Chronic Exposure of a Canadian Aboriginal Community to Uranium in Drinking Water: Chemical Toxicity and Radiation Dose

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Abstract. A study of an aboriginal community was conducted to determine if kidney function had been affected by the chronic ingestion of uranium in drinking water from drilled wells whose uranium concentrations varied from <1 to 1,418 ppb. This non-invasive study relied on the measurement of a combination of indicators of kidney function and markers for cell toxicity measured in urine samples collected from 39 females and 15 males. Ages ranged from 12 to 73 years. Correlation of uranium excreted in urine with bio-indicators at p ≤ 0.05 indicates interference with the kidney’s reabsorptive function. Because of the community’s concerns regarding cancer incidence, cumulative radiation doses were also calculated using uranium intake in drinking water over the preceding 15-year period. The average dose was 0.2 mSv and the highest dose, 1.7 mSv with the risk of cancer from the former being 1.4 in 100,000 while that from the latter is 12 in 100,000. Both would be difficult to detect in the community studied (population: 1,480).

Introduction

Uranium is the heaviest of the naturally occurring metals. As with Pb and Hg; it has been identified as a nephrotoxin [1]. Although ingested uranium may also have a radiological effect on the kidneys and other target organs such as bone, its nephrotoxicity is more likely due to its chemical properties than its radioactivity. Studies of the toxic effects of uranium intake through various routes were conducted in the 1940s as part of the war effort in the United States and in postwar experiments. The criteria for oral toxicity included mortality, a decrease in growth rate and histological changes. The principal histological finding was atrophic changes in the renal tubule.

Since the late 1950s, there have been no major efforts to update the toxicology of uranium as a nephrotoxin. Isolated studies were conducted on the mechanisms for the toxic effects of uranium at moderate to high acute dosing of experimental animals. Only a few studies were done on the bioeffects of chronic uranium intake by humans. Clarkson and Kench [2] studied a group of ten workers exposed to a gaseous uranium compound, while Thun et al. [3] evaluated kidney function in a group of uranium mill workers exposed primarily by inhalation. Moss and co-workers [4] studied a Canadian community that relied on private wells used for drinking water, which contained elevated levels of uranium. These investigators all reported uranium-correlated kidney bioeffects. A pilot epidemiological study was conducted by another group of Canadian investigators [5] of three Saskatchewan communities where uranium levels in drinking water varied from 0.48 to 50 ppb. Another human study was conducted by the present authors [6] in two communities whose drinking water contained 0.02 to 780 ppb uranium. More recently, a report was published on a study of 325 Finnish subjects [7] chronically exposed to elevated levels of uranium (up to 1,920 µg/L) in their drinking water. Like the previous human studies, the findings of this study support the uranium-induced involvement of the kidneys.

Historical background

The present study was conducted in Kitigan Zibi, an Algonquin community located 120 kilometres north of Ottawa, Canada’s capital. In 1993, a routine analysis of groundwater samples conducted by the Medical Services Branch of Health Canada showed that five wells in the community exceeded the Canadian uranium guideline of 100 ppb for drinking water. This was followed by an extensive survey conducted in 1994, which showed that of 331 wells sampled, 10 contained water with a uranium concentration exceeding 100 ppb, 11 with concentrations between 50 and 100 ppb, 36 wells with uranium levels between 20 and 50 ppb and 56 with concentrations between 10 and 20 ppb. The highest value observed
was 1,418 ppb. Between 1995 and 1997, subsequent to a request made for water treatment devices to be installed in the wells of concern, these devices were installed in 17 homes with the highest uranium levels and concentrations were reduced to less than 2 ppb. In 1996, the community expressed ongoing concerns about the potential health effects of consuming ground water with elevated uranium levels. Most concerns centered on cancer incidences in the community, which was believed to have been caused by uranium radioactivity.

