

March 1, 2000

UNITED STATES OF AMERICA
NUCLEAR REGULATORY COMMISSION

BEFORE THE COMMISSION

In the Matter of:)

HYDRO RESOURCES, INC.)

P.O. Box 15910)

Rio Rancho, New Mexico 87174)

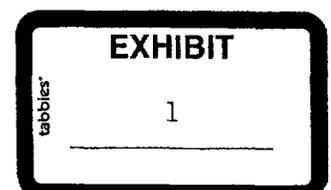
Docket No. 40-8968-ML

AFFIDAVIT OF DR. JOHN D. FOGARTY
IN SUPPORT OF ENDAUM/SRIC MOTION
TO REOPEN AND TO SUPPLEMENT THE RECORD

I, John D. Fogarty, being duly sworn, submit this affidavit on behalf of Eastern Navajo Diné Against Uranium Mining ("ENDAUM") and Southwest Research and Information Center ("SRIC") in support of ENDAUM's and SRIC's Motion to Reopen and to Supplement the Record of this proceeding related to the licensing of Hydro Resources, Inc.'s ("HRI's") Crownpoint Uranium Project ("CUP").

1. My name is John D. Fogarty. I am a family practice physician at the Crownpoint Healthcare Facility ("CHF") in Crownpoint, New Mexico. I reside in Crownpoint, where I have lived since August 1999. My mailing address is P.O. Box 179, Crownpoint, N.M., 87313.

2. I am qualified and competent to make this affidavit, and the factual statements herein are true and correct to the best of my knowledge, information and belief. The opinions expressed herein are based on my best professional judgment.



3. This affidavit repeats and expands upon the information and concerns that I presented in a January 28, 2000, letter that I wrote and sent to the Commissioners of the U.S. Nuclear Regulatory Commission ("NRC") and to Administrative Judge Peter B. Bloch. In my letter, I expressed my professional concerns about the safety of the NRC's proposed uranium restoration standard for the CUP. Since I sent that letter, representatives of ENDAUM and SRIC asked me if I would agree to set forth those same concerns in an affidavit to support ENDAUM's and SRIC's motion that the Commission reopen the record in this case and supplement the record with my testimony and its supporting documentation. I agreed to prepare this affidavit for that purpose because I now understand that certain rules of procedure adopted by the NRC must be followed for my information and views to be considered in the context of the Commission's licensing decision regarding the CUP. Furthermore, I am willing to present this information in a public hearing and answer questions regarding this testimony.

Professional Qualifications

4. My qualifications to make this affidavit are described in my résumé, a copy of which is appended hereto as **Exhibit A**. To summarize, I received my bachelor of science degree *magna cum laude* from the University of Iowa in 1987, and my medical doctorate from the University of Washington in 1993. I completed my family practice residency at the University of New Mexico ("UNM") in 1997, and I am board-certified in family practice by the American Board of Family Practice. The University of Washington has been ranked consistently as the best primary care medical school in the country, and the UNM family practice residency has been ranked consistently as one of the top three family practice residencies in the U.S. by *U.S. News and World Report*. I have completed the majority of course work for a masters degree in

public health ("MPH") at UNM, and expect to receive my degree next year. In my MPH course work, I have taken classes in epidemiology and environmental health. I am experienced in and well-versed in reviewing, understanding and interpreting the biomedical literature.

5. In 1995, I worked for the New Mexico Department of Health ("NMDOH") on issues related to improving the health of communities. While with NMDOH, I critiqued existing training programs in public health epidemiology and community medicine for primary care physicians, and made recommendations to create a more comprehensive training program. This program is now being used to train family practice residents in epidemiology, public health and community medicine at UNM and NMDOH. In recognition of my contribution in this area, I received the Mead Johnson Award from the American Academy of Family Practice in 1996. This award is given annually to 20 family practice residents across the nation on the basis of community involvement, scholastic achievement, patient care, and leadership.

6. I have been involved in both basic science and clinical research for more than 15 years. I was the coauthor of two articles in *Brain Research* and numerous abstracts about neuroanatomy, and was the principal investigator on a study about hypertension and pheochromocytoma that was published in *Archives of Family Medicine*. From 1997 to 1999, I served as the supervisory physician for the diabetes mellitus program at the Santa Fe Indian Health Service Hospital, which serves more than 20,000 people. As a result of this experience I am highly aware of the long-term effects of diabetes, including kidney disease. I believe my background in basic science, clinical research, public health, community medicine and clinical medicine has more than adequately prepared me to address a wide range of public health issues, including the adequacy of the NRC's proposed uranium restoration standard for the CUP.

7. I have had training and formal education in a wide range of chemical toxins. In preparing both my January 28 letter and this affidavit, I reviewed more than two dozen papers, articles and reports referenced in the biomedical literature on the chemical toxicity of uranium. I also spoke personally with several government scientists who are working on uranium toxicity studies or developing new or revised approaches to regulating uranium in drinking water. While I am not a health physicist, I do not believe that one has to have expertise in radiation science to review and evaluate the relevant literature on the *chemical toxicity* of uranium. Furthermore, as a physician, I am trained to evaluate and consider the chemical toxicities of numerous substances. Each drug that I prescribe has potential chemical toxicities about which I must be aware of and knowledgeable about.

8. As a physician employed at a major federal healthcare facility on the largest Native American reservation in the U.S., I have a professional duty to ensure the health and well-being of my patients, who include residents of Crownpoint, Church Rock and surrounding communities. This duty includes an obligation to point out circumstances, conditions and government policies which could place at risk the health and safety of the people who use this hospital for preventive, screening and treatment purposes. Hence, I am obligated to point out what I believe is a grievous error made by the NRC staff in adopting a groundwater restoration standard that, on its face, is inadequate to protect the health of my patients and of residents of the Crownpoint and Church Rock areas. The NRC staff is, in my view, wrong to have accepted a uranium "cleanup" level that is at least 20 times greater than the level of uranium in drinking water that has been shown to cause kidney impairment in chronically exposed individuals.

9. Prior to coming to Crownpoint in August 1999, I had no detailed knowledge of

the HRI project or the standards that NRC would apply to it. I became aware of HRI's proposed solution mines soon after I arrived in Crownpoint because they are a matter of frequent and often intense local public discussions, including extensive discussions among members of the medical, support and administrative staffs at CHF. I soon learned that the hospital's local health board, the Eastern Navajo Health Board ("ENHB"), which is made up of representatives of each of the Navajo chapters that are served by the Crownpoint Service Unit, had adopted a resolution opposing the HRI project in January 1995.¹ I also learned that the hospital's Safety Committee, which is made up of representatives of each of the major departments and divisions of the Service Unit, had issued a written statement in May 1997 raising numerous concerns about the CUP.² Against this background, and with regard for ensuring the health and well-being of my patients, I was concerned about an activity that could affect the integrity of the water supplies in Crownpoint, Church Rock and surrounding communities. Since then, I have conducted my own research on this matter.

Literature Reviewed

10. My initial research consisted of reviewing the *Final Environmental Impact*

¹Eastern Navajo Health Board (1995). Resolution of the Eastern Navajo Health Board Opposing Proposal of Hydro-Resources, Inc. (HRI) to Conduct Well Field Drilling and Uranium Production in the Crownpoint, New Mexico Area. January 4. Transmitted by letter from J. Vice, Chairperson, Eastern Navajo Health Board, to Chief, High-level Waste and Uranium Recovery Branch, USNRC, January 6. (NRC ACN 9501120327.)

²Crownpoint Healthcare Facility Safety Committee (1997). Position Statement Regarding the In-Situ-Leach Uranium Mine Proposed by Hydro-Resources, Inc. Department of Health and Human Services, Public Health Service, Indian Health Service (Crownpoint, N.M.), May 23.

Statement ("FEIS") for the CUP,³ reviewing the available literature on the chemical toxicity of uranium, and discussing why NRC chose a uranium restoration standard of 0.44 mg/l for the CUP with Mr. Christopher McKenney of the NRC staff. In preparing this affidavit, I also re-reviewed the February 20, 1998, affidavit given by Mr. McKenney; spoke with several officials of state, federal and Canadian agencies that regulate drinking water quality; and reviewed recently published papers on the effects of uranium on laboratory animals and human populations. References to specific citations of literature I reviewed are given in the text and footnotes of this affidavit.

Overall Conclusions

11. Based on my review of the current and historic literature on uranium's well-documented chemical toxicity; on the additional materials set forth in Paragraph 10 above; on relevant portions of the *FEIS*; on information gained from speaking at length on two occasions with Mr. McKenney, and on information gained from speaking with officials of several agencies who are familiar with uranium chemical toxicity and drinking water standards, I conclude that the groundwater restoration standard for uranium incorporated in the HRI license poses a significant threat to the health of the public, and is highly likely to cause irreparable harm to the current and future residents of Crownpoint and the surrounding communities who drink water from the Crownpoint municipal water supply or who obtain water now or in the future from wells that tap the Westwater Canyon Aquifer in the Church Rock area.

³U.S. Nuclear Regulatory Commission (1997). *Final Environmental Impact Statement to Construct and Operate the Crownpoint Uranium Solution Mining Project, McKinley County, New Mexico*, NUREG-1508, February.

12. Uranium has been known to be a toxin to the kidneys since the 19th century, and has been widely used by researchers in animal studies to generate models or estimates of adverse kidney effects in humans. Unfortunately, the research studies that the NRC staff used as a scientific basis for their assertion that the groundwater restoration standard of 0.44 mg/l is safe are outdated and flawed. More contemporary studies of adverse uranium effects in both exposed human populations and in laboratory animals are of higher quality and are more generalizable to human populations. These more recent studies, most of which have been published since 1995, demonstrate that humans show signs of kidney damage after consuming water over long periods of time with levels of uranium as low as 0.014 mg/l. They document important subclinical effects that could potentially lead to later kidney disease and even kidney failure. The NRC staff apparently did not consider the earlier of these relevant studies in its January 1998 decision to license the HRI project, nor consider the more recent of these studies in responding to ENDAUM's and SRIC's concerns about the adequacy of the uranium standard in their early-1999 written presentations on groundwater and environmental justice concerns. My conclusions are based on the analysis that follows.

Expert Analyses and Opinions

13. In early September 1999, I learned from reading the *FEIS* that NRC's "secondary restoration goal" for residual uranium levels in the Westwater Canyon Aquifer after mining would be about 63 to 440 times greater than the background uranium concentrations in the town of Crownpoint's well water.⁴ The NRC's proposed uranium standard seemed quite high

⁴This range is derived by dividing the proposed uranium restoration standard of 0.44 mg/L (or 440 μ g/L), by the minimum and maximum values for uranium concentrations in the

compared to the native water quality, so I began compiling and reading literature on the chemical toxicity of uranium. Five recent studies — two on human populations chronically exposed to uranium in drinking water (Mao, et al., 1995, and Zamora, et al., 1998) and three involving lab animals exposed to uranium compounds in drinking water (Gilman, et al., 1998a, 1998b, and 1998c) — caught my eye. The two human population studies documented toxic effects of chronic uranium ingestion on kidney functions at levels considerably below the NRC's restoration standard. The three animal studies could not detect a level of exposure below which no adverse kidney effects would be observed. I discuss the importance and implications of those studies at length later in this affidavit.

14. On December 29 and December 30, 1999, I spoke by telephone with Mr. McKenney. He told me how the NRC had determined the restoration standard of 0.44 mg/l for uranium, and he sent me a copy of an affidavit he said he had prepared in February 1998.⁵ On page 6 of his affidavit, Mr. McKenney wrote that in his opinion, “the secondary groundwater restoration goal for uranium of 0.44 mg/L (300 pCi/L) is protective of public health and safety with respect to chemical toxicity to the kidneys.” During our first conversation, I asked him how the NRC had reached the conclusion that a restoration standard of 0.44 mg/l would be safe for human consumption. He stated that there is not much information regarding the chemical toxicity of uranium in water. He also stated that he had used research studies listed on the

town of Crownpoint water wells, as those values (0.001 mg/L to 0.007 mg/L) were reported in the FEIS (Table 3.12 at 3-26).

⁵Affidavit of Christopher A. McKenney, United States Nuclear Regulatory Commission, in the matter of Hydro Resources, Inc., Docket No. 40-8968-ML, February 20, 1998.

Integrated Risk Information System (IRIS) provided by the U.S. Environmental Protection Agency (“USEPA”) to reach the conclusion that 0.44 mg/l of uranium is safe.

15. Mr. McKenney stated in his affidavit that ingestion of drinking water with 0.44 mg/l of uranium is protective of public health because it is below the ingestion standard of 10 mg/week of soluble uranium set by the NRC. He further stated that ingestion of 6.2 mg of uranium per week is “well below the exposure level at which renal failure would reasonably be expected to occur.” I found this statement alarming; to use “renal failure” as a health outcome measure that is “protective of public health and safety” is appalling and medically unacceptable. There is an enormous spectrum of disease that occurs before renal failure. A physician does not wait until an organ fails before intervening. For example, physicians know that high blood sugar from poorly controlled diabetes can cause microscopic damage to the kidneys and eventually complete loss of kidney function. A day, or week, or month of high blood sugar levels does not cause “renal failure,” but high blood sugar over the course of many years causes microalbuminuria, then chronic renal disease, which in turn causes high blood pressure, bone damage, anemia, acidosis, and malnutrition. All of these adverse physiological changes and diseases occur before “renal failure.” Thus, physicians attempt to intervene years and decades before kidney failure occurs in order to protect the patient’s health. Mr. McKenney fails to understand these clinical disease processes in the kidney. To protect “public health and safety with respect to chemical toxicity to the kidneys” (McKenney February 1998 Affidavit at 6), the NRC should be choosing much lower ingestion levels of uranium that are known, or at least predicted, *not* to cause adverse effects, including microscopic or subclinical damage.

16. I obtained and reviewed the studies that Mr. McKenney cited to support the

opinions he expressed to me and in his affidavit. I found that each of these studies, which were performed between 1949 and 1973, are methodologically flawed, poorly generalizable to human populations exposed to chronic ingestion of uranium, and outdated in light of more modern studies. My critique of each of those studies follows:

16a. Maynard and Hodge, 1949.⁶ This study involved animals including rats, dogs, and rabbits and had an exposure time of 30 days. The outcome measure of renal disease used in this study was based on histological examination of renal tissue. This method of examination (i.e., microscopic study of tissue structure) is misleading in that it provides evidence only of anatomical damage, and not functional damage, meaning the kidney could look normal but not work properly. The researchers did not look at markers for functional toxicity such as urinary proteins or enzymes. Furthermore the study used animals exposed only to short duration of uranium ingestion. It is next to impossible to reach conclusions about safe thresholds of uranium ingestion in human populations using this study.

16b. Hursh, et al., 1969.⁷ This study involved four hospital patients exposed to a single oral dose of uranium. The outcome measure chosen was urinary protein. The validity of this study is questionable because of the extremely small number of subjects studied. The generalizability of the results to humans exposed to chronic ingestion of uranium is also questionable because of the single dose exposure and the use of hospitalized patients.

⁶Maynard EA, Hodge HC (1949). Studies of the toxicity of various uranium compounds when fed to experimental animals. In: *The Pharmacology and Toxicology of Uranium Compounds* (C. Voegtlin and H.C. Hodge, eds., McGraw Hill, New York, N.Y.), 309-376.

⁷Hursh JB, Neuman WR, Toribara T, Wilson H, Waterhouse C (1969). Oral ingestion of uranium by man. *Health Physics*, 17:619-621.

16c. Hursh and Spoor, 1973.⁸ This study looked at seven subjects who were exposed to a single injection of intravenous uranium. The study's outcome measures included urinary catalase, nitrogen, and glomerular filtration rate. Again, the validity of this study is poor as it involved a small number of subjects and employed insensitive markers of renal damage. The generalizability of the study is certainly poor as these were ill patients that received intravenous and not oral doses of uranium.

16d. Novikov and Yudina, 1970.⁹ This study looked at female rabbits exposed to oral doses of uranium for 12 months. The outcome measures were serum urea, creatinine clearance, and enzyme levels in tissue. This study had poor validity because it used insensitive markers of disease.

17. The NRC has relied primarily on animal studies and a very small number of human experiments involving high doses of uranium given over very short periods of time to support the basis for the restoration standard. In light of newer findings, all of these studies contained at least two implicit assumptions that should be questioned: namely, that (1) there is no difference between acute and chronic exposure to uranium, and (2) valid and sensitive biomarkers for disease were used.

18. These assumptions are likely incorrect in light of studies conducted in the early-to-mid 1990s and reported in 1995 and 1998 by researchers with the Laboratory Centre for

⁸Hursh JB, Spoor NL (1973). Data on Man. In: *Uranium, Plutonium, Transplutonium Elements* (HC Hodge, JN Stannard, JB Hursh, eds., Springer-Verlag, Berlin), 197-239.

⁹Novikov YV, Yudina TV (1970). Data on the biological effect of small amounts of natural uranium in water. *Hyg. Sanit.*, 35:225-216.

Disease Control of Health Canada in Ottawa and with the Ottawa Department of Health. These studies raise significant concerns about the safety of the NRC's uranium restoration level for CUP. Two studies (Mao, et al., 1995, and Zamora, et al., 1998, which are attached hereto as **Exhibit B** and **Exhibit C**) were performed on healthy human populations that were exposed, over long periods of time, to levels of uranium in water (and, to a lesser extent, in food) that are considered to be quite low. These human studies employed more sensitive and sophisticated markers of kidney dysfunction and kidney cellular damage. Three other studies by A.C. Gilman and colleagues (1998a, b and c, attached hereto as **Exhibit D**, **Exhibit E** and **Exhibit F**) were performed on rats and rabbits using sensitive indicators of biological damage. Brief descriptions of the methodologies and findings of these Canadian studies follow:

18a. Mao Y., et. al., 1995 (see **Exhibit B**).¹⁰ This study evaluated the presence of certain biomarkers of kidney impairment in three groups of people (100 subjects in all) who had been exposed to varying concentrations of naturally occurring uranium in their drinking water. These researchers found a statistically significant association between increasing uranium exposure in water and levels of microalbumin in the urine of the study subjects. Subjects who drank water containing levels of uranium as low as 0.014 mg/l — or 31 times lower than the NRC restoration standard — were found to have higher levels of microalbumin in their urine. Microalbuminuria, or the condition of having small amounts of protein in one's urine, is a known

¹⁰Mao Y, Desmeules M, Schaubel D, Bérubé D, Dyck R, Brûlé D, Thomas B (1995). Inorganic Components of Drinking Water and Microalbuminuria. *Environ. Res.* 71:135-140.

risk factor for stroke, heart attack, and kidney failure.¹¹ In other words, the presence of virtually any level of microalbumin in urine is evidence of biological damage in the kidney *before* disease symptoms are evident in the individual. The medical profession refers to these effects as the *subclinical stage* of the spectrum of disease.

18b. Zamora ML, et. al., 1998 (see, **Exhibit C**).¹² This study also assessed bioindicators of kidney function in people (50 subjects) who were exposed to varying concentrations of uranium in their drinking water from periods ranging from 1 to 59 years. These researchers selected 30 subjects from a Nova Scotia community who drank well water containing uranium concentrations ranging from 0.002 mg/L to 0.780 mg/L and 20 subjects from Ottawa whose drinking water, which was obtained from a municipal distribution system, had a uranium concentration of 0.00002 mg/L, or 0.02 µg/L. The Nova Scotia subjects were categorized as the "high-exposure group" while the Ottawa subjects were categorized as the "low-exposure group." About half of the subjects in the high exposure group drank water exceeding the Canadian health guideline of 100 µg/l (i.e., 0.1 mg/l).¹³ Zamora and colleagues analyzed urine samples collected from the study subjects for four biomarkers of kidney function

¹¹Luft FC (1997). Microalbuminuria and essential hypertension: renal and cardiovascular implications. *Current Opinion in Nephrology and Hypertension*, 6(6):553-557.

¹²Zamora ML, Tracy BL, Zielinski JM, Meyerhof DP, Moss MA (1998). Chronic Ingestion of uranium in drinking water: a study of kidney bioeffects in humans. *Toxicological Sciences*, 43:68-77.

¹³See Zamora, et al., at 71 and 77, citing "Guidelines for Canadian Drinking Water Quality," Health and Welfare Canada, 6th ed., H48-10/1996E.

and four biomarkers of cellular toxicity.¹⁴ They found an association between increasing uranium ingestion and the presence of elevated levels of urinary glucose, alkaline phosphatase ("ALP," an enzyme localized in the brush-border membranes of the proximal tubules) and β_2 -microglobulin (a low-molecular weight protein that is reabsorbed and digested in the lining of the proximal tubules). These biomarkers are indicators of injury to the kidney's proximal tubules, which process, collect and transmit wastes from the blood.¹⁵ This association was observed only in the *high-exposure group* in which the subjects had total daily uranium consumption from both food and water ranging from 3 to 570 μg , with the percentage of uranium intake through water varying from 31% to 98%. Abnormally high urinary glucose was observed in subjects having a total daily uranium consumption as low as 21 μg , and abnormal levels of the

¹⁴The biomarkers of kidney function were creatinine, glucose, protein and β_2 -microglobulin (BMG); the biomarkers of cellular toxicity were alkaline phosphatase (ALP), γ -glutamyl transferase, lactate dehydrogenase, and N-acetyl- β -glucosaminidase. These markers are described in the paper by Zamora, et al., at 69-70 (*see, Exhibit C attached*).

¹⁵Zamora and colleagues' findings are entirely consistent with descriptions of the biological mechanisms by which uranium induces renal tubular impairment given throughout the relevant biomedical literature. Particularly instructive in this regard was R. W. Leggett's review paper in the September 1989 edition of *Health Physics* ("The behavior and chemical toxicity of U in the kidney: a reassessment," 57:365-383). Leggett reported, for instance, that glucosuria, or the presence of elevated glucose levels in urine, is known to be caused by direct interaction of uranium with the brush-border membranes of cells that line the proximal tubules of the kidney. ALP is present in the brush-border membranes, and BMG, a low-molecular-weight protein, is reabsorbed and digested in the lining of the proximal tubules. Zamora, et al. (at 73-77), reasoned that the mechanism indicated by the study results was disruption or structural damage of the cellular membranes by prolonged exposure to tubular fluid, resulting in release of ALP into the developing urine and decreased reabsorption of BMG.

enzyme ALP were observed in subjects having a total daily uranium consumption of 220 μg .^{16,17}

19. The Mao and Zamora studies are important in several ways. First, they were performed on humans who had ingested uranium chronically in their drinking water; as such, they give results that are more appropriate when generating standards for drinking water. Second, they found signs of renal injury at concentrations of uranium far below the NRC restoration standard of 0.44 mg/l (or 440 $\mu\text{g/l}$). Third, they employed more sensitive markers of injury than those the studies cited by and relied upon by the NRC. And fourth, in the case of the Zamora study, adverse renal effects were observed in subjects who had total daily uranium ingestion ranging from 21 μg to 220 μg , which equates to a weekly range of 0.147 mg to 1.54 mg — or 4 to 40 times less ingested uranium than Mr. McKenney has inferred is an entirely safe level.

20. Studies by Gilman, et al. (1998a,¹⁸ 1998b¹⁹ and 1998c²⁰). Three papers by A.P.

¹⁶See, Zamora, et al., at 71-72 and Table 3.

¹⁷The total daily intake of uranium from ingestion of water alone is based on an average daily consumption of 2 liters of water per person. Hence, a total daily intake of 220 μg of uranium would result from drinking water having a maximum concentration of 110 $\mu\text{g/l}$. The actual concentration would be less because some of the total daily uranium intake for subjects in the Zamora study was from uranium in contained in both food and water.

¹⁸Gilman AP, Villeneuve DC, Secours VE, Yagminas AP, Tracy BL, Quinn JM, Valli VE, Willes RJ, Moss MA (1998a.). Uranyl nitrate: 28-day and 91-day toxicity studies in the Sprague-Dawley Rat. *Toxicological Sciences*, 41:117-128. (**Exhibit D**).

¹⁹Gilman AP, Villeneuve DC, Secours VE, Yagminas AP, Tracy BL, Quinn JM, Valli VE, Moss MA (1998b.). Uranyl nitrate: 91-day toxicity studies in the New Zealand White Rabbit. *Toxicological Sciences*, 41:129-137. (**Exhibit E**).

²⁰Gilman AP, Moss MA, Villeneuve DC, Secours VE, Yagminas AP, Tracy BL, Quinn JM, Long G, Valli VE (1998c.). Uranyl nitrate: 91-day exposure and recovery studies in the New Zealand White Rabbit. *Toxicological Sciences*, 41:138-151. (**Exhibit F**).

