

## Chronic Ingestion of Uranium in Drinking Water: A Study of Kidney Bioeffects in Humans

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A study was conducted of the chemical effects on the human kidney induced by the chronic ingestion of uranium in drinking water. Subjects were divided into two groups: *The low-exposure group*, whose drinking water was obtained from a municipal water system and contained  $<1 \mu\text{g}$  uranium/L, and *the high-exposure group*, whose drinking water was obtained from private drilled wells and contained uranium levels that varied from 2 to 781  $\mu\text{g/L}$ . Years of residence varied from 1 to 33 years in *the low-exposure group* and from 3 to 59 years in *the high-exposure group*. The indicators of kidney function measured in this study included glucose, creatinine, protein, and  $\beta_2$ -microglobulin (BMG). The markers for cell toxicity studied were alkaline phosphatase (ALP),  $\gamma$ -glutamyl transferase (GGT), lactate dehydrogenase (LDH), and *N*-acetyl- $\beta$ -D-glucosaminidase (NAG). Urinary glucose was found to be significantly different and positively correlated with uranium intake for males, females, and pooled data. Increases in ALP and BMG were also observed to be correlated with uranium intake for pooled data. In contrast, the indicators for glomerular injury, creatinine and protein, were not significantly different between the two groups nor was their urinary excretion correlated to uranium intake. These results suggest that at the intakes observed in this study (0.004  $\mu\text{g/kg}$  to 9  $\mu\text{g/kg}$  body wt), the chronic ingestion of uranium in drinking water affects kidney function and that the proximal tubule, rather than the glomerulus, is the site for this interference. © 1998 Society of Toxicology.

In Canada, the federal Department of Health along with the Provinces and Territories, under the auspices of the Federal-Provincial Subcommittee on Drinking Water, establishes the guideline for uranium in drinking water. The Radiation Protection Bureau has measured the uranium concentration in the water supplies of 17 cities since 1975 and has found levels for monthly composite samples to usually be less than 1  $\mu\text{g/L}$  (Health and Welfare, Canada, 1979–1988). However, elevated

levels have been found in uranium mining as well as in non-uranium-producing communities. In the latter case, the uranium has been introduced into drinking water, not through human activity, but through contact with naturally occurring deposits of uranium minerals.

Uranium, the heaviest of the naturally occurring elements, is a metal whose biological effects were described in the literature as early as the 1820s (Stannard, 1988; Meyer and Pietsch, 1936). As with other heavy metals (Pb, Hg, Cd), it has been identified as a nephrotoxin (WHO, 1991). Its nephrotoxic effects are more likely due to its chemical properties rather than its radioactivity, although ingested uranium may have a radiological effect on other tissues of deposition such as bone.

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. These were carried out largely on laboratory animals (Voegtlin and Hodge, 1949). The criteria for oral toxicity included mortality, a decrease in growth rate, and histopathological changes. The principal histological finding was atrophic changes in the renal tubules. Some postwar human studies were conducted with hospital patients at the University of Rochester (Bassett, 1948; Terepka *et al.*, 1965) and Boston–Oak Ridge (Luessenhop *et al.*, 1958; Struxness *et al.*, 1955; Hursh *et al.*, 1969), although their primary purpose was to study the metabolism of uranium, rather than its health effects.

There have been no major efforts since the late 1950s to update the toxicology of uranium as a nephrotoxin (Leggett, 1989). Many isolated studies were conducted on the mechanisms for the toxic effects of uranium at moderate to high acute doses on experimental animals (Benscome *et al.*, 1960; Carafoli *et al.*, 1971; Galle, 1974; Schwartz and Flamenbaum, 1976; Blantz *et al.*, 1985). However, only a few studies were done on the bioeffects of chronic uranium intakes by humans. Clarkson and Kench (1956) studied a group of 10 workers exposed to a gaseous uranium compound while Thun *et al.*, (1985) evaluated kidney function in a group of uranium mill workers exposed primarily by inhalation. Moss and co-workers (1983) studied a Canadian community that relied on private wells containing elevated levels of uranium for their drinking

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water. These investigators all reported uranium-correlated kidney bioeffects. Three studies were conducted by the Health Canada to examine the structural changes in the kidneys of New Zealand rabbits and Sprague–Dawley rats exposed for 91 days to uranyl nitrate hexahydrate in their drinking water (Gilman *et al.*, 1998a,b,c). And concurrently with the present study, a pilot epidemiological study was conducted in three Saskatchewan communities where uranium levels in drinking water varied from 0.48 to 50  $\mu\text{g/L}$  (Mao *et al.*, 1995). The findings in both the epidemiological and animal studies suggest uranium-induced involvement of the kidneys.

Due to the paucity of human data, most standard settings for occupational and environmental contaminants have required the extrapolation to humans of conclusions derived from animal data. The present investigation was undertaken to obtain direct evidence from human subjects to determine if the bioeffects observed in animal studies would also be detected in humans.

Two communities were selected: The first community had private wells supplied from a groundwater source whose uranium content was well above the current Canadian drinking water guideline of 100  $\mu\text{g/L}$  (Health and Welfare Canada, 1996). The second had drinking water, supplied by surface water through the municipal distribution system, which contained less than 1  $\mu\text{g/L}$ . Only urinary biochemical indices were used. A combination of several indicators was used to detect uranium-induced bioeffects on kidney function such as loss of tubular reabsorptive ability or increased glomerular permeability (glucose, creatinine, total protein, and  $\beta_2$ -microglobulin) and provide insight into the site for these effects (alkaline phosphatase (ALP),  $\gamma$ -glutamyl transferase (GGT), *N*-acetyl- $\beta$ -D-glucosaminidase (NAG), lactate dehydrogenase (LDH)).

#### SELECTED MARKERS OF RENAL INJURY

Two types of biomarkers were used in this study: indicators of kidney function and markers for cell toxicity. Kidney function was assessed by creatinine, glucose, total protein, and  $\beta_2$ -microglobulin. Cell toxicity markers included the enzymes ALP, GGT, LDH, and NAG.

Glucose, a small molecule (MW = 180), is filtered by the glomerulus and is completely or almost completely reabsorbed into the blood by active processes in the proximal tubules of the kidney. Creatinine, a waste product of metabolism, and also a small molecule (MW = 113), is not reabsorbed in the tubules at all. Virtually all creatinine that is filtered in the glomerulus passes on through the tubular system and is excreted in the urine.

Under normal conditions, only small amounts of protein are detected in urine. Increased urinary excretion of low molecular weight proteins is primarily the result of an increase in plasma concentration or a decrease in tubular absorption of small proteins. If tubular absorption is not impaired, part of an increased load of small proteins will be absorbed, and any increase in urinary excretion will be minimized.

When the permeability of the capillaries is increased, high molecular weight proteinuria develops (Lillehoj and Poulik, 1986). Small changes in glomerular permeability will lead to large increases in the filtered load of larger proteins, e.g., albumin (MW = 69,000). Due to the low affinity of the tubular absorption process, these proteins appear in urine after escaping the mechanisms of tubular reabsorption. Thus, increased urinary excretion of serum proteins with molecular weight in excess of 50,000 such as albumin is an early indicator of glomerular injury (Lillehoj and Poulik, 1986).

