD DURING THE PAST MONTH OF SAMPLING		
Wild meat	31 (36)	
Wild berries	28 (33)	
Veggies or fruits from garden	37 (44)	
Caught fish	11 (13)	
Any of the above	**59 (69)**	
USE OF FINGERNAIL POLISH DURING SAMPLING PERIOD		
Yes	20 (24)	
No	65 (76)	
WORKING IN INDUSTRIAL FIELD [d]		
Yes	8 (9)	
No	77 (91)	
NUMBER OF PARTICIPANTS WHO HAVE THE CLOEST UOG WELLSWHO HAVE AT LEAST ONE UNG WELL TO A GIVEN DISTANCE FROM HOME FROM THEIR HOME		
within 2.5 km	30 (35)	
from 2.5-5 km	43 (51)	
from 5-10 km	9 (11)	
from ≥ 10 km	3 (3)	

NUMBER OF WELLS WITHIN A GIVEN DISTANCE FROM HOME	
within 2.5 km	**0 (0; 50)**
within 5 km	**9 (0; 138)**
within 10 km	**90 (0; 346)**
IDW	
within 2.5 km	0 (0; 47)
within 5 km	3 (0; 67)
within 10 km	13 (0; 94)
no buffer (all wells)	281 (168; 354)

[a] More than one source of drinking water involves a consumption of both tap and bottled water for 2 participants, and the consumption of both tap water and filtered tap water for 1 participant.

[b] More than one source of cooking water involves the use of both tap and bottled water for 3 participants, and the use of both tap and filtered tap water for 1 participant.

[c] More than one source of tap water at home includes a mix of water coming from a private well and a cistern for 1 participant.

[d] Working in any of the following workplaces : mine, natural gas, construction, forestry, pipeline, manufacturing metals, plastics, petroleum, rubber, textiles, glass, ceramics, paper, electronics, hot-type printing, batteries and fiberglass.

3.3.2 Comparisons of measured trace element concentrations in the EXPERIVA participants with reference concentrations

Measured concentrations of trace elements in EXPERIVA participants are displayed in Table 2. Some trace elements had detection frequencies below 60%: Li in nails and hair, Be in all matrices, Se, Ag and Cd in water, Tl in nails, hair and water, Pb in hair, and U in urine samples. It is noteworthy that in the second sample of tap water as compared to the first, measured concentrations of Cu, Zn, Ag, Cd, and Pb were considerably lower; this difference might capture the possible additional water contamination of the first sample from the pipes and the trace metal reduction in the second sample of tap water due to flushing (71,72).

We observed lower or similar urinary concentrations of Ni, Cu, Zn, As, Cd, Tl, Pb compared to the ones of women from CHMS (cycle 2). We observed higher measured urinary concentrations of V, Mn, Co, and Se compared to the ones from CHMS (cycle 2), of V, As and Se compared to FNBI (2011), of Sr and Ba compared to the ones from NHANES (fourth report), and of Li, Al, and Ga compared to the ones from Goullé et al. (2005) (Table 2). In Table 2, the density of gray highlight provides a visual for how many times in a relative scale the measured values exceeded the reference values (from 1-5 times for our data). For instance, for V which was not detected in urine from CHMS participants, median urinary levels and 95th percentile in our study participants were respectively 1.7 times and 3.8 times higher than the

LOD of 0.1 µg/L in CHMS (Table 2). Median concentrations were higher than those from reference populations for urinary Al (3.1 times), Ba (2.8 times), Li and Ga (2.4 times), Sr and Co (about 2 times), V (1.7 times), and Se (about 1.1 times compared to CHMS and 1.2 times compared to FNBI) (Table 2). Results differed for urinary As: the median concentration for urinary As was about 1.3 times lower than the one from CHMS cycle 2, and 3.2 times lower compared to Goullé et al. (2005), but was about 1.5 times higher compared to the ones from FNBI (2011) and NHANES. In table 2, As levels are preferentially compared to CHMS cycle 2 (see 3.4.5 Limitations). 95th percentiles of urinary concentrations in this study were higher than those from reference populations for V (3.8 times compared to CHMS, 2.5 times compared to FNBI), Co (2.3 times), Mn and Ba (1.8-1.9 times), Ga (1.4 times), and Al and Sr (1.2 times). Some participants had average urinary concentrations exceeding the 95th percentiles from reference populations: 64 study participants (75%) for V, 25 (29%) for Co, 17 (20%) for Mn, 19 (22%) for Ba, 15 (18%) for Ga, 7 (8%) for Al, and 12 (14%) for Sr.

Median urinary concentrations in our study were lower than health-based guidance values for Zn, Se, Cd and Tl (Appendix A2, Table 2). For Zn, the 95th percentile in this study (588 µg/L; 577 µg/g creatinine) was within the range of published biomonitoring equivalents (BEs) (values ranging from 481 to 3489 µg/L and from 564 to 4488 µg/g creatinine); it exceeded some BEs and was below some others (Appendix A2, Table 2) (122). For Se, the 95th percentile in this study (121 µg/L) was higher than the published BEs which ranged from 90 to 110 µg/L (Appendix A2, Table 2) (123). The 95th percentile for urinary Cd (0.25 µg/L; 0.26 µg/g creatinine) in our study was lower than published BEs, HBM-I, HBM-II and HBM-GV values, which ranged from 0.5 to 4 µg/L and from 1 to 2 µg/g creatinine (124). Similarly, the 95th percentile of urinary Tl (0.34 µg/L) in our participants was lower than the HBM-I value of 5 µg/L (125).

We observed lower or similar hair concentrations of Li, Al, Cr, Ni, Cu, Zn, As, Se, Ag, Cd, Pb, and U compared to the ones of women from CHMS (cycle 5). We observed higher measured hair concentrations of V, Co, and Ba compared to the ones from CHMS (cycle 5), and of Mn, Ga and Sr-compared to the ones from Goullé et al. (2005) (Table 2). Median concentrations in this study were higher for Ga (5.0 times), Ba (2.0 times), Co and Sr (1.5 times) (Table 2). 95th percentiles were also higher for Ga (3.7 times), for Mn (1.6 times), for Ba (1.4 times), Sr (1.3 times), V (1.2 times), and Co (1.1 times) (Table 2). Some participants had hair concentrations exceeding the 95th percentiles from CHMS or Goullé et al. (2005): 35 study participants (41%) for Ga, 7 (8%) for Ba, 8 (9%) for Sr, and 5 (6%) for V and Co. Reference hair values were missing for Fe.

For tap water, measured concentrations for Al, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Cd, Ba, Pb, and U in the study were considerably lower than the maximum acceptable concentrations from Canadian and British Columbia's drinking water guidelines (Table 2). For some of the trace elements, guidance values were not available for comparisons, that is, for Li, Be, V, Fe, Ga, Ag, and Tl.

Table 2. Concentrations of trace elements measured in the EXPERIVA study compared to concentrations measured in reference populations

	URINE (µg/L) [a]			HAIR (µg/g of hair) [b]			NAILS (µg/g of toenail) [c]			Tap water sample 1 (µg/L) [d]			Tap water sample 2 (µg/L) [d]		
	DF(%)	P50%	P95%	DF(%)	P50%	P95%	DF(%)	P50%	P95%	DF(%)	P50%	P95%	DF(%)	P50%	P95%
Li	100	29	55	4	<LOD	0.01	49	0.08	0.31	100	4.3	52	100	3.9	45
Be	27	<LOD	0.03	0	NA	NA	3	<LOD	0.00	26	<LOD	0.01	23	<LOD	0.01
Al	98	6.0	14	100	2.7	12.0	100	79	222	97	20	90	91	19	124
V	96	0.17	0.38	98	0.01	0.04	100	0.21	0.66	100	0.10	0.22	100	0.09	0.21
Cr	99	0.22	0.48	100	0.02	0.12	97	0.71	2.9	100	0.03	0.09	98	0.03	0.08
Mn	70	0.13	0.64	100	0.10	0.92	100	1.3	5.2	99	0.67	32	100	0.24	17
Fe	100	5.5	16	100	5.7	16	100	99	312	85	1.4	24	92	1.30	26
Co	100	0.70	2.8	96	0.02	0.15	100	0.05	0.11	100	0.23	1.00	100	0.09	0.43
Ni	100	1.2	3.6	100	0.09	0.42	99	11	99	100	1.3	11	100	0.69	2.5
Cu	100	8.4	23	100	14	66	100	4.9	9.6	92	43	349	76	10	269
Zn	100	192	588	100	153	255	100	99	131	95	30	643	76	4.5	407
Ga	97	0.17	0.39	100	0.05	0.25	100	0.14	0.43	100	3.3	5.9	100	3.2	5.8
As	100	5.9	40.0	72	0.01	0.02	100	0.09	0.25	94	0.24	0.81	98	0.23	1.1
Se	100	56	121	100	0.52	0.67	100	0.89	1.10	1	<LOD	1.2	0	NA	NA
Sr	100	173	343	100	1.3	5.9	100	1.2	3.5	100	169	240	99	165	245
Ag	0	NA	NA	60	0.01	0.14	85	0.02	0.19	39	<LOD	0.01	29	<LOD	0.01
Cd	98	0.12	0.25	76	0.00	0.02	93	0.02	0.07	15	<LOD	0.02	9	<LOD	0.02
Ba	98	2.9	8.9	100	1.2	5.7	100	3	10	100	68	127	100	68	119
Tl	99	0.17	0.34	0	NA	NA	0	NA	NA	16	<LOD	0.00	13	<LOD	0.00
Pb	94	0.18	0.49	23	<LOD	0.30	97	0.25	1.07	91	0.12	0.88	73	0.05	0.80
U	2	<LOD	0.01	99	0.02	0.09	100	0.01	0.02	100	0.52	0.89	100	0.54	0.75

LEGEND: C EXPERIVA > C ref. pop.

x1 x2 x3 x4 x5

Samples with DF< 60% (i.e., LODs >40%) were excluded from further analysis.

DF detection frequency
P% percentile
C concentration

[a] Urinary concentrations were preferentially compared to data from CHMS (2nd cycle), followed by NHANES (9th cycle) and Goullé et al. 2005.

[b] Hair concentrations were compared preferentially to data CHMS (5th cycle), followed by Goullé et al. 2005.

[c] No reference concentrations available for nails.

[d] Water concentrations were compared to Guidelines for Canadian Drinking Water Quality 2020 and to Source Drinking Water Quality Guidelines of Britich Columbia.

Note 1: Trace element concentrations were compared to reference populations based on availability.