Gilman and colleagues, published in the journal *Toxicological Sciences* in 1998, examined the multiple hematological, biochemical, and histopathological changes in rats and rabbits fed water containing the soluble uranium compound, uranyl nitrate. (These papers are attached to this affidavit as **Exhibits D, E and F.**) Five groups of 30 animals were exposed for 28 to 91 days to drinking water containing uranium concentrations ranging from 0.96 mg/l to 600 mg/l. Lesions developed in the kidneys and livers of the experimental rats in all exposure groups; lesions were even observed in the renal tubules and glomeruli in the lowest exposed rat group (Gilman, et al., 1998a at 117). A lowest-observed-adverse-effect level (“LOAEL”) of 0.96 mg uranyl nitrate per liter was observed in male rabbits (Gilman, et al., 1998b); the animals were *not* exposed to uranium-in-water concentrations of less than 0.96 mg/l. Importantly, the researchers did not find a no-observed-adverse-effects-level (“NOAEL”) in the rats since renal lesions were seen in the lowest exposed groups (Gilman, et al., 1998a).

21. The Gilman studies were somewhat limited by the small number of animals tested. However, the quality of these studies was improved over the animal studies of the 1940s and 1970s. Indeed, the studies’ findings led Gilman and colleagues to conclude, “the ability of uranium to produce specific tubular injury followed by basement membrane injury at relatively low doses in even a small proportion of exposed animals suggests that human exposure to soluble uranium over prolonged periods needs to be monitored” (Gilman, et al., 1998c, at 151; *see Exhibit F*).

22. In its decision to use 0.44 mg/l as a restoration standard, the NRC has also overlooked or ignored recommendations of several major state and national environmental and health agencies, including the USEPA, the California EPA’s Office of Environmental Health

Hazard Assessment (“OEHHA”), the Canadian national health agency, Health Canada, and the World Health Organization (“WHO”). These agencies have reviewed the current and historical literature and have revised or are in the process of revising *downward* their existing standards or guidelines on allowable levels of uranium in drinking water. A summary of those agencies’ recommendations follows:

22a. USEPA Uranium Regulation. EPA scientists who studied uranium toxicity concluded as early as 1983 that “it is deemed prudent to consider setting the health effects guidance level for uranium in drinking water at 10 pCi/l based primarily on health considerations.”²¹ Based in part on the data it had already collected and on the published literature available at the time, EPA in 1991 proposed a maximum contaminant level goal (“MCLG”) of zero for uranium in drinking water, and a maximum contaminant level (“MCL”) of 30 pCi/l, or 20 $\mu\text{g/l}$.²² While this standard has not been promulgated as a final regulation, an official in the EPA’s Office of Drinking Water in Washington, D.C., told me recently that EPA plans on finalizing a uranium MCL of 0.020 mg/l by 2001. (Personal communication, Dr. Ambika Bathija, December 23, 1999.) Dr. Bathija stated to me that EPA has reviewed results of the recent Canadian uranium studies in the context of its consideration of adopting the 0.020 mg/l uranium level as a final drinking water standard.

22b. California’s Uranium Standard and Public Health Goal. The California

²¹Cothem CR, Lappenbusch WL, Cotruvo JA (1983). Health effects guidance for uranium in drinking water. *Health Physics*, 44:337-384.

²²U.S. Environmental Protection Agency (1991). National Primary Drinking Water Regulations; Radionuclides. Notice of Proposed Rulemaking. *56 Federal Register* 33050-33127, July 18.

Department of Health Services's existing standard for uranium in drinking water is 20 pCi/l, which, according to OEHHA, was based in part on kidney toxicity derived from the 1970 rabbit study by Novikov and Yudina (1970). OEHHA, which is a division of the California Environmental Protection Agency that is charged with making recommendations for allowable levels of more than 80 toxic substances solely on the basis of known or suspected human health effects, is proposing a revised Public Health Goal ("PHG") of 0.2 pCi/l of uranium in drinking water (*Id.* at 1-2). The proposed revised PHG is "based on a study showing changes in indicators of kidney function in a human population." OEHHA's announcement, which is attached to this affidavit as **Exhibit G**, states that previous studies upon which the current California standard is based "has been superseded by more recent studies of the effects of uranium on kidney function in laboratory animals and in human populations." Those recent studies, according to the OEHHA announcement, include the Gilman and Zamora studies that I have summarized in this affidavit.

22c. Canadian Guidelines. Health Canada's Federal-Provincial Subcommittee on Drinking Water in January 1999 proposed a revised guideline for uranium in drinking water of 0.01 mg/l, or 10 μ g/l. This proposed guideline, which is discussed at length in a Health Canada document titled "Uranium in Drinking Water," which is attached to this affidavit at **Exhibit H**, would replace the current guideline of 0.1 mg/l, or 100 μ g/l. Michelle Giddings, a scientist with Health Canada's Bureau of Chemical Hazards, told me recently that the current guideline "is not acceptable" from a health-protection perspective, and that her agency has used the recent Canadian human and animal studies to support its efforts to revise the current guideline. (Personal communication, Michelle Giddings, February 29, 2000.) Ms. Giddings stated to me

that her office will finalize a new guideline of 0.02 mg/l in the coming months.²³ She stated that the proposed guideline of 0.02 mg/l was increased from the previous proposed level of 0.01 mg/l as a result of public comments received on the January 1999 draft guideline. Having reviewed the rationale for the 0.01 mg/l guideline (*see Exhibit H*), it is my professional opinion that Health Canada made a persuasive and scientifically credible case for the lower uranium level.

22d. World Health Organization Guidelines. In 1993, WHO adopted a uranium guideline for drinking water of 140 $\mu\text{g/l}$, based on the radiological characteristics of uranium.²⁴ In 1995, the organization's coordinating committee for updating the 1993 and 1996 drinking water guidelines recommended revising the 1993 uranium level in light of "new data on the chemical toxicity of uranium."²⁵ Citing the 1995 study by Mao, et al., and prepublication versions of Gilman's papers, WHO in 1998 published a provisional uranium guideline of 2 $\mu\text{g/l}$ (or 0.002 mg/l), "based on associations for subclinical renal effects reported in preliminary epidemiological studies." (*Id.* at 91.) A copy of the relevant addendum to the WHO drinking water guidelines that includes a discussion of the organization's bases for the revised uranium level is attached to this affidavit as **Exhibit I**.

22e. As shown in the table below, NRC's proposed uranium restoration standard of

²³The proposed Health Canada uranium guideline of 0.02 mg/l is equivalent to USEPA's 1991 proposed U.S. drinking water standard of 20 $\mu\text{g/l}$.

²⁴World Health Organization (1996). Guidelines for drinking-water quality (2nd ed.): Volume 2, Health criteria and other supporting information. WHO, International Programme on Chemical Safety (Geneva), 374-381.

²⁵World Health Organization (1998). Guidelines for drinking-water quality (2nd ed.): Addendum to Volume 2, Health criteria and other supporting information. WHO/EOS/98.1 (Geneva), 81-94.

0.44 mg/l for the Crownpoint Uranium Project exceeds *existing* state and federal limits and guidelines for uranium in drinking water, and will exceed by an even larger margin new and revised regulations and guidelines that are likely to be adopted in the coming year or two. The existing uranium limits and guidelines of the California Department of Health Services, Health Canada and the World Health Organization are being revised in response to the findings of the recent Canadian studies, which demonstrate kidney impairments at what to now have been considered to be “low” levels of uranium, i.e., concentrations at or below 0.020 mg/l. The

**Comparison of NRC’s Uranium Restoration Standard
With Other Agencies’ Uranium-In-Drinking Water Guidelines and Regulations**

Agency/Institution	Existing Limits and Guidelines for Uranium in Drinking Water	Proposed or Revised Limits and Guidelines for Uranium in Drinking Water
USNRC (for CUP)	0.44 mg/l	0.44 mg/l
USEPA	none	0.02 mg/l, or 30 pCi/l
California DHS; Cal EPA	20 pCi/l	0.2 pCi/l
Health Canada	0.1 mg/l, or 100 µg/l	0.01-0.02 mg/l, or 10-20 µg/l
World Health Organization	0.14 mg/l, or 140 µg/l	0.002 mg/l, or 2 µg/l

important message here is that the trend in standards setting for uranium in drinking water among regulatory agencies is a steady *lowering* of allowable levels in recognition of new evidence of *increasing risk* of human health impairment.

23. The Navajo people that I serve already suffer from inordinate amounts of renal

disease stemming from diabetes. The prevalence of end-stage renal disease²⁶ in Native Americans in the Southwestern United States is 4.6 times that of all other Americans, and the number of Native Americans with renal disease is growing — from 1988 to 1994, the number of Native Americans requiring dialysis or kidney transplant rose 107%.²⁷ Exposing the Navajo people of Crownpoint and Church Rock to another known kidney toxin is inviting disaster. Contemporary studies and recommendations from major state and federal environmental and health agencies in the U.S. and Canada demonstrate that the NRC's restoration goal of 0.44 mg/l for uranium in groundwater at the proposed HRI solutions mines in Church Rock and Crownpoint is not protective of public health and safety and is tantamount to malpractice.

24. For the reasons set forth in this affidavit, I believe that the license issued to HRI for the Crownpoint Uranium Project (CUP) is not adequate to protect public health and safety and therefore should be suspended immediately. On the basis of my professional judgment and the analysis contained herein, I believe that the project should be halted and the groundwater restoration standard be amended so that the standard requires HRI to return the uranium concentration in the restored water back to baseline levels at the conclusion of mining operations.

25. This concludes my testimony.

²⁶End-stage renal disease is defined as a condition in which a person's kidney has ceased to function, or is functioning at such a diminished level, to necessitate a kidney transplant or treatment by kidney dialysis.

²⁷Narva A (1997). Course in End-Stage Renal Disease for the Primary Indian Health Service Provider. (Albuquerque, N.M.), September 10.

AFFIRMATION

I declare on this 1st day of March 2000, at Crownpoint, New Mexico, under penalty of perjury that the foregoing is true and correct to the best of my knowledge, and the opinions expressed herein are based on my best professional judgment.

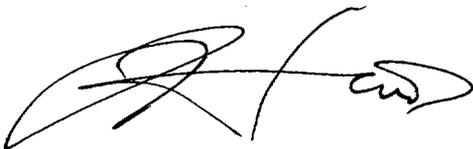

John D. Fogarty, M.D.

Sworn and subscribed before me, the undersigned, a Notary Public in and for the State of New Mexico, on this 1st day of March 2000, at Crownpoint, New Mexico.

My Commission expires on 8/8/2000.


Notary Public

Respectfully Submitted,


John D. Fogarty, M.D.

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- Education & Training:** University of New Mexico, Family Medicine Residency, Albuquerque, 6/93-1/97.
University of New Mexico, Masters of Public Health Program, 1994-present.
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University of Wisconsin. Foreign exchange program, London, England, 1987.
- Professional Experience:** Family Physician, Indian Health Service; Crownpoint Service Unit, 8/99-present.
Family Physician, Indian Health Service; Santa Fe Service Unit, 4/97-8/99.
Physician, New Mexico Department of Health; Albuquerque, NM, 1/95-6/95.
Locum Tenens Physician; Portales, Estancia, Farmington, Santa Fe, and Albuquerque, New Mexico and Dallas, Texas; 1/95-4/98.
Medical Volunteer, Kripa Foundation, Bombay, India, spring 1993.
Medical Volunteer, Lucea, Jamaica, winter 1991.
Volunteer, Geseundeit Medical Institute, Hillsboro WV, summer 1990.
- Licensure:** Diplomate, American Board of Family Practice, 1997-2004.
Diplomate, National Board of Medical Examiners.
New Mexico Medical License, #95-54.
Texas Medical License, #TL 050396100196.
- Honors:** Mead Johnson Award, American Academy of Family Physicians (AAFP), 1996.
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Resident Award Speaker, "Community Oriented Primary Care", AAFP, New Mexico, 1997.
Resident Award Speaker, "Overpopulation and Environmental Degradation", AAFP, New Mexico, 1996.
Magna Cum Laude, University of Iowa.
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- Activities:** Diabetes Coordinator, Santa Fe IHS Service Unit, 6/97-8/99.
Medical Student Preceptor, UNM School of Medicine, 1993-97.
AIDS Educator, East San Jose Elementary School, Albuquerque NM, 1994-95.
Cofounder and President, University of Washington Health Sciences Council for Environmental Awareness, 1992-93.
Chairperson, Media and Marketing Committee, Zero Population Growth, Seattle, 1992-93.
Program Developer, Interactive Computer Teaching Modules for Neuroanatomy Students, University of Washington, 1989.
- Presentations:** Lecturer, "Conducting Community Assessments", UNM School of Medicine, 1995-96.
Lecturer, "Epidemiology, Biostatistics, and Community Medicine", UNM, 1996.
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EXHIBIT

A

tabbles

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Publications:

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Inorganic Components of Drinking Water and Microalbuminuria

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Relatively little is known of the chronic effects attributable to the ingestion of inorganic components such as uranium and silicon. Although ingestion of large amounts of U can cause acute renal damage through a chemical effect, studies on humans have typically considered inhalation the route of exposure. We investigated the association between drinking water concentration levels of U and Si, and microalbuminuria, a sensitive biological indicator of renal dysfunction. Linear regression analysis revealed a statistically significant association between U cumulative exposure index and albumin per mmol creatinine ($P = 0.03$). No such significant relationship appeared for Si, although a positive trend was witnessed. Since normal but increasing levels of microalbuminuria were observed at U concentration levels below the Canadian Maximum Allowable Concentration (MAC), it is suggested that further study be undertaken. © 1995

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INTRODUCTION

Incidence rates for end-stage renal disease (ESRD), the limiting form of renal dysfunction, are reportedly increasing in North America (Canadian Institute for Health Information, 1994; National Institutes of Health, 1991), with future increases being projected (Desmeules *et al.*, 1995; Wood *et al.*, 1987). As the etiology for a large percentage of ESRD cases is poorly understood, it is prudent to evaluate agents suspected of promoting even subtle degrees of renal damage, including contaminants in drinking water.

The acute toxicity of uranium (U) has been studied extensively in animals and, to some extent, in humans (Tannenbaum, 1951; Thun *et al.*, 1985; Harvey *et al.*, 1986; Domingo *et al.*, 1987). Little is known about the chronic effects of U ingestion. Drinking water as a means of U ingestion has not

been extensively investigated (Cothorn and Lappenwush, 1983; Cothorn *et al.*, 1983), since inhalation has historically been considered the main route of exposure (Ortega *et al.*, 1989). Nephritis is the primary chemically induced health effect of uranium (Hursh and Spoor, 1973), with evidence existing that U-induced renal damage can occur following even moderate U dosage (Votegtlin and Hodge, 1949, 1953; Luessenhop *et al.*, 1958). The toxic action of U on the kidneys is well documented, with a comprehensive review provided by Leggett (1989).

Since inorganic components have been implicated as etiological agents in nephropathies, this investigation was designed to examine the relationship between drinking water concentration levels of U and other elements such as Si and urine albumin, a biological indicator of the early stages of renal dysfunction.

MATERIALS AND METHODS

Participant Selection

Between August and October 1993, inhabitants of three Saskatchewan communities were asked to participate in this study. Water sources within these communities had previously been shown to contain different uranium concentration levels. Forty participants from study site 1 (which served as a control site), and 30 from each study site 2 and site 3, were contacted by telephone using a random digit method. The first adult contacted at each residence was asked to participate. An appointment was then set up, at which time a nurse research assistant administered a questionnaire, and obtained blood and urine samples. In addition, water samples were obtained. If a telephone call did not result in an agreement to participate, the process was continued until the final total number of participants was obtained.

Urine Analysis

A first morning urine sample was collected using a sterile container. Biochemical analyses of the specimens employed standard nephelometric methodology (specifically, the Beckman array).

Water Analyses

The water source used for sampling, for each participant, was the drinking water outlet used by each participant, be it a private well or town water supply. Seventy of the 100 outlets were sampled in triplicate for unfiltered water. The sampling also included field-filtered (0.45 μm) water at 30 of the outlets and replication of 15 of latter outlets, as well as QA/QC blanks and spikes. The analysis targeted uranium (U) since preliminary studies had revealed high concentrations of this element (32–38 $\mu\text{g/liter}$) in these areas. Uranium concentration was determined on preserved samples (HNO_3 0.18%, w/w) using a Perkin-Elmer Elan 5000 ICP-MS equipped with a pneumatic nebulization system. All measurements were performed at mass 238 (and 235). Lead, cadmium, and mercury were measured using a similar method. Silicon concentration was determined using an ARL SpectraSpan7 DCP equipped with a pneumatic nebulization system, using the 251.611-nm emission line. All measurements were satisfactory for accuracy (Standard Reference Materials comparison within 5%), method precision (within 8% RSD), spike recoveries (90–110%), and blanks.

Statistical Analysis

A crude index of the cumulative exposure to uranium (hereafter symbolized by U^*) was calculated for each study participant as the product of the U concentration level in the drinking water supply, the reported average number of cups of water consumed at the residence each day, and the total number of years lived at the current residence. A cumulative silicon exposure index (hereafter represented by Si^*) was calculated similarly.

Data were available for the following biological renal function measurements: urine albumin concentration (mg/liter), and urine albumin scaled by urine creatinine concentration ($\mu\text{g}/\text{mmol}$ urine creatinine), the latter considered to be a more accurate measurement in a spot urine sample, since it accounts for the concentration of the urine, and serum creatinine. Linear regression was utilized to assess the relationship among the three biological renal function measurements, and (U and Si concentration) and (U^* and Si^*) separately. Treating the urine

and serum measurements as continuous variables was considered most appropriate since, for each, the cut-points defining normality are rather arbitrary. In this context, categorizing of these outcomes would represent an unnecessary loss of information. For each model, terms were entered for age and diabetes status, since both variables are known to increase the risk of renal dysfunction. The U (U^*) effect was controlled for Si (Si^*), and vice versa. Sex, smoking status, use of water filters and softeners, and previous occupational exposure to U, Cd, or Hg were not found to have an effect on any of the outcome measures, nor did they influence the magnitude of the effect of any of the variables of interest.

RESULTS

Response Rate

To obtain 40 subjects in the control site (site 1), 182 different phone numbers were dialed for a response rate of 22%. In site 2, 77 phone numbers were dialed to obtain a sample of 30 for a response rate of 39%. In site 3, 60 numbers were called to obtain the sample of 30 for a response rate of 50%. Thus, the overall response rate was 31.5%, in line with response rates typically found for health surveys requiring biological measurements.

Questionnaires were successfully administered to all 100 participants. In addition, there was 100% success rate in obtaining water samples and urine specimens. Blood samples were not obtainable, for technical reasons only, for 9 participants (9%).

Water Samples

None of the sites showed analyte concentrations above the Canadian Maximum Allowable Concentration (MAC) given in the Guidelines for Canadian Drinking Water Quality (Health and Welfare Canada, 1993), the MACs being 100 $\mu\text{g/liter}$ for U, 10 $\mu\text{g/liter}$ for Pb, 5 $\mu\text{g/liter}$ for Cd, and 1 $\mu\text{g/liter}$ for Hg. In fact, Pb, Cd and Hg were found at low concentration levels. Thus, the Pb mean concentration was below 1.0 $\mu\text{g/liter}$; Cd was found at concentrations below 0.1 $\mu\text{g/liter}$ for all outlets except two (at 0.3 and 0.8 $\mu\text{g/liter}$); Hg was not detected, consistent with concentrations well below 0.1 $\mu\text{g/liter}$.

Although the concentrations of U and Si were within ranges accepted as normal, their concentration levels were high, especially when compared to the median values previously reported for drinking water (typical values being 0.15 $\mu\text{g/liter}$ for U and 3 mg/liter for Si) (Cothorn and Lappenwush, 1983; U.S. Geological Survey, 1990; Livingstone, 1963).

Thus, an evaluation of the association between U and Si concentration levels, and the occurrence of chronic nephropathies was desired. The mean uranium and silicon concentration levels, and their ranges, are listed by site in Table 1. The control site (1) had the lowest U and Si concentration levels (0.71 and 0.75 mg/liter, respectively). Site 2 had the highest U concentration level (19.6 μ g/liter), while the highest Si concentration level was measured at site 3 (8.88 mg/liter).

No indication of the presence of U or Si in particulate matter was found. As well, no difference was noted between samples when outlets were replicated. In fact, the concentration of both elements appeared stable for samples originating from the same water sources, sampled at different distribution outlets or over intervals of many days.

Findings

Demographic characteristics of the 100 study participants are displayed in Table 2. The sex ratio was relatively low (56.3 males per 100 females), although males are typically underrepresented in surveys. The participants ranged in age from 18 to 84.

A frequency distribution of the urine albumin levels of the study participants is depicted in Fig. 1. Values ranged from 0.165 to 16.1 mg/mmol creatinine. For 8 participants, "elevated" urine albumin concentration levels were detected (i.e., >3.0 mg/mmol creatinine). Serum creatinine concentration levels ranged from 50 to 170 μ mol/liter, with 3 (4.4%) participants having values said to be indicative of prevalent renal damage (i.e., >120 μ mol/liter). For 9 participants, serum creatinine concentration levels were unavailable, since blood samples could not be obtained for technical reasons. Due to the lack of clinically significant variability in serum creatinine concentration among the study population, and since serum creatinine is not a sensitive biomarker for subtle renal damage, the statistical analysis focused on urine albumin. Microalbuminuria has been shown to be a sensitive indicator of early renal disease, particularly in patients with diabetic glomerulosclerosis.

TABLE 1
Uranium and Silicon Levels of Drinking Water

Study site	U (μ g/liter) mean (range)	Si (mg/liter) mean (range)
1 (control)	0.71 (0.48, 0.74)	0.75 (0.70, 0.84)
2	19.6 (<0.1, 48)	8.68 (<0.1, 11.9)
3	14.7 (<0.1, 50)	8.88 (<0.1, 22.5)

TABLE 2
Demographic Characteristics of Study Population, by Site ($n = 100$)

Age group	Region			Total
	Site 1	Site 2	Site 3	
≤ 39	21 (52.5)	14 (46.7)	11 (36.7)	46 (46.0)
40-59	8 (20.0)	8 (26.7)	4 (13.3)	20 (20.0)
≥ 60	11 (27.5)	8 (26.7)	15 (50.0)	34 (34.0)
Sex				
Male	12 (30.0)	12 (40.0)	12 (40.0)	36 (36.0)
Female	38 (70.0)	18 (60.0)	18 (60.0)	64 (64.0)
Total	40	30	30	100

Age-adjusted partial correlation coefficients (ρ) for the covariables used in the analysis are displayed in Table 3. Strong correlations were observed between U and Si ($\rho = 0.66$) and between U* and Si* ($\rho = 0.59$). Urine albumin (when measured as either mg/liter or mg/mmol creatinine) increased significantly with U* and Si*.

A summary of the results of the linear regression analysis can be found in Table 4. A statistically significant association ($P = 0.03$) was found between the uranium exposure index, U*, and urine albumin, when the latter was measured as milligrams per millimole creatinine. However, most participants had urine albumin levels within the normal range. Urine albumin, when measured as milligrams per liter, showed an association with U* ($P = 0.07$). No association between Si or its exposure index and any of the renal function biomarkers was observed. No association was found between serum creatinine and U, Si, U*, or Si* (data not shown).

DISCUSSION

This preliminary study found a significant ($P = 0.03$) positive association between increasing, but normal levels of urine albumin (units: mg per mmol creatinine) and uranium exposure index U* (calculated for each study participant as the product of the uranium concentration level in drinking water, the number of cups of water consumed per day, and the number of years lived at the current residence). No important association was found between urine albumin and silicon, or its corresponding exposure index, Si*, which was calculated analogously to that of uranium.

The statistically significant U effect is particularly noteworthy considering the relatively crude measure of exposure and small sample size ($n = 100$). Self-reports were not utilized with respect to the ascertainment of either exposure (U and Si concentration levels) or outcome (albumin levels), with the exception that the number of cups of water con-

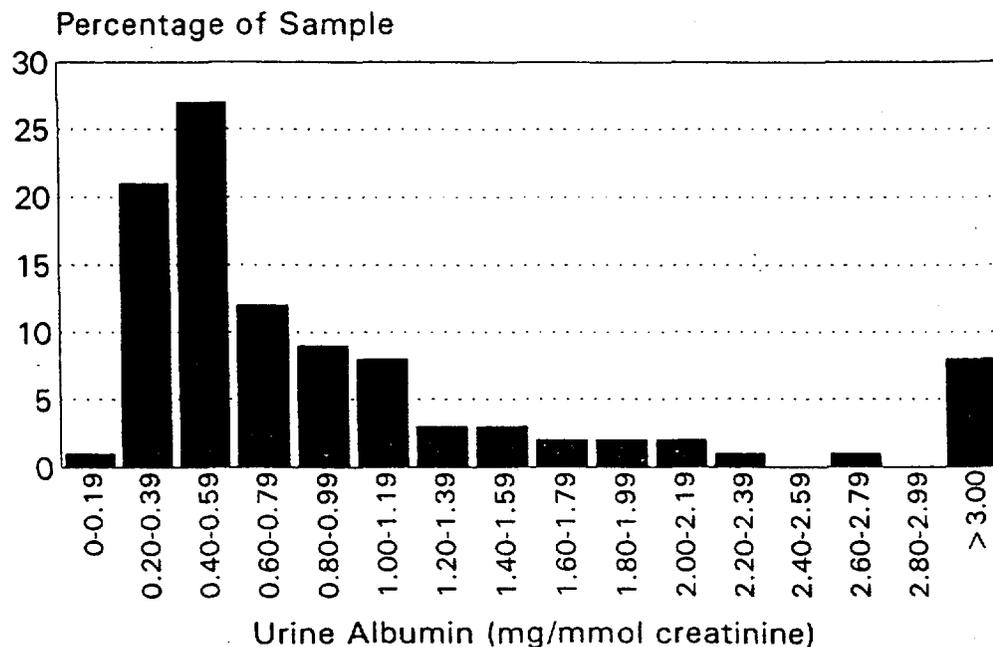


FIG. 1. Urine albumin levels of study population (mg/mmol creatinine). Generally, normal values are considered to be 0–1.14 mg/mmol creatinine.

sumed per day was reported by the participants themselves. The fact that the uranium exposure index described in the preceding paragraph, U^* , proved to be a significant predictor of urine albumin, while the U concentration level alone did not, adds credence to our results. That is, the positive relationship between albumin and uranium appears more genuine, given that consideration of the actual quantity of water consumed strengthens the association between the two variables. However, this association hinges on the assumption that levels of

uranium in drinking water are constant over time. In many cases where serial measurements are taken in a community, wide fluctuations occur.