The majority of animal studies have shown that the primary site of injury resulting from uranium intoxication is the renal tubule (Gilman *et al.*, 1998a,b,c; Diamond *et al.*, 1989). However, some questions have been raised about whether the glomerulus is also affected. In this study, it was anticipated that the differential effect on the indicators chosen would shed light on this subject. The renal tubule, in particular the proximal tubule, is the principal site for reabsorption of water and small molecules filtered at the glomerulus from the blood. If the tubule is the principal site of injury, increases in urinary excretion of the smaller molecules, such as glucose and BMG, will be observed with no change in the levels of larger protein molecules and creatinine which will be filtered at normal rates by healthy glomeruli. If, on the other hand, there is glomerular involvement, without tubular injury, levels of the larger protein molecules and creatinine will rise in the urine without significant increases in the smaller molecules. If both the tubule and the glomerulus are involved, then there would be a general increase in all these biomarkers.

Kidney tissue is the main source of urinary enzymes. Other investigators have used enzymes in the assessment of uranium nephrotoxicity. Zalups *et al.* (1988) used lactate dehydrogenase, catalase, and aspartate aminotransferase in a study of  $\text{UO}_2\text{F}_2$ -treated rats. Diamond and co-workers (1989) used *N*-acetyl- $\beta$ -D-glucosaminidase, alkaline phosphatase,  $\gamma$ -glutamyl transferase, lactate dehydrogenase, and aspartate aminotransferase as indicators of renal injury in uranyl fluoride-treated rats. Stroo and Hook (1977) used the lysosomal enzymes acid phosphatase and  $\beta$ -galactosidase and the brush-border markers maltase and alkaline phosphatase in a study of rats treated with uranyl acetate.

The diagnostic potential of urinary enzymes is often enhanced by the simultaneous assay of more than one enzyme, particularly if the activities of the individual enzymes used are high in different regions of the nephron (Price, 1982). The selection of kidney tissue enzymes for this study was therefore based on their site of maximal concentration in the nephron. GGT is maximal in the membrane of the proximal tubules and the loop of Henle (Albert *et al.*, 1961). ALP has been demonstrated in the membrane of epithelial cells of the proximal tubules (Butterworth *et al.*, 1965) and is located more superficially in the membrane than GGT (Jung *et al.*, 1993). NAG is found in lysosomes and is present in greatest concentration in the glomerulus and the proximal tubule (Bourbouze *et al.*,

TABLE 1  
Biomarkers and Analytical Methods Used

Analyte/biomarker	Analytical method	Detection limits
Uranium	Inductively coupled plasma/mass spectrometry	$6 \times 10^{-4}$ $\mu\text{g/L}$
Creatinine	Modified Jaffé (colorimetric)	0.01 mmol/L
Glucose	Hexokinase (enzymatic)	6.34 mg/L
Protein	Bio-Rad Coomassie blue protein assay	0.0007 mg/ml
$\beta_2$ -Microglobulin (BMG)	Phadebas competitive radioimmunoassay	12.7 nmol/L
Alkaline phosphatase (ALP)	Kinetic	0.06 U/L <sup>a</sup>
$\gamma$ -Glutamyl transferase (GGT)	Kinetic	2.04 U/L <sup>b</sup>
Lactate dehydrogenase (LDH)	Kinetic	0.04 U/L <sup>c</sup>
<i>N</i> -Acetyl- $\beta$ -D-glucosaminidase (NAG)	Fixed point	0.37 U/L <sup>d</sup>

<sup>a</sup> 1 unit will hydrolyze 1.0  $\mu\text{mol}$  of *p*-nitrophenyl phosphate per minute at pH 10.4 at 37°C.

<sup>b</sup> 1 unit will liberate 1.0  $\mu\text{mol}$  of *p*-nitroaniline from *L*- $\gamma$ -glutamyl-*p*-nitroanilide per minute at pH 8.5 at 25°C.

<sup>c</sup> 1 unit will reduce 1.0  $\mu\text{mol}$  of pyruvate to *L*-lactate per minute at pH 7.5 at 37°C.

<sup>d</sup> 1 unit will hydrolyze 1.0  $\mu\text{mol}$  of *p*-nitrophenyl *N*-acetyl- $\beta$ -D-glucosaminide to *p*-nitrophenol and *N*-acetyl-D-glucosamine per minute at pH 4.25 at 25°C.

1984). LDH is found in the cytoplasm and is maximal in the distal tubule (Bonting *et al.*, 1960).

It was anticipated that the combination of biomarkers chosen for the present study would provide information on which site within the kidney is affected by uranium.

## MATERIALS AND METHODS

**Selection of study populations.** A village in Nova Scotia was selected for this study. Previous analysis by the province had shown elevated uranium levels in water from private wells in this community. Some wells exceeded the present Canadian uranium guideline of 100  $\mu\text{g/L}$  for drinking water (Health and Welfare Canada, 1996). Residents were approached to participate in this study based on the uranium levels in their well water. Adult participants were recruited with as even a distribution between males and females and as good a spread of age between 20 and 70 years, as possible. Several teenagers were also included in the study.

A group of healthy subjects, who were gender and age-matched to the first group, were selected from a pool of volunteers residing in Ottawa, Ontario, which is supplied by surface water whose uranium concentration was less than 1  $\mu\text{g/L}$ .

Questionnaires were administered to participants to help establish such parameters as age, years of residence in the community, and health status. To avoid confounding factors in the statistical analysis of the results, candidates with a history of renal, heart, or liver disease; hypertension; and diabetes mellitus were excluded from the study as were individuals who were on any medication that might interfere with the biomarker measurements.

**Sample collection.** A separate sample of tapwater was collected from each household at the beginning of the study and analyzed for uranium content.

Duplicate portions of each drink and food consumed by each subject was collected over a 3-day period to determine uranium intake in water and through food. Uranium intake is the product of the concentration in water or food and the amount consumed.

Twenty-four-hour urine samples were collected for uranium, glucose, protein, and creatinine, while one 8-h sample was collected from each subject from approximately 22:00 h in the evening to 6:00 h the following morning for the enzyme and BMG measurements. The uranium concentrations in urine were used to determine the fractional uptake of uranium by the GI tract. These results are being reported in a separate publication, now in preparation.

**Analytical methods.** Water taken as such or in tea or coffee was evaporated to dryness on a hotplate, ashed in a muffle furnace at 450°C for 20 h,

cooled, and then taken up in warm 1% HCl to complete dissolution of the residue. Urine samples were acidified with 1% HCl and analyzed without further pretreatment. Food samples were homogenized. An aliquot of the homogenate was dried overnight at 105°C and ashed in a muffle furnace at 600°C. The ash was then dissolved in hot aqua regia and diluted to volume.

Uranium in the digestates was measured by inductively coupled plasma/mass spectrometry (ICP/MS). Tracer recovery for the protocol used to prepare the food and water samples was  $99.9 \pm 1.2\%$ . The detection limit was  $6 \times 10^{-4}$   $\mu\text{g/L}$  for water and the aqueous solutions of food digestates.

For the biomarkers, all urine samples were centrifuged prior to analyses. Glucose, creatinine, and total protein were measured in the supernatant without further treatment. Phosphate buffer (pH 7.6) was added to the supernatant to adjust the pH for the  $\beta_2$ -microglobulin assay. For the enzyme assays, the supernatant was dialyzed against water for 2 h to remove low molecular mass inhibitors (Laszlo and Szabo, 1982).