Note 2: The density of gray highlight is used to give a visual for how many times in a relative and approximate (rounded) scale the measured values exceeded the reference values (from more than 1 to 5 times).

3.3.3 Correlations between trace element concentrations in the different matrices

We assessed correlations between concentrations of trace elements measured in the biological and water samples and presented them in a heatmap (Figure 2). The Spearman's rank correlation coefficients (r_s) are presented in Supplemental Material Appendix A3 Figure 1, and ranged from -0.25 to

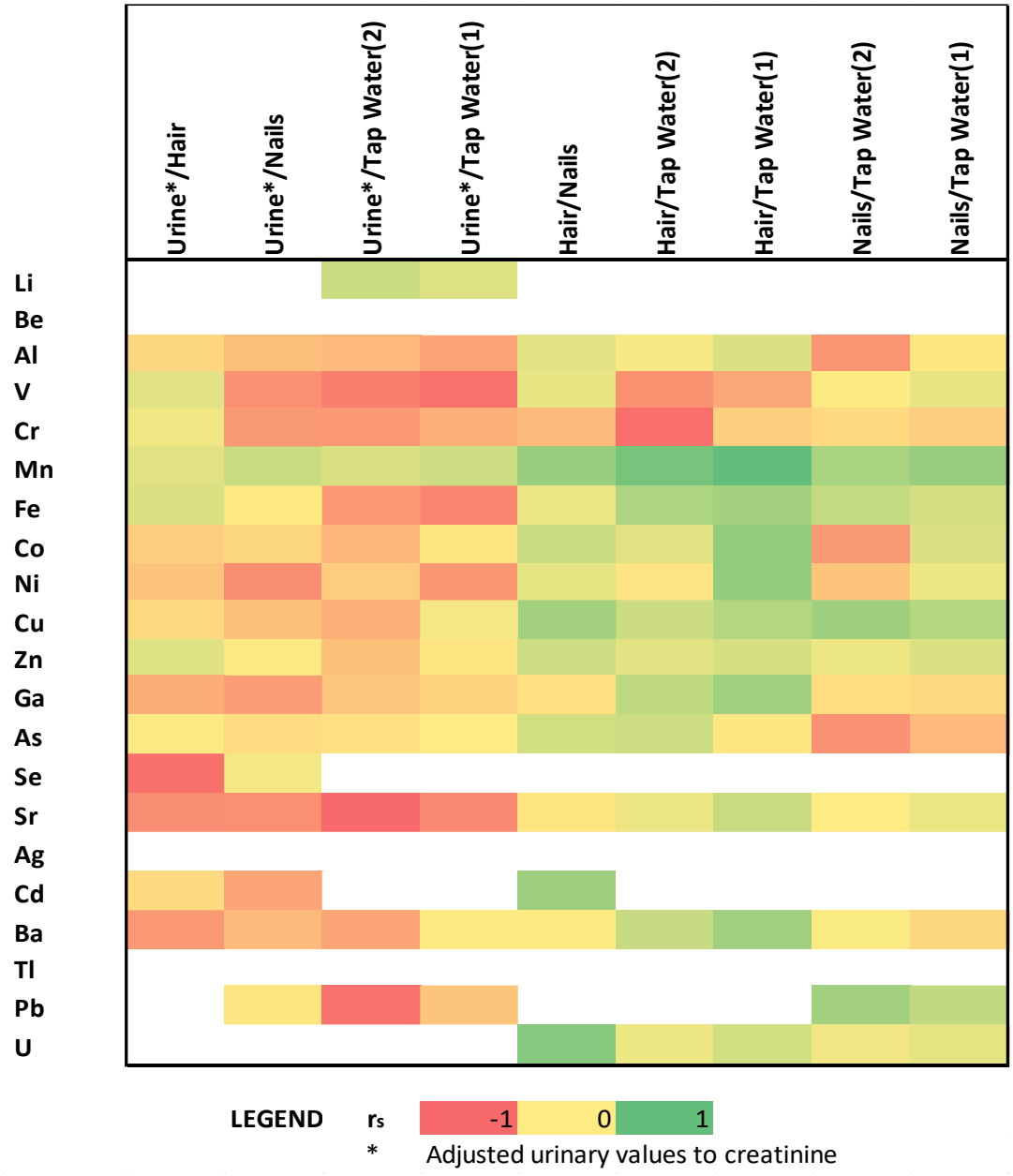
0.65 (very weak to moderate correlations). In general, correlations with tap water concentrations were strongest for hair, followed by nails, and urine. Nail concentrations of trace elements best correlated with concentrations in hair.

With urinary concentrations of trace elements expressed on a creatinine basis (in µg/g creatinine), we observed very weak (r_s -0.25 to 0.26) and mostly statistically insignificant correlations with other matrices (Figure 2 and Appendix A3, Figure 1). Positive correlations were observed for concentrations of urinary Mn with the ones in nails and in tap water (first sample), and, for concentrations of urinary Li with the levels in tap water (second sample), with coefficients ranging from 0.25 to 0.26. Negative correlations were observed for concentrations of urinary Se with the ones in hair, and for urinary V with tap water (first sample), as well as for urinary Sr and Pb with tap water (second sample), with coefficients ranging from -0.22 to -0.25.

We observed mostly very weak to weak correlations but also some moderate correlations with tap water, hair, and nails. With trace element concentrations in tap water, positive correlations were observed with concentrations in nails (Mn, Fe, Cu, Pb) and in hair (Mn, Fe, Co, Ni, Cu, Ga, As, Sr, Ba, U), with coefficients ranging from 0.23 to 0.65. We observed a negative correlation for Cr between levels in tap water (sample 2) and hair (r = -0.23). Finally, for trace elements concentrations in hair and nails, positive correlations were observed with Mn, Co, Cu, Zn, Cd and U (coefficients ranging from 0.25 to 0.44).

Of note, overall, Mn showed positive correlations across all matrices.

Figure 2. Heatmap of Spearman's rank correlations between trace element concentrations in biological and tap water samples

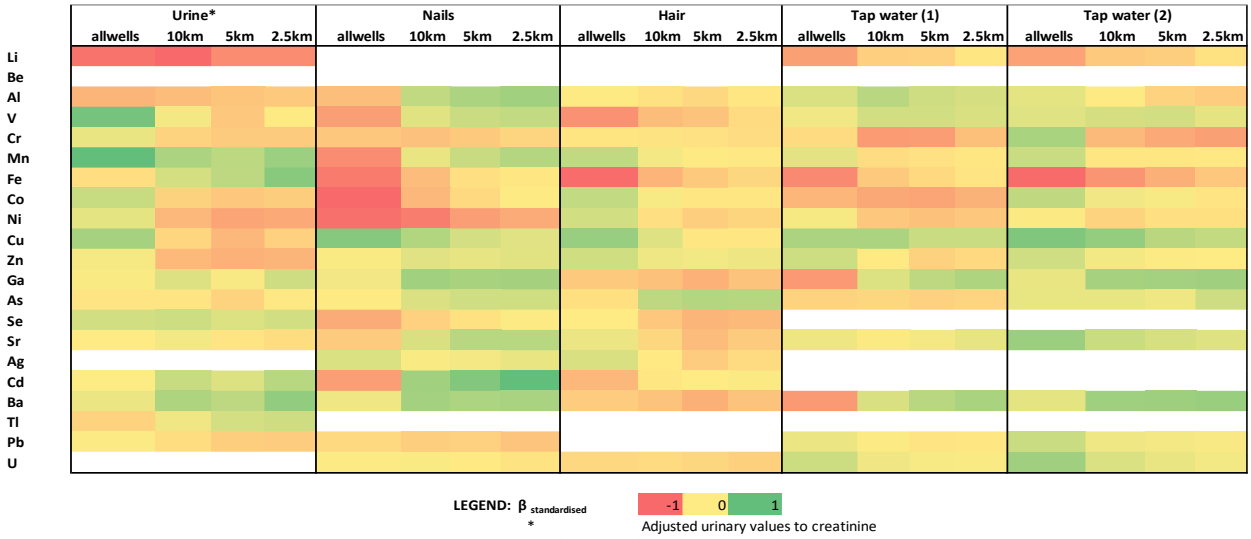


3.3.4 Associations between trace element concentrations in the different matrices and density/proximity of UOG wells

We assessed associations between IDW metrics and trace element concentrations in the different matrices (Figure 3). Standardized beta coefficients (β) adjusted for covariates are displayed in

Supplemental Material Appendix A3, Figure 2, and ranged from -0.23 to 0.39. The strongest associations with IDW metrics were observed with concentrations in nails and in the second sample of tap water. IDW metrics were not associated with trace element concentrations in urine and in the first sample of tap water. Results were similar for wet-weight and creatinine-adjusted urinary concentrations. The only significant associations between IDW metrics and trace elements concentrations in hair were observed for Cu (β: 0.26; 95% CI: [0.05, 0.48]) and for Fe (β: -0.23; 95% CI: [-0.44, -0.01]) (no buffer zone). For the second sample of tap water, we found positive and statistically significant associations between IDW metrics for the following trace elements concentrations: Cr, Sr, and U (with no buffer zone), Cu (no buffer zone and 10-km buffer zone), Ga and Ba (10-km, 5-km, and 2.5-km buffer zones). Fe in the second sample of tap water was negatively associated with the IDW without a buffer zone (β: -0.23; 95% CI: [-0.42, -0.04]). Finally, several significant positive associations were also observed between IDW metrics and trace element concentrations in toenails: for Al (5km and 2.5-km buffer zones), Mn (2.5-km buffer zone), Cu (no buffer zone), Ga (10-km, 5-km, and 2.5-km buffer zones), Cd (10-km and 5-km buffer zones), and Ba (10-km and 2.5-km buffer zones). Co in toenails was negatively associated with the IDW without a buffer zone (β: -0.23; 95% CI: [-0.47,-0.00002]. Overall, Cu was the only element throughout all matrices with systematically positive associations with IDW metrics.

Figure 3. Heatmap of the associations between trace element concentrations in samples and UOG well density/proximity metrics (IDW)[a]



[a] For biological matrices (hair, urine, and nails), multiple regression models were adjusted for the following covariables: source of tap water (municipal, cistern, private well, or other), cooking water (bottled vs. other), drinking water (bottled vs. other), working in industry, smoking status at time of recruitment, and consumption of local food. For water, multiple regression models were adjusted for the source of tap water (cistern, private well, or other).