The impact of known risk factors for renal dysfunction, such as age and diabetes status, were factored into the statistical models utilized in the analysis. Sex, self-reported occupational exposure, use of water filters and softeners, and having a first-degree relative with end-stage renal disease did not distort the association between U^* and urine albumin. Since all participants were asymptomatic, re-

TABLE 3
Age-Adjusted Partial Correlations among Study Covariables

	U	Si	U^{**}	Si^{**}	Urine albumin/creatinine ^b	Urine albumin ^c	Serum creatinine
U	1.00	0.66	0.61	0.33	0.05	0.03	-0.14
Si		1.00	0.43	0.59	0.16	0.05	0.12
U^*			1.00	0.67	0.39	0.37	0.09
Si^*				1.00	0.32	0.38	0.26
Urine albumin per unit creatinine					1.00	0.91	0.18
Urine albumin						1.00	0.17
Serum creatinine							1.00

^a U^* (Si^*) were calculated as the product of U (Si) concentration, as measured in the drinking water supply, daily water consumption (cups), and duration of stay at current residence (years).

^b Units: mg urine albumin per mmol creatinine.

^c Units: mg/liter.

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

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Chronic Ingestion of Uranium in Drinking Water: A Study of Kidney Bioeffects in Humans. Zamora, M. L., Tracy, B. L., Zielinski, J. M., Meyerhof, D. P., and Moss, M. A. (1998). *Toxicol. Sci.* 43, 68-77.

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 β_2 -microglobulin (BMG). The markers for cell toxicity studied were alkaline phosphatase (ALP), γ -glutamyl transferase (GGT), lactate dehydrogenase (LDH), and *N*-acetyl- β -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 β_2 -microglobulin) and provide insight into the site for these effects (alkaline phosphatase (ALP), γ -glutamyl transferase (GGT), *N*-acetyl- β -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 β_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 UO_2F_2 -treated rats. Diamond and co-workers (1989) used *N*-acetyl- β -D-glucosaminidase, alkaline phosphatase, γ -glutamyl transferase, lactate dehydrogenase, and aspartate amino transferase as indicators of renal injury in uranyl fluoride-treated rats. Stroo and Hook (1977) used the lysosomal enzymes acid phosphatase and β -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
β_2 -Microglobulin (BMG)	Phadebas competitive radioimmunoassay	12.7 nmol/L
Alkaline phosphatase (ALP)	Kinetic	0.06 U/L ^a
γ -Glutamyl transferase (GGT)	Kinetic	2.04 U/L ^b
Lactate dehydrogenase (LDH)	Kinetic	0.04 U/L ^c
<i>N</i> -Acetyl- β -D-glucosaminidase (NAG)	Fixed point	0.37 U/L ^d

^a 1 unit will hydrolyze 1.0 μmol of *p*-nitrophenyl phosphate per minute at pH 10.4 at 37°C.

^b 1 unit will liberate 1.0 μmol of *p*-nitroaniline from *L*- γ -glutamyl-*p*-nitroanilide per minute at pH 8.5 at 25°C.

^c 1 unit will reduce 1.0 μmol of pyruvate to *L*-lactate per minute at pH 7.5 at 37°C.

^d 1 unit will hydrolyze 1.0 μmol of *p*-nitrophenyl *N*-acetyl- β -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 β_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 ^a		Low-exposure group ^b	
	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

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

^b 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 μg 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 μg , while the smallest amount was 0.34 μg . 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^a

Sex	Age	Total Uranium Intake ($\mu\text{g}/\text{day}$)	Exposure Level ^b	Glucose (mg/d)	Creatinine (mM/d)	Protein (mg/d)	ALP ^c	GGT ^d (U/g Creatinine)	LDH ^e (U/g Creatinine)	NAG ^f	BMG ^g ($\mu\text{g}/\text{g}$ Creatinine)
F	48	570	H	79.1	15 ^Δ	29.6	9.7	17	19	2.9	120
M	36	410	H	388 ^Δ	20 ^Δ	89.7 ^Δ	23 ^Δ	13	12	2.7	50
F	23	300	H	128	6.5	38.4	57 ^Δ	21	17	4.0	97
F	37	220	H	251 ^Δ	8.9	33.1	22 ^Δ	14	69 ^Δ	3.2	82
F	78	220	H	43.4	3.0 *	0.9	21 ^Δ	0 *	35	0.0 *	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 ^Δ	12	35	29	6.5	140
F	22	150	H	56.8	9.7	58.5	7.8	23	410 ^Δ	4.0	21
F	45	98	H	43.3	8.1	40.3	7.8	38	290 ^Δ	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 ^Δ	20 ^Δ	69.5	2.0	19	16	4.1	21
F	21	62	H	32.7	5.2	28.6	6.4	38	98 ^Δ	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 ^Δ	13	57.4	17	20	29	2.8	61
M	36	20	L	88.7	13	46.6	6.1	31	62 ^Δ	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 ^Δ	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 ^Δ	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	26	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 ^Δ	6.1	270
M	37	0.59	L	111	18 ^Δ	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	

^a Values exceeding 95th-percentile in reference range are indicated with Δ ; values below the 5th-percentile are indicated with *.

^b 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 $1 \mu\text{g}/\text{L}$.

^c Alkaline phosphatase: 1 unit will hydrolyze $1.0 \mu\text{mol}$ of *p*-nitrophenyl phosphate per minute at pH 10.4 at 37°C .

^d Gamma-glutamyl transferase: 1 unit will liberate $1.0 \mu\text{mol}$ of *p*-nitroaniline from L - γ -glutamyl-*p*-nitroanilide per minute at pH 8.5 at 25°C .

^e Lactate dehydrogenase: 1 unit will reduce $1.0 \mu\text{mol}$ of pyruvate to *L*-lactate per minute at pH 7.5 at 37°C .

^f *N*-acetyl- β -D-glucosaminidase: 1 unit will hydrolyze $1.0 \mu\text{mol}$ of *p*-nitrophenyl *N*-acetyl- β -D-glucosaminide to *p*-nitrophenol and *N*-acetyl-D-glucosamine per minute at pH 4.25 at 25°C .

^g β -2-microglobulin.

TABLE 4
Intersubject Variability^a in Biomarker Data

Biomarker	High-exposure group ^b	Low-exposure group ^c
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)

^a This table gives median values with minimum and maximum values in brackets.

^b Number of subjects, 30.

^c 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 μg U/kg body wt followed by three injections of 240 μg 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	p 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 (Nomiyama and Foulkes, 1968; Haley *et al.*, 1982; Diamond *et al.*, 1989).

TABLE 6
Spearman Correlation Coefficients^a for Uranium Intake for Male, Female, and Pooled Data

Biomarker	Males ^b	Females ^c	Pooled data ^d
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)

^a Numbers in parentheses are the corresponding p values.

^b Number of observations, 17, except for BMG where $N = 13$.

^c Number of observations, 33, except for BMG where $N = 27$.

^d Number of observations, 50, except for BMG where $N = 40$.

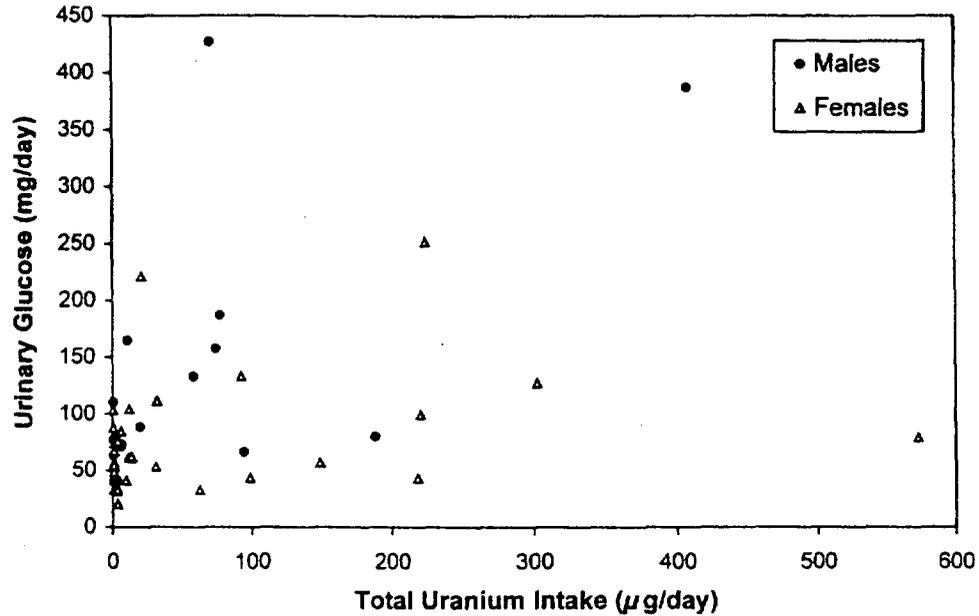


FIG. 1. Variation of urinary glucose with total daily uranium intake. The Y coordinate represents the mean of six measurements in 8-h urine specimens. Uranium intake is the mean of amounts ingested in water and through food over a 3-day period.

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

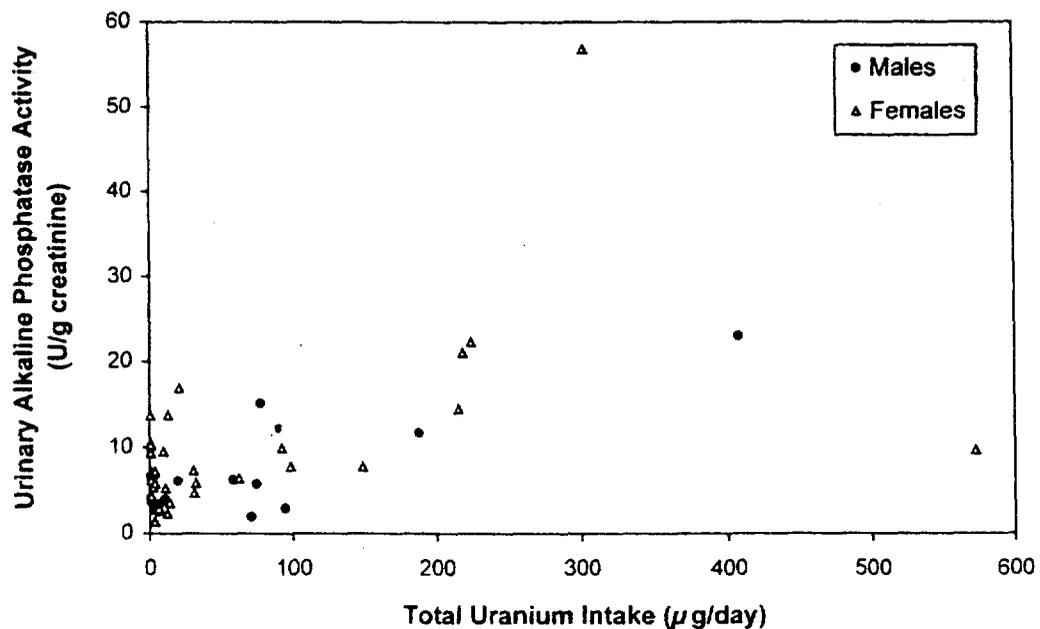


FIG. 2. Variation of urinary alkaline phosphatase activity with total daily uranium intake. The Y coordinate represents the mean of six measurements in 8-h urine specimens. Uranium intake is the mean of amounts ingested in water and through food over a 3-day period.

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 β_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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Uranyl Nitrate: 28-Day and 91-Day Toxicity Studies in the Sprague-Dawley Rat¹

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Although uranium (U) is a classic experimental nephrotoxin, there are few data on its potential long-term chemical toxicity. These studies were undertaken to derive a no-observed-adverse-effect level (NOAEL) in male and female Sprague-Dawley rats following 91-day exposure to uranium (as uranyl nitrate hexahydrate, UN) in drinking water. Following a 28-day range-finding study, five groups of 15 male and 15 female weanling rats were exposed for 91 days to UN in drinking water (0.96, 4.8, 24, 120, or 600 mg UN/L). A control group was given tap water (<0.001 mg U/L). Daily clinical observations were recorded. Following the study, animals were euthanized and exsanguinated, and multiple hematological and biochemical parameters were determined. Necropsies were conducted, and multiple tissues were sampled for histopathological examination. The hematological and biochemical parameters were not affected in a significant exposure-related manner. Although there were qualitative and slight quantitative differences between males and females, histopathological lesions were observed in the kidney and liver, in both males and females, in all groups including the lowest exposure groups. Renal lesions of tubules (apical nuclear displacement and vesiculation, cytoplasmic vacuolation, and dilation), glomeruli (capsular sclerosis), and interstitium (reticulin sclerosis and lymphoid cuffing) were observed in the lowest exposure groups. A NOAEL was not achieved in this study, since adverse renal lesions were seen in the lowest exposed groups. A lowest-observed-adverse-effect level of 0.96 mg UN/L drinking water can be reported for both the male and the female rats (average dose equivalent 0.06 and 0.09 mg U/kg body wt/day, respectively). © 1998 Society of Toxicology.

Key Words: uranium; uranyl nitrate hexahydrate; subchronic exposure; drinking water; Sprague-Dawley rat; nephrotoxicity.

Naturally occurring uranium (U) salts are common to

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many drinking water supplies, with levels ranging from 0.015 to 980 $\mu\text{g U/L}$ of water and an estimated average of 3 $\mu\text{g/L}$ (Drury *et al.*, 1981). The wide range of levels of uranium in drinking water, together with the observation of consistently higher levels in certain community water supplies, has raised concerns regarding the potential hazard of such sources of uranium to human health (Cothorn *et al.*, 1983; Cothorn and Lappenbusch, 1983; Moss *et al.*, 1983).

Two different types of health hazards could potentially be associated with exposure to uranium: radiation toxicity and chemical toxicity (Novikov, 1972; Cooper *et al.*, 1982; Haley *et al.*, 1982; Cothorn *et al.*, 1983). The isotopic mixture of naturally occurring uranium results in about 10 pCi of alpha radiation per 1.5 μg of uranium. The disposition of uranium in the body is such that the risk of adverse effects in kidney or bone associated with the radioactivity is considered less than the risk of chemical toxic effects (Novikov, 1972; Priest *et al.*, 1982; Moss, 1989). Therefore, a major focus in assessing potential health hazards associated with

TABLE 1

Mean Uranium Residues ($\mu\text{g/g}$) in Kidney and Bone Tissues of Female Rats after 28 Days Treatment with Uranyl Nitrate (UN)^a

Group number:	1	5	6
Exposure (mg UN/L):	0	120	600
TWA uranium equivalent dose ^b (mg U/kg body wt/day):	<0.0001	7.82	40.00
Number of animals studied:	10	5	7
Kidney	<0.2	<0.2	0.92 ^c (0.19) ^d
Bone	<0.2	1.78 (0.40)	4.60 ^c (1.08)

^a Reported in Tracy *et al.* (1992).

^b Time-weighted average uranium equivalent dose. Based on terminal body weight and Week 4 fluid consumption data: uranyl nitrate hexahydrate $\times 0.474 =$ uranium equivalent.

^c Significantly different from control group ($p < 0.05$); Duncan procedure.

^d \pm standard error of mean.

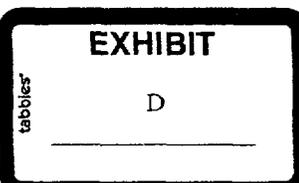


TABLE 2
Mean Terminal Body Weight, Body Weight Gain, Food and Water Intake, and Kidney Weights of Female and Male Rats after 91 Days Treatment with Uranyl Nitrate (UN)

Group number:	1	2	3	4	5	6
Exposure (mg UN/L):	0	0.96	4.8	24	120	600
Mean terminal body weight (g)						
Females	310.3 (7.7) ^b	311.9 (7.9)	315.1 (8.5)	320.1 ^a (6.7)	336.9 ^a (8.9)	309.5 (6.8)
Males	487.0 (13.94)	521.8 (11.94)	513.7 (14.69)	494.9 (15.23)	496.2 (9.30)	503.2 (12.29)
Mean body weight gain (g)						
Females	206.7 (5.7)	210.6 (8.7)	215.8 (8.3)	216.7 (6.5)	234.7 ^a (6.6)	210.1 (7.7)
Males	389.07 (11.81)	417.33 (10.94)	408.93 (14.79)	391.80 (15.01)	393.67 (7.39)	398.53 (13.83)
Mean feed intake (g/animal/day)						
Females	19.79 (0.50)	20.07 (0.49)	20.07 (0.33)	20.32 (0.53)	20.44 (0.65)	19.56 (0.54)
Males	21.23 (2.52)	23.21 (2.26)	24.14 (2.97)	22.76 (1.94)	23.03 (2.20)	22.53 (2.31)
Mean fluid consumption (ml/animal/day)						
Females	34.75 (1.56)	35.71 (1.53)	35.69 (1.77)	34.65 (1.21)	35.91 (1.35)	34.43 (1.77)
Males	36.76 (1.44)	35.37 (1.55)	38.75 (1.55)	35.92 (2.06)	34.05 (1.12)	36.84 (1.37)
Mean kidney weight (% body weight)						
Females	0.34 (0.02)	0.33 (0.01)	0.32 (0.01)	0.32 (0.01)	0.31 (0.01)	0.33 (0.01)
Males	0.28 (0.01)	0.29 (0.01)	0.29 (0.01)	0.30 (0.005)	0.30 (0.01)	0.30 (0.01)

^a Significantly different from group 1: $p < 0.05$; Duncan's multiple range test.

^b \pm standard error of the mean with 15 rats per group.

uranium has been upon its chemical toxicity. The U.S. EPA guidance levels for uranium were formerly estimated based upon the radiation hazard (Cothorn *et al.*, 1983), but chemical toxicity is now also taken into consideration (U.S. EPA, 1991). Uranium is a classic experimental nephrotoxin, and its use in high dosage for the induction of acute nephrotoxicity in animals has been well established (Cothorn *et al.*, 1983; Haley, 1982; Haley *et al.*, 1982; Moss, 1989). However, there are few data on the potential long-term toxicity of uranium (Haley *et al.*, 1982; Cothorn *et al.*, 1983; Leggett, 1989). This study was undertaken to provide an estimation of the no-observed-adverse-effect level (NOAEL) in rats following exposure to uranium for 91 days.

METHODS

28-day range-finding study. Five groups of 10 male and 10 female weanling specific pathogen-free (SPF)-derived (about 60 g body wt) Sprague-Dawley rats (obtained from Charles River Breeding Laboratories Inc.) were exposed for 4 weeks to uranyl nitrate hexahydrate, $UO_2(NO_3)_2 \cdot 6H_2O$ (CAS No. 13520-83-7), in their drinking water. Simultaneously, control rats, 10 males and 10 females (group 1), were administered drinking water containing less than 0.001 mg U/L (concentration range over four determinations: 0.00078 to 0.00087 mg U/L). Exposed groups 2, 3, 4,

5, and 6 received drinking water with uranyl nitrate hexahydrate (UN: supplier British Drug House) added to concentrations of 0.96, 4.8, 24, 120, and 600 mg UN/L of water, respectively. UN was readily soluble up to these concentrations using gentle agitation from a magnetic stirrer.

All animals were housed individually in stainless-steel mesh cages with free access to food (Purina Rat Lab Chow; $U < 0.10 \mu\text{g/g}$) and drinking water, both during the standard quarantine period and during the study. Detailed clinical observations were conducted daily. Body weights were measured weekly. Feed intake and fluid consumption data were recorded. For the 28-day study, time-weighted average (TWA) doses were calculated from water intake data collected over Week 4 and from terminal body weights. Uranium equivalents were obtained by multiplying the uranyl nitrate hexahydrate value by 0.474.

After 4 weeks of treatment, all animals were anesthetized with ether and exsanguinated via the abdominal aorta. The following hematological parameters were determined for each animal: hemoglobin, packed cell volume (PCV), red blood cell counts (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and total white blood cell count (WBC). Cell counts were determined with a Baker 7000 cell counter. Differential WBC counts were conducted on blood samples from groups 1 (control) and 6 (600 mg UN/L).

Biochemical determinations were conducted on serum, using a Technicon SMA 12/60 microanalyzer, and included measurements of sodium, potassium, inorganic phosphate, total bilirubin, alkaline phosphatase (AP), aspartate aminotransferase (AST), total protein, calcium, cholesterol, glucose, uric acid, and lactic dehydrogenase (LD). Sorbitol dehydrogenase (SDH)

TABLE 3
Uranyl Nitrate Dosage (mg UN/kg Body wt/Day^a) at Weeks 1, 6, and 12 of Treatment of Female and Male Rats with Uranyl Nitrate (UN)

Group number:		1	2	3	4	5	6
Exposure (mg UN/L):		0	0.96	4.8	24	120	600
Study week							
1	Females	0.0003 (0.00001)	0.27 (0.01)	1.32 (0.06)	6.85 (0.23)	32.09 (0.42)	167.21 (7.57) ^b
	Males	0.0002 (0.00001)	0.20 (0.006)	1.05 (0.03)	5.29 (0.04)	27.63 (2.00)	124.62 (2.88)
6	Females	0.0001 (0.00001)	0.15 (0.01)	0.75 (0.07)	3.56 (0.12)	18.01 (0.90)	100.19 (7.05)
	Males	0.0001 (0.0001)	0.10 (0.005)	0.56 (0.03)	2.66 (0.17)	13.08 (0.66)	67.76 (3.58)
12	Females	0.0001 (0.00002)	0.14 (0.01)	0.68 (0.04)	3.16 (0.13)	16.13 (1.28)	84.43 (4.77)
	Males	0.0001 (0.0002)	0.07 (0.005)	0.43 (0.04)	2.18 (0.18)	9.83 (0.58)	49.77 (3.87)
TWA uranium equivalent dose (mg U/kg body wt/day) ^c							
	Females	<0.0001	0.09	0.42	2.01	9.98	53.56
	Males	<0.0001	0.06	0.31	1.52	7.54	36.73

^a Calculated from weekly fluid consumption data and body weights.

^b ± standard error of the mean.

^c TWA dosage: time-weighted average dosage, calculated from the area under the dose-time curve assuming a linear relationship of dose and time between Study Weeks 1 to 6 and 6 to 12. Uranium equivalent = uranyl nitrate hexahydrate × 0.474.

activity was determined according to an automated method (Yagminas and Villeneuve, 1977).

Gross pathological examinations were conducted on all animals at necropsy. Organ weights were obtained for brain, heart, liver, spleen, and

kidneys. The following tissues were fixed in 10% buffered formalin (pH 7.4): adrenal, bone marrow, brain (three sections), bronchi, colon, duodenum, epididymis, esophagus, stomach (gastric cardia, fundus, and pylorus), heart, kidney, liver, lungs, mesenteric and mediastinal lymph nodes.

TABLE 4
Selected Hematological and Biochemical Parameters of Female and Male Rats after 91 Days Treatment with Uranyl Nitrate (UN)

Group number:		1	2	3	4	5	6
Exposure (mg UN/L):		0	0.96	4.8	24	120	600
Mean hemoglobin (g/L)							
	Females	145 (1) ^a	147 (1)	149 (2)	150 ^b (2)	147 (1)	148 (1)
	Males	145 (3)	146 (1)	145 (1)	148 (1)	145 (1)	143 (2)
Mean erythrocytes (×10 ¹² /L)							
	Females	6.69 (0.08)	7.16 (0.07)	7.18 (0.10)	7.36 ^b (0.11)	7.22 (0.10)	7.26 (0.10)
	Males	7.59 (0.18)	7.84 (0.10)	7.74 (0.08)	7.84 (0.08)	7.64 (0.10)	7.62 (0.12)
Mean corpuscular hemoglobin (pg)							
	Females	20.9 (0.2)	20.5 (0.2)	20.7 (0.2)	20.3 ^b (0.1)	20.3 ^b (0.2)	20.3 ^b (0.1)
	Males	19.2 (0.2)	18.7 (0.2)	18.8 (0.1)	18.8 (0.1)	19.0 (0.2)	18.8 (0.2)
Mean glucose (mmol/L)							
	Females	8.9 (0.2)	8.2 (0.2)	8.4 (0.3)	8.5 (0.2)	8.5 (0.3)	8.8 (0.3)
	Males	8.7 (0.2)	8.8 (0.2)	9.5 ^b (0.3)	9.0 (0.2)	9.4 ^b (0.2)	9.1 (0.3)

^a ± standard error of the mean with 15 rats per group.