The National Advisor, Drinking Water Safety Program, Medical Services Branch approached the Radiation Protection Bureau (RPB) to request a study to determine if the health problems observed in Kitigan Zibi residents were uranium intake-related. RPB staff explained that the observed health problems were not known health effects for uranium toxicity. It was agreed that a study should take place in any case and that the study would be similar to one previously conducted by RPB in another community in Canada whose drinking water is supplied by wells [6]. That study was designed to detect adverse effects on kidney function as a result of consuming drinking water with elevated levels of uranium and not to determine if a link existed between uranium exposure and cancer incidence.

**Materials and methods**

**Selection of study participants:**

Adult participants were recruited among males and females to obtain as good a spread of age between 20 and 70 years as possible. Several teenagers were also included in the study. To have as wide a range as possible of uranium intake levels, volunteers were chosen from three groups: (1) homes where wells supplied water with uranium levels exceeding the federal guideline of 100 ppb uranium, but which after installation of treatment devices were in compliance at the time of sampling; (2) residences where uranium levels were below the guideline but well above background and had not yet been remediated; and (3) homes where the uranium concentrations were at or very close to background levels.

**Selection of indicators of renal bio-effects:**

Two types of bio-markers were used: (1) indicators of kidney function and (2) markers for cell toxicity. Kidney function was assessed by changes in urinary volume, specific gravity, glucose, albumin and B2 obulin, BMG. The kidney filters blood at the glomerulus and then forms urine in the tubule by concentrating the glomerular filtrate and reabsorbing certain solutes derived from blood, such as glucose and small proteins like BMG. The increased appearance in urine of large molecules from blood such as albumin is an early indicator of glomerular injury while increased urinary excretion of low molecular weight proteins such as BMG is primarily the result of an increase in plasma concentration and/or a decrease in tubular reabsorption. The increased excretion of dilute urine is also indicative of decreased tubular reabsorption. Kidney tissue is the main source of urinary enzymes, which are present in larger amounts in some parts of the kidney than others and therefore, act as good markers for the site of kidney injury. The enzymes used in this study and the sites where they are present maximally include alkaline phosphatase, ALP (proximal tubule), \( \gamma \)-glutamyl transferase, GGT (proximal tubule, loop of Henle), lactate dehydrogenase, LDH (distal tubule) and N-aceteyl- \( \beta \)-glucosaminidase, NAG (proximal tubule, the glomerulus).

**Indicators of exposure:**

In this survey, four indicators of exposure were considered to determine the correlation between uranium intake and urinary bio-marker levels. These include: (1) \( \text{U}_{\text{Excr}} \), the uranium concentration in 24-hour urine samples collected at the time of the survey, (2) \( \text{U}_{\text{Last}} \), intake during the most recent period of exposure (up to 2.8 years), taken to be from the time of sampling back to the date of water treatment plus one year, (3) \( \text{U}_{\text{Max}} \), the highest concentration of uranium in drinking water ingested by each subject.
over the 15-year period preceding the study, and (4) Tot_In, total time-integrated uranium intake over a period of up to 15 years preceding the study. The equations used to calculate Tot_In included intake from all sites from which each participant had taken drinking water and included the participant’s home, place of work, a neighbour’s well, the Health Centre, or the Band Office.

The investigators recognized that the participants’ urinary uranium levels was the most direct indicator of uranium kidney exposure, but it was desired to establish a link between this exposure index and uranium intake in drinking water. Thus, three other indicators were considered in this study. Since treatment of some wells to reduce uranium levels, in effect, altered uranium intake profiles, it was thought that using the intake during the most recent period (U_Last) of exposure (up to 2.8 years, which encompassed the time interval since the earliest treatment date prior to sampling plus an arbitrarily chosen cushion of one year) would put all study subjects on a “level playing field”.

Responses to the questionnaires indicated that the population was quite mobile and, in most cases, changed residences over short periods of time and while at the same residence, obtained their drinking water from various sources. People who were thought to be good candidates for inclusion in a Reference Group turned out to have had previous intakes of uranium in their drinking water. Thus, information was gathered regarding residential and work water location for a long enough period prior to the time this survey was undertaken. The fifteen-year period prior to the date of sample collection (Tot_In) was chosen for this purpose. This choice would allow the investigators not only to obtain a more comprehensive intake profile, but, although it was not the primary objective of the study, to also calculate a cumulative radiation dose since this period encompassed both the short-term and the long-term component ($T_{1/2} = 5000$ days) for uranium retention in bone.