3.4. Discussion

To the best of our knowledge, following our pilot study, this is the first study to report concentrations of trace elements in an extensive set of hair, urine, nails, and tap water samples in a region of unconventional oil and gas operations. In this study, we measured the levels of 21 trace elements in biological and tap water samples collected from 85 pregnant women living in the Peace River Valley in Northeastern British Columbia. We found higher urinary and hair concentrations of certain trace elements in study participants compared to reference populations (e.g., Mn, Sr, Co, Ba). Correlations with tap water concentrations were strongest for hair, followed by nails, and urine. Positive (e.g., Cu, Ga, Ba) and negative (e.g., Fe) associations were observed between IDW metrics and the concentrations of certain trace elements in water, hair, and nails.

3.4.1 Comparison with values from reference populations

Study participants had higher hair and urinary levels of certain trace elements compared to reference populations, when comparing the differences for 50^{th} and 95^{th} percentiles. Namely, this was the case both for urinary and hair levels of V, Mn, Co, Sr, Ba and Ga. In addition, urinary concentrations of Li, Se and Al were also higher compared to these reference populations. Concentrations of these trace elements in hair and urine were not correlated. Our results were consistent with the results of the previous pilot study conducted by our research team in this same area in 2019 (n=29), where higher concentrations of Mn in both urine and hair, and Ba, Al, and Sr in hair were measured when compared to the same reference populations (101). Some study participants also had slightly higher urinary levels of Zn and Se compared to some health-based guidance values. Of note, health-based guidance values used herein mostly represent levels at which risk is considered to be negligible/minimal, except for HBM-II values which are considered as levels above which adverse health effects may occur. Overall, when we evaluated one trace element at the time, concentrations in our study participants do not suggest health risks, but exceedances of some health-based guidance values and higher concentrations relative to reference populations warrant further evaluation, namely in terms of identifying sources of exposure.

3.4.2 Correlations of trace element levels across biological and water samples

Correlations with tap water concentrations were strongest for hair, followed by nails, and urine concentrations. Our correlation analyses showed no correlation between urinary and hair concentrations except the one very weak negative correlation for Se. Although we collected repeated urine samples to account for day-to-day variations in urinary concentrations, average urinary concentrations are still likely influenced by recent exposures. In contrast, hair concentrations reflect past exposures over longer periods (48,80). The lack of correlation between urinary and hair concentrations of trace elements have been reported also previously by other studies (62,101). Also, urinary concentrations were not correlated with toenails concentrations, except for one very weak positive correlation for Mn. Similarly, this may be due to the fact that nails also reflect the body burden months before collection, making correlations unlikely unless exposure tracks well through time (62,78). Except for this one element (Mn), our findings were consistent with the findings of another study evaluating occupational exposure to V, Cr, Fe, Co, Ni, As, Se, Cd, Ba, Pb, U, Mn and Cu using toenail samples from Qatar farm workers (63). Urinary concentrations in our study were also not correlated with concentrations of trace elements in tap water (both samples), except for some elements (e.g., Li and Mn with positive correlations and V, Sr, and Pb with negative correlations). A recent study conducted in China by Tong et al. (2022), also studied Se, As, Cd, Cr, Cu, Mn, Ni, Pb, and Zn, and, similarly, no significant correlations were observed for all of these trace elements (save the difference of the results for Mn, as well as for As for which they found a positive correlation) between urinary and drinking water concentrations (41,126). Along with this study, another study conducted in Pakistan also reported significant moderate positive correlations between urinary As levels and concentrations of As in drinking water (126).

Correlations were strongest between trace elements concentrations in hair and water (both samples). Hair and tap water concentrations were positively correlated for Mn, Fe, Co, Ni, Cu, Ga, As, Sr, Ba, and U, and negatively correlated for Cr. Consistent with our findings, results of one recent study in Finland by Kousa et al. (2021) showed that the concentrations of Ni and Mn in hair correlated with those in Ni- and Mn-contaminated drinking water (127). Similarly, findings in another study in the Mekong River basin of Cambodia by Phan et al. (2013) showed significant positive correlations between concentrations of As, Mn and Ba in hair and drinking water (47). Contaminated groundwater As concentrations were shown to

be correlated with As in hair in another study in Vietnam (128). Chanpiwat et al. (2015) also reported moderate statistically significant positive correlations between groundwater and hair levels of for As, Ba, Fe (129).

In our study, positive correlations were also found between trace elements concentrations in nails and tap water for Mn, Fe, Cu and Pb. Positive significant correlations were also reported by Chanpiwat et al. (2015) for concentrations of Fe, but also for As and Ba in nails and tap water (129).

Our analyses showed correlations between toenail and hair concentrations of Mn, Cu, Cd, U, Co, and Zn. A study by Rodushkin and Axelsson (2000) reported positive correlations for Cd and Pb concentrations in hair and nails, and weaker correlations for Se, Sr, V and Zn (90). Yet, a study by Sahoo et al. (2015) reported a lack of correlations for Cd, Cu, Mn, Ni, Sr, and Th in nails and hair (78). In general, hair and nails, although both reflecting long-term exposures, have very different elemental compositions, including for trace elements; we observed that research findings with regards to correlations across matrices are sometimes conflicting and vary from lack of correlations to varying relationships (78,90,129).

3.4.3 Associations of trace element levels with IDW density/proximity metrics

Concentrations of various trace elements in nails and tap water were associated with IDW well density/proximity metrics in multiple regression models. Namely, positive associations with IDW metrics were observed both for the second sample of tap water and nail concentrations of Ga, Cu and Ba. Cushowed positive associations with IDW metrics across all matrices except urine (hair, nails and tap water). Fe concentrations in both tap water (second sample) and hair showed negative associations with IDW metrics. In addition, nail levels of Al, Mn, and Cd, tap water (second sample) levels of Cr, Sr, and U, and hair levels of Cu were also positively associated with IDW metrics.

Associations are observed with the second sample of tap water as compared to the first sample; this may be because the second sample better reflects the contamination from the source, while to the first sample contains additionally trace metal contamination from the pipes. To date, no other studies explored associations of trace element concentrations in a region of intensive UOG operations with the density and proximity of UOG wells. However, some past studies have assessed other exposures (VOCs, radon, etc.) and explored their associations with well density/proximity (27). Namely, a recent study

using EXPERIVA data and published by Caron-Beaudoin et al. (2021) found associations between the density of UOG wells and chloroform, acetone and BTEX concentrations in indoor air, and with trihalomethane concentrations in tap water. Another study by Xu et al. (2019), reported that proximity of UOG wells was strongly associated with increased indoor radon concentrations (130).

3.4.4 Potential sources and pathways of exposure to trace elements

Unconventional oil and gas activities may lead to environmental contamination of soil and water (28,131). The release of trace elements into the environment through wastewaters from unconventional oil and gas activity is determined namely by the content of geological formations (13,22). In the Montney formation in the Peace River Valley, relatively high levels of several trace elements like Al, Ba, Sr, and Mn have been reported in the rock contents in previous studies (28,131), while others appear in relatively lower concentrations (e.g., Ba, Ag, Cd, Co, Cr, Ni, Pb, Sr, V) (28,29). Although, it has been reported that food serves as a major source of exposure to most trace elements, drinking water has been recognized to also be an important exposure pathway for humans (39,41). Drinking water contamination (e.g., surface water, groundwater, private drinking water) has been previously observed in regions with intensive UOG operations, e.g., increased water levels of Ba have been observed in Peace River Valley groundwaters, and As, Ba and Sr in Texas in private drinking water (44–46).

Our study results showed that all the trace element concentrations in tap water were significantly lower than the Canadian and British Columbia drinking/source water guideline values (maximum acceptable concentrations). Nevertheless, we observed increased levels of various trace elements in EXPERIVA participants, e.g., higher urinary and hair concentrations of Co, Ba, Sr, Mn, V, Ga, Li, Se, and Al compared to reference populations. Considering the above discussion regarding the natural presence of trace elements in rock formations and their increased concentrations in water contents in regions with intense UOG operations, our results suggest that the observed higher concentrations of certain trace elements in EXPERIVA participants may be related to the intense UOG activities in the region. Results of the evaluation of the associations in our study (e.g., positive associations for Al, Mn, Cu, Ga, Cd, Ba, Cr, Sr, and U) also suggest that the presence of various trace elements in biological matrices (nails and hair) and in the environment (tap water) may be associated with the density and proximity of UOG wells. However, many different factors could affect estimating and measuring the exposure of trace elements in our participants; to be able to establish a verifiable link between the observations in our study and

the proximity of the hydraulic fracture activities, it is necessary to further investigate exposure pathways, exposure factors, and attributable exposures, in much more details.

3.4.5 Limitations

Our study has several limitations. First, although we tried to locate recent reference concentrations and comparable reference populations (e.g., Canadian, pregnant women, ages 18-40), their availability was limited, and some important limitations were encountered in this aspect. To start, biomonitoring data from the general Canadian population lacked for several trace elements, namely, for Sr, Ba, Li, Al, and Ga. Trace element concentrations in biological samples (including hair, nails and urine) vary across regions and countries due to various different environmental and lifestyle conditions (132). Age, sex, hair colour, self-care routines, smoking, racial and ethnic factors, all play a role in the trace element content of hair and nails (62,63,73,90). Pregnancy can affect urinary content and mobilize trace elements in the body, hence it would have been preferable to use reference data from pregnant women as well (33). Also, the comparisons of the measured concentrations of trace elements to the reference populations has been done without the consideration of variation (e.g., confidence intervals) of the CHMS/NHANES biomarker estimates. These determinations were done as previously described in the pilot study (101). Hence, the comparison results should be interpreted with caution for the elements which have small differences from the reference concentrations (e.g., Co in hair) with consideration of variations this small might be insignificant.

In addition, data was not collected regarding the intake of dietary supplements by the EXPERIVA participants. This is an important limitation especially considering that participants were pregnant women and dietary supplementation in Canada is often administered during pregnancy (e.g., Fe, Ca, Zn) (133). These supplements may contain trace elements as part of the ingredients, and this may interfere with the trace element body burden, potentially altering the urinary trace element levels and hence affecting the results of our study. In addition, another limitation comes with the multiple testing in this study which leads to findings that can be affected by random chance. For that reason, results from our study are best interpreted in terms of patterns of association rather than based on significance testing. Finally, another limitation in our study is the relatively small number of participants (n=85), which is inherent to research in remote areas, for the study of such diversified relationships. All these limitations hampered drawing further conclusions in this study regarding the possible sources and pathways of exposures but provided insight into the potential association with UOG activity.