^b Significantly different from group 1: $p < 0.05$; Duncan's multiple range test.

TABLE 5
Uranium Residues ($\mu\text{g/g}$) in Selected Tissues of Female and Male Rats after 91 Days Treatment with Uranyl Nitrate (UN)^a

Group Number:	1	5	6
Exposure (mg UN/L):	0	120	600
TWA uranium equivalent dose ^b (mg U/kg body wt/day)			
Females:	<0.0001	9.98	53.56
Males:	<0.0001	7.54	36.73
Animals sampled (female/male):	15/15	6/5	6/6
<hr/>			
Kidney			
Females	<0.2	0.42 (0.04) ^c	1.67 (0.37)
Males	<0.2	0.42 (0.07)	2.12 (0.81)
Bone			
Females	<0.2	0.50 (0.14)	0.73 (0.13)
Males	<0.2	0.40 (0.15)	0.97 (0.14)
Brain			
Females	na	na	nd
Males	na	na	0.43 (0.53)
Liver and spleen			
Females and males	na	na	nd

Note. na, not analyzed; nd, not detected.

^a Reported (kidney and bone) in Tracy *et al.* (1992).

^b Time-weighted average uranium equivalent dose. Calculated from the area under the dose-time curve assuming a linear relationship of dose and time between Study Weeks 1 to 6 and 6 to 12. Uranium equivalent = uranyl nitrate hexahydrate \times 0.474.

^c \pm standard error of mean.

ovary, pancreas, parathyroid, pituitary, salivary glands, skeletal muscle, spleen, testes, thoracic aorta, thymus, thyroid, trachea, and uterus. All tissues were processed through paraffin embedding, sectioned at 6 μm , and stained with hematoxylin and eosin (H&E). The blocks containing renal tissue were subsequently recut at 5 μm and stained with H&E, Heidenhain's iron hematoxylin (HN), and periodic acid-Schiff, methenamine-silver (PAMS) for more specific identification of cytoplasmic and basement membrane changes. Fatty change in the liver was confirmed in frozen sections as previously described (Villeneuve *et al.*, 1979). The animals and tissues were examined by a pathologist without knowledge of the experimental protocol, according to a predetermined and standardized scoring system provided in the Lab Cat program for histopathology (Innovative Programming Associates, NJ) which incorporated a severity scale from normal or minimal change (1), to mild (2), moderate (3), and marked change (4) for each tissue examined. Tissues were also evaluated within these categories as to whether the changes were focal, locally extensive, multifocal, or diffuse.

Uranium residues were examined in samples of brain, liver, spleen, blood, kidney, and bone from all female dose groups but not males, as reported in Tracy *et al.* (1992), with a lower limit of detection of about 0.03 $\mu\text{g/g}$.

Statistical analyses of all data other than the pathology scores were carried out using a one-way analysis of variance, followed by a Duncan's multiple range test to assess which groups were significantly different ($p < 0.05$) from the controls (Nie *et al.*, 1977). The incidence scores of histopathological lesions were analyzed by the Fisher exact test. Kidney and liver lesion severity scores were evaluated by an analysis of variance followed by Duncan's multiple range test and by Dunnett's t test.

91-day subchronic study. The exposure levels for the 91-day study were identical to those in the 28-day range-finding study. The number of animals was increased to 15 per sex per group. The animals were necropsied

after 13 weeks of treatment. Water and food intake were measured during three weekly periods (Weeks 1, 6, and 12). Otherwise, the experimental conditions of the study and the collection and analyses of various samples were as described for the 28-day study.

The TWA doses were calculated from the area under the dose-time curve, assuming a linear relationship of dose and time between Study Weeks 1-6 and 6-12. Body weights measured at these times were used in the calculations.

RESULTS

28-Day Range-Finding Study

The time-weighted average equivalent dose of uranium, expressed as mg U/kg body wt/day, for groups 1-6, respectively, were <0.0001, 0.07, 0.33, 1.65, 7.82, and 40.00 for females, and <0.0001, 0.05, 0.27, 1.34, 6.65, and 35.30 for males. No differences in clinical signs were observed between the exposed and control rats. No significant dose-related effects were observed on body weight gain, feed intake, or fluid consumption.

No dose-related effects were evident in hematological parameters, including hemoglobin, PCV, RBC, MCV, MCHC, platelet count, WBC, and differential white blood cell counts. Serum uric acid levels appeared to increase with the level of uranyl nitrate treatment, although only group 6 females showed uric acid levels significantly greater than controls (i.e., 1.64 vs

TABLE 6
Kidney and Liver Lesion Incidence Summary in Male Rats after 91 Days Treatment with Uranyl Nitrate (UN)

Group number:	1	2	3	4	5	6
Exposure (mg UN/L):	0	0.96	4.8	24	120	600
Animals examined per group:	15	15	15	15	15	15
Kidney						
Glomerular adhesions	2 ^a	4	10	10	10	11
Tubular						
Cytoplasmic shedding	13	9	12	12	14	13
Cytoplasmic inclusions	14	14	13	15	14	13
Apical displacement of nuclei	0	6	1	5	0	2
Cytoplasmic vacuolation	0	9	7	12	9	7
Nuclear vesiculation	0	6	10	6	6	5
Tubular dilation	0	4	5	10	4	5
Tubular atrophy	0	0	0	1	3	1
Cytoplasmic degranulation	0	2	11	13	7	7
Interstitial						
Collagen sclerosis	0	1	2	1	2	12
Reticulin sclerosis	4	5	8	5	8	10
Lymphoid cuffing	0	6	6	2	7	10
Liver						
Accentuation of zonation	0	2	1	0	8	10
Nuclei						
Anisokaryosis	0	5	10	14	15	15
Vesiculation	0	0	7	2	0	0
Hyperchromicity	0	0	1	12	15	15
Cytoplasm						
Increased portal density	1	1	1	5	15	13
Perivenous vacuolation	0	5	1	3	0	0
Increased perivenous homogeneity	3	14	14	13	15	15

^a Number of animals in group with indicated abnormalities.

1.18 mg/dl; $p < 0.05$). Other clinical chemistry parameters were not affected in a dose-related manner.

Compared to controls there were no significant differences in organ weights of males or females, in any dose group at the conclusion of this study. Significantly increased tissue uranium levels were detected in the kidney and bone in group 6 females (Table 1). Tissue uranium levels were not measured in males in the 28-day study.

Gross pathological examination was performed in all animals, but histopathological evaluation was performed only on the control group and the highest exposure group (600 mg UN/L). Quantitative analysis of the histopathological data did not identify any significant differences between control and high-dose animals in either the male or the female group, although there was a very small increase in the number of affected animals in the high-dose group. Based upon these observations, the 91-day toxicity study was designed using the same dose levels as the 28-day study.

91-Day Subchronic Study

No treatment-related clinical signs were observed in the exposed animals. Terminal body weights were statistically

greater in groups 4 and 5 than in control (group 1) females. Group 5 females had a significantly greater body weight gain than group 1. However, these differences did not appear to be dose-related. There were no significant dose-related differences in kidney weight expressed as a percentage of body weight (Table 2). No significant differences were observed between control and exposed groups in average feed intake or water consumption (Table 2). Mean fluid consumption increased between Weeks 1 and 6 of the study; however, there were no significant dose-related differences. Since the concentrations of uranyl nitrate in the drinking water remained constant throughout the study, the dosage of uranyl nitrate per kilogram body weight decreased with age (Table 3). This decrease was most pronounced during the first 6 weeks of the study. The time-weighted average equivalent dose of uranium, expressed as mg U/kg body wt/day, for groups 1 through 6, respectively, were <0.0001 , 0.09, 0.42, 2.01, 9.98, and 53.56 for females and <0.0001 , 0.06, 0.31, 1.52, 7.54, and 36.73 for males (Table 3).

Hemoglobin and RBCs were significantly increased in group 4 females, and MCH values were slightly but significantly decreased in groups 4, 5, and 6 females (Table 4). Serum glucose levels were significantly increased in groups

TABLE 7
Kidney and Liver Lesion Incidence Summary in Female Rats after 91 Days Treatment with Uranyl Nitrate (UN)

Group number:	1	2	3	4	5	6
Exposure (mg UN/L):	0	0.96	4.8	24	120	600
Animals examined per group:	15	15	15	15	14	14
Kidney						
Glomerular adhesions	12 ^a	15	13	15	14	14
Capsular sclerosis	0	5	4	3	6	5
Tubular						
Cytoplasmic inclusions	14	13	15	14	14	14
Cytoplasmic vacuolation	1	3	6	2	4	2
Anisokaryosis	0	4	3	8	4	7
Nuclear vesiculation	0	6	6	7	4	7
Pyknosis	0	3	2	3	7	3
Tubular dilation	0	0	1	1	0	3
Tubular atrophy	0	0	2	1	0	1
Cytoplasmic degranulation	1	6	3	5	9	5
Interstitial						
Collagen sclerosis	1	3	2	1	0	2
Reticulin sclerosis	1	9	8	7	6	5
Liver						
Accentuation of zonation	0	5	3	4	4	3
Nuclei						
Anisokaryosis	0	11	15	15	14	14
Vesiculation	7	15	13	12	8	11
Hyperchromicity	0	0	2	6	13	14
Cytoplasm						
Increased portal density	0	11	15	14	11	12
Increased perivenous homogeneity	4	10	14	13	9	14

^a Number of animals in group with indicated abnormalities.

3 and 5 males (Table 4), and serum sodium levels were decreased in group 3 females. No dose-related trends were evident in these parameters, and no significant differences were observed in other biochemical parameters. Uranium residues were detected in bone and kidneys from males and females in groups 5 and 6; the kidney uranium concentrations in group 6 were about fivefold greater than those measured in group 5 (Table 5). Brain samples from males in group 6 also had detectable levels of uranium, whereas levels in liver and spleen samples were undetectable.

The incidence and severity of selected kidney and liver lesions are summarized, by exposure group, for males and females, in Tables 6-9. Significant renal histological changes (Figs. 1-6) were observed in males in tubules at the lowest exposure level with dilation (Fig. 2), apical displacement (Fig. 3), and vesiculation of tubular nuclei (Fig. 6), and cytoplasmic vacuolation (Fig. 3) and degranulation (Fig. 4). Other lesions, including glomerular adhesions and focal tubular degranulation, became significantly different above the 4.8 mg UN/L exposure level. Females had significant changes to both glomerular and tubular elements, the most important consisting of focal sclerosis of glomerular capsules (Fig. 5) and anisokaryosis and vesiculation of tubular nuclei. Reticulin changes were more significant than collagen changes in the renal supporting connective

tissues. Overall, the most important changes in the female study were sclerosis of glomerular capsules and reticulin sclerosis of tubular basement membranes and interstitial scarring, both of which are nonreparable lesions and occurred at the lowest exposure level.

Adaptive and likely reversible changes occurred in the liver of male and female rats including accentuation of zonation and anisokaryosis. Sinus hyperplasia was observed in the spleen of both males and females in a treatment-related manner which reached a significant difference from controls in group 6 (data not shown).

Nonspecific and likely reversible changes of the thyroid gland were similar in both sexes, but were more severe in males. Multifocal reduction of follicular size with increased epithelial height was a common observation in both sexes. Only males had decreased amount and density of colloid. These differences were significantly different from controls in groups 3-6 (males) and in groups 4 and 6 (females) (data not shown).

Although histopathological findings were generally less severe in the female animals, statistically significant changes to the liver and kidney were detected at the lowest exposure level in both females and males. In terms of permanent injury, the renal changes are the most significant in both male and female rats. It would appear that on the basis of

TABLE 8
Statistical Evaluation of Kidney and Liver Lesion Incidence Data for Male and Female Rats
after 91 Days Treatment with Uranyl Nitrate (UN)^a

Group number:	2	3	4	5	6
Exposure (mg UN/L):	0.96	4.8	24	120	600
Kidney					
Glomerular adhesions		**	**	**	**
Capsular sclerosis	×	×		×	×
Tubular					
Anisokaryosis	×		×	×	×
Pyknosis				×	×
Apical displacement of nuclei	**		*		
Nuclear vesiculation	**	***	**	**	*
	×	×	×	×	×
Cytoplasmic vacuolation	***	**	***	***	**
		×			
Cytoplasmic degranulation		***	***	**	**
	×			×	
Tubular dilation	*	*	***	*	*
Interstitial					
Collagen sclerosis					***
Reticulin sclerosis					*
Lymphoid cuffing	×	×	×	×	×
	**	**		**	***
Liver					
Accentuation of zonation				**	***
	×		×	×	
Nuclei					
Anisokaryosis	*	***	***	***	***
	×	×	×	×	×
Vesiculation	×	×	×	×	×
	×	×	×	×	×
Hyperchromicity			***	***	***
			×	×	×
Cytoplasm					
Increased portal density				***	***
	×	×	×	×	×
Perivenous vacuolation	*				
Increased perivenous homogeneity	***	***	**	***	***
	×	×	×	×	×

^a Fisher exact test.

Male	Female	Significantly different from control group
*	×	$p < 0.05$
**	×	$p < 0.01$
***	×	$p < 0.001$

these histopathological findings, a no-observed-effect-level for uranyl nitrate was not observed in this study.

DISCUSSION

Exposure-related signs of toxicity or modifications in normal appearance or behavior were not found in rats exposed to UN for 91 days at levels up to 600 mg/L drinking water (equivalent to a time-weighted average equivalent dose of 37 or 54 mg U/kg body wt/day for males and females, respectively).

Histopathological lesions were observed primarily in the liver, thyroid, and kidney in the rats exposed to UN. The lesions of the liver which were generally nonspecific nuclear and cytoplasmic reactive changes apparent in both males and females of all exposure groups were directly related to uranium exposure. The incidence and severity of liver lesions increased with dose, even though the uranium levels in the liver were below detection. Thyroid lesions similar to those observed in this study have also been reported in other subacute and subchronic rat studies involving different toxicants (Chu *et al.*, 1983, 1984), and would not seem to be

TABLE 9
Statistical Evaluation of Kidney and Liver Lesion Severity Data for Male and Female Rats
after 91 Days Treatment with Uranyl Nitrate (UN)^a

Group number:	2	3	4	5	6
Exposure (mg UN/L):	0.96	4.8	24	120	600
Kidney					
Glomerular adhesions			*		*
Glomerular congestion					xx
Tubular					
Cytoplasmic shedding	*				
Cytoplasmic inclusions			*	**	
Cytoplasmic vacuolation			*	*	**
Cytoplasmic degranulation				*	*
Apical displacement of nuclei	*				
Anisokaryosis			xx		xx
Nuclear vesiculation					xx
Tubular dilation			**		
Tubular atrophy				**	
Protein casts		x			
Interstitial					
Collagen sclerosis					**
Lymphoid cuffing				**	**
Liver					
Accentuation of zonation				*	**
Nuclei					
Anisokaryosis		*	**	**	**
Vesiculation	x	xx	xx	xx	xx
Hyperchromicity	xx	x	x	**	**
Cytoplasm					
Increased portal density				**	**
Perivenous vacuolation	x	xx	xx	xx	xx
Increased perivenous homogeneity	**	**	**	**	**
Increased perivenous and midzone homogeneity		xx	xx	xx	xx

^a Dunnett's *t* test.

Male	Female	Significantly different from control group
*	x	$p < 0.05$
**	xx	$p < 0.01$

specific to uranium exposure. As reported by others (Haley, 1982; Haley *et al.*, 1982; Morrow *et al.*, 1982; Cothorn *et al.*, 1983), the major tissue affected was the kidney. In males, the major kidney lesions were vesiculation and apical displacement of the proximal tubular nuclei and cytoplasmic vacuolation and tubular dilation. These tubular changes may result in permanent injury to basement membranes with loss of nephrons and reduced renal function. Similar kidney lesions have been reported in acute exposure studies to UN and other uranium compounds in the rat (Haley, 1982; Haley *et al.*, 1982; Lim *et al.*, 1987). Thickened tubular basement membranes which may be not uranyl related were also identified (Fig. 2).

In the exposed females, the most important changes were glomerular capsular sclerosis and reticulin sclerosis of the interstitial connective tissues. These focal changes, while not severe, are important because they are nonreparable, and would limit the kidney's functional reserve. Sustained exposures would likely increase the number of damaged glomeruli and ultimately would impair renal function. The finding of increased severity for cytoplasmic inclusions in the group 5 males which was reduced to a mild level in group 6 may result from uranyl inhibition of hepatic α_2 -microglobulin production, resulting in reduced renal accumulation. This was not found in females, and may reflect androgen dependence for the deposited protein. Lesions of the glomerular apparatus

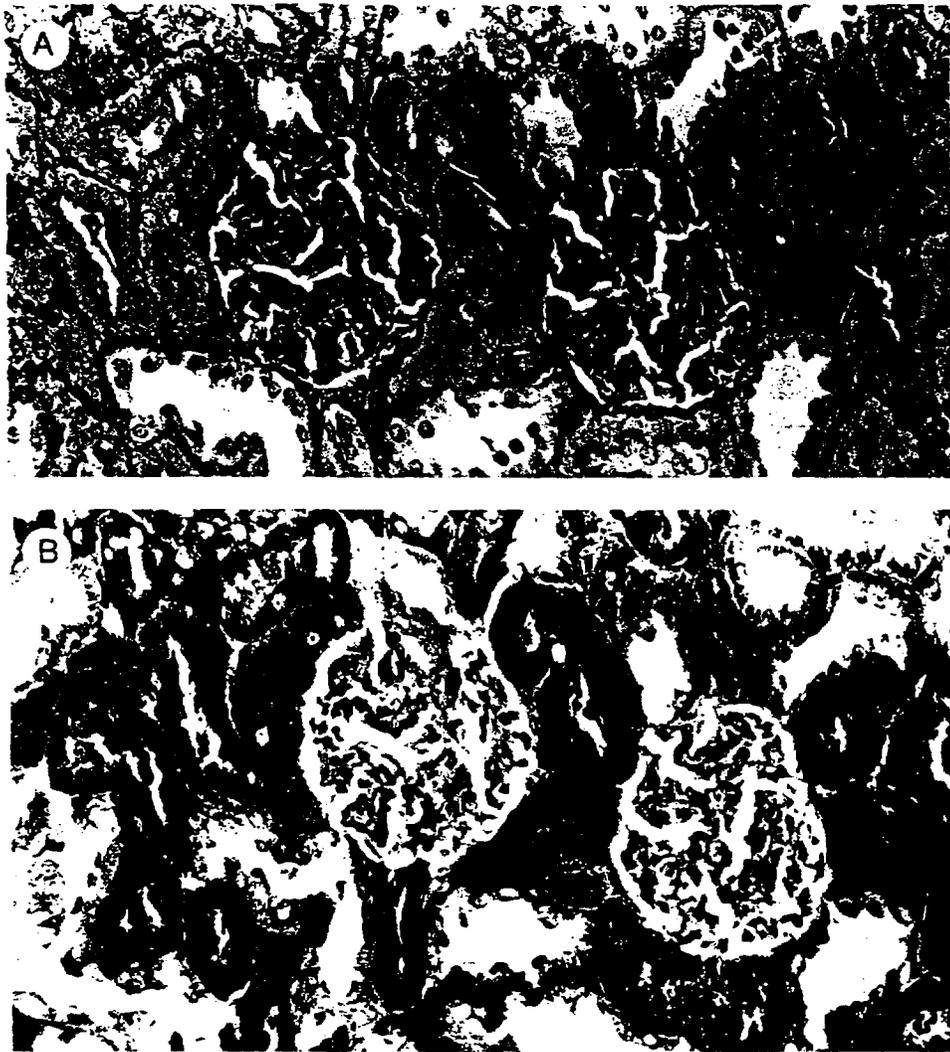


FIG. 1. Renal cortex from a female rat in the control group of the 91-day study. (A) Outer stripe cortex. Glomerular and tubular basement membranes are of normal and uniform thickness (arrow) and proximal and distal tubules have uniform diameter, cytoplasmic volume, and nuclei. PAMS $\times 320$. (B) Section to adjacent (A) demonstrating normal depth of cytoplasmic staining in proximal (dark) and distal (light) tubules. HN $\times 320$.

have been reported elsewhere following acute exposures to UN in male Sprague-Dawley rats (Haley, 1982) and in female Sprague-Dawley rats (Avasthi *et al.*, 1980), and to uranyl acetate in white rabbits (Kobayashi *et al.*, 1984).

In addition to the contrast between males and females in the type of kidney lesions observed, there were quantitative differences in their sensitivity to uranyl nitrate, which cannot be accounted for on the basis of uranium dosage: females consumed a higher time-weighted average dose (mg U/kg body wt/day) than males in every exposure group (Table 3).

Despite these qualitative and quantitative differences, kidney toxicity was evident at the lowest exposure level used in both males and females (i.e., 0.96 mg UN/L drinking water for 91 days; equivalent to time-weighted average doses of 0.06 or 0.09 mg U/kg body wt/day for males and females, respectively). Transient proteinuria and marked renal mor-

phological changes have been reported in rats following a single exposure of 50 μg UN/kg body wt, but this was following parenteral administration (Bentley *et al.*, 1985).

The explanation of the observed qualitative and moderate quantitative differences in sensitivity between male and female rats in the development of kidney lesions following UN exposure is not readily apparent. The renal toxicity of administered nephrotoxins may depend in part upon the sex of the animal (Ackerman and Hook, 1984). Adaptive responses of dog kidney to acute intravenous and inhalation exposure of uranyl fluoride has been described (Morrow *et al.*, 1982). The possibility of an acquired tolerance to uranium on the basis of morphological changes observed in regenerated proximal tubular cells has been recently reviewed (Leggett, 1989). It was suggested that the reduction in microvilli on luminal surfaces would result in reduced

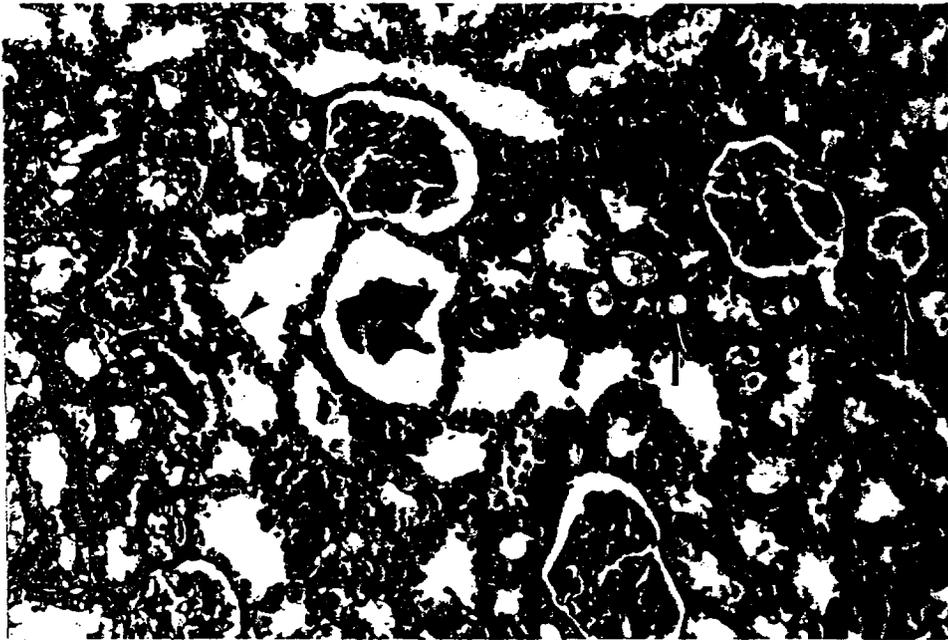


FIG. 2. Outer stripe renal cortex from a male rat which received 0.96 mg UN/L for 91 days. Multiple profiles of markedly dilated distal convoluted tubules. Epithelium is irregularly flattened and nuclei are irregularly spaced (arrowhead) and focally protruding into the lumen. Basement membranes are normal surrounding the dilated tubules and thickened around adjacent proximal tubules of reduced diameter (arrows). PAMS $\times 160$.

attachment of uranium to cell surfaces and thereby reduce its entry into cells. This may partly explain some experimental observations of apparent tolerance, but the significance of any such long-term protective effects remains uncertain.