The U_Max information was collected to compare its merits as a determinant of adverse kidney bio-effects to total exposure (Tot_In) or the most recent exposure (U_Last). It was thought that this information might be of interest to the agencies involved in setting the Canadian Uranium in Drinking Water Guideline.

**Information gathering:**

The Kitigan Zibi Community Health Representative and Community Health Nurse obtained signed consent forms, and administered questionnaires that captured data related to water supply history (source, treatment, etc.), residential history, health history, as well as fluid consumption. The fluid consumption questionnaire developed by Health Canada and used in a previous study [8] captured information on intake of tap water at home and at work, as well as non-tap water beverages such as beer, pop, fruit juice, etc. The information was based on recall of fluid intake on the day preceding urine collection and on the day of urine collection itself.

The Medical Services Branch (MSB) provided the information on uranium levels in drinking water at all locations identified in the questionnaires. Where uranium levels were variable for a particular well, means of the reported uranium levels were used in the calculations. Two assumptions were made in calculating the total intake (TOT_IN) of uranium over this period: (1) That the mean values recorded by the Medical Services Branch for a particular well applied throughout the period of use and (2) That the volume of daily tap water intake recorded by each individual in his/her fluid consumption questionnaire applied throughout the 15-year period preceding this study.

**Sample collection:**

A water sample was taken from the home kitchen tap according to instructions published in Health Canada (1996). Three urine samples were collected from each participant: (1) A spot urine sample to screen for casts, as well as for blood and urinary tract infections which might cause interferences in
subsequent bio-indicator measurements; (2) An 8-h urine sample collected between approximately 22:00h in the evening and 6:00h the following morning for $\beta_2$-microglobulin and enzyme (ALP, GGT, NAG and LDH) measurements; (3) A 24-hour urine sample for measurements of urine volume, specific gravity, glucose, albumin, creatinine and uranium.

Samples were refrigerated during transport from Kitigan Zibi to Ottawa. Preservation and prevention of the plating out of uranium on containers was accomplished by acidification of both water and urine samples with concentrated HCl to a concentration of 1% (v/v).

Analytical methods:

Uranium in water and urine samples were analyzed by Elemental Research Laboratory using inductively coupled plasma – mass spectrometry (ICP-MS). Both water and urine samples were analyzed without any prior chemical separation of uranium from the sample matrix. The detection limit was $6 \times 10^{-4} \mu g/L$ for water and $1.8 \times 10^{-3} \mu g/L$ for urine samples.

With the exception of urine volume and specific gravity, all bio-indicators were quantified using a Varian Cary IE UV/visible spectrophotometer. These methods used were chosen for their specificity for the analyte, their sensitivity and their resistance to interferences. They are identical to those used in a previous study conducted by the present investigators [6]. Urine volume was measured using a 2L graduated cylinder for which the error tolerance was ±12 mL. Specific gravity was measured using a Fisher Precision Specific Gravity hydrometer to an accuracy of 0.001.

For the bio-indicators all urine samples were centrifuged prior to analyses. Glucose, creatinine and total protein were measured in the supernatant without further treatment. To prevent $\beta_2$-microglobulin degradation at pH<5.6, sample pH was adjusted by the addition of phosphate buffer (pH 7.6) to the supernatant. For the enzyme assays, the supernatant was dialyzed against water for two hours to remove inhibitors of low molecular mass [9].

Quality assurance:

Calibration curves were established with appropriate standards for each bio-marker and all equipment (pipettes, spectrophotometer, analytical balance) were calibrated to an accuracy of 5%. For water samples, uranium measurements by Elemental Research Laboratory ERL were verified by the MSB laboratory. The relative difference between the ERL and MSB measurement was 0.09 ± 0.21. Splits of 10% of the urine samples analyzed by (ERL) were sent to RPB for blind duplicate uranium analysis by laser phosphorimetry. The relative difference between ERL’s results and that of the in-house (RPB) results was 0.11 ± 25.15. These values satisfy relative bias and repeatability requirements of the Canadian regulatory standard [10] as well as those of the international standard, ISO 12790 [11] for bioassay measurements.