3.5. Conclusions

To date, there is limited data on human exposure to environmental contaminants like trace elements, emitted by UOG activities, which could impact health, especially in vulnerable populations like pregnant women and the fetus. Our study results from the Peace River Valley show that trace element levels in the tap water were below the maximum acceptable limits of Health Canada and British Columbia guideline values. Nonetheless, we observed higher concentrations of certain trace elements in urinary and hair samples from our participants compared to reference populations for V, Mn, Co, Sr, Ba, Li, Al, and Ga. Some study participants had also slightly higher urinary levels of Zn and Se compared to some health-based guidance values. Concentrations of various trace elements in tap water, nails and hair water were associated with well density/proximity (IDW metrics): positively for Al, Mn, Cu, Ga, Cd, Ba, Cr, Sr, U and negatively for Fe. Whether the measured trace element concentrations in this study are related to UOG activity remains uncertain. Given the growing hydraulic fracturing activities in this region, assessing further exposure levels in this region and their associations with the density of wells, in the larger picture of the interplay of various exposure factors and diversified relationships and impacts of these factors, is crucial.

CRediT authorship contribution statement

Lilit Gasparyan: Conceptualization, Methodology, Software, Validation, Statistical analysis, Investigation, Data curation, Writing of the article, Visualization, Project administration. **Juliette Duc :** Statistical analysis**,** Software, Writing – review & editing. **Lucie Claustre :** Writing – review & editing. **Delphine Bosson-Rieutort :** Methodology, Statistical analysis**,** Software, Supervision, Writing – review & editing. **Michèle Bouchard :** Resources, Chemical determination of trace elements in the samples, Writing – review & editing. **Maryse Bouchard :** Methodology, Writing – review & editing. **Élyse Caron-Beaudoin :** Sampling, Data collection, Conceptualization, Methodology, Writing – review & editing, Resources, Funding acquisition. **Marc-André Verner:** Conceptualization, Methodology, Supervision, Writing – review & editing, Resources, Funding acquisition.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplemental Material

Title: Density and proximity of unconventional oil and gas wells and concentrations of trace elements in urine, hair, nails and tap water samples from pregnant women living in Northeastern British Columbia

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Appendix A1 – Limits of Detection

Chemical determination of trace elements was performed at the laboratory of Michèle Bouchard at the Université de Montréal. The limit of detection (LOD) is defined as the minimum concentration of the chemical that is greater than zero and is measured and reported at 95% statistical confidence level. LOD (LDM) determination corresponds to 3xSD of 10 blanks. The method is inspired by that of the DR12-VMC, of the MELCC (134).

Table S1. Limits of detection (LODs) for trace elements measured in the biological and water samples

	LOD			
	Urine (µg/L)	**Nails (µg/g of toenail)**	**Hair (µg/g of hair)**	**Water (µg/L)**
Li	0.773	0.078	0.013	0.036
Be	0.020	0.007	0.007	0.001
Al	1.448	0.715	0.121	0.335
V	0.013	0.003	0.001	0.003
Cr	0.044	0.137	0.003	0.003
Mn	0.032	0.014	0.004	0.011
Fe	0.278	0.400	0.072	0.339
Co	0.018	0.004	0.001	0.002
Ni	0.024	0.038	0.003	0.012
Cu	0.048	0.207	0.611	4.503
Zn	0.955	3.566	0.869	1.712
Ga	0.022	0.005	0.001	0.011
As	0.057	0.019	0.003	0.018
Se	0.517	0.296	0.038	1.085
Sr	0.390	0.056	0.035	0.568
Ag	0.000	0.005	0.002	0.003
Cd	0.022	0.004	0.002	0.009
Ba	0.021	0.018	0.012	0.319
Tl	0.026	0.006	0.001	0.001
Pb	0.017	0.032	0.157	0.023
U	0.014	0.002	0.001	0.005

Appendix A2 – Reference Trace Element Concentrations

Table S2. URINE: Measured urinary trace element concentrations in the EXPERIVA participants and concentrations in the reference populations

	EXPERIVA URINE (µg/L)			[a] CHMS cycle 2 (µg/L)		[b] NHANES (µg/L)		[c] Goullé et al. (µg/L)		[d] FNBI 2011 (µg/L)	
	DF(%)	P50%	P95%	P50%	P95%	P50%	P95%	P50%	P95%	P50%	P95%
Li [c]	100	29	55	NA	NA	NA	NA	12	219	NA	NA
Be [b]	27	<LOD (0.02)	0.03	NA	NA	< LOD (0.072)	< LOD (0.072)	0.01	0.04	NA	NA
Al [c]	98	6.0	14	NA	NA	NA	NA	1.9	11	NA	NA
V [a]	96	0.17	0.38	< LOD (0.1)	< LOD (0.1)	NA	NA	3.3	10	<LOD (1.6)	0.15
Cr	99	0.22	0.48	NA	NA	NA	NA	NA	NA	NA	NA
Mn [a]	70	0.13	0.64	< LOD (0.2)	0.34	< LOD (0.13)	0.35	0.31	1.3	<LOD (2.3)	0.65
Fe	100	5.5	16	NA	NA	NA	NA	NA	NA	NA	NA
Co [a]	100	0.70	2.8	0.33	1.2	0.43	1.8	0.30	1.1	NA	NA
Ni [a]	100	1.2	3.6	1.3	5.3	NA	NA	1.8	4.1	1.3	4.7
Cu [a]	100	8.4	23	12	27	NA	NA	6.9	12	11	34
Zn [a]	100	192	588	280	870	NA	NA	195	499	289	1109
Ga [c]	97	0.17	0.39	NA	NA	NA	NA	0.07	0.28	NA	NA
As [a]	100	5.9	40.0	7.4	73	5.1	43	19	161	4	42*
Se [a]	100	56	121	53	140	NA	NA	20	46	48	139
Sr [b]	100	173	343	NA	NA	87	283	90	413	NA	NA
Ag	0	NA	NA	< LOD (0.1)	< LOD (0.1)	NA	NA	NA	NA	NA	NA
Cd [a]	98	0.12	0.25	0.37	1.1	0.14	1.1	0.16	0.79	0.60	2.2
Ba [b]	98	2.9	8.9	NA	NA	1.04	5.0	0.89	3.9	NA	NA
Tl [a]	99	0.17	0.34	0.24	0.62	0.15	0.43	0.15	0.84	NA	NA
Pb [a]	94	0.18	0.49	0.40	1.7	0.26	1.3	0.55	2.1	0.44	2.0*
U [a]	2	<LOD (0.01)	0.01	< LOD (0.01)	0.02	0.00	0.03	0.00	0.01	<LOD (0.031)	NA

Samples with DF< 60% (i.e., LODs >40%) were excluded from further analysis.

DF detection frequency

P% percentile

*Estimates are presented, but high sampling variablility associated with these estimates.

Note 1: Measured urinary concentrations are compared preferentially to data from CHMS cycle 2, if unavaiilable, then to data from NHANES, if unavailable, then to data from Goullé et al. (FNBI contains information for the same elements as CHMS)

Note 2: Numbers have been rounded to two significant figures

Table S3. URINE: Measured urinary trace element concentrations in the EXPERIVA participants and health-based guidance/reference values.

	EXPERIVA URINE (ug/L)			Biomonitoring equivalents (BE) dossiers	HBM Comission (2011)
	DF(%)	P50%	P95%	(ug/L)	(ug/L)
Zn	100	192	588	439 (ATSDR 2005) 481 (US EPA 2005) 658 (EFSA 2006) 909 (IOM 2001) 1316 (EC 2004) 1585 (JECFA 1982) 3489 (EC 2004)	
				(µg/g creatinine)	
		227	577	564 (ATSDR 2005) 619 (US EPA 2005) 847 (EFSA 2006) 1170 (IOM 2001) 1693 (EC 2004) 2040 (JECFA 1982) 4488 (EC 2004)	
Se				**(ug/L)**	**(ug/L)**
	100	56	121	90 (ATSDR 2003) 90 (US EPA 1991) 110 (IOM 2000)	
Cd				**(ug/L)**	**(ug/L)**
	98	0.12	0.25	1.2 (ATSDR 1999) 1.5 (US EPA 1994)	1 (HBM-I) 4 (HBM-II)
				(µg/g creatinine)	
		0.12	0.26	2 (US EPA 1994) 1.7 (ATSDR 1999)	
Tl					**(ug/L)**
	99	0.17	0.34		5 (HBM-I)

DF detection frequency

P% percentile

Table S4. HAIR: Measured trace element concentrations in the hair samples from the EXPERIVA participants and concentrations in the reference populations.

	EXPERIVA HAIR (µg/g hair)			[a] CHMS cycle 5 (µg/g hair)		[b] Goullé et al. (µg/g hair)	
	DF(%)	P50%	P95%	P50%	P95%	P50%	P95%
Li	4	<LOD (0.01)	0.01	0.012	0.081*	0.016	0.042
Be	0	NA	NA	<LOD (0.030)	<LOD (0.030)	0.007	0.012
Al	100	2.7	12.0	4.6	16	1.630	5.300
V	98	0.01	0.04	0.0085	0.031*	0.016	0.051
Cr	100	0.02	0.12	0.1	0.52	0.200	0.520
Mn	100	0.10	0.92	0.13	NA**	0.067	0.570
Fe	100	5.7	16	NA	NA	NA	NA
Co	96	0.02	0.15	0.014*	0.14*	0.023	0.140
Ni	100	0.09	0.42	0.3	1.2*	0.230	0.900
Cu	100	14	66	16	70*	20.300	61.300
Zn	100	153	255	190	390	162.000	209.000
Ga	100	0.05	0.25	NA	NA	0.011	0.068
As	72	0.01	0.02	0.018	0.052	0.050	0.080
Se	100	0.52	0.67	0.63	0.84	0.540	1.370
Sr	100	1.3	5.9	NA	NA	0.890	4.630
Ag	60	0.01	0.14	0.029	0.20*	0.080	1.310
Cd	76	0.00	0.02	<LOD (0.010)	0.024	0.011	0.170
Ba	100	1.2	5.7	0.6	4.0*	0.280	1.580
Tl	0	NA	NA	0.00039	0.0041*	0.0002	0.0004
Pb	23	<LOD (0.16)	0.30	0.12	0.70*	0.410	4.570
U	99	0.02	0.09	0.024*	0.13	0.009	0.030

Samples with DF< 60% (i.e., LODs >40%) were excluded from further analysis.

DF detection frequency

P% percentile

*Estimates are presented, but high sampling variablility associated with these estimates.