Differences between males and females in sensitivity of the kidney to uranium do not seem to correlate with tissue uranium residue levels. Uranium concentrations in the kidney were only moderately higher in males than in females

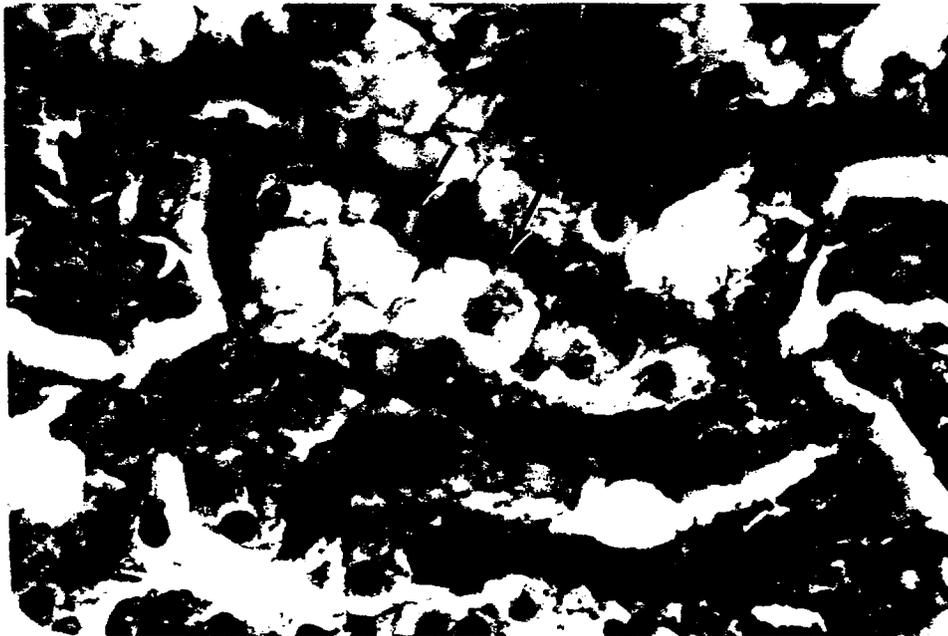


FIG. 3. Inner stripe renal cortex from a male rat which received 0.96 mg UN/L in drinking water for 91 days. Multifocal vacuolation of cytoplasm in a segment of proximal convoluted tubule with normal basement membranes. The vacuoles variably surround the nuclei and extend to the basement membrane (arrows). H&E $\times 720$.

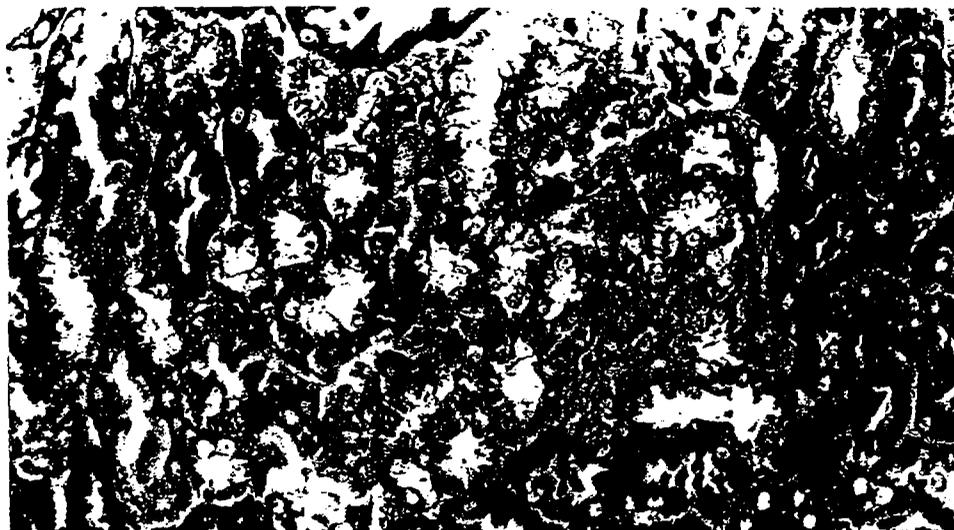


FIG. 4. Renal cortex from a male rat which received 120 mg UN/L in drinking water for 91 days. Multiple profiles of tubular injury with typical loss of density of cytoplasmic staining due to degranulation. HN $\times 320$.

in group 6, and similar in both sexes in group 5. This observation is surprising in view of the larger time-weighted average dose of uranium received by females. The increase in uranium residue level in kidney from group 5 to group 6 is proportional to the increase in dose level between those groups (Table 5). This suggests that basic pharmacokinetic parameters are similar between the sexes, and does not account for the reduced sensitivity in females.

A no-observed-adverse-effect level (NOAEL) was not achieved in male or female rats in the current study, since adverse renal and hepatic changes were seen in all exposed

groups. Based upon the frequency and degree of nonreparable degenerative lesions in the renal proximal convoluted tubule in males, and in the glomerulus of females, a lowest-observed-adverse-effect-level (LOAEL) of 0.96 mg UN/L drinking water can be reported for both the male and the female rats (TWA uranium equivalent dose 0.06 and 0.09 mg U/kg body wt/day, respectively). A simple linear interpolation of the kidney residue data available in Table 5 suggests that kidney tissue uranium concentrations would be close to the limit of quantitation ($0.2 \mu\text{g/g}$) at this exposure concentration.

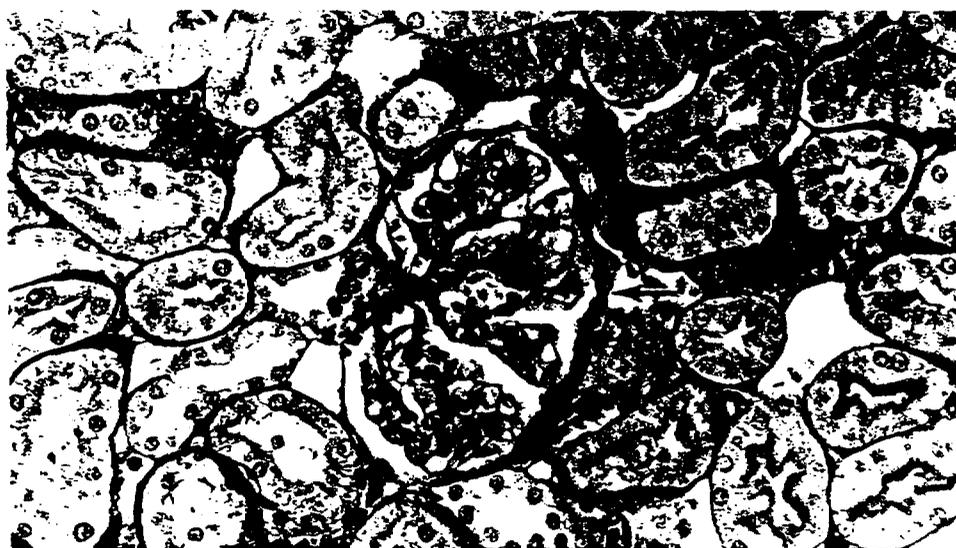


FIG. 5. Renal cortex from a female rat which received 600 mg UN/L of drinking water for 91 days. There is marked and irregular thickening of the parietal layer of Bowman's capsule typically opposite the vascular pole of the glomerulus (arrow). PAMS $\times 320$.



FIG. 6. Renal cortex from a male rat which received 600 mg UN/L of drinking water for 91 days. Focus of glomerular injury with accumulation of protein in the urinary space of the glomerulus and protein casts irregularly dilating adjacent proximal tubules (arrow). There is irregular vesiculation and pyknosis of nuclei with loss of tubular cytoplasmic staining density. Affected tubules have normal basement membranes (arrowhead). PAMS $\times 320$.

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Uranyl Nitrate: 91-Day Toxicity Studies in the New Zealand White Rabbit¹

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These studies were undertaken to derive a lowest-observed-adverse-effect level (LOAEL) in the New Zealand White rabbit following a 91-day exposure to uranium (U, as uranyl nitrate hexahydrate, UN) in drinking water. Males were exposed for 91 days to UN in their drinking water (0.96, 4.8, 24, 120, or 600 mg UN/L). Subsequently, females were similarly exposed for 91 days (4.8, 24, or 600 mg UN/L). Control groups were given tap water (<0.001 mg U/L). Regular observations were recorded, and urine was collected periodically. Four males showed evidence of *Pasteurella multocida* infection and were excluded from the study. Following the study, all animals were euthanized, and multiple hematological and biochemical parameters were determined. Necropsies were conducted, and histopathological examination was performed. The hematological and biochemical parameters were not affected in a significant exposure-related manner. Dose-dependent differences consisted of histopathological changes limited primarily to kidney. Changes in renal tubules were characteristic of uranium toxicity. Based on changes in the tubular nuclei, the 91-day LOAEL for males in this study is 0.96 mg UN/L drinking water. The females drank 65% more water than the males, yet appeared to be less affected by the exposure regimen, although they also developed significant tubular nuclear changes in their lowest exposure group, deriving a LOAEL of 4.8 mg UN/L. Tissue uranium residue studies suggested that pharmacokinetic parameters for the males and females differ, possibly accounting for the difference in observed sensitivity to UN. An adverse effect of *P. multocida* infection cannot be excluded. © 1998 Society of Toxicology.

Key Words: uranium; uranyl nitrate hexahydrate; subchronic exposure; drinking water; New Zealand White rabbit; nephrotoxicity.

In a related publication (Gilman *et al.*, 1998), we have reported 91-day studies of uranium (U) exposure in the Sprague-Dawley rat, where a lowest-observed-adverse-effect level (LOAEL) of 0.96 mg UN/L drinking water was derived for both males and females. The present studies were undertaken to provide an estimation of the LOAEL in the New Zealand White rabbit following exposure to uranium for 91 days.

METHODS

Five groups of 10 male weanling (initial body weight about 3200 g) New Zealand White rabbits (obtained from Charles River Breeding Laboratories Inc.) were exposed for 91 days to uranyl nitrate hexahydrate, $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (CAS No. 13520-83-7) in their drinking water. Concurrently, 10 control male rabbits (group 1) were given drinking water containing less than 0.001 mg U/L. Exposed animals (groups 2-6, respectively) received drinking water with uranyl nitrate hexahydrate (UN) added to concentrations of 0.96, 4.8, 24, 120, or 600 mg/L.

Similarly, female weanling New Zealand White rabbits (10 per group; initial body weight about 3100 g) from the same supplier were exposed to 4.8, 24, or 600 mg UN/L drinking water for 91 days (groups 2-4). Ten control females received drinking water containing less than 0.001 mg U/L (group 1). Female animals were purchased as specific pathogen free (SPF); males were not.

All animals were acclimated for 3 weeks prior to the start of the study and housed individually in stainless-steel mesh cages with free access to food (Purina Rabbit Lab Chow; U < 0.5 $\mu\text{g/g}$) and drinking water. The possibility of coprophagia was minimized through the use of wire mesh floors in the cages. Detailed clinical observations were conducted daily. Body weights were recorded weekly. Food and water intake were measured four times throughout the experiment.

After 30, 60, and 91 days of exposure, urine was collected from four male rabbits from each group. Volume was recorded and levels of the following analytes were determined: uranium, glucose, creatinine, urea nitrogen, total protein, albumin, lactic dehydrogenase (LD), γ -glutamyl transpeptidase (γ -GT), leucine aminopeptidase (LAP), and *N*-acetyl- β -D-glucosaminidase (NAG).

After 30 and 90 days of exposure in the female study, urine was collected from all the animals in group 4 (600 mg UN/L) and from all the controls. The following analytes were determined: glucose, creatinine, urea nitrogen, total protein, albumin, and NAG.

Dye clearance tests were performed 1 week before the termination of

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the studies, using standard bromsulphothalein (BSP) and phenolsulphothalein (PSP) test procedures to assess liver and kidney function, respectively (Boldrini and Tirone, 1980). Four male animals from each exposure group (three from group 3) were injected with BSP and PSP, and timed blood samples were collected subsequently to determine dye clearance. For the female study, six animals from each exposure group were similarly studied for PSP clearance only.

After 91 days of exposure, all animals were anesthetized with sodium pentobarbital and exsanguinated via the abdominal aorta. Routine hematological parameters were determined for each animal as previously described (Gilman *et al.*, 1998). Comprehensive biochemical determinations were conducted on serum from male and female rabbits, as described previously (Gilman *et al.*, 1998) but with the addition of urea nitrogen and creatinine.

Gross pathological examinations were conducted on all animals at necropsy. Organ weights were measured on brain, heart, liver, spleen, and kidneys. Histopathological examination was performed as previously described (Gilman *et al.*, 1998). All tissues were processed through paraffin embedding, sectioned at 6 μm , and stained with hematoxylin and eosin (H&E). The blocks containing renal tissue were subsequently recut at 5 μm and stained with H&E, Heidenhain's iron hematoxylin (HN), and periodic acid-Schiff, methenamine-silver (PAMS) for more specific identification of cytoplasmic and basement membrane changes. In the male study, fatty change in the liver was determined in frozen sections as described elsewhere (Villeneuve *et al.*, 1979). The animals and tissues were examined by a pathologist without knowledge of the experimental protocol, according to a predetermined and standardized severity scoring system as previously described (Gilman *et al.*, 1998).

Uranium residues were determined in samples of kidney and femur from five to six males in each exposure group and from all the female rabbits using the method described by Tracy *et al.* (1992). The detection limit for the analytical procedure for uranium measurement in these tissues was 0.03 $\mu\text{g/g}$.

Statistical analysis of the data was carried out as previously described (Gilman *et al.*, 1998).

RESULTS

Although all rabbits were expected to be pathogen free, male rabbits were not SPF derived, and four of the males were shown to have developed *Pasteurella multocida* infection during the course of the study. Two of these died during the study, and the other two presented with clinical signs (submandibular abscess) which were treated. All four infected males were removed from the study and statistical analysis. Two other animals died prematurely; necropsy findings were consistent with mucoid enteritis in one and ischemic or toxic renal failure in the other. These animals were also excluded from statistical analysis; however, it is possible that their signs represented treatment-related injury, since both were from group 6. In addition, two other animals were removed from the study because of gastric obstruction due to hair ball formation. Thus statistical analyses on the males are based on the surviving 52 animals that did not require any medical treatment. There was no evidence of *Pasteurella* infection in the female study.

The average dose of uranium in the exposed groups ranged from 0.05 to 28.7 mg U/kg body wt/day for males and from 0.49 to 43.02 for females (Table 1).

No significant differences in weight gain, food consumption, or water intake occurred between control and exposed groups in either sex throughout the experiments (Tables 2-3). Water and food consumption in controls and all exposed groups was lower at Week 12 than at

TABLE 1
Uranium Dosage Levels in Male and Female New Zealand White Rabbits during 91 Days Treatment with Uranyl Nitrate Hexahydrate (UN)

Group number	Exposure (mg UN/L)	Theoretical ^a U content (mg/L)	Measured ^b average U content (mg/L)	Average ^c water intake (ml/day)	Average ^d body weight (kg)	Average U intake (mg U/kg/day)
Males						
1	0	0.005	0.0	342	4.085	0.00
2	0.96	0.46	0.6	351	4.054	0.05
3	4.8	2.3	2.3	347	4.051	0.20
4	24.0	11.4	10.1	339	3.903	0.88
5	120.0	56.9	52.0	365	3.933	4.82
6	600.0	284.4	323.0	343	3.864	28.70
Females						
1	0	0.005	0.0	564	3.885	0.00
2	4.8	2.3	3.4	561	3.894	0.49
3	24.0	11.4	9.6	530	3.856	1.32
4	600.0	284.4	306.4	566	4.029	43.02

^a Uranyl nitrate hexahydrate \times 0.474 = uranium equivalent.

^b Average of three samples taken at intervals during the study.

^c Average of four consumption studies undertaken during Weeks 3, 7, 10, and 12 (males) and Weeks 3, 6, 8, and 12 (females).

^d Calculated from average of initial and final body weights.

TABLE 2

Body Weight and Food and Water Consumption in Male New Zealand White Rabbits during 91 Days Treatment with Uranyl Nitrate Hexahydrate

Group number (male)	Exposure (mg UN/L)	Week 3	Week 12	
1	0	Bw (kg)	3.825 ± 0.295 ^a	4.345 ± 0.402 ^a
		Food (g/day)	181 ± 28 ^a	148 ± 20 ^a
		Water (ml/day)	382 ± 72 ^a	318 ± 79 ^a
2	0.96	Bw (kg)	3.779 ± 0.398 ^b	4.329 ± 0.547 ^b
		Food (g/day)	183 ± 37	142 ± 27
		Water (ml/day)	381 ± 77	334 ± 86
3	4.8	Bw (kg)	3.704 ± 0.299	4.397 ± 0.446
		Food (g/day)	180 ± 36	153 ± 17
		Water (ml/day)	358 ± 80	325 ± 93
4	24.0	Bw (kg)	3.620 ± 0.249 ^b	4.185 ± 0.451 ^b
		Food (g/day)	173 ± 37 ^b	157 ± 18 ^b
		Water (ml/day)	366 ± 48 ^b	320 ± 49 ^b
5	120.0	Bw (kg)	3.660 ± 0.206 ^b	4.205 ± 0.307 ^b
		Food (g/day)	186 ± 39 ^b	152 ± 23 ^b
		Water (ml/day)	407 ± 44 ^b	349 ± 71 ^b
6	600.0	Bw (kg)	3.531 ± 0.280 ^c	4.196 ± 0.302 ^c
		Food (g/day)	176 ± 36 ^c	154 ± 19 ^c
		Water (ml/day)	378 ± 63 ^c	321 ± 71 ^c

Note. Bw, body weight; food, food consumption; water, water consumption. All data are expressed as means ± SD, and $n = 10$ unless otherwise noted. Exposed group means and controls were not significantly different within weeks.

^a $n = 9$.

^b $n = 8$.

^c $n = 7$.

Week 3. However, the females consumed about 65% more water overall than the males, resulting in the difference in average dose received.

Water and food consumption declined in the controls and in all exposure groups, both male and female, as the study proceeded. Nevertheless, food and water intake was similar among all groups of the same sex in the study, and this decline did not appear to be dose dependent (Tables 2-3).

In male rabbits, no urinary parameters were affected after 30 days of treatment (data not shown). At the end of 60 days, group 4 rabbits had significantly higher NAG activity ($p < 0.05$), and group 6 rabbits had a significantly higher total protein ($p < 0.05$) than the controls (data not shown). Following 91 days of treatment, there were no significant differences between the urine parameters of any exposed male group and controls.

There were no significant differences between urinary parameters of female rabbits in the control group and group 4 (600 mg UN/L), after either 30 or 90 days of exposure (data not shown).

In males, there was no significant relationship between BSP retention and the UN exposure level. There was, however, a significant ($p < 0.01$) linear relationship between exposure level and rate of PSP excretion, expressed as the reduction of log PSP concentration (data not shown). This effect was most pronounced in the highest exposure group, and detectable in the second highest exposure group. In females, no significant effects on the rate of excretion of PSP were observed.

A number of changes were observed in various hematological and biochemical parameters, none of which appeared to be dose dependent (data not shown). In the male rabbits, the hematocrits in groups 2 and 3 were significantly lower than that of the control group ($p < 0.05$). The percentage of polymorphonuclear leukocytes in group 5 males was significantly higher than that in the controls ($p < 0.05$). In the female rabbits, hemoglobin was found to be significantly lower in group 4 (600 mg UN/L) than in the control group ($p < 0.05$). Biochemical analysis of serum samples in male rabbits showed LD activity to be significantly increased in group 4 compared

TABLE 3

Body Weight and Food and Water Consumption in Female New Zealand White Rabbits during 91 Days Treatment with Uranyl Nitrate Hexahydrate

Group number (female)	Exposure (mg UN/L)	Week 3	Week 12	
1	0	Bw (kg)	3.460 ± 0.142	4.309 ± 0.209
		Food (g/day)	240 ± 16	227 ± 27
		Water (ml/day)	645 ± 177	523 ± 118
2	4.8	Bw (kg)	3.565 ± 0.262 ^a	4.222 ± 0.359
		Food (g/day)	228 ± 34	211 ± 39
		Water (ml/day)	697 ± 184	515 ± 140
3	24.0	Bw (kg)	3.505 ± 0.183	4.206 ± 0.237
		Food (g/day)	241 ± 31	229 ± 31
		Water (ml/day)	590 ± 104	500 ± 66
4	600.0	Bw (kg)	3.638 ± 0.188	4.420 ± 0.184
		Food (g/day)	245 ± 35	243 ± 49
		Water (ml/day)	667 ± 169	560 ± 134

Note. Bw, body weight; food, food consumption; water, water consumption. All data are expressed as means ± SD, and $n = 10$ unless otherwise noted. Exposed group means and controls were not significantly different within weeks.

^a $n = 9$.

TABLE 4
Uranium Residues ($\mu\text{g/g}$) in Kidney and Bone of Male and Female New Zealand White Rabbits
after 91 Days Treatment with Uranyl Nitrate

Group number	Exposure (mg UN/L)	Uranium concentration ($\mu\text{g/g}$)			
		<i>n</i>	Kidney	<i>n</i>	Femur
Males					
1	0	5	0.05 \pm 0.01	5	0.05 \pm 0.01
2	0.96	6	0.04 \pm 0.01	5	0.09 \pm 0.05
3	4.8	6	0.50 \pm 0.22	5	0.05 \pm 0.01
4	24.0	6	0.73 \pm 0.20	5	0.30 \pm 0.15
5	120.0	6	2.97 [†] \pm 1.45	5	0.76 \pm 0.24
6	600.0	5	4.98 [†] \pm 0.82	5	4.04 [†] \pm 0.55
Females					
1	0	10	0.010 \pm 0.005	10	0.013 \pm 0.002
2	4.8	10	0.019 \pm 0.004	10	0.053 \pm 0.004
3	24.0	10	0.07 \pm 0.01	10	0.15 \pm 0.01
4	600.0	10	1.03 [†] \pm 0.15	10	3.06 [†] \pm 0.31

Note. All errors are standard errors of the mean.

[†] Significantly different from control group: $p < 0.05$; Duncan procedure.

to the control group ($p < 0.05$). In females, group 2 (4.8 mg UN/L) had significantly decreased serum glucose levels compared to the control group ($p < 0.05$).

For both male and female rabbits, neither organ weights nor organ to body weight ratios were significantly affected by the administration of UN (data not shown). The

tissue levels of uranium showed qualitative and quantitative differences between the males and females (Table 4). In males, levels of uranium in kidney tended to be slightly higher than in bone. In females, the converse was the case, and the levels of uranium in female kidneys was generally one-fifth of that detected in males.

TABLE 5
Tabular Summary of Kidney Lesion Incidence of Male New Zealand White Rabbits
after 91 Days Treatment with Uranyl Nitrate (UN)

Group number:	1	2	3	4	5	6
Exposure (mg UN/L):	0	0.96	4.8	24	120	600
Animals examined per group:	10	10	9	10	10	9
Glomerular						
Adhesions	8 ^a	10	9	10	10	9
Tubular						
Cytoplasmic inclusions	9	10	9	10	10	7
Apical displacement of nuclei	0	0	0	1	0	4
Cytoplasmic vacuolation	0	5	3	6	4	6
Anisokaryosis	0	5	8	9	9	9
Hyperchromicity	0	0	6	8	9	9
Nuclear vesiculation	0	8	8	10	10	9
Nuclear pyknosis	0	3	4	6	7	5
Tubular dilation	0	3	2	3	4	4
Protein casts	0	1	2	1	0	4
Atrophy	0	2	2	1	4	5
Interstitial						
Collagen sclerosis	0	1	0	2	5	7
Reticulin sclerosis	0	3	3	6	10	8

^a Number of animals in group with indicated abnormalities.



FIG. 1. Renal cortex from a male rabbit in the control group. Tubular diameter, cytoplasmic volume and density, nuclear size, and basement membranes are normal and relatively uniform throughout. PAMS $\times 320$.

Exposure to UN was associated with significant dose-related histopathological changes most prominent in the kidney and thyroid gland and to a lesser extent in the liver. A summary of the incidence of kidney lesions is presented in Tables 5 and 6. The statistical analyses of incidence and severity evaluations are presented in Tables 7 and 8.

TABLE 6

Tabular Summary of Kidney Lesion Incidence of Female New Zealand White Rabbits after 91 Days Treatment with Uranyl Nitrate (UN)

Group number:	1	2	3	4
Exposure (mg UN/L):	0	4.8	24	600
Animals examined per group:	10	10	10	9
Glomerular				
Adhesions	10 ^a	10	10	9
Tubular				
Cytoplasmic inclusions	10	8	10	9
Cytoplasmic vacuolation	0	3	3	3
Anisokaryosis	0	4	8	9
Nuclear hyperchromicity	0	0	0	9
Nuclear vesiculation	0	5	8	9
Nuclear pyknosis	0	1	0	5
Tubular dilation	3	10	6	9
Protein casts	5	6	2	3
Atrophy	0	7	5	3
Tubular pigmentation	0	2	1	5
Interstitial				
Collagen sclerosis	2	2	3	9
Reticulin sclerosis	5	7	10	9

^a Number of animals in group with indicated abnormalities.

Histopathological Findings in Male and Female Rabbits

Histopathological changes occurred in the kidneys of male and female rabbits (Figs. 1-3). These consisted of foci of cytoplasmic vacuolation in proximal renal tubular epithelium (Fig. 2A) resting on normal basement membranes (Fig. 2B) in animals at the lowest exposure level. The cytoplasmic changes were accompanied by both vesiculation and pyknosis of tubular nuclei both at the lowest (Fig. 2A) and at higher dose levels (Fig. 3A) where the epithelium was injured prior to any detectable change in the tubular basement membranes (Fig. 3B).