All bioindicators were quantified using a Bausch and Lomb Spectronic 2000 UV/visible spectrophotometer. Table 1 lists the methods used for the measurement of the biomarkers in this study with corresponding detection limits. These protocols were chosen for their specificity for the analyte, their sensitivity, and their resistance to interferences. All equipment (pipettes, spectrophotometer, analytical balance) were calibrated to an accuracy of  $\pm 5\%$ . Calibration curves were established with appropriate standards for each biomarker and all measurements corrected for spike recovery. Measurement reproducibility was within 11% RSD.

**Statistical methods.** In this study, total uranium intake from both water and food, averaged over the 3-day study period, was used as the indicator for establishing a correlation between the different biomarkers and uranium exposure.

Because the Ottawa subjects were also exposed to uranium, albeit at very low levels in food, the two groups could not be distinguished simply as "exposed" versus "unexposed." Instead, all subjects were pooled, regardless of place of residence or source of drinking water, and then grouped according to uranium levels in their drinking water. Those whose drinking water uranium concentration was  $\geq 1$   $\mu\text{g/L}$  were placed in the *high-exposure group*, while those whose drinking water uranium content was less than 1  $\mu\text{g/L}$  were assigned to the *low-exposure group*.

Data on biomarkers, as well as log-transformed data, were checked for normality, using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Since this test did not support the assumption of normality, and because of small sample sizes, it is appropriate to use nonparametric methods for the analysis. To test the equality of the means in different groups (subpopulations), the Kruskal-Wallis test (Lehman, 1975) was used. This test utilizes the ranks of the

TABLE 2  
Study Population Characteristics

	High-exposure group <sup>a</sup>		Low-exposure group <sup>b</sup>	
	Males	Females	Males	Females
Number of subjects	10	20	7	13
Age (years)	15–56	13–87	14–43	15–68
Age at initial exposure (years)	0–28	0–34	0–37	0–51
Years of residence	13–36	3–59	5–33	1–26
Uranium in drinking water ( $\mu\text{g/L}$ )	4–780	2–780	0.02	0.02
Uranium intake from all sources ( $\mu\text{g/day}$ )	6–410	3–570	0.3–20	0.4–10

<sup>a</sup> Uranium level in drinking water  $\geq 1 \mu\text{g/L}$ .

<sup>b</sup> Uranium level in drinking water  $< 1 \mu\text{g/L}$ .

measurements, rather than the actual values. To investigate the relationships between total uranium intake and the biomarkers of interest, the Spearman correlation coefficient (Lehman, 1975) 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 itself represents the degree of association. The test of significance for that coefficient determines, at a chosen level of probability, whether the association exists in the population from which the sample was drawn. In this study, the significance chosen for rejection of the null hypothesis was  $p \leq 0.05$ .

## RESULTS

### Study Population Characteristics

Table 2 summarizes the characteristics of the two study populations. Fifty-nine people volunteered to participate in this study. Nine were not included in the statistical analysis due to health problems that could interfere with the interpretation of results. The *high-exposure group* included 10 males and 20 females. Males in this group ranged in age from 15 to 56 years, while age among females varied from 13 to 87 years. Years of residence varied from 3 to 59 years, while age at the beginning of residence varied from 0 to 34 years.

The *low-exposure group* included 7 males and 13 females. The range in age was from 14 to 43 years among males and from 15 to 68 years among females. Years of residence varied from 1 to 33 years. Age at the beginning of residence in this group varied from 0 to 51 years.

### Levels of Uranium Intake

Uranium in the drinking water of the *high-exposure group* ranged from 2 to 780  $\mu\text{g/L}$ . Approximately half of the 30 donors in this group consumed drinking water with uranium levels exceeding the present Canadian Guideline (Health Canada, 1996) of 100  $\mu\text{g/L}$ . The range of total daily uranium consumption through both food and drinking water was from 3

to 570  $\mu\text{g}$  uranium with the percentage of intake through water varying between 31 and 98%.

The uranium concentration in the municipal drinking water supply of the *low-exposure group* was  $0.02 \pm 0.004 \mu\text{g/L}$ . The largest amount of uranium ingested daily in this group was 20  $\mu\text{g}$ , while the smallest amount was 0.34  $\mu\text{g}$ . The percentage of intake through water varied from 1 to 9%.

Total daily intake of uranium per kilogram of body weight varied from 0.058 to 8.5  $\mu\text{g/kg}$  among the *high-exposure group* participants and 0.004 to 0.20  $\mu\text{g/kg}$  in the *low-exposure group*.

### Bioindicator Measurements

Table 3 lists the individual results for the biomarker measurements. Of these, four glucose, four ALP, and seven LDH values exceeded the published 95th percentile ranges (Lentner, 1981; Niwa *et al.*, 1993) for the method used in our analyses. One GGT result and one NAG value were below the 5th percentile literature values (Niwa *et al.*, 1993) for these biomarkers. Three protein results exceeded the published “at rest” value of 80 mg/day (Burtis and Ashwood, 1994). Differences between published creatinine reference intervals (Burtis and Ashwood, 1994) and some creatinine results (one below and four above) were borderline in nature.

ALP values exceeded reference ranges (Niwa *et al.*, 1993) for uranium intakes ranging from 220 to 410  $\mu\text{g/day}$ , while for glucose, this occurred when intakes varied from 21 to 410  $\mu\text{g/day}$  and for LDH with intakes which varied from 0.63 to 220  $\mu\text{g/day}$ .

The GGT result below the lower limit of the published reference range was for an individual whose daily intake was 220  $\mu\text{g/day}$ . The NAG result below the published NAG reference range was for the same individual.

Glucose, protein, and creatinine values were based on successive 24-h urine sample measurements and intrasubject variability ranged from 3 to 62, 2 to 54, and 1 to 3% RSD, respectively. For other biomarkers, results were based on measurements on a single 8-h sample.

Table 4 summarizes the data in Table 3 and gives an indication of intersubject variability.

### Statistical Analysis

Table 5 summarizes the outcome of the Kruskal–Wallis test for significance of difference between the *high-exposure group* and the *low-exposure group*. At the chosen significance level of  $p \leq 0.05$ , the biomarkers that are significantly different between the two groups are glucose for male ( $p < 0.05$ ), female ( $p < 0.02$ ), and pooled male and female data ( $p < 0.001$ ) and LDH for males ( $p < 0.02$ ).