**95th percentile for Mn was unreliable to be published in CHMS cycle 5, thus, it is compared to Goullé et al.

The median for Mn is compared to CHMS cycle 5.

Note: Measured hair concentrations are compared preferntially to data from CHMS cycle 5, if unavailable, then to data from Goullé et al.

Table S5. TAP WATER: Measured trace element concentrations in the tap water samples and reference concentrations

	EXPERIVA Tap water sample 1 (µg/L)			EXPERIVA Tap water sample 2 (µg/L)			Guidelines for Canadian Drinking Water Quality	Source Drinking Water Quality Guidelines of British Columbia
	DF(%)	P50%	P95%	DF(%)	P50%	P95%	MAC (ug/L)	MAC (ug/L)
Li	100	4.3	52	100	3.9	45	NA	NA
Be	26	<LOD (0.001)	0.01	23	<LOD (0.001)	0.01	NA	NA
Al	97	20	90	91	19	124	NA	9500
V	100	0.10	0.22	100	0.09	0.21	NA	NA
Cr	100	0.03	0.09	98	0.03	0.08	50	50
Mn	99	0.67	32	100	0.24	17	120	120
Fe	85	1.4	24	92	1.30	26	NA	NA
Co	100	0.23	1.00	100	0.09	0.43	NA	1
Ni	100	1.3	11	100	0.69	2.5	NA	80
Cu	92	43	349	76	10	269	2000	2000
Zn	95	30	643	76	4.5	407	NA	3000
Ga	100	3.3	5.9	100	3.2	5.8	NA	NA
As	94	0.24	0.81	98	0.23	1.1	10	10
Se	1	<LOD	1.2	0	NA	NA	50	10
Sr	100	169	240	99	165	245	7000	7000
Ag	39	<LOD (0.003)	0.01	29	<LOD (0.003)	0.01	NA	NA
Cd	15	<LOD (0.009)	0.02	9	<LOD (0.009)	0.02	7	5
Ba	100	68	127	100	68	119	2000	NA
Tl	16	<LOD (0.001)	0.00	13	<LOD (0.001)	0.00	NA	NA
Pb	91	0.12	0.88	73	0.05	0.80	5	5
U	100	0.52	0.89	100	0.54	0.75	20	20

Samples with DF< 60% (i.e., LODs >40%) were excluded from further analysis.

DF detection frequency

P% percentile

MAC maximum acceptable concentration

Note: Water was sampled from the participant's kitchen tap with 2 samples. The first was collected when turning on the tap and the second after 5 minutes of water running.

Appendix A3 – Heatmaps

Figure S1. Heatmap of Spearman's rank correlations between trace element concentrations in biological and drinking water samples

	Urine*/Hair	Urine*/Nails	Urine*/Water(2)	Urine*/Water(1)	Hair/Nails	Hair/Water(2)	Hair/Water(1)	Nails/Water(2)	Nails/Water(1)
Li			**0.26**	0.19					
Be									
Al	0.01	-0.05	-0.06	-0.11	0.18	0.09	0.20	-0.14	0.05
V	0.18	-0.16	-0.19	**-0.22**	0.14	-0.15	-0.10	0.07	0.15
Cr	0.12	-0.13	-0.13	-0.08	-0.05	**-0.23**	-0.01	0.02	-0.01
Mn	0.18	**0.26**	0.20	**0.25**	**0.44**	**0.57**	**0.65**	**0.38**	**0.45**
Fe	0.20	0.05	-0.14	-0.18	0.13	**0.37**	**0.41**	**0.28**	0.22
Co	-0.01	0.01	-0.06	0.04	**0.26**	0.17	**0.47**	-0.13	0.20
Ni	-0.04	-0.16	-0.02	-0.14	0.16	0.04	**0.47**	-0.03	0.13
Cu	0.02	-0.04	-0.08	0.10	**0.41**	**0.26**	**0.34**	**0.43**	**0.34**
Zn	0.18	0.07	-0.04	0.04	**0.25**	0.17	0.22	0.13	0.20
Ga	-0.09	-0.12	-0.03	0.00	0.03	**0.30**	**0.42**	0.02	0.01
As	0.07	0.02	0.04	0.06	0.23	**0.25**	0.05	-0.15	-0.06
Se	**-0.23**	0.10							
Sr	-0.16	-0.16	**-0.25**	-0.17	0.04	0.14	**0.27**	0.07	0.13
Ag									
Cd	0.02	-0.11			**0.43**				
Ba	-0.14	-0.05	-0.10	0.06	0.06	**0.28**	**0.42**	0.08	0.01
Tl									
Pb		0.04	**-0.22**	-0.03				**0.41**	**0.30**
U					**0.52**	0.13	**0.23**	0.11	0.16

LEGEND: r_s -1 0 1

Bold statistically significant ($p < 0.05$)

* Adjusted urinary values to creatinine

Figure S2. Heatmap of the associations between trace element concentrations in the samples and the well density/proximity metrics (IDW): standardized beta coefficients from multiple linear regression models adjusted to covariables

	Urine*				Nails				Hair				Tap water (1)				Tap water (2)			
	allwells	10km	5km	2.5km	allwells	10km	5km	2.5km	allwells	10km	5km	2.5km	allwells	10km	5km	2.5km	allwells	10km	5km	2.5km
Li	-0.16	-0.18	-0.13	-0.13									-0.13	-0.03	-0.03	0.01	-0.12	-0.04	-0.04	0.00
Be																				
Al	-0.08	-0.06	-0.05	-0.05	-0.07	0.17	**0.22**	**0.24**	0.02	0.00	-0.01	0.01	0.11	0.19	0.14	0.12	0.09	0.02	-0.02	-0.04
V	0.12	0.01	-0.05	0.00	-0.13	0.09	0.15	0.16	-0.15	-0.07	-0.06	-0.01	0.06	0.13	0.13	0.11	0.09	0.12	0.13	0.08
Cr	0.02	-0.04	-0.05	-0.05	-0.05	-0.06	-0.05	-0.02	0.01	0.01	0.00	-0.01	-0.01	-0.14	-0.13	-0.06	**0.22**	-0.07	-0.11	-0.13
Mn	0.14	0.07	0.06	0.08	-0.17	0.07	0.15	**0.20**	0.17	0.05	0.02	0.02	0.09	-0.01	0.00	0.01	0.15	0.01	0.01	0.02
Fe	-0.02	0.03	0.06	0.10	-0.20	-0.07	0.00	0.01	**-0.23**	-0.09	-0.04	-0.02	-0.17	-0.04	-0.01	0.01	**-0.23**	-0.15	-0.09	-0.05
Co	0.05	-0.04	-0.05	-0.04	**-0.23**	-0.08	-0.01	0.02	0.17	0.04	0.01	0.01	-0.08	-0.11	-0.12	-0.09	0.17	0.05	0.04	0.01
Ni	0.02	-0.07	-0.10	-0.09	-0.22	-0.19	-0.13	-0.10	0.13	0.00	-0.03	-0.02	0.04	-0.05	-0.06	-0.05	0.03	-0.02	0.00	0.00
Cu	0.08	-0.03	-0.07	-0.04	**0.30**	0.20	0.13	0.10	**0.26**	0.10	0.01	0.01	0.22	0.22	0.15	0.15	**0.32**	**0.27**	0.18	0.16
Zn	0.00	-0.07	-0.08	-0.08	0.04	0.10	0.08	0.09	0.13	0.06	0.05	0.06	0.14	0.02	-0.03	-0.01	0.13	0.05	0.03	0.03
Ga	0.00	0.03	0.00	0.04	0.05	**0.24**	**0.22**	**0.23**	-0.04	-0.06	-0.09	-0.06	-0.14	0.10	0.18	0.21	0.07	**0.23**	**0.23**	**0.24**
As	-0.01	-0.01	-0.03	-0.01	0.02	0.11	0.13	0.13	0.00	0.18	0.20	0.19	-0.03	-0.02	-0.03	-0.02	0.08	0.08	0.06	0.14
Se	0.04	0.04	0.03	0.04	-0.10	-0.03	0.00	0.03	0.03	-0.05	-0.08	-0.07								
Sr	0.00	0.01	-0.01	-0.02	-0.04	0.11	0.19	0.19	0.07	-0.02	-0.07	-0.04	0.06	0.03	0.05	0.08	**0.26**	0.15	0.12	0.10
Ag					0.11	0.04	0.05	0.07	0.11	0.02	-0.04	-0.01								
Cd	0.00	0.05	0.03	0.06	-0.13	**0.24**	**0.31**	0.39	-0.08	0.01	0.02	0.03								
Ba	0.02	0.07	0.06	0.09	0.06	**0.24**	0.21	**0.22**	-0.04	-0.06	-0.09	-0.06	-0.14	0.11	0.19	0.22	0.09	**0.25**	**0.25**	**0.26**
Tl	-0.03	0.01	0.04	0.04																
Pb	0.00	-0.02	-0.04	-0.04	-0.01	-0.03	-0.03	-0.05					0.07	0.03	0.01	0.01	0.15	0.06	0.05	0.04
U					0.03	0.03	0.02	0.01	-0.02	-0.01	-0.02	-0.03	0.14	0.06	0.04	0.04	**0.24**	0.11	0.07	0.05

LEGEND: $\beta_{standardised}$ -1 0 1

Bold statistically significant ($p < 0.05$)

* Adjusted urinary values to creatinine

4. General Discussions and Conclusions

4.1 The initiation and the necessity of the project

It is due to the raised concerns and the request of the communities living near UOG sites in British Columbia that, originally, the project in this region (Peace River Valley) has been initiated by our research team. These communities have been wondering about the possible consequences and health impacts associated with living near such industrial sites, and have been raising questions and demanding clear answers to this question. This led our research team, in collaboration with First Nations and public health authorities in the region, to set up and conduct the pilot study in 2016 as discussed previously, which led our team to conduct a larger biomonitoring study (EXPERIVA) in 2018. Our research team has been the first to evaluate the importance of this exposure in this population.

4.2 Review of the main findings

Our study results showed that trace element levels in the tap water were below the maximum acceptable limits of Canadian and British Columbia guideline values. Nonetheless, we observed higher concentrations of certain trace elements in urinary and hair samples from our participants compared to reference populations : Co, Ba, Sr, Mn, V, and Ga . Our results were consistent with the results of the previous pilot study conducted by our research team in this same area in 2019 (n=29), where similarly higher concentrations of Mn in both urine and hair, and Ba, Al, and Sr in hair of participants were observed compared to the same reference populations (101). Study participants had also slightly higher urinary levels of Zn and Se compared to some health-based guidance values. Concentrations of various trace elements in tap water, nails and hair water were weakly associated with well density/proximity (IDW metrics), positively for Al, Mn, Cu, Ga, Cd, Ba, Cr, Sr, and U, and negatively for Fe. Whether the measured trace element concentrations in this study are related to UOG activity remains uncertain.