Changes in the thyroid gland consisted of an irregular increase in epithelial height from flattened or cuboidal epithelium to epithelium that was approximately three to four times normal height, with clear foamy cytoplasm and with vesiculation of nuclei. Hepatic changes were minimal, consisting of accentuation of zonation occurring at the architectural level as well as hepatocellular anisokaryosis.

Metaplastic changes occurred in the aorta in a small number of treated and control animals. They ranged from focal laminar mineralization of the media to formation of well-developed bony plates with, in one case, a bone marrow cavity. These lesions were generally accompanied by other minor changes in the endothelium and underlying elastic membrane, suggesting a general metabolic etiology rather than the result of a prior atherosclerotic plaque. These changes did not appear to be dose related.

DISCUSSION

The highest dose of UN (600 mg UN/L) administered to male rabbits under these study conditions was toxic

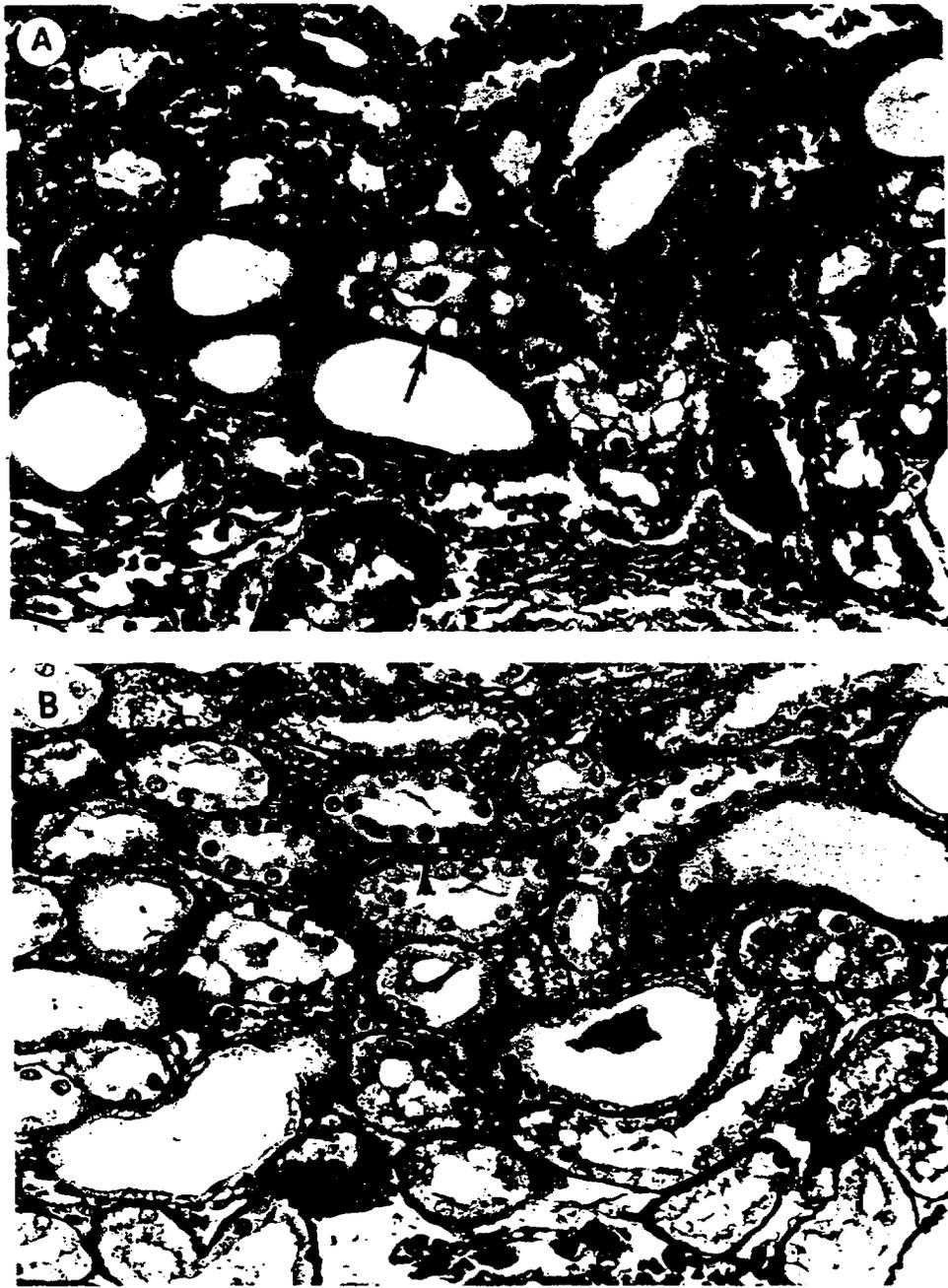


FIG. 2. Renal corticomedullary region from a male rabbit which received 0.96 mg UN/L of drinking water for 91 days. (A) There is marked multifocal vacuolation of thick ascending tubular cytoplasm with focal nuclear pyknosis (arrow) and luminal debris (above arrow). There is multifocal flattening of tubular epithelium with moderate vesiculation and anisokaryosis of tubular nuclei. H&E $\times 320$. (B) Adjacent section of same area. Basement membranes (arrowhead) are normal. PAMS $\times 320$.

and in some cases may have been lethal. Significant effects on kidney physiology and pathology were observed. The histopathological findings we observed in the kidney tubules were consistent with typical UN-induced nephrotoxicity (Moss, 1989). Dose-related changes in tubular nuclei were significantly different ($p < 0.05$) from con-

trols even in the 0.96 mg UN/L group (Tables 7 and 8). Significant changes in severity ($p < 0.01$) were found in 10 different morphological indicators of tubular injury in the highest exposure group (Table 8). In addition, significant changes were found in a treatment-related manner in renal interstitial connective tissue. In the ag-

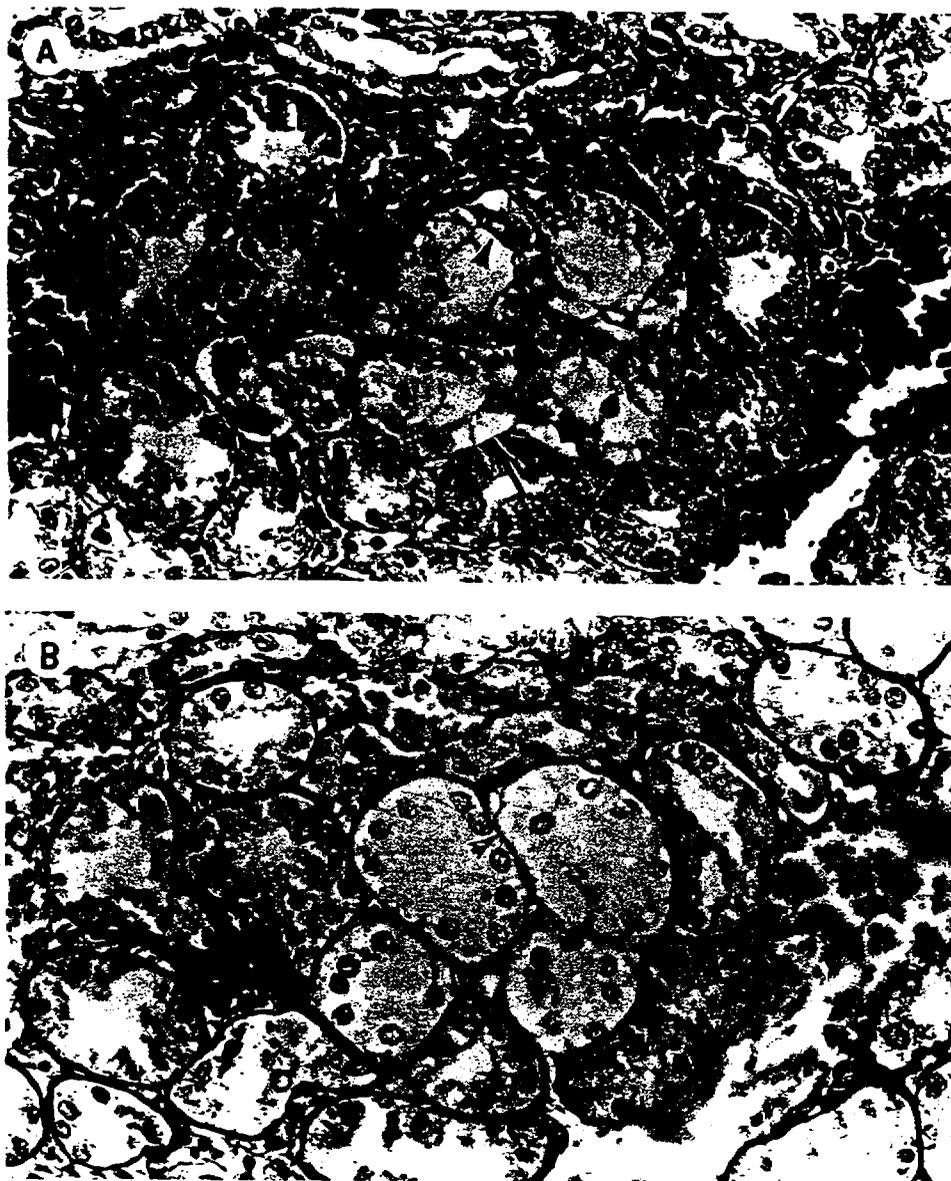


FIG. 3. Renal cortex of a male rabbit which received 24 mg UN/L of drinking water for 91 days. (A) Outer renal cortex with multiple profiles of proximal convoluted tubules with marked vacuolation of cytoplasm (arrow), reduced staining density, and vesiculation of nuclei (arrowhead). H&E $\times 320$. (B) Section adjacent to (A) demonstrating normal basement membranes (arrowhead). PAMS $\times 320$.

gregate, these changes indicate that all the male rabbits exposed to UN were affected seriously enough that some permanent kidney functional impairment was likely.

The changes in the aorta appeared to be sporadic and were not dose dependent. No similar aortic changes had been observed in our previous UN 91-day studies in rats (Gilman *et al.*, 1998).

Female animals in this study appeared much less affected by the exposure regimen than males, although significant nuclear changes (anisokaryosis and vesiculation) were seen in the lowest female exposure group (4.8 mg

UN/L). These changes, and other indicators of tubular injury, significantly increased with increasing exposure.

It is possibly significant that the female rabbits were specific pathogen free, and may have responded differently from the males, where 4 animals of 60 were shown to have contracted *P. multocida* infection. All animals known to be infected were excluded from the study, but the possibility that some remaining animals may have had subclinical infection cannot be excluded.

The sex of an animal can have a marked effect on the renal toxicity of administered nephrotoxins (Ackerman

TABLE 7

Statistical Evaluation of Kidney Lesion Incidence Data for New Zealand White Rabbits after 91 Days Treatment with Uranyl Nitrate (UN)^a

	2	3	4	5	6
Group number (male):	2	3	4	5	6
Group number (female):	—	2	3	—	4
Exposure (mg UN/L):	0.96	4.8	24	120	600
Glomerular					
Adhesions					
Tubular					
Cytoplasmic inclusions					*
Apical displacement of nuclei					**
Cytoplasmic vacuolation	*		**	*	**
Anisokaryosis	*	***	***	***	***
Nuclear hyperchromicity	—	◇	◇◇◇	—	◇◇◇
Nuclear vesiculation	***	***	***	***	***
Nuclear pyknosis	—	*	**	**	*
Tubular dilation	—			*	*
Protein casts	—	◇◇		—	◇◇
Atrophy	—			*	*
Tubular pigmentation	—			—	◇
Interstitial					
Collagen sclerosis	—			*	***
Reticulin sclerosis	—		**	***	***

^a Fisher exact test.

Male	Female	Significantly different from control group
*	◇	$p < 0.05$
**	◇◇	$p < 0.01$
***	◇◇◇	$p < 0.001$
—	—	No exposed group

and Hook, 1984). A similar effect was observed in 91-day rat studies, where female Sprague-Dawley rats exhibited less sensitivity to the nephrotoxic effects of UN than did males in equivalent dosage groups (Gilman *et al.*, 1998).

The female rabbits consumed on average 65% more water than the males (Tables 2-3). The reason for this is not readily apparent. The male study was conducted during January-March, while the female study was during the period August-November. Although the facility was climatically controlled, it is possible that there were minor temperature and humidity differences between the two studies. It is noteworthy that although the females consumed about 65% more water than the males and their average uranium intake was about 50% greater on a mg/

kg body wt/day basis (Table 1), their tissue uranium levels were not similarly raised for the highest exposure group. For the highest exposure group, the average kidney uranium level in females was only 20% of that in the males, while the average bone uranium level in females was 76% of that in the males (Table 4). These qualitative and quantitative differences between the males and females suggest that their pharmacokinetic parameters differ. This contrasts with our findings in rat studies, where differences in male/female sensitivity to UN could not be attributed to pharmacokinetic parameters (Gilman *et al.*, 1998). If the renal tissue levels of uranium in the female rabbits were below or close to the threshold concentration for renal injury, this could account for their reduced sensitivity compared to the males.

TABLE 8

Statistical Evaluation of Kidney Lesion Severity Data for New Zealand White Rabbits after 91 Days Treatment with Uranyl Nitrate (UN)^a

	2	3	4	5	6
Group number (male):	2	3	4	5	6
Group number (female):	—	2	3	—	4
Exposure (mg UN/L):	0.96	4.8	24	120	600
Glomerular					
Adhesions	—		**	**	
Tubular					
Cytoplasmic inclusions					**
Apical displacement of nuclei					**
Cytoplasmic vacuolation					**
Anisokaryosis	—		**	**	**
Nuclear vesiculation	*	**	◇◇	—	◇◇
Nuclear pyknosis	—		◇◇	—	◇◇
Hyperchromicity	—		**	**	**
Tubular dilation	—	◇◇	◇	—	◇◇
Atrophy	—			—	**
Tubular pigmentation	—			—	◇◇
Protein casts	—			—	**
Interstitial					
Collagen sclerosis	—			*	**
Reticulin sclerosis	—		*	**	**

^a Dunnnett's *t* test.

Male	Female	Significantly different from control group
*	◇	$p < 0.05$
**	◇◇	$p < 0.01$
—	—	No exposed group

Although rabbits have been used in uranyl-induced acute renal failure studies (Kobayashi *et al.*, 1984), no published studies could be found reporting an estimated renal threshold concentration for uranium (U_{tc}) in the rabbit. Various estimates for U_{tc} have been published for rats, suggesting a value in the range of 0.7–1.3 $\mu\text{g/g}$, where much more marked changes were observed with renal tissue uranium levels in the range 1.3–3.5 $\mu\text{g/g}$ (Diamond *et al.*, 1987). Estimates of U_{tc} in other species have been reported as low as 0.3 $\mu\text{g U/g}$ in dogs (Morrow *et al.*, 1982). On the basis of current information, it has been suggested recently that the level of 3 $\mu\text{g U/g}$ used as a guidance level for limiting human occupational exposures should be lowered by about an order of magnitude (Leggett, 1989). The lowest-observed-adverse-effect level (LOAEL) of 0.96 mg UN/L reported here for males corresponded to a mean renal tissue uranium concentration of 0.04 $\mu\text{g/g}$, while the LOAEL of 4.8 mg UN/L for females corresponded to a concentration of 0.02 $\mu\text{g U/g}$. These estimates are considerably less than the U_{tc} noted above for rats and dogs.

In terms of nuclear changes in particular, a no-observed-adverse-effect level (NOAEL) was not identified in either the male or the female rabbit study, and a LOAEL was at the 0.96 mg UN/L exposure level (average dose equivalent 0.05 mg U/kg body wt/day) in males and at the 4.8 mg UN/L exposure level (average dose equivalent 0.49 mg U/kg body wt/day) in females. We have reported elsewhere a 91-day LOAEL of 0.96 mg UN/L drinking water for male and female Sprague-Daw-

ley rats, equivalent to doses of 0.06 and 0.09 mg U/kg body wt/day, respectively (Gilman *et al.*, 1998).

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Uranyl Nitrate: 91-Day Exposure and Recovery Studies in the Male New Zealand White Rabbit

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This study was undertaken to examine the reversibility of renal injury in the male New Zealand White rabbit subsequent to a 91-day exposure to uranyl nitrate (UN) in drinking water, followed by various recovery periods. Specific pathogen-free (SPF) animals were exposed for 91 days to UN in their drinking water (24 or 600 mg UN/L). Control groups were given municipal tap water (<0.001 mg U/L). Regular clinical observations were recorded, and urine was collected periodically. Recovery periods between the last UN exposure and termination were 0, 8, 14, 45, or 91 days. Following the study, all animals were anesthetized and terminated by exsanguination, and multiple hematological and biochemical parameters were determined. Necropsies were conducted, and histopathological examination was performed. Exposure-related histopathological changes were observed only at much higher doses than in our previous male rabbit study where non-SPF-free animals had been used. Minor increases in kidney to body weight ratios were observed in the high-dose groups following exposure and early recovery. Renal tubular injury with degenerative nuclear changes, cytoplasmic vacuolation, and tubular dilation was seen in the high-dose group, without consistent resolution even after 91 days recovery. Animals ingested approximately 33% more uranium per day in this study than did males in a comparable dose group in the previous study, yet their kidney tissue uranium residues were 30% lower. These results suggest that SPF rabbits are less sensitive to uranyl injury than the non-SPF animals. The lowest-observed-adverse-effect level is estimated to lie at or below 24 mg UN/L. © 1998 Society of Toxicology.

Key Words: uranium; uranyl nitrate hexahydrate; subchronic exposure; drinking water; New Zealand White rabbit; nephrotoxicity.

Previous studies in Sprague-Dawley rats suggested a 91-day lowest-observed-adverse-effect level (LOAEL) of 0.96

mg uranyl nitrate hexahydrate (UN)/L drinking water in both male and female rats (Gilman *et al.*, 1998a). In further studies, a LOAEL of 0.96 mg UN/L drinking water in the male New Zealand White rabbit was also derived following 91 days exposure (Gilman *et al.*, 1998b), based upon nuclear changes in the renal proximal tubular epithelium. The present study was undertaken to observe the reversibility of such pathological changes subsequent to a 91-day exposure to UN in drinking water, followed by various recovery periods.

METHODS

Seven groups (initial body weight about 3000 g) of male weanling specific pathogen-free (SPF) New Zealand White (NZ) rabbits (obtained from Charles River Breeding Laboratories Inc.) were exposed for 91 days to uranyl nitrate hexahydrate, $UO_2(NO_3)_2 \cdot 6H_2O$ (CAS No. 13520-83-7, in their drinking water. Exposed groups received drinking water with UN added to concentrations of 24 or 600 mg/L. Concurrently, three control groups were given municipal tap water containing less than 0.001 mg uranium (U) /L. After the 91-day exposure period, each group was allowed a recovery period of 0, 8, 14, 45, or 91 days (UN-treated water replaced with regular municipal tap water) before the scheduled termination (Fig. 1).

All animals were acclimated for 3 weeks prior to the start of the study and housed individually in stainless-steel mesh cages with free access to food (Purina Rabbit Lab Chow; U < 0.5 μ g/g) and drinking water. The possibility of coprophagia was minimized through the use of wire mesh floors in the cages. Detailed clinical observations were conducted daily, and body weights were recorded weekly. Food and water intake were measured eight times throughout the experiment.

A 48-h urine void was collected three times during the exposure phase and four times during the recovery phase. Volume was recorded and levels of various analytes were determined as described previously (Gilman *et al.*, 1998b). Phenolsulfonphthalein (PSP) dye and uranium clearance tests were performed on selected groups of animals on Days 50 and 84 of exposure, and on Days 43 and 84 of recovery as described previously (Gilman *et al.*, 1998b).

In order to study *in vivo* uranium clearance, whole-body counting studies were performed on one group of animals on the first day of the recovery period, and repeated at 24-h intervals thereafter for 6 days. This group was euthanized 8 days after the end of the exposure period. The results of these clearance studies have been reported elsewhere (Tracy *et al.*, 1992).

Following the postexposure recovery period, all animals were anesthetized with sodium pentobarbital and exsanguinated via the abdominal aorta.

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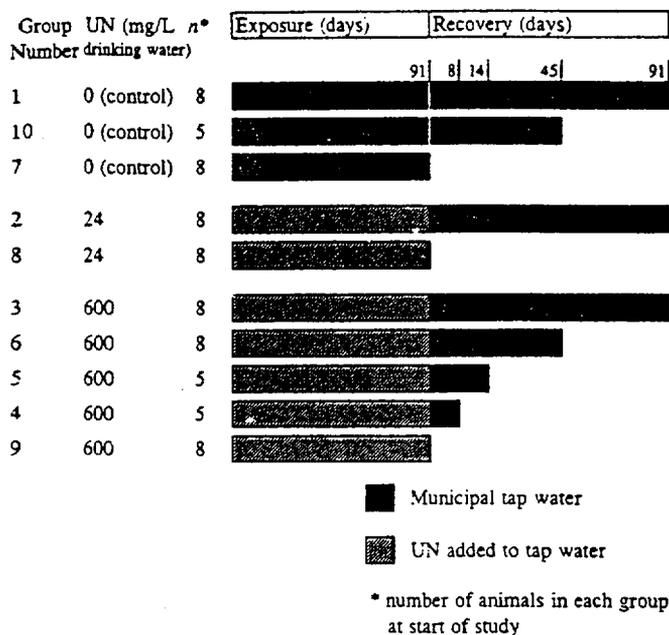


FIG. 1. Treatment levels and exposure times for uranyl nitrate in drinking water and recovery periods on untreated drinking water for male New Zealand White rabbits exposed to uranyl nitrate.

Routine hematological parameters were determined for each animal as previously described (Gilman *et al.*, 1998a). Biochemical determinations were conducted on serum, as described previously (Gilman *et al.*, 1998a,b).

Gross pathological examinations were conducted on all animals at necropsy. Organ weights were measured on heart, liver, spleen, and kidneys. Histopathological examination was performed as previously described (Gilman *et al.*, 1998a,b). All tissues were processed through paraffin embedding, sectioned at 6 μm , and stained with hematoxylin and eosin (H&E). The blocks containing renal tissue were subsequently recut at 5 μm and stained with H&E, Heidenhain's iron hematoxylin (HN), and periodic acid-Schiff, methenamine-silver (PAMS) for more specific identification of cytoplasmic and basement membrane changes. The animals and tissues were examined by a pathologist without knowledge of the experimental protocol, according to a predetermined and standardized scoring system as previously described (Gilman *et al.*, 1998a).

Uranium residues were determined in samples of kidney and femur, using the method described by Tracy *et al.* (1992). Results from uranium residue determinations on feces and urine have been reported previously (Tracey *et al.*, 1992).

Statistical analysis of the data was carried out as previously described (Gilman *et al.*, 1998a).

RESULTS

The average dose of uranium in the exposed animals was 1.36 or 40.98 mg U/kg body wt/day for the 24 and 600 mg UN/L groups, respectively (Table 1). No significant dose-related differences in food and water consumption or body weight gain occurred between the exposed and control groups throughout the experiment (Table 2). Significant ($p < 0.05$) dose-related changes are described below:

Urinary parameters. Group 3 animals (91 days exposure to 600 mg UN/L followed by 91 days recovery) showed the following changes in urine parameters: an initial decrease in output detected at Week 1, accompanied by increased excretion of glucose and protein and increased leucine aminopeptidase activity (Table 3). These latter changes persisted through Week 4. Seven days after the end of the 91-day exposure period (Study Week 14), urinary volume was increased, while among other parameters the only abnormality detected was continuing increased glucose excretion. At Weeks 16, 18, and 26 (i.e., 3, 5, and 13 weeks of postexposure recovery), there were no significant differences in urinary parameters between exposed animals and controls. Urinary glucose excretion continued to be elevated in the exposed animals, but was below the $p < 0.05$ level (Table 3). There were no significant dose-related differences in urinary parameters between the 24 mg UN/L exposed groups and controls (data not shown).

Hematology. No significant differences were detected either in the 24 or 600 mg UN/L exposed groups euthanized at the end of the 91-day exposure period, nor in the other 24 mg UN/L group euthanized after the 91-day recovery period. The groups exposed to 600 mg UN/L showed increases in percentage and total lymphocyte counts after 91 days recovery, accompanied by a proportionate decrease in the percentage and total polymorphonuclear counts (Table 4). The total WBC was not significantly different from controls.

Blood chemistry. Blood glucose was significantly increased in the 600 mg UN/L exposed group at the end of the 91-day exposure period, but did not remain significantly elevated in exposed groups sampled following any subsequent recovery period (Table 5). In the 600 mg UN/L group euthanized after 91 days exposure, several differences were observed in comparison with other similarly exposed groups following subsequent recovery periods: calcium became progressively elevated, while total protein, total bilirubin, lactic dehydrogenase, and sodium decreased. It should be noted that all of these latter changes, although significantly different between the 600 mg UN/L exposed groups, were not significantly different from the applicable recovery date control groups, and therefore did not seem to be exposure related. The only significant difference detected between a 600 mg UN/L exposed group following recovery and a control group was an elevated alkaline phosphatase at 91 days recovery (Table 5).