The results of the analysis of correlation with total daily uranium intake are presented in Table 6. Of the markers for proximal tubular injury studied, glucose, ALP, and BMG were significantly correlated with uranium intake for pooled male

TABLE 3  
Biomarker Measurement Results<sup>d</sup>

Sex	Age	Total Uranium Intake ( $\mu\text{g}/\text{day}$ )	Exposure Level <sup>b</sup>	Glucose ( $\text{mg}/\text{d}$ )	Creatinine ( $\text{mM}/\text{d}$ )	Protein ( $\text{mg}/\text{d}$ )	ALP <sup>c</sup>	GGT <sup>d</sup> (U/g Creatinine)	LDH <sup>e</sup>	NAG <sup>f</sup>	BMG <sup>g</sup> ( $\mu\text{g}/\text{g}$ Creatinine)
F	48	570	H	79.1	15 <sup>Δ</sup>	29.6	9.7	17	19	2.9	120
M	36	410	H	388 <sup>Δ</sup>	20 <sup>Δ</sup>	89.7 <sup>Δ</sup>	23 <sup>Δ</sup>	13	12	2.7	50
F	23	300	H	128	6.5	38.4	57 <sup>Δ</sup>	21	17	4.0	97
F	37	220	H	251 <sup>Δ</sup>	8.9	33.1	22 <sup>Δ</sup>	14	69 <sup>Δ</sup>	3.2	82
F	76	220	H	43.4	3.0 <sup>*</sup>	0.9	21 <sup>Δ</sup>	0 <sup>*</sup>	35	0.0 <sup>*</sup>	340
F	87	220	H	99.6	8.4	20.8	14	11	8.8	6.6	210
M	56	190	H	80.2	9.8	135 <sup>Δ</sup>	12	35	29	6.5	140
F	22	150	H	56.8	9.7	58.5	7.8	23	410 <sup>Δ</sup>	4.0	21
F	45	98	H	43.3	8.1	40.3	7.8	38	290 <sup>Δ</sup>	5.8	53
M	36	94	H	66.5	11	41.8	3.0	27	7.7	3.6	45
F	40	92	H	133	8.8	68.7	9.9	47	31	11	79
M	41	77	H	187	7.5	15.7	15	11	11	0.5	92
M	15	74	H	158	10	39.9	5.8	25	9.2	3.3	67
M	34	70	H	427 <sup>Δ</sup>	20 <sup>Δ</sup>	69.5	2.0	19	16	4.1	21
F	21	62	H	32.7	5.2	28.6	6.4	38	98 <sup>Δ</sup>	2.6	97
M	15	58	H	133	9.0	35.4	6.3	25	11	3.6	46
F	13	32	H	112	8.4	29.1	5.9	25	18	3.6	34
F	52	31	H	111	8.7	27.2	4.7	27	0.0	2.4	38
F	54	31	H	53.3	13	33.8	7.3	20	0.0	1.7	55
F	17	21	H	221 <sup>Δ</sup>	13	57.4	17	20	29	2.8	61
M	36	20	L	88.7	13	46.6	6.1	31	62 <sup>Δ</sup>	5.4	74
F	45	14	H	62.3	6.8	0.2	3.5	22	6.2	2.0	97
F	41	13	H	60.9	9.2	51.1	14	26	52	3.9	35
F	33	12	H	105	8.4	33.6	2.3	23	26	5.0	26
F	24	11	H	61.5	12	48.4	5.2	28	18	2.8	53
M	35	11	H	165	12	41.5	4.0	31	15	4.0	62
F	68	9.6	L	41.1	12	57.2	9.5	38	58 <sup>Δ</sup>	5.2	11
M	43	6.6	H	73.3	9.7	33.2	3.4	29	11	4.3	32
M	15	6.2	H	71.3	8.6	95.9 <sup>Δ</sup>	2.6	30	5.1	4.5	66
F	68	6.1	H	84.6	9.2	22.7	2.8	19	15	3.6	28
F	35	3.8	H	75.6	8.4	29.3	5.8	25	19	3.1	58
F	39	3.7	L	20.2	6.5	25.2	7.2	28	27	3.0	63
F	17	3.4	H	32.8	7.2	20.6	1.4	32	0.0	3.5	15
M	14	3.1	L	40.5	10	55.0	5.5	23	34	4.0	22
F	40	2.7	L	35.2	6.1	20.8	6.9	24	55	5.0	65
F	15	2.7	L	31.8	6.9	20.6	5.3	23	28	4.0	51
M	43	2.5	L	80.4	11	33.6	2.8	17	25	5.1	33
F	43	1.3	L	66.9	13	45.3	4.3	23	22	3.4	36
F	26	1.0	L	54.4	6.5	14.3	7.1	26	7.6	2.1	
F	37	1.0	L	74.2	6.3	17.7	6.9	36	14	2.4	
F	22	0.80	L	48.6	9.1	30.6	6.1	24	32	3.1	35
F	67	0.63	L	57.3	7.4	41.4	10	26	66 <sup>Δ</sup>	6.1	270
M	37	0.59	L	111	18 <sup>Δ</sup>	35.9	4.1	11	13	1.2	
F	46	0.52	L	52.8	8.0	44.6	14	28	37	4.2	
F	17	0.51	L	104	12	44.6	9.3	25	28	2.2	
F	33	0.43	L	87.7	8.5	26.4	11	26	23	2.2	
F	54	0.43	L	32.7	5.8	19.3	9.5	25	21	4.9	
M	15	0.37	L	63.3	8.3	52.4	6.6	40	16	2.3	
M	42	0.34	L	77.5	12	32.5	3.6	27	17	2.3	
M	33	0.34	L	39.9	9.7	29.8	9.9	23	11	2.8	

<sup>a</sup> Values exceeding 95th-percentile in reference range are indicated with  $\Delta$ ; values below the 5th-percentile are indicated with  $*$ .

<sup>b</sup> H, Concentration of uranium in drinking water is greater than or equal to  $1 \mu\text{g}/\text{L}$ . L, Concentration of uranium in drinking water is less than  $1 \mu\text{g}/\text{L}$ .

<sup>c</sup> Alkaline phosphatase; 1 unit will hydrolyze  $1.0 \mu\text{mol}$  of *p*-nitrophenyl phosphate per minute at pH 10.4 at  $37^\circ\text{C}$ .

<sup>d</sup> Gamma-glutamyl transferase; 1 unit will liberate  $1.0 \mu\text{mol}$  of *p*-nitroaniline from *L*- $\gamma$ -glutamyl-*p*-nitroanilide per minute at pH 8.5 at  $25^\circ\text{C}$ .

<sup>e</sup> Lactate dehydrogenase; 1 unit will reduce  $1.0 \mu\text{mol}$  of pyruvate to *L*-lactate per minute at pH 7.5 at  $37^\circ\text{C}$ .

<sup>f</sup> *N*-acetyl- $\beta$ -D-glucosaminidase; 1 unit will hydrolyze  $1.0 \mu\text{mol}$  of *p*-nitrophenyl *N*-acetyl- $\beta$ -D-glucosaminide to *p*-nitrophenol and *N*-acetyl-D-glucosamine per minute at pH 4.25 at  $25^\circ\text{C}$ .

<sup>g</sup>  $\beta$ -2-microglobulin.

TABLE 4  
Intersubject Variability<sup>a</sup> in Biomarker Data

Biomarker	High-exposure group <sup>b</sup>	Low-exposure group <sup>c</sup>
Glucose	82.4 (32.7–427)	55.9 (20.2–111)
Creatinine	9.0 (3.0–20)	8.8 (5.8–18)
Protein	34.6 (0.2–135)	33.0 (14.3–57.2)
BMG	56 (15–340)	43 (11–270)
ALP	6.3 (1.4–57)	6.9 (2.8–14)
GGT	25 (0.0–47)	26 (11–40)
LDH	17 (0.0–410)	25 (7.6–66)
NAG	3.6 (0.0–11)	3.2 (1.2–6.1)

<sup>a</sup> This table gives median values with minimum and maximum values in brackets.

<sup>b</sup> Number of subjects, 30.