4.3 Sources of trace elements considered in this study, some limitations, and considerations

Many different factors could affect estimating and measuring exposure to trace elements in our participants. In this study, associations were assessed between the density/proximity of UOG wells and the concentrations of trace elements in the biological and tap water samples using multiple linear regression models adjusted for several covariates for each of the matrices. Various potential covariates -

that is, factors potentially affecting the trace element concentrations in these different matrices and predictors for increased proximity to UOG wells were first identified using the published literature. Identified potential factors included smoking status, source of drinking water, the rate of water consumption, the source of cooking water, the source of bathing water, use of nail polish, body mass index (BMI), all of which could affect the attributable trace element levels to the exposure and hence the exposure assessment (62,73,75,78,90,91,97–99). Not all these potential covariates were explored in this study (e.g., racial and ethnic factors, hair color, underlying diseases). Many of these potential predictors available from the EXPERIVA questionnaire were preliminarily tested for association with the trace element concentrations in different matrices and with the IDW metrics using non-parametric Wilcoxon and Kruskal Wallis tests. All the identified potential predictors from EXPERIVA questionnaire showed associations with at least one trace element concentration in the matrices. Not all of them however showed associations with IDW density/proximity metrics (e.g., educational status, BMI). In bivariate analyses, if a covariate showed a statistically significant association with a p value of <0.2 with at least one of the trace element concentrations and simultaneously with at least one of the IDW density/proximity metrics, it was included in the multiple linear regression model (e.g., for water sources of kitchen tap water, for biological matrices smoking status and consumption of local food) (27).

It is to note that some factors affecting the trace element concentrations were taken into consideration in multiple linear regression models as covariables, and others were accounted for in the sampling strategy. For example, Cd and Pb in tap water can come as well from deterioration of linings of pipes (especially in low occupancy sites and old residencies), resulting in higher concentrations of these trace elements in the tap water (71,72). For this reason, two samples of tap water were collected with an interval, letting the water to run for five minutes between the first and second sample, which has been shown in several studies to be a low-cost exposure prevention technique (71). Prevailing guidelines suggest flushing for 30 seconds to 2 minutes, however, studies have also shown that levels of lead can vary and the time necessary for lead reduction in the tap can vary as well (e.g., even after 6 min of flushing no significant decrease was observed), hence there are no flush recommendations for consistent effective exposure prevention or trace metal reduction in tap (71). Thus, contamination of drinking water from pipes can increase exposures and affect the measured concentrations of trace elements in our study.

On the other hand, our study results showed that all the trace element concentrations in tap water were significantly lower than the Canadian and British Columbia drinking/source water guideline values

(maximum acceptable concentrations). Different water consumption patterns were revealed from the data collected in the EXPERIVA questionnaire. For drinking water, 37 (44%) of the participants drank bottled water, 22 (26%) filtered tap water and only 23 (27%), drank tap water directly from the kitchen; the other 3 (4%) participants used water for drinking from more than one source. The collected data from questionnaires showed that the participants drank from one to twenty cups of water (250 mL) daily. On average the participants intake was about seven cups daily, but there was high variability in their daily intakes. Other sources of exposure to trace elements were not fully assessed in this study. It would be of further interest to account for other sources of exposure to estimate total daily doses for the population in this region. Exposure from nutrition (e.g., from local food such as caught fish, consumption of veggies, fruits and wild berries, wild and organ meat) is another important source of trace elements. Trace element contamination of fish has been a documented concern in industrial contexts (135). Evaluating also external exposure, the trace element burden added from nutrition (e.g., the intake of fish), drinking water, and other above-discussed factors, would allow to have a better and more complete picture and estimate of exposure and help with the interpretation of the observations in this study as well. To establish a verifiable link between the observations in this study and the proximity of unconventional oil and gas activities, it is necessary to explore further in details the sources and pathways of exposure.

4.4 Limitations and strengths of the study

In this study, assessment of trace elements from three biomarkers (urine, hair and nails) allowed to draw an image on both past and present exposures with non-invasive methods (80). Hair, nails and urine are biomaterials that present several advantageous for their use in the laboratory: they involve painless and easy collection and transportation, high stability at room temperatures, and often contain higher concentrations of interested substances relative to other body tissues or fluids (75,78). This made the sampling process easier for the participants and presented less of hinders for their participation. However, these biomarkers present some disadvantages as well: in nail and hair sampling, exogenous contamination (e.g., from dust, pharmaceutical use) can affect the exposure measures (75,78,97–99). On the other hand, studies show that different mediums are best fit for specific trace element measures. For instance, urine sampling may be best for recent dietary intakes of Se, As, and Cd, while blood sampling may be best for Fe, Zn, Cu, Se, Cd, and Pb (136). For example, for As, urinary levels better reflect recent dietary intake and internal exposure as 80% is excreted in urine after 3 days (136). In contrast, serum and whole blood are not good indicators for this trace element because it is cleared

rapidly within a couple of hours (136). Another example is Fe; since Fe is part of hemoglobin, blood could be a better Fe body-status indicator. In addition, external exposure to trace elements – the trace element burden added from nutrition, drinking water, and other discussed factors – was not fully assessed in this study and this was also limiting the interpretation of the results in the larger context of diversified relationships. Other limitations regarding the reference populations used in this study, including the small number of participants, the diversified relationships between trace element levels in different tissues in the body, and the multiple testing in this study, were also discussed.

4.5 Scientific contribution of our results and future implications

This study was conducted as part of a larger biomonitoring study in the Peace River Valley, the Exposures in the Peace River Valley (EXPERIVA) study exploring exposure of the local people to various contaminants associated with UOG activity. In addition to the samples collected and considered in the frame of this study (biological hair, urine, and nails samples, and environmental tap water samples), as part of the EXPERIVA study, other measurements were made (e.g., indoor airvolatile organic compounds and radon) and other studies were conducted as well by other members of our research team. As such, this biomonitoring initiative, including the data and results from this study, will serve as reference values and building blocks in future epidemiological studies, to monitor the evolution of populational exposure in this region. As natural gas industry is continuing to grow very fast with increasing local and global demands, and with British Columbia accelerating its liquified natural gas production, assessing further exposure levels in this region and their associations with the density of hydraulic fracturing wells is crucial. The results of this study also provide a portrait of gestational exposure to trace elements in this region, which can allow fetal exposure to the various trace elements to be estimated from maternal exposure. Ultimately, the results of this study should help regulatory bodies evaluate the health risks associated with unconventional oil and gas extraction and to take regulatory actions as necessary.

4.6 Recommendations for future research

The findings and the limitations from this study lead us to some recommendations provided hereafter for further research. A direct recommendation for subsequent research would be to do a follow up in this region. Our results suggested that the higher concentrations of certain trace elements in EXPERIVA participants may be related to the intense UOG activities. To establish whether the proximity of the

hydraulic fracture activities truly increases exposure to trace elements, further investigation of the sources and pathways of exposures would be necessary (including external exposures like from nutrition as discussed previously). Based on previous discussion, it would also be useful to collect blood samples (including cord blood) to better assess prenatal exposure and exposure of pregnant women to the trace elements for which blood is the best matrix. This would also allow an easier comparison of the measured values with those from reference population as nationally representative data for trace elements is available for blood. For example, Fe reference data lacked for sampled matrices, but nationally representative blood concentration data is available in CHMS cycle 2. In the latter, Fe levels were preferentially measured in blood through serum ferritin levels because of its affinity for this protein.

Additionally, it would be necessary to follow up with the communities and authorities in this region, to address their concerns with up-to-date information based on the results of this research, and to share with them the upcoming steps.

It would also be useful to sample tap water from residences with private wells, which are more likely to be impacted by the surrounding UOG wells. Another recommendation would be to conduct epidemiological studies on birth outcomes measuring both density/proximity of wells (which has already been associated with birth outcomes) and measuring exposure through biomarkers to determine if observed associations may be due to contaminant exposures. Finally, it would be interesting to explore the exposome, including exposures to the light, to the noise and to traffic in such regions of active UOG operations.

5. Conclusion

In sum, our study results show that certain trace elements are encountered in higher concentrations in the EXPERIVA participants and that the levels of some trace elements in different matrices are associated with density/proximity of UOG wells. Although the results of our studies point towards the existence of associations, further study improving the various limitations of this study is necessary. Some of the major limitations encountered in our study were the small number of participants, the lack of comparable reference populations for the trace elements and the lack of consideration of supplemental intakes by pregnant women. Assessing further exposure levels in this region and their associations with the density of wells, considering also various external exposure factors and the

diversified relationships and impacts of these factors, is also crucial. In addition, collecting blood samples from this region would allow access to more complete comparable national data for the trace elements, and to better assess exposure to certain elements like Fe. The results of this study will serve as basis for future epidemiological studies in the region and others, and should help regulatory bodies to evaluate the health risks associated with UOG operations.

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Annex A: Ethical approval from the Health Research Ethics Committee of the University of Montreal

Université de Montréal

Comité d'éthique de la recherche clinique (CERC)

5 décembre 2018

Objet: Approbation éthique – « Gestational exposure to chemicals related to hydraulic fracturing and their endocrine disrupting potencial in Northeastern British Columbia »

M. Marc-André Verner, Mme Michèle Bouchard, Mme Maryse Bouchard & Mme Élyse Caron-Beaudoin,

Le Comité d'éthique de la recherche clinique a étudié le projet de recherche susmentionné et a délivré le certificat d'éthique demandé suite à la satisfaction des exigences précédemment émises. Vous trouverez ci-joint une copie numérisée de votre certificat; copie également envoyée au Bureau Recherche-Développement-Valorisation.

Notez qu'il y apparaît une mention relative à un suivi annuel et que le certificat comporte une date de fin de validité. En effet, afin de répondre aux exigences éthiques en vigueur au Canada et à l'Université de Montréal, nous devons exercer un suivi annuel auprès des chercheurs et étudiants-chercheurs.