In vivo studies. The PSP tissue clearance studies showed no significant dose-related effects (data not shown).

Organ weights. No significant dose-related effects were detected in organ weights in the 24 mg UN/L exposed groups (data not shown). Kidney weight, expressed as a percentage of total body weight, was increased in the 600 mg UN/L group (0.27%) immediately following 91 days exposure

TABLE 1
Uranium Dosage Levels in Male New Zealand White Rabbits during 91 Days Treatment with Uranyl Nitrate Hexahydrate (UN)

Group number	Exposure (mg UN/L)	Theoretical ^a U content (mg/L)	Measured ^b average U content (mg/L)	Average ^c water intake (ml/day)	Average ^d body weight (kg)	Average U intake (mg U/kg/day)
1 + 7 + 10	0 ^{e,f}	0.00474	0.0	553	3.582	0.00
2 + 8	24.0 ^{e,g}	11.4	9.3	508	3.476	1.36
3 + 4 + 5 + 6 + 9	600.0 ^{e,h}	284.4	302.4	476	3.513	40.98

^a Uranyl nitrate hexahydrate \times 0.474 = uranium (U) equivalent.

^b Average of four samples taken at intervals during the study.

^c Average of four consumption studies undertaken during Weeks 2, 5, 8, and 11 of the exposure period.

^d Calculated from average of Week 3 and Week 12 body weight.

^e Pooled data; differences between groups were not significant.

^f Experimental groups 1, 7, 10; $n = 20$.

^g Experimental groups 2, 8; $n = 16$.

^h Experimental groups 3, 4, 5, 6, 9; $n = 33$.

compared with the exposed 24 mg UN/L group and applicable control (both 0.25%). This percentage steadily decreased during the recovery period and was not significantly different from controls by the 45th day of recovery or thereafter. No significant dose-related effects were detected following 91 days recovery postexposure to 600 mg UN/L.

Histopathology. Histopathological changes were observed in the kidney, thyroid gland, liver, and aorta. Major histological changes occurred in the kidneys of male rabbits (Figs. 2–7, Table 6). Focal dilation of renal proximal tubules resulted from exposure to both 24 mg UN/L, group 8 (Fig. 3), and 600 mg UN/L, group 9 (Fig. 5). Tubular dilation

TABLE 2
Body Weight and Food and Water Consumption in Male New Zealand White Rabbits during 91 Days Treatment with Uranyl Nitrate Hexahydrate (UN) and during Subsequent 91-Day Recovery Period

Group number	Exposure (mg UN/L)	Week 2	5	8	11	15	18	21	24	
1	0 (control); $n = 8$	Body weight (g) ^a	3072	3628	3815	4092	4141	4286	nd	4488
			(143) ^b	(168)	(176)	(218)	(197)	(272)	(—)	(210)
		Food (g/day)	158	313	293	246	217	208	222	219
			(11)	(47)	(44)	(33)	(34)	(34)	(39)	(43)
2	24.0; $n = 8$	Water (ml/day)	431	634	613	534	457	442	464	469
			(25)	(144)	(117)	(82)	(82)	(104)	(77)	(92)
		Body weight (g) ^a	3048	3501	3708	3903	3919	4063	nd	4277
			(169)	(188)	(207)	(173)	(122)	(165)	(—)	(179)
3	600.0; $n = 8$	Food (g/day)	160	308	280	235	194	200	208	200
			(7)	(36)	(22)	(19)	(24)	(21)	(19)	(23)
		Water (ml/day)	416	595	540	479	407	422	430	413
			(43)	(106)	(137)	(91)	(106)	(86)	(60)	(95)
3	600.0; $n = 8$	Body weight (g) ^a	3052	3543	3749	3973	4069	4223	nd	4437
			(192)	(250)	(323)	(373)	(416)	(426)	(—)	(457)
		Food (g/day)	168	307	267	228	185	217	202	215
			(6)	(48)	(45)	(38)	(74)	(36)	(37)	(36)
3	600.0; $n = 8$	Water (ml/day)	398	546	515	445	494	511	520	480
			(32)	(65)	(80)	(57)	(61)	(109)	(97)	(118)

Note. nd, no data.

^a Body weight was measured at Weeks 3, 6, 9, 12, 14, 16, 19, and 26.

^b Data are means (\pm SD).

TABLE 3
Biochemical Changes in Urine of Male New Zealand White Rabbits Exposed to 600 mg/L Uranyl Nitrate Hexahydrate for 91 Days, Followed by a 91-Day Recovery Period

	Week:	Exposure			Recovery			
		1	4	13	14	16	18	26
Mean urine volume (ml/48 h)								
Exposed*		248 ^a (95) ^b	255 (45)	116 ^c (4)	265 (75)	228 ^d (133)	193 ^d (79)	269 (98)
Control*		381 (111) ^b	338 (86)	140 ^e (52)	216 (43)	138 ^d (56)	129 ^d (65)	225 (63)
Mean glucose (mmol/24 h/kg body wt)								
Exposed		12.3 ^a (5.0)	4.4 ^a (1.6)	2.7 (0.9)	3.0 ^a (1.0)	2.3 ^f (1.1)	3.0 ^d (1.2)	3.3 (1.5)
Control		2.7 (1.5)	2.6 (0.7)	2.5 (1.2)	1.7 (0.5)	2.6 ^e (1.1)	2.5 ^d (0.9)	2.2 ^f (0.8)
Mean total protein (mg/24 h/kg body wt)								
Exposed		2120 ^a (2293)	615 ^a (37)	449 (288)	305 (107)	281 ^f (102)	477 ^d (219)	110 (170)
Control		379 (189)	520 (142)	325 (135)	338 (255)	236 ^e (100)	738 ^d (310)	30 ^f (20)
Mean leucine aminopeptidase (mUnits/24 h/kg body wt)								
Exposed		4.592 ^a (2.344)	2.15 ^a (0.924)	nd	nd	nd	nd	nd
Control		0.852 (1.337)	1.423 (0.801)	nd	nd	nd	nd	nd

Note. ^aSignificantly different from control group ($p < 0.05$). ^b(\pm SD). $n = 6$ unless otherwise noted: ^c 2; ^d 3; ^e 4; ^f 5. nd, no data. *Control is Group 1; exposed is Group 3.

was accompanied by cytoplasmic vacuolation (Figs. 3 and 4) and shedding (Fig. 6). Concurrent nuclear changes included apical displacement and irregular placement (Figs. 3 and 4) with vesiculation, anisokaryosis (Figs. 4, 6, and 7A), and pyknosis (Fig. 3). Tubular basement membranes were normal in areas of epithelial injury in early lesions (Fig. 5) but became thickened focally in animals in recovery (Fig. 7B) and in locally extensive areas (Fig. 7C). Thyroid changes were most pronounced in the controls and not treatment related. For the liver, an irregular accentuation of zonation was present at the architectural level, accompanied by increased variation in hepatocellular nuclear size, nuclear pyknosis, and extensive cytoplasmic vacuolation. Foci of degenerative change in the aorta were not treatment related and occurred as described previously (Gilman *et al.*, 1998b).

The statistical analyses of incidence and severity evaluations are presented in Tables 7 and 8. In summary, exposure to 600 mg UN/L was associated with significant changes in renal proximal tubules and basement membranes which persisted after a period of recovery as long as 45 days, and in some cases, 91 days.

Tissue retention studies. Significant elevations of uranium in kidney and femur were demonstrated following 91 days exposure to 24 and 600 mg UN/L compared to controls

(Table 9). Uranium clearance from kidney during the subsequent 91-day recovery period was much more rapid than from bone (Fig. 8, Table 10).

DISCUSSION

Based upon changes in renal proximal tubular nuclei, a 91-day LOAEL for male rabbits of 0.96 mg UN/L was derived in a previous study of New Zealand White rabbits (Gilman *et al.*, 1998b). While females ingested on average 65% more water than the males, with correspondingly higher UN doses, they appeared less affected by this exposure, and a LOAEL of 4.8 mg UN/L was derived (Gilman *et al.*, 1998b). Based upon the differing tissue uranium residues, it was speculated that a difference in pharmacokinetic parameters between the males and females might have accounted for the difference in their observed sensitivity to UN: it is known that the sex of an animal can have a marked effect upon the renal toxicity of administered nephrotoxins (Ackerman and Hook, 1984).

The rabbits exposed to 24 mg UN/L in this study showed few dose-related changes (tubular atrophy or dilation and cytoplasmic vacuolation or pigmentation), contrasting markedly with the findings of the previous study in male rabbits

TABLE 4
Hematological Changes in Male New Zealand White Rabbits Exposed to 600 mg/L Uranyl Nitrate Hexahydrate for 91 Days, Followed by Various Recovery Periods up to 91 Days

	Exposure		Recovery			
	Week:	13	14	17	20	26
Exposed <i>n</i> :		6	5	5	6	8
Group number:		9	4	5	6	3
Control <i>n</i> :		8	—	—	4	7
Group number:		7			10	1
Mean white blood cells ($\times 10^9/L$)						
Exposed		4.7 ^a (1.5) ^b	6.8 (2.3)	5.9 (1.7)	7.2 (1.3)	7.6 (1.6)
Control		5.5 (2.0) ^b	nd	nd	8.3 (2.8)	7.7 (2.1)
Mean lymphocytes (%)						
Exposed		44 ^c (3)	55 (8)	55 (12)	56 (8)	65 ^d (6)
Control		41 (14)	nd	nd	52 (10)	49 (9)
Mean total lymphocytes ($\times 10^9/L$)						
Exposed		2.00 ^a (0.54)	3.56 (0.80)	3.09 (0.35)	4.05 (0.98)	4.97 ^d (1.36)
Control		2.10 (0.87)	nd	nd	4.34 (1.76)	3.68 (0.87)
Mean polymorphonuclear cells (%)						
Exposed		40 ^a (3)	35 (8)	31 (7)	30 (12)	23 ^d (6)
Control		45 (16)	nd	nd	35 (8)	37 (12)
Mean total polymorphonuclear cells ($\times 10^9/L$)						
Exposed		1.87 (0.64)	2.49 (1.30)	1.90 (0.95)	2.18 (0.89)	1.74 ^d (0.53)
Control		2.58 (1.46)	nd	nd	2.92 (1.32)	2.90 (1.43)

Note. ^a Significantly different from other exposed groups following 45 days recovery ($p < 0.05$). ^b (\pm SD). ^c Significantly different from other exposed groups following 8 days recovery ($p < 0.05$). ^d Significantly different from control group ($p < 0.05$). nd, no data.

(Gilman *et al.*, 1998b). The animals in the 600 mg UN/L exposure group in the present study ingested an average of 41 mg U/kg body wt/day, compared to an average of 29 mg U/kg body wt/day in the comparable male exposure group in the previous study. In spite of this larger ingested dose, the kidney residual uranium levels in the present study were 3.48 μ g/g immediately following 91 days exposure, compared to 4.98 μ g/g in the previous study (Table 10). Lower residual uranium levels were also seen in bone in the present study (2.89 μ g/g) compared to 4.04 μ g/g in the previous study (Table 10). These findings suggest that the animals in the two studies handled the ingested dose of uranium differently, clearing it much more effectively in the present study and reducing the accumulated uranium burden in both kidney and bone. The lower renal uranium concentrations in the present study are also consistent with the observed

reduction in regressive and presumably toxicant-induced histopathological findings compared with the previous study.

The female rabbits in the previous study were SPF-derived animals (Gilman *et al.*, 1998b). The 600 mg UN/L exposure group ingested an average of 43 mg U/kg body wt/day, and had a residual bone uranium concentration of 3.1 μ g/g. These values compare closely with those of the males in the present study, although the females had lower kidney residual uranium levels (1.0 μ g/g).

The male rabbits in the present study were SPF, while 4 of the 60 male animals of the previous study were found to be infected with *Pasteurella multocida* (Gilman *et al.*, 1998b). Although all known affected animals were subsequently excluded from that study, the possibility exists that some remaining males may have had a subclinical infection which may have affected their response to administered uranium.

TABLE 5
Serum Biochemical Changes in Male New Zealand White Rabbits Exposed to 600 mg/L Uranyl Nitrate Hexahydrate for 91 Days, Followed by Various Recovery Periods up to 91 Days

	Exposure		Recovery		
	Week:	13	14	20	26
	Exposed data n:	8	5	7	7
	Group number:	9	4	6	3
	Control data n:	8	—	4	8
	Group number:	7		10	1
Mean glucose (mmol/L)					
Exposed		7.5 ^a (1.2) ^b	9.8 (4.3)	7.9 (1.1)	8.6 (0.5)
Control		6.2 ^c (0.6) ^b	nd	8.2 ^e (0.2)	9.3 (1.5)
Mean calcium (mmol/L)					
Exposed		2.9 ^d (0.2)	3.1 (0.9)	3.6 (0.2)	4.0 ^b (0.8)
Control		2.9 ^c (0.1)	nd	3.5 (0.1)	3.4 (0.2)
Mean alkaline phosphatase (Units/L)					
Exposed		72 (22)	61 (22)	55 (9)	63 ^a (19)
Control		82 ^c (21)	nd	43 ^e (8)	43 (7)
Mean bilirubin (μ mol/L)					
Exposed		10 ^d (3)	9 (3)	5 (0)	5 (2)
Control		10 ^c (3)	nd	5 ^e (0)	5 (0)
Mean lactic dehydrogenase (Units/L)					
Exposed		292 ^d (122)	192 (120)	36 ^f (22)	69 ^e (49)
Control		332 ^c (189)	nd	nd	45 (21)
Mean sodium (mmol/L)					
Exposed		149 ^d (3)	150 (3)	135 (2)	132 (10)
Control		149 ^c (5)	nd	135 ^e (1)	135 (2)

Note. ^a Significantly different from control group ($p < 0.05$). ^b (\pm SD). ^c Significantly different from subsequent control groups ($p < 0.05$). ^d Significantly different from other exposed groups after 45 days recovery or more ($p < 0.05$). n, as shown in table heading, except where otherwise noted: ^e 3; ^f 4; ^g 6; ^h 8. nd, no data.

Animals under subclinically expressed disease stress may be more susceptible to the effects of a toxicant.

No significant dose effects were found in PSP clearance in the present study. In the previous study (Gilman *et al.*, 1998b), there was a significant linear relationship between exposure level and rate of reduction in excretion of PSP in the male rabbits. This tends to further support the notion that the non-SPF rabbits exhibited some UN dose-related impairment of renal clearance.

Although there remain some sex differences in the bone-kidney uranium ratios (females in previous study vs males in the present study: Table 10), the present findings tend to

dismiss any such explanation for the male-female differences observed in the previous rabbit study; the SPF animals appear to be less sensitive to the toxic effects of uranium.

A summary of administered uranium dose and tissue uranium residues in this and our previously reported studies in male and female Sprague-Dawley rats and male and female NZ white rabbits is shown in Table 8, and includes some ratios derived from these data. Examination of the dose/kidney U ratios shows that female rats accumulated less kidney U residue per equivalent dose than did males. Similarly, the female rabbits show less kidney U than did male rabbits (0-day recovery group from the present study), while



FIG. 2. Outer stripe renal cortex from a male rabbit in the control group. Glomerular tuft and parietal Bowman's capsule as well as tubular cytoplasm, nuclei, and basement membranes are normal. PAMS $\times 320$.

the male rabbits from the previous study (which may have included animals with subclinical *Pasteurella* infection) showed a much higher kidney U retention. These data are presented graphically in Fig. 9.

The histopathological changes observed were similar to

those seen in the female rabbits in the previous study (Gilman *et al.*, 1998b). The thyroid changes did not appear to be dose or treatment related, whereas the hepatic changes were treatment related but not dose related. Focal degenerative changes of the aorta consisting of mineralization of the

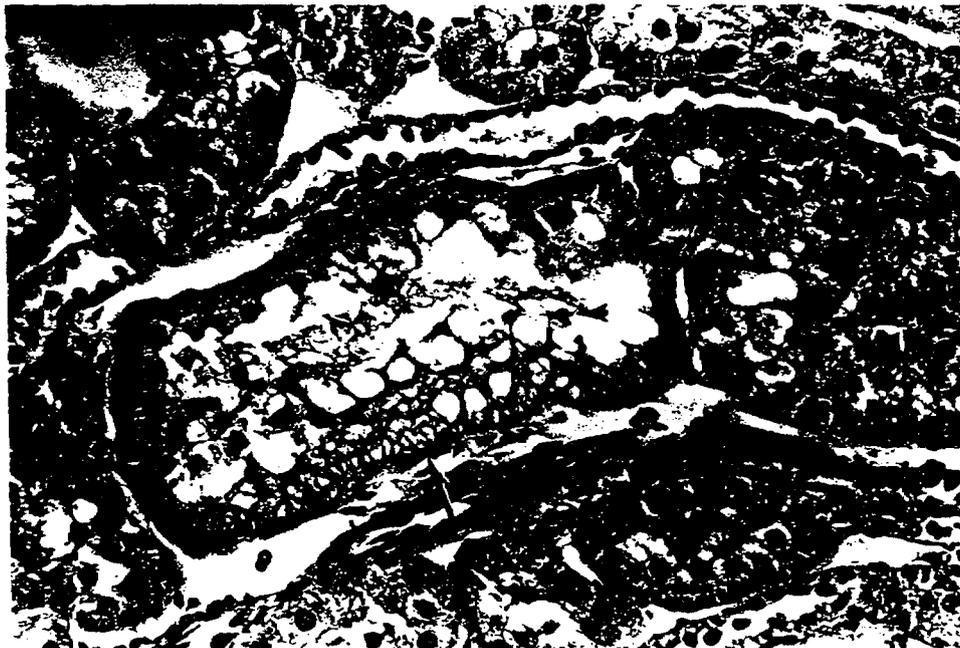


FIG. 3. Outer stripe renal cortex from a male rabbit which received 24 mg UN/L of drinking water for 91 days (group 8). There is moderate focal dilation of a proximal tubule with moderate multifocal cytoplasmic vacuolation (arrow), apical displacement of nuclei, and focal nuclear pyknosis (arrowhead). H&E $\times 320$.

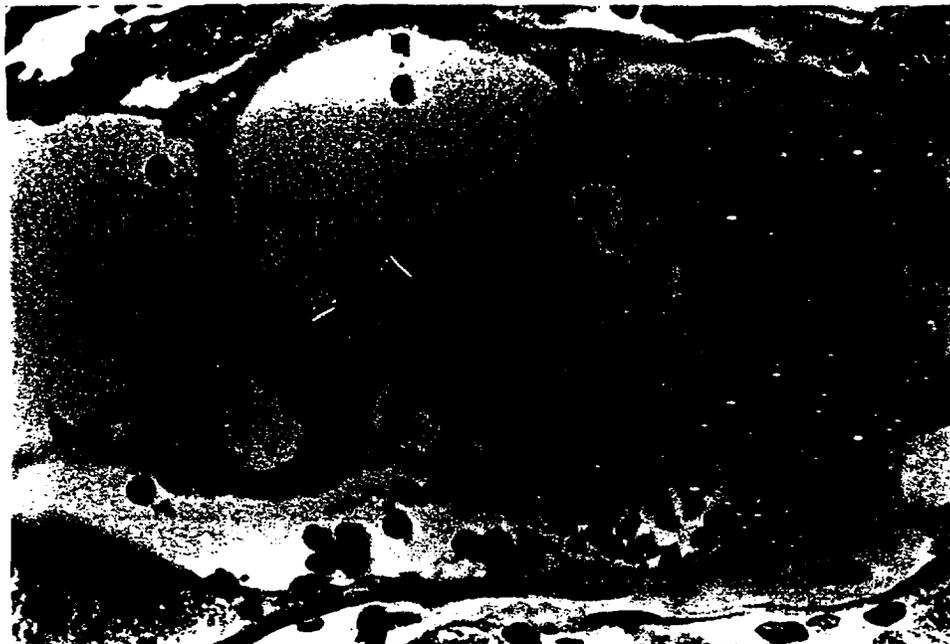


FIG. 4. Renal corticomedullary junction from a female rabbit which received 24 mg UN/L drinking water for 91 days (group 8). There is mild multifocal basal vacuolation (arrowheads) with irregular placement of nuclei which have moderate vesiculation and anisokaryosis. H&E $\times 720$.

medial layers with giant cell reaction were observed in both controls and treated animals and appeared to be sporadic lesions exacerbated by UN exposure.

The early biochemical changes observed in urine during the exposure period of the 600 mg UN/L animals (reduc-

tion in urine flow accompanied by glycosuria, gross proteinuria, and enzymuria) are typical of those associated with acute uranyl nephrotoxicity (Moss, 1989). During the recovery period, continuing glycosuria was noted, accompanied by slightly increased urine volumes (although the

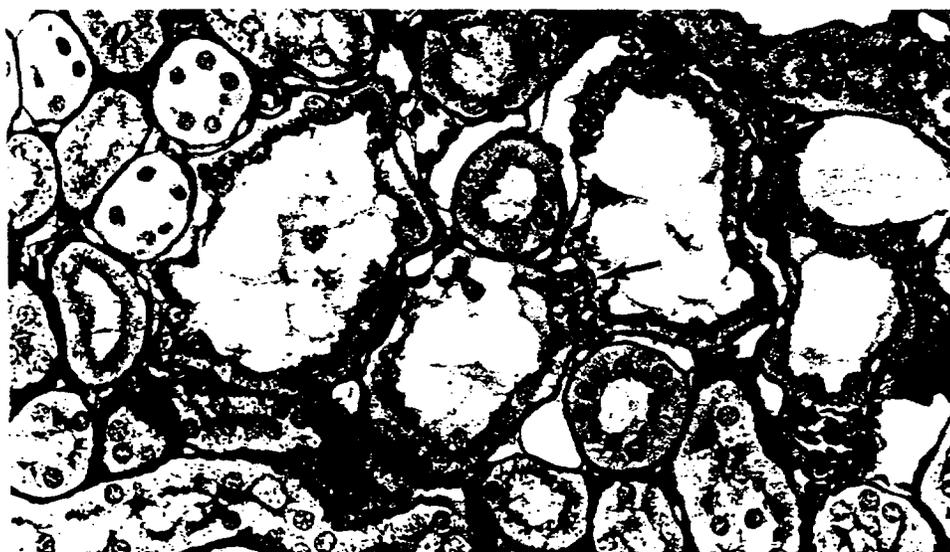


FIG. 5. Outer stripe renal cortex from a male rabbit which received 600 mg UN/L of drinking water for 91 days (group 9). Moderate to marked dilation of tubules with partial collapse demonstrated by folding and wrinkling of basement membrane (arrow) that is of normal thickness. PAMS $\times 320$.

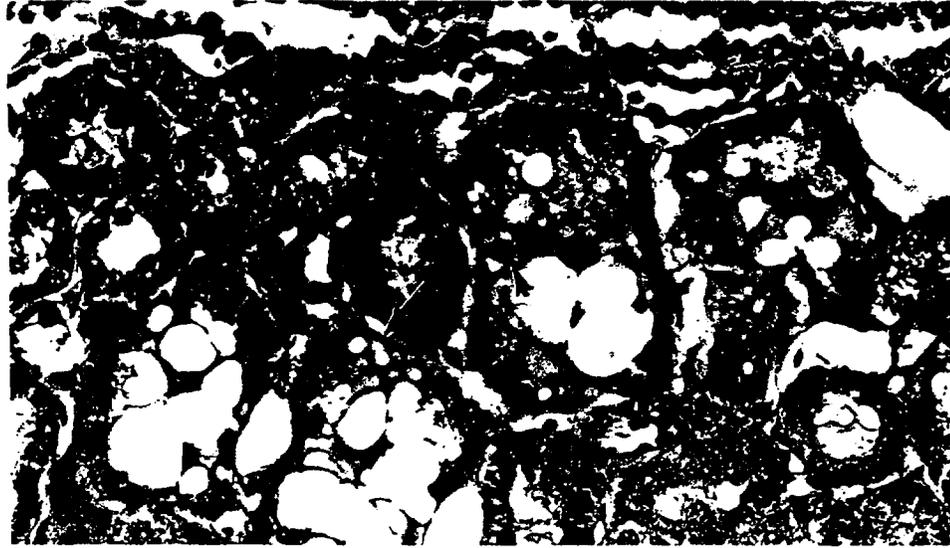


FIG. 6. Outer stripe renal cortex from a male rabbit which received 600 mg UN/L of drinking water for 91 days (group 9). There is moderate multifocal dilation of proximal convoluted tubules with marked cytoplasmic vacuolation (arrow), multifocal cytoplasmic shedding (arrowhead), and irregular placement of nuclei with vesiculation and anisokaryosis. H&E $\times 320$.

latter did not reach a $p < 0.05$ significance level compared to controls).