<sup>c</sup> Number of subjects, 20, except for BMG, 9.

and female data. The positive correlation with glucose was weak to moderate (with  $r_s$  varying from 0.34 to 0.57) but statistically significant for pooled data ( $p < 0.001$ ), males ( $p < 0.02$ ), and females ( $p < 0.05$ ). The positive correlation between uranium intake and ALP and BMG was also weak (with  $r_s = 0.28$  and  $r_s = 0.39$ , respectively) but statistically significant ( $p < 0.05$  and  $p < 0.01$ , respectively) for pooled data. BMG was also found to be positively correlated with uranium intake for female data ( $r_s = 0.38$ ,  $p < 0.05$ ).

No significant correlation was observed with NAG, and the negative correlation with GGT was marginally significant for female data ( $p < 0.06$ ) only. No significant correlation with uranium intake was found for either creatinine ( $p = 0.45$ , 0.57, and 0.46, respectively, for pooled data, males, and females, respectively) or total protein ( $p = 0.23$ , 0.11, and 0.36), the markers used for glomerular injury in this study.

## DISCUSSION

Upon comparison of the *high-exposure group* with the *low-exposure group*, only glucose and LDH showed a statistically significant difference at  $p \leq 0.05$ .

Glucose excretion increased with increasing daily uranium intake (Fig. 1) at a statistically significant level for both males and females. In contrast, there was no significant difference shown between the two groups in the excretion of creatinine or protein, nor did these biomarkers correlate significantly with daily uranium intake. Note that the method of measurement used for urinary protein in this study measures primarily large protein molecules such as albumin (MW = 69,000).

In a study conducted by Diamond *et al.* (1989) of rats injected with uranyl fluoride solution (five injections of 120  $\mu\text{g}$  U/kg body wt followed by three injections of 240  $\mu\text{g}$  U/kg body wt intermittently over a 33-day period), glucose was found to be the most sensitive of the biochemical indicators of renal injury used, exhibiting a 150-fold elevation in treated rats over controls. If the uranium-correlated trend observed in our

TABLE 5  
Test for Significance of Difference between Biomarker Levels in the High-Exposure and Low-Exposure Groups

Biomarker	<i>p</i> values for Kruskal–Wallis test		
	Males	Females	Pooled data
Glucose	0.05*	0.02*	0.001*
Creatinine	0.70	0.27	0.62
Protein	0.33	0.44	0.45
BMG	0.50	0.66	0.38
ALP	0.92	0.94	0.98
GGT	0.77	0.15	0.42
LDH	0.02*	0.38	0.08
NAG	0.49	0.78	0.80

\* Statistically significant difference at  $p \leq 0.05$ .

study was due to the excessive filtration of glucose by damaged glomeruli, then a similar trend should be observed for creatinine which is freely filtered at the glomerulus but is not reabsorbed at the tubules. A similar observation would also be made of the protein results, since high molecular weight proteinuria is often associated with glomerular injury (Lillehoj and Poulik, 1986). Compromised glomeruli would filter larger proteins easily and these would be reabsorbed with difficulty at the proximal tubules which have a low affinity for high molecular weight proteins. However, neither value for creatinine or protein exhibited this trend for pooled data or for males or females. The glucose, creatinine, and total protein data taken together appear to suggest that at the levels of uranium intake observed in this study (2 to 410  $\mu\text{g}/\text{day}$  among males and 2 to 570  $\mu\text{g}/\text{day}$  for females), the segment of the nephron most at risk to injury is the proximal tubule rather than the glomerulus. This finding is in agreement with animal data obtained by previous investigators (Nomiya and Foulkes, 1968; Haley *et al.*, 1982; Diamond *et al.*, 1989).

TABLE 6  
Spearman Correlation Coefficients<sup>a</sup> for Uranium Intake for Male, Female, and Pooled Data

Biomarker	Males <sup>b</sup>	Females <sup>c</sup>	Pooled data <sup>d</sup>
Glucose	0.57 (0.02)	0.34 (0.05)	0.40 (0.001)
Creatinine	0.15 (0.57)	0.13 (0.46)	0.11 (0.45)
Protein	0.40 (0.11)	0.17 (0.36)	0.17 (0.23)
BMG	0.43 (0.14)	0.38 (0.05)	0.39 (0.01)
ALP	0.25 (0.33)	0.26 (0.14)	0.28 (0.05)
GGT	-0.03 (0.92)	-0.33 (0.06)	-0.22 (0.12)
LDH	-0.22 (0.39)	0.06 (0.75)	0.02 (0.89)
NAG	0.19 (0.46)	0.05 (0.79)	0.15 (0.29)

<sup>a</sup> Numbers in parentheses are the corresponding *p* values.

<sup>b</sup> Number of observations, 17, except for BMG where  $N = 13$ .

<sup>c</sup> Number of observations, 33, except for BMG where  $N = 27$ .

<sup>d</sup> Number of observations, 50, except for BMG where  $N = 40$ .

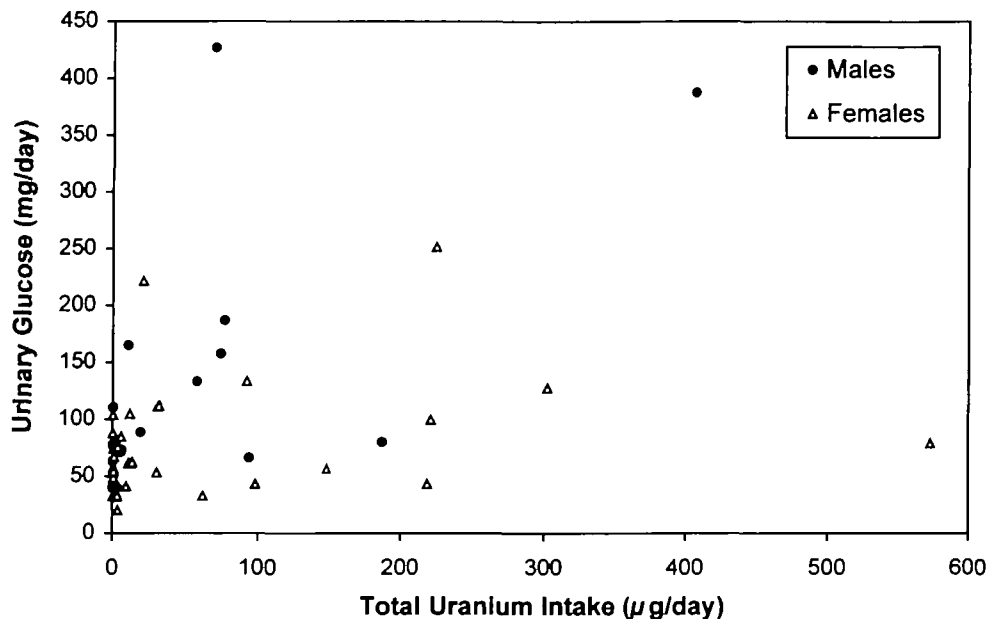


FIG. 1. Variation of urinary glucose with total daily uranium intake. The Y coordinate represents the mean of six measurements in 8-h urine specimens. Uranium intake is the mean of amounts ingested in water and through food over a 3-day period.

Diamond and co-workers (1989) also found LDH to be a sensitive bioindicator of renal injury. In their study, LDH increased 11 times in treated rats over controls. In our study, although four of the values in the *high-exposure group* exceeded the published upper limit for the reference range and a statistically significant difference was observed between this group and the *low-exposure group*, urinary LDH

did not correlate significantly with uranium intake ( $r_s = -0.22$ ,  $p = 0.39$ ).