Statistical methods:

Even participants in Group (3) above with the lowest concentration of uranium in their residential drinking water (0.01 $\mu g/L$) were potentially exposed to uranium at previous addresses or through intake from other sources. As a result, no clear Reference Group could be identified from the study population, and it was decided to pool all results for the three groups regardless of place of residence or source of drinking water.

Prior statistical analysis showed that several of the indicators of tubular toxicity chosen for this study were sensitive to the volume of liquid intake; thus, results for their measurement were normalized to this parameter. These biomarkers included: urine volume ($r_s = 0.33$, $p = 0.016$ for pooled data), urine specific gravity ($r_s = 0.42$, $p = 0.0079$ for females and $r_s = -0.32$, $p = 0.019$ for pooled data); alkaline phosphatase ($r_s = 0.27$, $p = 0.046$); and $\gamma$-glutamyltransferase ($r_s = 0.32$, $p = 0.047$) for females. The normalization
procedure was also applied to the other markers to allow comparisons on the same basis; thus, two sets of data were subjected to exposure-response correlation analysis. The data adjusted for the volume of daily fluid intake are referred to as Fluid Intake-Adjusted Data in the tables, while the unadjusted data are labelled Unadjusted Data.

The Shapiro-Wilk test [12] was used to check the normality of distribution of biomarker measurements as well as log-transformed data. Since the Shapiro-Wilk test did not support the assumption of normality, and sample sizes were small, nonparametric methods were used for bio-statistical analysis. The Kruskal-Wallis test [13] was used to test the equality of the medians of male and female results. To investigate the dose-response relationship between uranium exposure indicators and the bio-markers of interest, the Spearman correlation coefficient [13] was used. The Spearman correlation coefficient provides an alternative to the Pearson correlation coefficient when data do not come from a bivariate normal distribution. The correlation coefficient represents the degree of association while the test of significance for the coefficient determines, at a chosen level of probability, whether the association exists in the population from which the sample was drawn. In this investigation, the significance chosen for rejection of the null hypothesis was \( p \leq 0.05 \). The same level of significance was used for the previously cited RPB study [6].

**Results**

*Study population characteristics:*

A total of 77 Kitigan Zibi residents (23 males, 54 females) were recruited with as broad an age variance as possible (20-70 years). Of these, five were teenagers (3 males, 2 females). Eight of the male and 15 of the female volunteers had health problems including diabetes mellitus, hypertension, and renal, heart or liver disease which could cause confounding factors in the statistical analysis of results. Measurements from these individuals were excluded from subsequent statistical analysis. Thus, 54 of the original 77 volunteers (15 males and 39 females) were included in the statistical analysis of results. Male ages ranged from 12 to 61 years, while the age among females varied from 12 to 73 years.

*Levels of uranium exposure indicators:*

For the uranium concentration in 24-h urine (U_Excr) samples collected at the time of survey, the range was 0.02 to 1.3 µg U/day, while the range for the highest concentration of uranium in drinking water (U_Max) was 0.4 to 845 µg U/L of water over the 15-year period of exposure. Total intake over the 2.8 years immediately preceding the study (U_Last) varied from 0 to 302 mg U, while the total uranium intake over the 15-year period preceding the study (Tot_In) varied from 0 to 1761 mg U.

*Bioindicator measurements:*

When uranium-correlated, bio-indicator measurements (unadjusted for fluid-intake) were compared to published values for normal or reference ranges, 21 results exceeded the range for urine volume [14] and 26 were below normal for specific gravity [14]. For GGT, 16 values were above the normal range. All BMG values were within the reference range provided by the supplier of the test kit used for this study.