De manière à rendre ce processus le plus simple possible, nous avons élaboré un court questionnaire qui vous permettra à la fois de satisfaire aux exigences du suivi et de nous faire part de vos commentaires et de vos besoins en matière d'éthique en cours de recherche. Ce questionnaire de suivi devra être rempli annuellement jusqu'à la fin du projet et pourra nous être retourné par courriel. La validité de l'approbation éthique est conditionnelle à ce suivi. Sur réception du dernier rapport de suivi en fin de projet, votre dossier sera clos.

Il est entendu que cela ne modifie en rien l'obligation pour le chercheur, tel qu'indiqué sur le certificat d'éthique, de signaler au CERC tout incident grave dès qu'il survient ou de lui faire part de tout changement anticipé au protocole de recherche.

Nous vous prions d'agréer, Mesdames, Messieurs, l'expression de nos sentiments les meilleurs,

Nathalie Folch, Présidente
Comité d'éthique de la recherche clinique
Université de Montréal

c.c. Gestion des certificats, BRDV

Cochercheurs

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Université de Montréal

Comité d'éthique de la recherche clinique (CERC)

CERTIFICAT D'APPROBATION ÉTHIQUE

Le Comité d'éthique de la recherche clinique, selon les procédures en vigueur, en vertu des documents qui lui ont été fournis, a examiné le projet de recherche suivant et conclu qu'il respecte les règles d'éthique énoncées dans la Politique sur la recherche avec des êtres humains *de l'Université de Montréal.*

Projet	
Titre du projet	**Gestational exposure to chemicals related to hydraulic fracturing and their endocrine disrupting potencial in Northeastern British Columbia**
Chercheurs requérants	**Marc-André Verner**, professeur associé, École de santé publique - Département de santé environnementale et santé au travail **Élyse Caron-Beaudoin**, chercheure postdoctorale, École de santé publique - Département de santé environnementale et santé au travail **Maryse Bouchard**, professeure agrégée, École de santé publique - Département de santé environnementale et santé au travail **Michèle Bouchard**, professeure titulaire, École de santé publique - Département de santé environnementale et santé au travail
Autres collaborateurs:	Katherine Frohlich et Sami Haddad (Université de Montréal); Jonathan Chevrier (McGill University); Pierre Ayotte (Université Laval); Géraldine Delbès, Thomas Sanderson et Cathy Vaillancourt (INRS Institut Armand-Frappier); Margot Parkes (University of Northern British Columbia); Kyle Powys Whyte (Michigan State University); Alice Muirhead (Canadian Partnership Against Cancer); Ray Copes (Dalla Lana School of Public Health University of Toronto)

Financement	
Organisme	IRSC
Programme	Subvention Projet
Titre de l'octroi si différent	Gestational exposure to chemicals related to hydraulic fracturing in Northeastern British Columbia, and their endocrine disrupting potential
Numéro d'octroi	390320
Chercheur principal	
No de compte	

MODALITÉS D'APPLICATION

Tout changement anticipé au protocole de recherche doit être communiqué au Comité qui en évaluera l'impact au chapitre de l'éthique. Toute interruption prématurée du projet ou tout incident grave doit être immédiatement signalé au Comité. Selon les règles universitaires en vigueur, un suivi annuel est minimalement exigé pour maintenir la validité de la présente approbation éthique, et ce, jusqu'à la fin du projet. Le questionnaire de suivi est disponible sur la page web du Comité.

[redacted]

Nathalie Folch, Présidente
Comité d'éthique de la recherche clinique
Université de Montréal

5 décembre 2018
Date de délivrance

1er janvier 2020
Date de fin de validité et du prochain suivi

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Annex B: Canadian Reference Values for Tolerable Upper Intake Levels of Available Trace Elements

Table 1 - Canadian Reference Values for trace element intake levels for females in 19-50y age group (39)

	EAR (µg/day)	RDA/AI (µg/day)	UL (µg/day)
As		ND	ND
Cr		25	ND
Cu	700	900	10 000
Mn		1800	11000
Ni		ND	1000
Se	45	55	400
V		ND	1800
Zn	6800	8000	40 000

EAR	Estimated Average Requirement (EAR)
RDA/AI	Recommended Dietary Allowance (RDA)/Adequate Intake (AI)
UL	Tolerable Upper Intake Level (UL)

Annex C: Summary of the method of measuring creatinine in urine

Résumé de la méthode de mesure de la créatinine dans les urines

1. Préparation de la courbe standard

   On prépare la courbe de calibration dans des tubes de verre :

Standard	Concentration (mmol/L)	Volume de standard (ul)	Volume d'eau Milli-Q (ul)
Blanc (0)	0	0	3000
1	0.0333	10	2990
2	0.0666	20	2980
3	0.0999	30	2970
4	0.1332	40	2960
5	0.1667	50	2950
6	0.3333	100	2900

2. On prépare les échantillons (dilution 1/100) :

   a) Mettre 2970 ul d'eau Milli-Q dans chaque tubes (+4 tubes pour les contrôles)

   b) Vortexer 10 sec chaque échantillon

   c) Ajouter 30 ul d'urine

   d) Agiter par resuspension

3. On prépare les 4 contrôles enrichis :

- On ajoute 30 ul des solutions 1 et 2 dans les tubes (2x C1 et 2x C2)

4. On prépare la solution de picrate alcalin

   - À faire quotidiennement lorsque les échantillons sont prêts car on ne peut le conserver plus de 30 minutes.

   - On évalue la quantité de réactif requis avant de commencer (nombre d'échantillons X 1.5 ml de picrate alcalin). + 4 contrôles et la courbe de calibration de 7 points.

   - Dans un bécher, avec un cylindre gradué, on mesure 1 volume de NaOH 2.5N et on ajoute 5 volumes d'acide picrique

   - Exemple : pour 3 travailleurs : 20 ml de NaOH 2.5N + 100 ml d'acide picrique.

   - Juste avant l'utilisation, on mélange dans un bécher, par agitation avec une barre magnétique, les deux solutions.

5. Dosage et lecture

   - On ajoute 1,5 ml de picrate alcalin à tous les échantillons (échantillons, contrôles et standards)

   - On laisse incuber les tubes à la température de la pièce pendant 10 minutes.

   - On transfère la solution dans les cuvettes

   - On lit au spectrophotomètre à 520 nm.

Annex D: EXPERIVA Questionnaire

EXPERIVA

Exposures in the Peace River Valley

STUDY QUESTIONNAIRE

#ID :

Department of Occupational and Environmental Health

Université de Montréal

University of Montreal Public Health Research Institute

IRSPUM

>> **INSTRUCTIONS** <<

∴ Answering this questionnaire should take 60 minutes of your time;

∴ The data collected will be used for research purposes only.
Data confidentiality is guaranteed;

∴ As far as possible, please answer all the questions in the questionnaire. If a question makes you uneasy, you can choose to not answer it. However, we would like your answers to be as honest as possible;

∴ Unless otherwise specified, please focus on your current pregnancy;

∴ If you have any question while filling out the questionnaire,
do not hesitate to contact us:

→ **Thank you for completing this survey!**

a/s **Marc-André Verner**, PhD
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We thank you for your participation in this study. Please answer each of the following questions:

INFORMATION ON YOUR PREGNANCY

1. How long have you been pregnant?

☐☐ Weeks

2. Have you ever been pregnant before the current pregnancy?

☐ No ☐ Yes ☐ I don't know

If yes, how many pregnancies leading to a living child have you had so far?

☐☐ Pregnancy/Pregnancies

3. What method do you plan to use to feed your new baby in the first few weeks?

☐ Breastfeeding only (baby will not be given formula)

☐ Formula only

☐ Both breastfeeding and formula

☐ I don't know

PHYSICAL MEASURES

4. What was your weight just before getting pregnant?	☐	Kg or	☐	Lbs.
5. What is your current weight?	☐	Kg or	☐	Lbs.
6. How tall are you?	☐	cm or	☐	feet/inches

RESIDENCE AND ENVIRONMENT

7. Including yourself, how many people currently live in your household?

☐☐ People

8. Are you the owner of the home you live in?

☐ No ☐ Yes

9. How many rooms are there in the home you live in? Please include all the rooms except the bathroom and hallways

☐☐ Rooms

10. Select the dwelling type

☐ Single detached ☐ Duplex ☐ Institution

☐ Double ☐ Low-rise apartment (fewer than 5 stories) ☐ Hotel or camp

☐ Row or Terrace ☐ High-rise apartment (5 stories or more) ☐ Mobile home

☐ Other: _____

11. Does your home have an attached garage?

☐ No ☐ Yes

If yes, during the days you collected urine, did you run the car, lawn mower or other machinery in the garage?

☐ Yes ☐ No ☐ I don't know

12. Does your home have an indoor fireplace?

☐ No ☐ Yes

If yes, during the days you collected urine, for how long did you use the indoor fireplace?

☐☐ Hours

13. Do you currently store paints or fuels inside your home? Include your basement and attached garage

☐ No ☐ Yes ☐ I don't know

14. Do you currently store or use moth balls, moth crystals or toilet bowl deodorizers inside your home?

☐ No ☐ Yes ☐ I don't know

15. During the days you collected urine, did you cook or bake with natural gas?

☐ No ☐ Yes ☐ I don't know

16. During the days you collected urine, did you pump gas into a car or other motor vehicle yourself?

☐ No ☐ Yes ☐ I don't know

17. During the days you collected urine, did you breathe fumes from fingernail polish?

☐ No ☐ Yes ☐ I don't know

18. How many times did you apply nail polish on your toes in the last 3 months?

☐☐ Times ☐ I don't know

19. How long have you been living at your current address?

☐ Less than 1 year ☐ Between 1-2 years ☐ Between 2-5 years ☐ More than 5 years

20. Do you work in any of these workplaces?

☐ Mines
☐ Natural gas
☐ Construction
☐ Forestry
☐ Pipeline
☐ Manufacturing metals
☐ Manufacturing plastics
☐ Manufacturing petroleum
☐ Manufacturing rubber
☐ Manufacturing textiles
☐ Manufacturing glass
☐ Manufacturing ceramics
☐ Manufacturing paper
☐ Manufacturing electronics
☐ Manufacturing hot-type printing
☐ Manufacturing batteries
☐ Manufacturing fiberglass
☐ No
☐ I don't know

21. Does someone living with you work in any of these workplaces?

☐ Mines
☐ Natural gas
☐ Construction
☐ Forestry
☐ Pipeline
☐ Manufacturing metals
☐ Manufacturing plastics
☐ Manufacturing petroleum
☐ Manufacturing rubber
☐ Manufacturing textiles
☐ Manufacturing glass
☐ Manufacturing ceramics
☐ Manufacturing paper
☐ Manufacturing electronics
☐ Manufacturing hot-type printing
☐ Manufacturing batteries
☐ Manufacturing fiberglass
☐ No
☐ I don't know