The positive correlation of ALP and BMG with uranium intake (Figs. 2 and 3) for pooled male and female data provide support to the conclusion regarding the involvement of the proximal tubule. The findings with BMG are in agreement with those of Moss *et al.* (1983) and Thun *et al.* (1985). Although

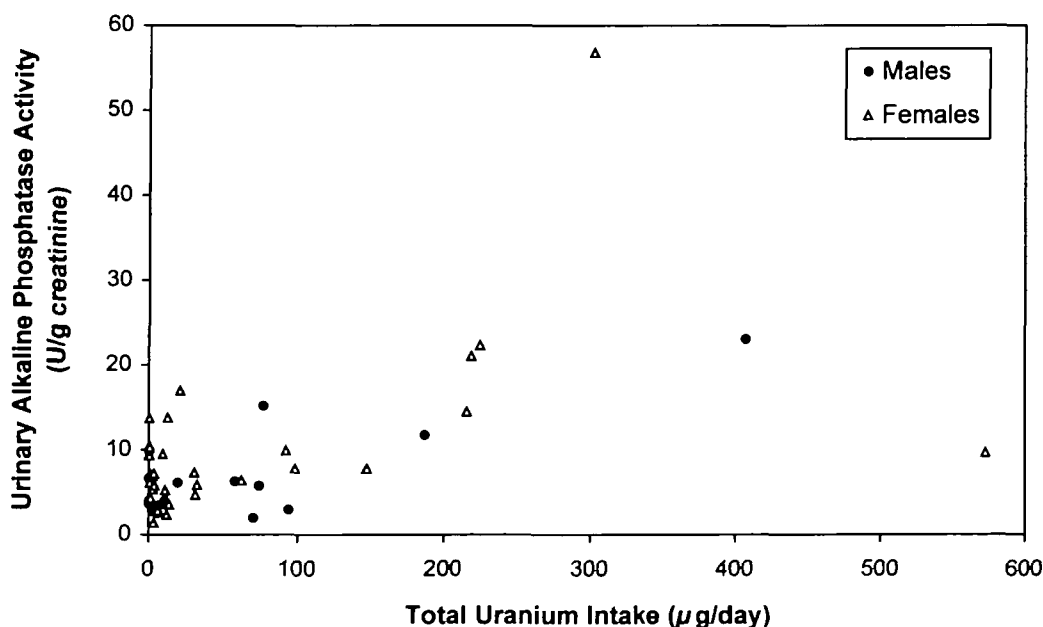


FIG. 2. Variation of urinary alkaline phosphatase activity with total daily uranium intake. The Y coordinate represents the mean of six measurements in 8-h urine specimens. Uranium intake is the mean of amounts ingested in water and through food over a 3-day period.

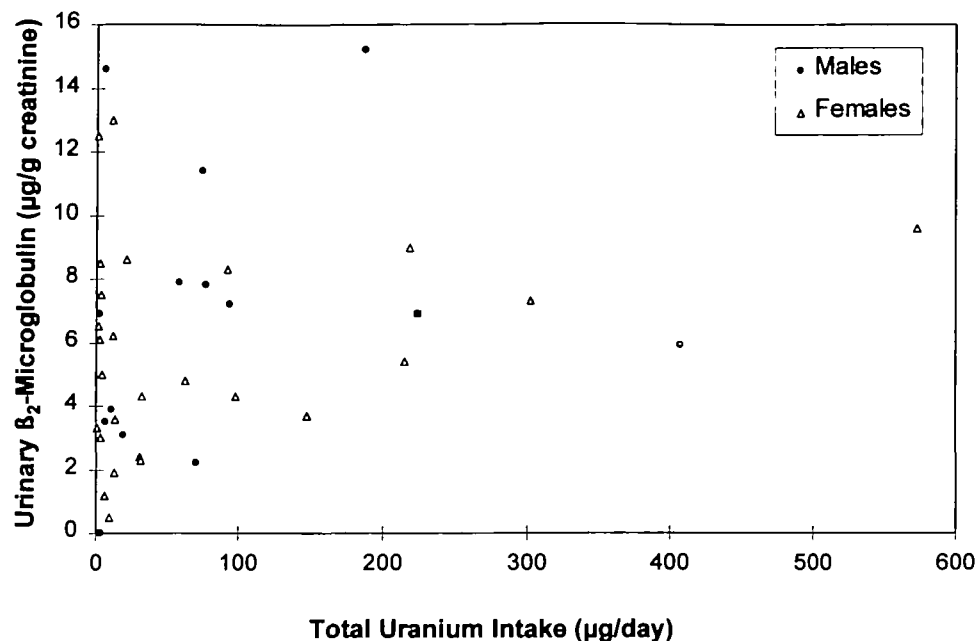


FIG. 3. Variation of urinary  $\beta_2$ -microglobulin with total uranium intake. The Y coordinate represents the mean of six measurements in 8-h urine specimens. Uranium intake is the mean of amounts ingested in water and through food over a 3-day period.

there was no significant difference between exposed groups and controls at the 95% confidence level, Moss and co-workers noted a trend of increasing urinary BMG excretion with increasing uranium exposure. Thun *et al.* also observed a positive correlation between length of exposure and urinary BMG clearance by uranium workers, although the observed values did not exceed the upper limit of normal provided by the manufacturer of the BMG test kit.

BMG is not of renal origin. It is a low molecular weight protein (MW = 11,800) filtered at 100% of the glomerular rate and then reabsorbed and digested in the lining of the proximal renal tubules. ALP is present on the membrane of the brush border of proximal tubule cells (Butterworth *et al.*, 1965). The mechanism for the results obtained with these two biomarkers may involve the disruption of the cell membrane by prolonged exposure to tubular fluid, resulting in a release of ALP into the developing urine and a decreased ability to reabsorb BMG. It is known that high dosing with uranium can lead to structural changes in the brush-border membrane of the proximal tubules (Haley *et al.*, 1982; Kirschbaum, 1982; Schwartz and Flamenbaum, 1976). Loss of microvilli has been observed as early as 1 h after injection of 10 mg U/kg into rats (Haley *et al.*, 1982). An increased activity of ALP has been observed in urine soon after acute exposure to uranium (Nomiya *et al.*, 1974; Yuile, 1973) and may be associated at least in part with such loss of microvilli of the proximal tubules, where ALP is known to be localized (Yuile, 1973). Although the daily dose rates in the present study are very much lower than the doses quoted in these reports, constant daily insult to the tubular epithelium may conceivably achieve the same effect.

Initially, the GGT and NAG results that were below published reference ranges for these biomarkers seemed puzzling. However, there have been reports in the literature where similar observations were made by other investigators in studies on the toxicity of uranium or other metals. Diamond *et al.* (1989) observed a trend of decreasing GGT with uranium treatment of male Wistar rats. Niwa *et al.* (1993) reported a similar observation with the renal tubular enzyme trehalase in a study of subjects residing in a Cd-polluted area in Japan and postulated that this decrease may be indicative not of cell injury but of the actual loss of enzyme-producing cell mass. Another possible mechanism for the decrease in GGT activity may be enzyme inhibition by uranium once it has penetrated the tubular cell membrane. In a study of canine kidney, Nechay *et al.* (1980) observed a reduction in the activity of  $\text{Na}^+$  and  $\text{K}^+$ -ATPase following treatment with heavy metals such as mercury and uranium.