*Statistical analysis:*

Uranium excreted in urine measures specifically the amount of uranium that reaches the kidney and is the most direct indicator of this organ’s exposure. The results of the correlation analysis with the other exposure indicators are as follows: For pooled (i.e., combined male and female data) fluid intake-adjusted data, significant correlation \( (r_s = 0.28, p = 0.04) \) was observed with uranium intake over the 15-year period preceding sample collection (Tot_In). For volume-adjusted female data, U_Excr also correlated
significantly \((r_s = 0.41, p = 0.01)\) with \(\text{Tot}_\text{In}\), as well as uranium intake, \(\text{U}_\text{Last}\), during the most recent period of up to 2.8 years preceding sampling, \((r_s = 0.32, p = 0.05)\). For male subjects, there was no significant correlation between \(\text{U}_\text{Excr}\) and the other uranium exposure indicators. Since uranium excreted in the urine \((\text{U}_\text{Excr})\) correlates best with the 15-year time-integrated intake \((\text{Tot}_\text{In})\) and is the most direct indicator of kidney exposure to uranium, conclusions in this study are drawn from outcomes of the correlation analyses between this parameter and the measured markers of kidney toxicity.

Results of the analysis performed on data adjusted for the volume of each subject’s liquid intake (Fluid-intake-adjusted data) and unadjusted data are presented in Table I for males, females and pooled male and female results. Correlations for pooled data were not considered when the Kruskal-Wallis test indicated that a statistically significant difference existed between male and female biomarker values. For unadjusted data, the gender-sensitive biomarkers and the \(p\) values for the Kruskal-Wallis test are: specific gravity \((p = 0.036)\), glucose \((p = 0.007)\), LDH \((p = 0.036)\) and GGT \((p = 0.048)\), while for data which had been normalized for the volume of fluid intake, glucose \((p = 0.0044)\) results showed gender-dependence. In these cases, conclusions were drawn separately for male and female populations. For all other biomarkers, conclusions were based on combined (pooled) male and female data.

| Table I: \(\text{U}_\text{Excr}\) Spearman correlation coefficients for unadjusted data and fluid-intake adjusted data\(^1\) |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Unadjusted data | Fluid-intake adjusted data |
|                  | Male            | Female           | Pooled           | Male            | Female           | Pooled           |
| Urine Volume     | 0.34 (0.22)     | 0.48 (0.0018)   | 0.46 (0.0004)   | 0.45 (0.10)     | 0.54 (0.0004)   | 0.50 (0.0001)   |
| Specific Gravity \(^2\) | -0.02 (0.94) | -0.37 (0.02) | -0.25 (0.0653) | 0.60 (0.02)     | 0.31 (0.05)     | 0.35 (0.0088)   |
| Glucose \(^3\)   | 0.29 (0.30)     | 0.13 (0.44)     | 0.14 (0.30)     | 0.15 (0.60)     | 0.12 (0.48)     | 0.14 (0.31)     |
| Albumin          | 0.34 (0.21)     | 0.20 (0.23)     | 0.25 (0.07)     | 0.08 (0.78)     | 0.26 (0.12)     | 0.23 (0.09)     |
| ALP              | 0.17 (0.54)     | -0.32 (0.85)    | -0.03 (0.81)    | 0.17 (0.54)     | 0.18 (0.28)     | 0.15 (0.29)     |
| LDH\(^2\)        | -0.19 (0.50)    | -0.08 (0.62)    | -0.11 (0.44)    | 0.12 (0.67)     | 0.17 (0.31)     | 0.16 (0.26)     |
| GGT\(^2\)        | 0.40 (0.14)     | 0.09 (0.61)     | 0.16 (0.26)     | 0.57 (0.03)     | 0.33 (0.04)     | 0.37 (0.0064)   |
| NAG              | -0.17 (0.55)    | -0.06 (0.72)    | -0.08 (0.58)    | 0.33 (0.24)     | 0.13 (0.44)     | 0.15 (0.29)     |
| BMG              | -0.12 (0.74)    | 0.09 (0.69)     | 0.12 (0.53)     | 0.47 (0.17)     | 0.52 (0.02)     | 0.49 (0.0047)   |

1 Numbers in parentheses are \(p\) values.
2 Biomarkers that are gender dependent (unadjusted data).
3 Biomarkers that are gender dependent (adjusted data).
4 Data in bold typeface are statistically significant correlations from which conclusions are drawn.