22. Have you ever done any of the following activities?

- ☐ Health service maintenance
- ☐ Chemical processing
- ☐ Electroplating
- ☐ Soldering
- ☐ Welding
- ☐ Metal cutting
- ☐ Leather tanning
- ☐ Fireworks
- ☐ Metal smelting
- ☐ Photographic darkroom work
- ☐ Hunting with lead ammunition
- ☐ No
- ☐ I don't know

YOUR HEALTH

23. Compared to other people your age, would you say that, in general, your physical health is

- ☐ Excellent
- ☐ Very good
- ☐ Pretty good
- ☐ Fair
- ☐ Poor
- ☐ I don't know

24. Compared to other people your age, would you say that, in general, your mental health is

- ☐ Excellent
- ☐ Very good
- ☐ Pretty good
- ☐ Fair
- ☐ Poor
- ☐ I don't know

25. Do you suffer from one of the following health problems: chronic bronchitis, persistent cough or asthma?

- ☐ No
- ☐ Yes
- ☐ I don't know

26. In your life, have you smoked a total of 100 cigarettes or more (around 4 packs)?

- ☐ No
- ☐ Yes → go to question 28

27. Have you ever smoked an entire cigarette?

- ☐ No → go to question 33
- ☐ Yes

28. During the past 30 days (past month), have you smoked part or all of a cigarette?

- ☐ No
- ☐ Yes

29. Currently, do you smoke cigarettes daily, occasionally or not at all?

☐ Daily

☐ Occasionally → go to question 31

☐ Not at all → go to question 33

If you smoke daily

30. How many cigarettes do you smoke each day now?

☐☐ cigarette(s) per day

If you smoke occasionally

31. On the days when you smoke, how many cigarettes do you usually smoke?

☐☐ cigarette(s) per day

32. In the past month, how many days did you smoke one cigarette or more?

☐☐ day(s)

If you never smoke

33. Have you ever smoked cigarettes daily?

☐ No ☐ Yes

34. If yes, when did you stop smoking every day?

☐ Less than a year ago

☐ From 1 year ago to less than 2 years ago

☐ From 2 years ago to less than 3 years ago

☐ 3 or more years ago

35. Before your pregnancy, how often did someone smoke inside the car you were riding and/or inside the home you live in? Include both household members and visitors

- ☐ Every day
- ☐ Almost every day
- ☐ At least once a week
- ☐ At least once a month
- ☐ Less than once a month
- ☐ Never

36. Since you became pregnant, how often does someone smoke inside the car you were riding and/or the home you live in? Include both household members and visitors

- ☐ Every day
- ☐ Almost every day
- ☐ At least once a week
- ☐ At least once a month
- ☐ Less than once a month
- ☐ Never

37. During the days you collected urine, on an average week day, how much time did you usually spend outside (for work, family time, outdoor activities, sports, walking)?

- ☐ None
- ☐ 1 to less than 30 minutes
- ☐ 30 minutes to less than 2 hours
- ☐ 2 hours or more

38. During the days you collected urine, on an average weekend day, how much time did you usually spend outside (for work, family time, outdoor activities, sports, walking)?

- ☐ None
- ☐ 1 to less than 30 minutes
- ☐ 30 minutes to less than 2 hours
- ☐ 2 hours or more

39. During the days you collected urine, did you inhale smoke from any source for 10 or more minutes? Inhaled smoke includes smoke from campfires, fireplaces, cannabis and tobacco products

☐ No ☐ Yes ☐ I don't know

DIET & NUTRITION

40. During the past month, have you eaten wild meat?

☐ No ☐ Yes ☐ I don't know

If yes, how many times have you eaten wild meat during this period?

☐☐ Times

If yes, have you eaten organ meat during this period?

☐ No ☐ Yes ☐ I don't know

41. During the past month, have you eaten wild berries?

☐ No ☐ Yes ☐ I don't know

If yes, how many times have you eaten wild berries during this period?

☐☐ Times

42. During the past month, have you eaten vegetables or fruits from yard gardens?

☐ No ☐ Yes ☐ I don't know

If yes, how many times have you eaten vegetables or fruits from yard gardens during this period?

☐☐ Times

43. During the past month, have you eaten caught freshwater fish?

☐ No ☐ Yes ☐ I don't know

If yes, how many times have you eaten caught freshwater fish during this period?

☐☐ Times

STORE-BOUGHT FOOD ITEMS

44. During the days you collected urine, have you eaten dairy products like cheese and yogurt?

☐ No ☐ Yes ☐ I don't know

If yes, how many times have you eaten dairy products during this period?

☐☐ Times

45. During the days you collected urine, have you eaten dried/smoked food (fruit, fish, meat)?

☐ No ☐ Yes ☐ I don't know

If yes, how many times have you eaten dried/smoked food during this period?

☐☐ Times

46. During the days you collected urine, have you eaten fermented and/or pickled vegetables?

☐ No ☐ Yes ☐ I don't know

If yes, how many times have you fermented and/or pickled vegetables during this period?

☐☐ Times

47. During the days you collected urine, have you eaten tomato products (e.g tomato soup, canned tomatoes, tomato pulp)?

☐ No ☐ Yes ☐ I don't know

If yes, how many times have you eaten tomato products during this period?

☐☐ Times

48. During the days you collected urine, have you drunk fruit juices and preserves?

☐ No ☐ Yes ☐ I don't know

If yes, how many times have you consume fruit juices and preserves during this period?

☐☐ Times

49. During the days you collected urine, have you eaten baked goods (e.g patries, cake, cupcakes, donuts)?

☐ No ☐ Yes ☐ I don't know

If yes, how many times have you eaten baked goods during this period?

☐☐ Times

WATER

50. When you drink water at home, what is your primary source of drinking water?

- ☐ Tap water
- ☐ Filtered tap water (e.g., Brita, reverse osmosis)
- ☐ Bottled water
- ☐ Other: _____

51. What kind of water do you use to cook with at home?

- ☐ Tap water
- ☐ Filtered tap water (e.g. Brita, reverse osmosis)
- ☐ Bottled water
- ☐ Other: _____

52. What is the source of the tap water in your home?

- ☐ Water supplied by your city, town or municipality → Which city/town/municipality:_____
- ☐ Water from a private well
- ☐ Water from a cistern
- ☐ Water from a surface source such as natural spring, lake or river
- ☐ Other: _____
- ☐ I don't know

53. During the days you collected urine, how many times did you take a hot shower, for 5 minutes or longer, or a hot bath, for 20 minutes or longer? (do not include cold baths or showers)

☐☐ Times

54. How much water, in cups, do you usually drink daily? (A cup is equivalent to the size of a measuring cup: 250 mL or 8 oz.)

☐☐ Cups

DEMOGRAPHIC INFORMATION

55. In what year were you born? (yyyy) ☐☐☐☐

56. What is the highest level of education you have completed (this does not include current studies)?

- ☐ No school, or only kindergarden
- ☐ Elementary school
- ☐ 10th grade or less
- ☐ 11th grade
- ☐ Diploma/certificate in trade school, technical institute, nursing school
- ☐ Bachelor's degree
- ☐ Degree in medicine, dentistry, veterinary medicine, optometry
- ☐ University graduate certificate
- ☐ Master's degree
- ☐ Earned doctorate

57. Do you identify as Indigenous?

- ☐ No
- ☐ Yes

58. Can you estimate in which of the following groups your total household income falls, before taxes and deductions?

- ☐ No personal income
- ☐ $1 to $19,000
- ☐ $20,000 to 49,000
- ☐ $50,000 to $99,999
- ☐ $100,000 and more
- ☐ I don't know

SEMI-STRUCTURED INTERVIEW ON ENVIRONMENT QUALITY

>> **INSTRUCTIONS** <<

∴ This interview will be recorded by the researcher;

∴ The data collected will be used for research purposes only.
Data confidentiality is guaranteed;

∴ As far as possible, please answer all the questions.
If a question makes you uneasy, you can choose to not answer it. However, we would like your answers to be as honest as possible;

∴ If you have any question, do not hesitate to contact us:

→ **Thank you for completing this survey!**

We thank you for your participation in this study. Please answer each of the following questions:

ENVIRONMENTAL ISSUES

1. Which of these environmental issues would you say is the most important to you?

- ☐ Water contamination
- ☐ Air pollution
- ☐ Climate change
- ☐ Water shortage
- ☐ None
- ☐ Other: _____

2. Which of these environmental issues affects your family the most?

- ☐ Water contamination
- ☐ Air pollution
- ☐ Climate change
- ☐ Water shortage
- ☐ None
- ☐ Other: _____

3. In your opinion, what is the cause of the environmental issue you ranked as the most serious?

4. In your opinion, what is the cause of the environmental issue you ranked as affecting your family the most?

ENVIRONMENT QUALITY

5. How would you describe the quality of your environment?

- ☐ Poor
- ☐ Below average
- ☐ Average
- ☐ Above average
- ☐ Excellent
- ☐ I don't know

6. What motivated your choice of area of residence?

7. How would you describe your drinking water quality?

- ☐ Poor
- ☐ Below average
- ☐ Average
- ☐ Above average
- ☐ Excellent
- ☐ I don't know

8. Have you ever experienced problems with your drinking water (e.g: unpleasant taste, colour, etc)?

☐ No ☐ Yes ☐ I don't know

If yes, describe the problems

9. In your view, has the quality of the environment ever affected your health?

- ☐ No
- ☐ Yes → Describe how
- ☐ I don't know

10. In your view, has the quality of the environment ever affected the health of any of your family or friends living in the same region?

- ☐ No
- ☐ Yes → Describe how
- ☐ I don't know

11. In your view, has fracking activity in the area affected your health, or the health of your family or friends?

☐ No ☐ Yes ☐ I don't know

INFORMATION ABOUT ENVIRONMENTAL ISSUES

12. How familiar are you with the causes of environmental issues we've discussed so far?

Not familiar at all Very familiar

☐ 1. ☐ 2. ☐ 3. ☐ 4. ☐ 5.

13. How familiar are you with the impacts of the environment on human health?

Not familiar at all Very familiar

☐ 1. ☐ 2. ☐ 3. ☐ 4. ☐ 5.

14. Where do you get the information on these issues (e.g., medical clinics, service care providers, school, media, Internet, environmental groups, scientific journals, government, friends and family)?