Proteins appear in urine only after escaping the efficient mechanisms of tubular reabsorption (Lillehoj and Poulik, 1986). Increased urinary excretion of serum proteins with molecular weight in excess of 50,000, such as albumin, is an early indicator of glomerular injury (Lillehoj and Poulik, 1986). Albumin constitutes the major protein in normal urine because it is the protein found in largest concentration in plasma. Values for this protein were not observed to differ significantly between the two groups and were not significantly correlated to uranium intake. In the Saskatchewan, Canada, study conducted by Mao and co-workers (1995), a uranium exposure index-correlated microalbuminuria was observed, which is defined as an elevated urinary albumin excretion rate



of 20–200  $\mu\text{g}/\text{min}$  that is below the range at which urinary protein is detected by conventional methods (Davison *et al.*, 1995; Mogensen *et al.*, 1992). The range of uranium concentrations in drinking water in this study was 0.48 to 50  $\mu\text{g}/\text{L}$ . Persistent microalbuminuria indicates a high probability of damage to the glomerular filtration capacity of the kidney and is of great diagnostic relevance for diabetic nephropathy. In the Mao study, diabetics were not excluded although diabetic status was corrected for in the statistical analysis of results. Their findings do not necessarily suggest glomerular damage, as proteinuria has also been observed in at least one animal study (Diamond *et al.*, 1989) where histopathology of rat kidney tissue revealed only damage initially to the S2 and S3 segments of the proximal tubule and, eventually, to the loop of Henle as treatment progressed further. No damage to the glomerulus was reported. These authors postulated that although glomerular injury could not be ruled out completely, the proteinuria observed in their experiment may also have arisen from peritubular plasma diffusing into the urine across a disrupted tubular epithelium.

At the levels of intake observed in this study, the results taken collectively point to the renal tubule, rather than the glomerulus, as the part of the nephron most at risk of injury from chronic uranium ingestion in drinking water. This finding is in agreement with histological evidence obtained in animal experiments. In both uranyl fluoride-treated rats (Diamond *et al.*, 1989) and uranyl nitrate hexahydrate-treated rabbits and rats (Gilman *et al.*, 1998a,b,c), significant dose-related injury was noted in the tubules rather than the glomeruli. The Diamond *et al.* (1989) study further establishes the S2 and S3 segments of the proximal tubule to be most at risk of injury from this type of exposure.

We have utilized total uranium intake through food and water as a measure of uranium exposure to the kidney since this is the quantity of interest in standard setting and the development of guidelines for drinking water quality. A more specific measure would undoubtedly have been the amount of uranium actually reaching the kidney. This quantity is being assessed in an upcoming publication, in which the balance between uranium intake and uranium excretion in urine is being used to determine the fractional uptake of uranium in the GI tract. Preliminary indications are that the average uranium uptake is about 1%. The difference between cases where the uptake is primarily from drinking water and cases where the uptake is primarily from food is being investigated.

### CONCLUSIONS

The nephrotoxicity of uranium has been established through numerous animal studies. The present investigation suggests that long-term ingestion of uranium by humans may produce interference with kidney function at the elevated levels of uranium found in some groundwater supplies.

The combined trend effects observed in this study of increas-

ing urinary glucose, alkaline phosphatase, and  $\beta_2$ -microglobulin with increasing chronic uranium ingestion suggest that the primary site for this interference is the proximal tubule.

These observed effects may represent a manifestation of subclinical toxicity which will not necessarily lead to kidney failure or overt illness. It may, however, be the first step in a spectrum which with the chronic intake of elevated levels of uranium may lead to progressive or irreversible renal injury.

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### REFERENCES

- Albert, A., Orlowski, M., and Szweczk, A. (1961). Histochemical demonstration of gamma-glutamyl transpeptidase. *Nature* **191**, 767–768.
- Bassett, W. H. (1948). The excretion of hexavalent uranium following intravenous administration. In *Studies on Human Subjects UR-37*. University of Rochester Atomic Energy Project, New York.
- Benscome, S. A., Stone, R. S., Latta, H., and Madden, S. C. (1960). Acute tubular and glomerular lesions in rat kidneys after uranium injury. *Arch. Pathol.* **69**, 122–128.
- Blantz, R. C., Pelayo, J. C., Gushwa, L. C., Myers, R., and Evan, A. P. (1985). Functional basis for the glomerular alterations in uranyl nitrate acute renal failure. *Kidney Int.* **228**, 733–743.
- Bonting, S. L., Pollack, V. E., Muehrke, R. C., and Kark, R. (1960). Quantitative histochemistry of the nephron. III. Lactic dehydrogenase activity in man and other species. *J. Clin. Invest.* **39**, 1381–1385.
- Bourbouze, R., Baumann, F. C., Bonvalet, J. P., and Farman, N. (1984). Distribution of N-acetyl- $\beta$ -D-glucosaminidase along the rabbit nephron. *Kidney Int.* **25**, 636–642.
- Burtis, C. A., and Ashwood, E. R. (Eds.) (1994). Reference intervals. In *Tietz Textbook of Clinical Chemistry*, pp. 2175–2211. Saunders, Philadelphia.
- Butterworth, P. J., Moss, E. W., Pitkanen, E., and Pringle, A. (1965). Patterns of urinary excretion of alkaline phosphatase in acute renal failure. *Clin. Chim. Acta.* **11**, 212–219.
- Carafoli, E., Tiozzo, R., Pasquali-Ronchetti, I., and Laschi, R. (1971). A study of  $\text{Ca}^{++}$  metabolism in kidney mitochondria during acute uranium intoxication. *Lab. Invest.* **25**, 516–527.
- Clarkson, T. W., and Kench, J. E. (1956). Urinary excretion of amino acids by men absorbing heavy metals. *Biochem. J.* **62**, 361–372.
- Davison, A. M., Cumming, A. D., and Swainson, C. P. (1995). Diseases of the kidney and urinary tract. In *Davidson's Principles and Practice of Medicine* (C. R. W. Edwards, I. A. D. Bouchier, and C. Haslett, Eds.), pp. 611–668. Churchill Livingstone, Edinburgh.
- Diamond, G. L., Morrow, P. E., Panner, B. J., Gelein, R. M., and Batt, R. B. (1989). Reversible uranyl fluoride nephrotoxicity in the Long Evans rat. *Fundam. Appl. Toxicol.* **13**, 65–78.