For unadjusted pooled data, urinary volume, which was found to be gender-independent, correlated positively with \(\text{U}_\text{Excr}\) \((r_s = 0.46, p = 0.0004)\). For unadjusted female results, significant correlation was observed between \(\text{U}_\text{Excr}\) and urine specific gravity \((r_s = -0.37, p = 0.02)\) and was found to be gender-dependent. For unadjusted male data, no correlation was observed at the level of significance chosen for this study \((p<0.05)\) with any of the gender-specific biomarkers. For the gender-insensitive biomarker albumin, no significant correlation was observed for combined male and female data \((r_s = 0.25, p = 0.07)\).

For fluid intake-adjusted pooled data, positive correlations with \(\text{U}_\text{Excr}\) were found for markers of tubular injury: urine volume, \((r_s = 0.50, p = 0.0001)\), specific gravity, \((r_s = 0.35, p = 0.0088)\), GGT, \((r_s = 0.37, p = 0.0064)\), and BMG \((r_s = 0.49, p = 0.0047)\), which though weak to moderately strong, were all highly statistically significant. For the gender-specific biomarker glucose, no correlation was observed for either male \((r_s = 0.15, p = 0.60)\) or female data \((r_s = 0.12, p = 0.48)\). No significant correlation was observed with albumin \((r_s = 0.23, p = 0.09)\) for pooled male and female data.

**Correction of male urine sample volumes to 24-hour value:**

The level of uranium in urine is indicative of exposure [27]. A truly representative sample should be obtained by collecting all the urine excreted during a 24-hour period. Because collection is often
technically difficult for individuals who work outside the home, it was decided to investigate whether the inability to observe uranium exposure-correlated changes for the male subjects was because the samples submitted were not true 24-hour samples. To test this hypothesis, the urinary uranium concentration in the male samples was normalized with the 24-hour creatinine value calculated using the methodology described in Z. Karpas et al. [28]. In subsequent biomarker analysis of unadjusted data, statistically significant moderate to strong correlation was obtained with the enzyme GGT ($r_s = 0.61, p = 0.0164$). For data adjusted for the volume of daily fluid intake, statistically significant moderate to strong correlation was also obtained for the other urinary enzymes, ALP ($r_s = 0.61, p = 0.0156$), LDH ($r_s = 0.55, p = 0.035$), GGT ($r_s = 0.66, p = 0.0078$), and NAG ($r_s = 0.60, p = 0.0172$). In both analyses, correlation was not observed for albumin – the indicator used in this study for glomerular dysfunction: Unadjusted data ($r_s = -0.30, p = 0.28$) and adjusted data ($r_s = -0.29, p = 0.30$).

**Discussion**

**Kidney bioeffects:**

The present investigation was designed to be completely non-invasive and depended solely on the use of a combination of several urinary bio-indicators of kidney dysfunction measured in the same urine specimens. Results of measurement of markers of tubular function were found to be more closely associated with urinary uranium concentration than the other indicators of uranium exposure. This could be because the other indicators may have included errors in recall by study volunteers as well as variation of uranium levels in groundwater over time, whereas urinary uranium is independent of these sources of uncertainty. Consequently, conclusions from this study are drawn from correlations observed with urinary uranium.

The analysis of the fluid intake-adjusted pooled data indicates that in the combined male and female population, the reabsorption capability of the kidney is decreased as shown by the enhancement of urine volume with increasing urinary uranium. The positive correlation of BMG with urinary uranium for pooled male and female data further supports this conclusion. The increased urinary excretion of BMG with increased uranium ingestion agrees with the findings of two previous Canadian studies [4, 6] of communities with elevated uranium levels in their drinking water. It is also consistent with an American study of uranium mill workers [3] who were exposed to yellowcake. The observed positive correlation with GGT for pooled adjusted data and male unadjusted data is consistent with the findings of a French study where GGT is the enzyme observed to have increased in the urine of workers exposed to uranium intakes in a refinery [15].

BMG is not of renal origin. It is a small protein (MW = 11,800) freely filtered by the glomerulus and then reabsorbed and digested in the lining of the kidney tubule. GGT is maximal in the membrane of the proximal tubules and the loop of Henle. Positive correlation of enzyme activity with uranium exposure suggests cell damage. The mechanism for the increased excretion of these two biomarkers may involve the disruption of the cell membrane by prolonged exposure to uranium in the tubular fluid, resulting in an enhanced release of GGT into the developing urine and a decreased ability to reabsorb BMG. High dosing with uranium can lead to structural changes in the brush-border membrane of the proximal tubules [16, 17, 18]. Loss of microvilli has been observed as early as one hour after injection of 10 mg U/kg into rats [16]. Although the daily dose rates in the present study are very much lower than doses reported in those studies, it is quite possible that repeated insult to the tubular epithelium over a prolonged period achieves the same effect in humans. Indeed, the observed increased excretion of GGT, which is maximal in the proximal tubule and the loop of Henle, with increased uranium exposure is consistent with the histological finding of Diamond et al. [19] who reported damage initially to the S2 and S3 segments of the proximal tubule of rat kidney, and eventually, to the loop of Henle as treatment progressed further.
Both the proximal tubule and the loop of Henle in the kidney nephron are involved in reabsorption processes for water and electrolytes, the former being the principal site for optional reabsorption and the latter being the site where further reabsorption takes place. For data unadjusted for fluid intake, the statistically significant negative correlation of urinary uranium (U_Excr) with specific gravity together with positive correlation with urine volume strongly suggest the dilution of urine with increasing levels of exposure, most likely due to the decreased ability of the kidney to reabsorb water. The positive correlation with urine specific gravity may seem puzzling, initially, but is not unreasonable in view of the observation of enhanced BMG and GGT excretion, which would increase the ratio of solutes to liquid in urine when compared to normal urine. The highly significant positive correlation of the observed specific gravity with BMG ($r = 0.60, p = 0.0003$) and GGT ($r = 0.64, p = 0.0001$) obtained upon subsequent statistical analysis supports this hypothesis.

Initially, the inability to observe a correlation between uranium exposure and glucose, which was observed by the present investigators in a previous human study [6], was puzzling. Glucose was found both in that study and in a rat study [19] to be the most sensitive of the bio-indicators used in those investigations. In the Limson Zamora study, urinary glucose was found to exceed the published normal range at a minimum total daily intake of 21 µg U. The daily uranium intakes from drinking water for the present population ranged from 0.01 to 119 µg per day. The observed difference may be of genetic origin. Note that it has been widely reported that Native Americans such as Zunis [20], Navajos [21], and Pimas [22] have exhibited poor glycemic control. Indeed, American Indians have been found to be susceptible to non-insulin dependent diabetes mellitus, NIDDM [23]. This study’s participants are members of the Algonquin First Nation. The prevalence of NIDDM in Algonquin communities in Quebec, Canada has also been reported [24]. Although volunteers with frank diabetes were excluded from the statistical analysis of results, it is quite possible that the reason for the difference observed between this study and that conducted by the present investigators on a Caucasian community is this widely-reported genetic difference in glucose metabolism.

The inability to detect a correlation between uranium and the enzyme ALP was even more puzzling, especially since ALP has been reported to be located more superficially than GGT in the cellular membrane of renal tubular epithelium [25]. Investigation of the effect of other variables, e.g., age, on urinary ALP excretion, did not yield any statistically significant uranium ingestion dose dependence for both fluid intake-adjusted and unadjusted data. The rationale for this observation would have to be established in a separate study, as no previously reported investigations offer an adequate explanation.

Albumin constitutes the major protein in normal urine because it is the protein found in largest concentration in plasma. Increased urinary excretion of serum proteins with molecular weight in excess of 50,000, such as albumin, is an early indicator of glomerular injury [26]. Values for this protein were not found to be significantly correlated to U_Excr, thus suggesting that there was no glomerular involvement at the levels of uranium intake observed in this study.