- Galle, P. (1974). Role des lysosomes et des mitochondries dans les phenomenes de concentration et d'elimination d'elements mineraux (uranium et or) par le rein. *J. Microscopie* **19**, 17–24.
- Gilman, A. P., Villeneuve, D. C., Secours, V. E., Yagminas, A. P., Tracy, B. L., Quinn, J. M., Valli, V. E., Wiles, R. J., and Moss, M. A. (1998a). Uranyl nitrate: 28-day and 91-day toxicity studies in the Sprague-Dawley rat. *Toxicol. Sci.* **41**, 117–128.
- Gilman, A. P., Villeneuve, D. C., Secours, V. E., Yagminas, A. P., Tracy, B. L., Quinn, J. M., Valli, V. E., and Moss, M. A. (1998b). Uranyl nitrate: 91-day toxicity studies in the New Zealand white rabbit. *Toxicol. Sci.* **41**, 129–137.
- Gilman, A. P., Villeneuve, D. C., Secours, V. E., Yagminas, A. P., Tracy, B. L., Quinn, J. M., Valli, V. E., and Moss, M. A. (1998c). Uranyl nitrate: 91-day exposure and recovery studies in the New Zealand white rabbit. *Toxicol. Sci.* **41**, 138–151.
- Haley, D. P., Bulger, R. E., and Doby, D. C. (1982). The long-term effects of uranyl nitrate on the structure and function of the rat kidney. *Virchows Arch. B* **41**, 181–192.
- Health and Welfare Canada (1996). *Guidelines for Canadian Drinking Water Quality*, 6th ed., H48-10/1996E. Canada Communications Group Publishing, Ottawa.
- Health and Welfare Canada (1979–1988). *Environmental Radioactivity in Canada* (Radiological Monitoring Annual Reports). Radiation Protection Bureau, Ottawa.
- Hursh, J. B., Neuman, W. R., Toribara, T., Wilson, H., and Waterhouse, C. (1969). Oral ingestion of uranium by man. *Health Phys.* **17**, 619–621.
- Jung, K., Kirschner, P., Wille, A., and Brien, G. (1993). Excretion of urinary enzymes after extracorporeal shockwave lithotripsy: A critical reevaluation. *J. Urol.* **149**, 1409–1413.
- Kirschbaum, B. B. (1982). Aggregation of renal brush border membranes by concanavalin A and heavy metals. *Toxicol. Appl. Pharmacol.* **64**, 10–19.
- Laszlo, S., and Szabo, A. (1982). Gamma-glutamyl transpeptidase activity in human urine. *Clin. Chim. Acta* **126**, 9–16.
- Leggett, R. W. (1989). The behavior and chemical toxicity of U in the kidney: A reassessment. *Health Phys.* **57**, 365–383.
- Lehman, E. L. (1975). *Nonparametrics: Statistical Methods Based on Ranks*. Holden-Day, San Francisco.
- Lentner, C. (Ed.) (1981). Units of measurement, body fluids, composition of the body, nutrition. In *Geigy Scientific Tables*, Vol. 1, pp. 81–83. Ciba-Geigy Ltd., Basle.
- Lillehoj, E. P., and Poulik, M. D. (1986). Normal and abnormal aspects of proteinuria. I. Mechanisms, characteristics and analyses of urinary protein. II. Clinical considerations. *Exp. Pathol.* **29**(1), 1–28.
- Luessenhop, A. J., Gallimore, J. C., Sweet, W. H., Struxness, G., and Robinson, J. (1958). The toxicity in man of hexavalent uranium following intravenous administration. *Am. J. Roentgenol. Radium Ther. Nucl. Med.* **79**, 83–100.
- Mao, Y., Desmeules, M., Schaubel, D., Berube, D., Dyck, R., Brule, D., and Thomas, B. (1995). Inorganic components of drinking water and microalbuminuria. *Environ. Res.* **71**, 135–140.
- Meyer, R. J., and Pietsch, E. (1936). *Gmelins Handbuch der anorganischen Chemie*, 8th ed., System No. 55. Verlag Chemie g.m.b.h., Leipzig/Berlin.
- Mogensen, C. E., Damsgaard, E. M., Froland, A., Nielsen, S., de Fine Olivarius, N., and Schmitz, A. (1992). Microalbuminuria in non-insulin-dependent diabetes. *Clin. Nephrol.* **38**(Suppl. 1), S28–39.
- Moss, M. A., McCurdy, R. F., Dooley, K. C., Givener, M. L., Dymond, L. C., Slater, J. M., and Courmeya, M. M. (1983). Uranium in drinking water—Report on clinical studies in Nova Scotia. In *Chemical Toxicology and Clinical Chemistry of Metals* (S. S. Brown, and J. Savory, Eds.), pp. 149–152. Academic Press, London.
- Nechay, B. R., Thompson, J. D., and Saunders, J. P. (1980). Inhibition by uranyl nitrate of adenosine triphosphatases derived from animal and human tissues. *Toxicol. Appl. Pharmacol.* **53**, 410–419.
- Niwa, T., Katzuzaki, T., Yazawa, T., Tatemichi, H., Emoto, Y., Miyazaki, T., and Maeda, K. (1993). Urinary trehalase activity in chronic glomerulonephritis. *Nephron* **63**(4), 423–442.
- Nomiyama, K., Yamamoto, A., and Sato, C. (1974). Assay of urinary enzymes in toxic nephropathy. *Toxicol. Appl. Pharmacol.* **27**, 484–490.
- Nomiyama, K., and Foulkes, E. C. (1968). Some effects of uranyl acetate on proximal tubular function in rabbit kidney. *Toxicol. Appl. Pharmacol.* **13**, 89–98.
- Price, R. G. (1982). Urinary enzymes, nephrotoxicity and renal disease. *Toxicology (Ireland)*, **23** (2–3), 99–134.
- Schwartz, J. H., and Flamenbaum, W. (1976). Uranyl nitrate and HgCl<sub>2</sub>-induced alterations in ion transport. *Kidney Int.* **10**, 123–127.
- Shapiro, S. S., and Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika* **52**, 591–611.
- Stannard, J. N. (1988). Uranium. In *Radioactivity and Health: A History* (R. W. Balmaan, Jr., Ed.), pp. 79–111. Office of Scientific and Technical Information, Springfield, VA.
- Stroo, W. E., and Hook, J. B. (1977). Enzymes of renal origin in urine as indicators of nephrotoxicity. *Toxicol. Appl. Pharmacol.* **39**, 423–434.
- Struxness, E. G., Luessenhop, A. J., Bernard, S. R., and Gallimore, J. C. (1955). The distribution and excretion of hexavalent uranium in man. In *Proceedings of the International Conference on the Peaceful Uses of Atomic Energy*, Vol. 10, pp. 186–196. United Nations, New York.
- Terepka, A. R., Toribara, T. Y., and Neuman, W. F. (1965). *Skeletal Retention of Uranium in Man*. Presented at the 46th Meeting of the Endocrine Society, San Francisco.
- Thun, M. J., Baker, D. B., Steenland, K., Smith, A. B., Halpern, W., and Berl, T. (1985). Renal toxicity in uranium mill workers. *Scand. J. Work. Environ. Health* **11**, 83–90.
- Voegtlin, C., and Hodge, H. C. (Eds.) (1949). *Pharmacology and Toxicology of Uranium Compounds*, National Nuclear Energy Series, Division VI, Volume I, Parts I and II. McGraw-Hill, New York.
- World Health Organization (WHO) (1991). *Principles and Methods for the Assessment of Nephrotoxicity Associated with Exposure to Chemicals*, Environmental Health Criteria 119, Geneva.
- Yuile, C. L. (1973). Animal experiments. In *Uranium, Plutonium, Transplutonic Elements* (H. C. Hodge, J. N. Stannard, and J. B. Hursh, Eds.), Vol. 36, pp. 165–196.
- Zalups, R., Gelein, R., Morrow, P., and Diamond, G. (1988). Nephrotoxicity of uranyl fluoride in uni-nephrectomized and sham-operated rat. *Toxicol. Appl. Pharmacol.* **94**, 11–22.