In this study, NAG was not found to be a gender-sensitive biomarker for adjusted or unadjusted data and is not used as a basis for inferences; however, the positive correlation observed between U_Excr and NAG, with the creatinine-normalized male samples, may raise questions regarding the involvement of the glomerulus since NAG is also found in high concentration in the glomerulus. It should be noted, however, that statistically significant correlation was not observed with albumin for the same samples. Further, although proteinuria was observed in at least one animal study [19], the histopathology of kidney tissue in that study revealed damage only to the S2 and S3 segments of the proximal tubule initially and as treatment progressed, the loop of Henle, as well. But no damage to the glomerulus was reported.

Although internally deposited uranium also has toxic effects on the cardiovascular system, liver, muscle and nervous system, extensive studies in experimental animals have clearly shown that renal toxicity is probably its most important detrimental chemical effect [27]. In all animals studied, the kidney is the
major deposition site for uranium entering the systemic circulation and a substantial fraction of the metal filtered by the kidneys is temporarily retained in the renal tubules before it passes into the urinary bladder.

The results of the present investigation point to the renal tubule as the primary site of insult to the kidney from chronic intake of uranium in drinking water. The involvement of the tubule agrees with histological evidence obtained in animal studies. In uranyl fluoride-treated rats [19], significant dose-related injury was noted in the tubules rather than in the glomeruli. The tubules are the site where small molecules such as water and electrolytes are reabsorbed after glomerular filtration in order to maintain water, electrolyte and acid-base homeostasis. Indeed, a recent study of the kidney bio-effects of long-term uranium intake in drinking water by a Finnish population [7] reported uranium-correlated enhanced urinary excretion of the electrolytes calcium and phosphate.

In healthy subjects, the volume and composition of the body fluids vary within narrow limits. The kidneys are largely responsible for maintaining this state. This study indicates that in humans, the primary kidney function adversely affected by long-term uranium ingestion is the reabsorption of small molecules by the tubules. This kidney function affects water balance, electrolyte balance and acid-base balance, all of which affect human health.

Cancer incidence:

Although this study was not designed to establish a link between uranium levels in the community and cancer incidence, the community’s ongoing concerns prompted the calculations below. This study provides valuable information on fifteen-year intakes of uranium for each of the 54 participants. These intakes were converted to a cumulative radiation dose in millisieverts (mSv) thus:

\[
\text{Radiation dose (mSv)} = 15\text{-year uranium intake (mg)} \times 25 \text{ (Bq/mg)} \times 0.000038 \text{ (mSv/Bq)}
\]

The average dose for the 54 participants was 0.2 mSv. Ninety percent of the cumulative 15-year doses were less than 0.6 mSv while the highest dose was 1.7 mSv. From information provided by the International Commission on Radiological Protection, 1 mSv of radiation is assumed to carry a risk of cancer and other serious diseases of 7 in 100,000. Thus, the highest dose of 1.7 mSv would give a total risk of 12 in 100,000. The average dose of 0.2 mSv would give a risk of 1.4 in 100,000. These increases in cancer would be very difficult to detect in a community as small as Kitigan Zibi (population 1,480). Furthermore, the types of cancer that would most likely be caused by these uranium doses would be bone cancer and possibly kidney cancer – types not commonly seen in the community.

Conclusion

The bio-effects reported here are mild and represent a manifestation of subclinical toxicity which will not necessarily lead to overt illness or kidney failure; however, the trends observed with both unadjusted and fluid intake-adjusted data suggest that the long-term ingestion of uranium in drinking water interfered with kidney function. It is noteworthy that these observations do not appear to be race-specific, as they are in agreement with human studies involving Caucasians. Although this study was not designed to investigate if long-term uranium ingestion in water is linked to cancers observed among some members of the Kitigan Zibi community, it was possible to show that the increased cancer risk was unlikely to be more than 12 in 100,000 for the highest 15-year exposure to uranium. Even this risk to the most exposed participant would be difficult to observe in a population of 1,480. Thus, at the levels of uranium intake reported for this community, our observations suggest that chemical toxicity would be a greater health concern than radiotoxicity.
References