Animals from the 600 mg UN/L exposed group euthanized after 14 days of recovery (group 5) had moderate hyperglycemia; unfortunately there was no control group for this

recovery group to determine whether this was a significant finding (Table 5). However, it is noted that the similarly exposed group 3 animals which were monitored for the full 91 days recovery had a significantly increased glycosuria at this point in the recovery (Table 3). Hyperglycemia may

TABLE 6
Kidney Lesion Incidence Summary of Male New Zealand White Rabbits after 91 Days Treatment with Uranyl Nitrate (UN) and Various Periods of Recovery

Exposure:	Control			24 mg UN/L		600 mg UN/L				
	7	10	1	8	2	9	4	5	6	3
Group number:	7	10	1	8	2	9	4	5	6	3
Days of recovery:	0	45	91	0	91	0	8	14	45	91
Animals examined per group:	6	4	8	8	8	8	5	5	6	8
Glomerular										
Adhesions	4 ^a	3	4	6	6	6	5	4	3	7
Tubular										
Cytoplasmic inclusions	6	4	8	8	8	8	5	5	5	7
Cytoplasmic vacuolation	0	0	0	0	1	4	1	1	6	4
Anisokaryosis	4	0	0	0	0	8	5	5	5	8
Nuclear hyperchromicity	1	0	0	0	0	8	5	4	2	5
Nuclear vesiculation	4	0	0	0	0	8	5	5	5	7
Nuclear pyknosis	1	0	0	0	0	3	3	0	0	1
Tubular dilation	1	1	1	1	3	7	4	4	3	7
Protein casts	0	1	0	2	0	2	0	1	4	2
Tubular atrophy	0	0	0	0	1	5	4	2	3	0
Tubular pigmentation	0	0	1	0	1	2	5	3	2	4
Interstitial										
Collagen sclerosis	0	1	0	1	1	5	5	4	5	3
Reticulin sclerosis	4	3	0	6	2	8	5	5	5	8

^a Number of animals in exposure group with specific abnormalities.

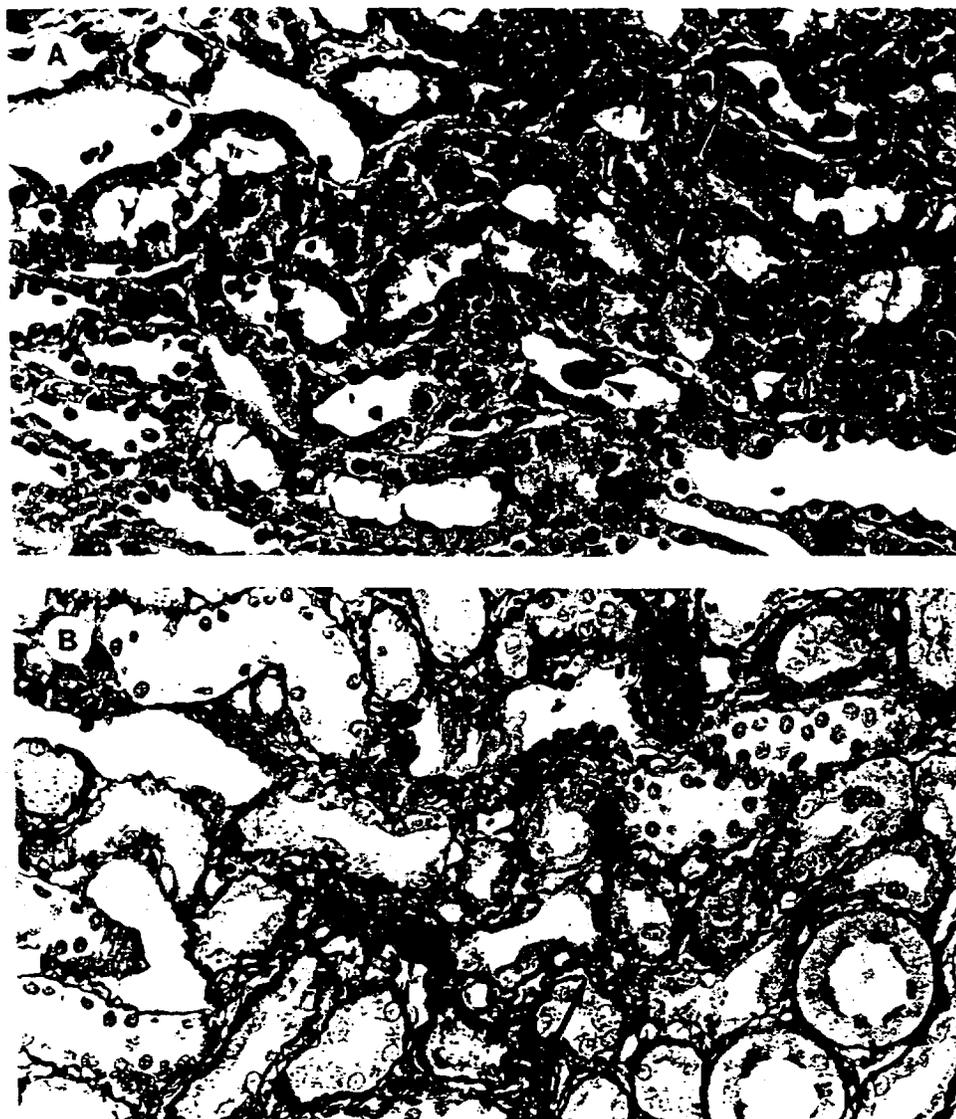


FIG. 7. Renal corticomedullary junction from a male rabbit which received 600 mg UN/L of drinking water for 91 days followed by 45 days of recovery on clean water (group 6). (A) There is mild irregular dilation of tubules with intratubular variation in the cytoplasmic volume and staining density. There is continuing focal shedding of cytoplasm with luminal protrusion of nuclei (arrowhead) and moderate vesiculation, anisokaryosis, and hyperchromicity of nuclei (arrow). H&E $\times 320$. (B) Section adjacent to (A) with moderate multifocal thickening and wrinkling of tubular basement membranes (arrow) and persistence of silver-positive granules in tubular cytoplasm. PAMS $\times 320$. (C) Corticomedullary junction of the same animal as (A) and (B) demonstrating mild generalized basement membrane thickening (arrow) in a medullary ray. PAMS $\times 160$.

account in part for the continuing glycosuria, but it is also possible that a tubular injury-associated change in the renal threshold for glucose was responsible. This could not be further elucidated by comparison with our previous rabbit studies, where neither significant hyperglycemia nor glycosuria were observed relative to controls (Gilman *et al.*, 1998b).

Following review of the histopathology data, the lowest-observed-adverse-effect-level (LOAEL) is estimated to be at or below 24 mg UN/L, associated with a kidney uranium

concentration of 0.18 $\mu\text{g/g}$ or less. We have previously estimated a 91-day LOAEL of 0.96 mg UN/L in male and female Sprague-Dawley rats, based primarily upon lesions of the renal tubular epithelium (Gilman *et al.*, 1998a). Unfortunately, kidney uranium concentrations were not determined in those exposed animals (because they were estimated to be below the limit of analytical detection based upon an initial range-finding study). However, a threshold concentration for uranium has been reported elsewhere for rats in the range of 0.7–1.3 $\mu\text{g U/g}$ based upon eight injec-

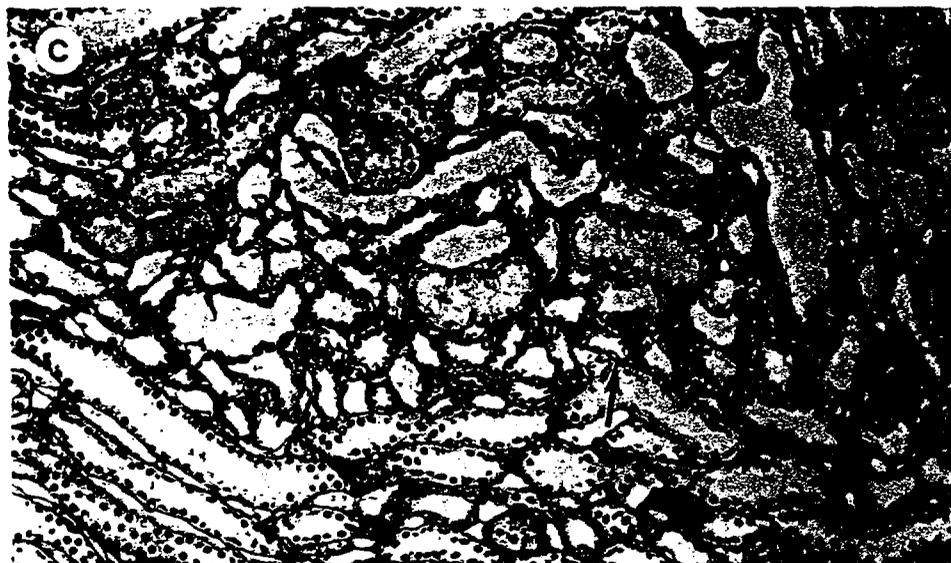


FIG. 7—Continued

tions of UO_2F_2 over a 24-day period (Diamond *et al.*, 1987). Severe tubular injury and associated abnormalities were also reported within the range of 1.3–3.5 μg U/g in that study.

A summary of the various LOAELs derived during our uranium toxicity studies is presented in Table 11. Our studies

suggest that, except for the non-SPF animals, the Sprague-Dawley rat is more sensitive to uranyl nitrate than the NZ White rabbit.

The present study was designed to examine the reversibility of the early renal lesions induced by exposure to uranium

TABLE 7
Statistical Evaluation of Kidney Lesion Incidence Data for Male New Zealand White Rabbits after 91 Days Treatment with Uranyl Nitrate (UN) and Various Periods of Recovery^a

	24 mg UN/L		600 mg UN/L				
	8	2	9	4	5	6	3
Exposure group:	8	2	9	4	5	6	3
Days of recovery:	0	91	0	8	14	45	91
Glomerular							
Adhesions							
Tubular							
Cytoplasmic inclusions							
Cytoplasmic vacuolation							
Anisokaryosis							
Nuclear hyperchromicity							
Nuclear vesiculation							
Nuclear pyknosis							
Tubular dilation							
Protein casts							
Tubular atrophy							
Tubular pigmentation							
Interstitial							
Collagen sclerosis							
Reticulin sclerosis							

^a Fisher exact test.

* Significantly different from control group ($p < 0.05$).

** Significantly different from control group ($p < 0.01$).

*** Significantly different from control group ($p < 0.001$).

TABLE 8
Statistical Evaluation of Kidney Lesion Severity Data for Male New Zealand White Rabbits after 91 Days Treatment with Uranyl Nitrate (UN) and Various Periods of Recovery^a

Exposure group: Days of recovery:	24 mg UN/L		600 mg UN/L				
	8 0	2 91	9 0	4 8	5 14	6 45	3 91
Glomerular							
Adhesions							
Tubular							
Cytoplasmic inclusions							
Cytoplasmic vacuolation			*			**	
Anisokaryosis			**	**	**	**	**
Nuclear hyperchromicity			**	**	**	**	**
Nuclear vesiculation			**	**	**	**	**
Nuclear pyknosis			*	**			
Tubular dilation			**	*	*		**
Protein casts						*	
Tubular atrophy			**	*			
Tubular pigmentation				**	*		
Interstitial							
Collagen sclerosis			**	**		**	
Reticulin sclerosis			**	**	**	**	**

^a Dunnett's *t* test.

* Significantly different from control group ($p < 0.05$).

** Significantly different from control group ($p < 0.01$).

in the rabbit. The kidney lesion incidence summary (Table 6) shows that in the 600 mg UN/L exposure groups, important lesions were still present after 91 days recovery. These findings suggest that some significant recovery occurred during the 91-day postexposure period. Cytoplasmic vacuolation was still present at 91 days recovery and nuclear vesiculation, anisokaryosis, and hyperchromicity in the tubular epithelium were more prevalent in the 91-day recovery group than in the 45-day group, as was tubular dilation. The sever-

TABLE 9
Uranium Residues ($\mu\text{g/g}$ Wet wt) in Kidney and Bone of Male New Zealand White Rabbits after 91 Days Treatment with Uranyl Nitrate Hexahydrate (UN)

Group number	Exposure (mg UN/L)	Mean uranium concentration ($\mu\text{g/g}$)	
		Kidney,* $n = 8/\text{group}$	Femur $n = 8/\text{group}$
7	0 (control)	0.01 (0.01) ^a	0.10 (0.15)
8	24.0	0.18 (0.14)	0.20 (0.05)
9	600.0	3.48 (1.54)*	2.89 (0.78)*

^a (\pm SD).

* Significantly different from control group: $p < 0.05$.

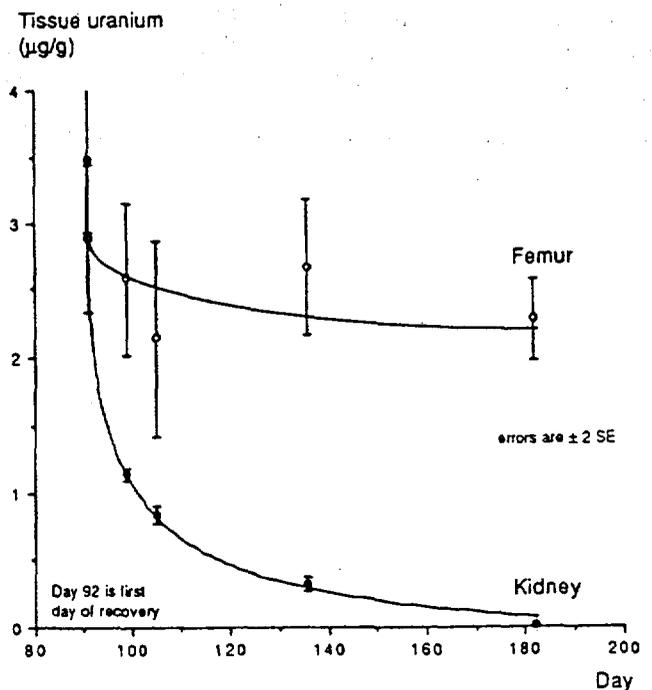


FIG. 8. Changes in tissue concentration of uranium in kidney and bone ($\mu\text{g/g}$ wet wt) of male New Zealand White rabbits previously exposed to uranyl nitrate in the drinking water for 91 days, followed by a period of recovery on untreated water. Lines of best fit produced by interpolation function of Cricket Graph (Cricket Software, PA).

TABLE 10
Summary of Dose and Tissue Uranium Residues in Rats and Rabbits Following 91 Days Exposure to Uranyl Nitrate Hexahydrate in Drinking Water (600 mg/L)

Study	n ^a	TWA U dose ^b	Kidney U ^c	Bone U ^c	Ratios		
					Dose/kidney U	Dose/bone U	Kidney U/bone U
Male rat ^d	6	3016.88 (184.04) ^e	2.12 (1.99) ^e	0.97 (0.33) ^e	1423.06	3110.19	2.19
Female rat ^d	6	4366.19 (952.10)	1.67 (0.91)	0.73 (0.33)	2614.49	5981.08	2.29
Male rabbit ^f	5	2677.87 (288.42)	4.98 (1.84)	4.04 (1.23)	537.72	662.84	1.23
Female rabbit ^f	10	3902.72 (745.46)	1.03 (0.46)	3.06 (1.00)	3789.05	1275.40	0.34
Male rabbit							
0 days recovery (group 9)	8	3557.25 (648.45)	3.48 (1.54)	2.89 (0.78)	1022.20	1230.88	1.20
8 days recovery (group 4)	5	3310.94 (400.43)	1.14 (0.11)	2.58 (0.64)	2904.33	1283.31	0.44
14 days recovery (group 5)	5	3806.85 (61.84)	0.84 (0.15)	2.14 (0.81)	4531.96	1778.90	0.39
45 days recovery (group 6)	7	3731.72 (668.58)	0.31 (0.19)	2.67 (0.67)	12037.8	1397.65	0.12
91 days recovery (group 3)	8	3696.63 (314.83)	0.02 (0.01)	2.28 (0.43)	184832.0	1621.33	0.01

^a Number of animals for which tissue uranium residue data were available, and may not include entire exposed group.

^b Time-weighted average dose of uranium (mg U/kg body wt/91 days). The TWA values for this table were calculated only for those animals for which tissue residue data were available, and may show small differences from group TWA values given elsewhere.

^c Tissue uranium residue, $\mu\text{g/g}$ wet wt.

^d Gilman *et al.* (1998a).

^e Data are means (\pm SD).

^f Gilman *et al.* (1998b).

ity of histological changes did not increase between the 45- and 91-day recovery groups, but the continuing prevalence during this time suggests that subchronic exposure to 600 mg UN/L caused an injury which was self-sustaining. It is

important to note that the presence of sclerotic changes in tubular basement membranes and renal interstitium persisted in recovery and likely represents permanent injury. Confir-

TABLE 11
Summary of Lowest-Observed-Adverse-Effect Levels (LOAELs) in Sprague-Dawley Rats and NZ White Rabbits Following 91 Days Exposure to Uranyl Nitrate Hexahydrate (UN) in Drinking Water

Study	Exposure group (mg UN/L)	LOAEL	
		Uranium dose equivalent (mg/kg body wt/day)	Kidney tissue uranium residue ($\mu\text{g/g}$ wet tissue)
Male rat ^a	0.96	0.06	0.2 ^b
Female rat ^a	0.96	0.09	0.2 ^b
Male rabbit ^{c,d}	0.96	0.05	0.04 (0.03) ^e
Female rabbit ^f	4.8	0.49	0.02 (0.01)
Male rabbit ^{g,h}	24	1.36	0.18 (0.13)

^a Gilman *et al.* (1998a).

^b No residue data for this exposed group were available; values are interpolations reported in Gilman *et al.* (1998a).

^c Gilman *et al.* (1998b).

^d Non-SPF animals; may include animals with subclinical *Pasteurella* infection.

^e Data are means (\pm SD).

^f Recovery study; animals were SPF.

^g The LOAEL is estimated to lie at or below 24 mg UN/L.

Kidney uranium / TWA 91 d dose ^{a,b}

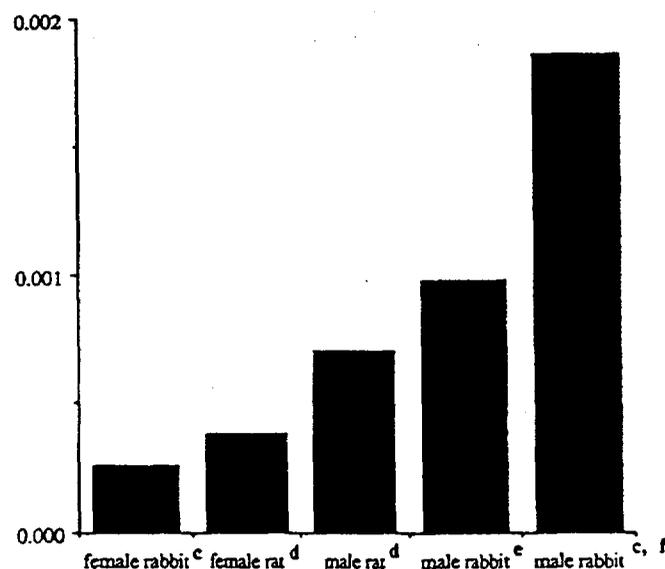


FIG. 9. Tissue concentration of uranium in kidney as a function of time-weighted average dose of uranium exposure in Sprague-Dawley rats and New Zealand White rabbits. ^a Kidney uranium concentration: $\mu\text{g/g}$, wet wt. ^b Time-weighted average uranium dose: mg uranium/kg body wt/91 days. ^c Gilman *et al.* (1998b). ^d Gilman *et al.* (1998a). ^e Present study. ^f This study may have included male animals with subclinical *Pasteurella* infection.

mation of the severity of these lesions using electron microscopy has already been reported (McDonald-Taylor *et al.*, 1997, 1992).

The reversibility of injury resulting from a lower dose of uranium remains to be explored. Further exposure-recovery studies using doses between 4.8 and 24 mg UN/L are required to better approximate both the LOAEL and the NOAEL. High-resolution proton nuclear magnetic resonance (^1H NMR) spectroscopy has been recently reported to reveal high concentrations of 3-D-hydroxybutyrate, in the absence of acetoacetate or acetone, in the urine of rats acutely exposed to UN by intraperitoneal injection (Anthony *et al.*, 1994). This is proposed as a novel marker of certain forms of proximal tubular nephrotoxicity, including that induced by exposure to UN, and may be a useful adjunct in future studies of this nature.

In summary, the ability of UN to produce specific tubular injury followed by basement membrane injury at relatively low doses in even a small proportion of exposed animals suggests that human exposure to soluble uranium over prolonged periods needs to be monitored.

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Public Health Goal for
URANIUM
In Drinking Water

SUMMARY

A Public Health Goal (PHG) of 0.2 ppb (0.2 pCi/L) is proposed for natural uranium in drinking water based on a study showing changes in indicators of kidney function (b 2-microglobulin and g -glutamyl transferase levels in the urine) in a human population. This proposed PHG has been calculated from an NOEL of 3 m g/day with an uncertainty factor of 10 for intrahuman variability. This proposed PHG is also protective against carcinogenic effects of ionizing radiation produced by uranium. The theoretical cancer risk at the proposed PHG is estimated at 4×10^{-7} . OEHHA considers theoretical risks below 10^{-6} to be negligible. Uranium is a naturally occurring radioactive element that is ubiquitous in the earth's crust. Uranium is found in ground and surface waters due to its natural occurrence in geological formations. The average uranium concentrations in surface, ground and domestic water are 1, 3 and 2 pCi/L, respectively. The uranium intake from water is about equal to the total from other dietary components. Natural uranium contains 99.27 percent ^{238}U , 0.72 percent ^{235}U and 0.006 percent ^{234}U by weight. The primary noncarcinogenic toxic effect of uranium is on the kidneys. Recently published studies in rats, rabbits, and humans show effects of chronic uranium exposure at low levels in drinking water. Effects seen in rats, at the lowest average dose of 0.06 mg U/kg-day, including histopathological lesions of the kidney tubules, glomeruli and interstitium are considered clearly adverse effects albeit not severe. Histopathological effects were also seen at the same exposure level in the liver including nuclear anisokaryosis and vesiculation. Effects on chemical indicators of kidney function

EXHIBIT

G

tabbles

were seen in urine of humans exposed to low levels of uranium in drinking water for periods up to 33 years. These effects, such as increased urinary glucose, b 2-microglobulin, and g -glutamyl transferase, are indicative of potential kidney injury rather than toxicity per se. Uranium is an emitter of ionizing radiation, and ionizing radiation is carcinogenic, mutagenic and teratogenic. A level of 0.2 ppb (0.2 pCi/L) is considered protective for both kidney toxicity and carcinogenicity and is therefore proposed as the PHG for natural uranium in California drinking water.

The U.S. Environmental Protection Agency (U.S. EPA) has not established a Maximum Contaminant Level (MCL) for natural uranium, but does have an MCLG of 0. The State of California has an MCL for uranium of 20 pCi/L based on earlier studies of toxicity to the kidney. The rabbit study used in the risk assessment on which the California MCL is based (Novikov and Yudina, 1970) has been superseded by more recent studies of the effects of uranium on kidney function in laboratory animals and in human populations (Gilman et al., 1998a-c; Zamora et al., 1998; Health Canada, 1998).

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Uranium

Purpose of Consultation

For the past several years, the Federal-Provincial Subcommittee on Drinking Water has been assessing the available information on uranium with the intent of revising the current guideline for uranium in drinking water. The purpose of this consultation is to solicit comments on the approach used in developing the proposed guideline as well as on the potential economic costs of implementing it. In reviewing this document, key questions you should consider include the following:

- Do the water quality monitoring data accurately reflect the current situation in Canada? Are additional water quality data available?
- Is the risk assessment valid? Are there other relevant studies that should be included in the Criteria Summary?
- At what value should the guideline be set to adequately protect public health and to provide a cost-effective solution?

The Subcommittee has requested that this document be made available to the public and open for comment. Comments are appreciated, with accompanying justification, if required. Comments can be sent via E-mail to water_eau@hc-sc.gc.ca or by mail to the DWS Secretariat, 12th Floor, Jeanne Mance Bldg., A.L. 1912A, Tunney's Pasture, Ottawa, Ontario K1A 0K9. ***All comments must be received before August 1st, 1999.***

It should be noted that this Criteria Summary on uranium in drinking water will be revised following evaluation of comments received, and a maximum acceptable concentration (MAC) for uranium in drinking water will be established. This document should be considered a *draft*.

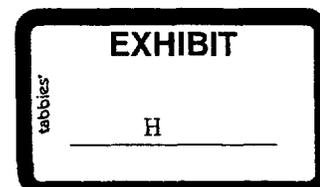


Uranium in Drinking Water - Document
for Public Consultation



January 11, 1999

Canada



Uranium in Drinking Water

Document for Public Comment

Prepared by the Federal-Provincial
Subcommittee on Drinking Water

Comment Period Ends
August 1st, 1999

Uranium in Drinking Water

Public Comment Document
January 1999

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November 1998
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URANIUM*

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Proposed Guideline

The proposed guideline for uranium in drinking water is 0.01 mg/L (10 µg/L).

Identity, Use and Sources in the Environment

Uranium occurs naturally in the +2, +3, +4, +5 or +6 valence states, but most commonly in the hexavalent form. In nature, hexavalent uranium is commonly associated with oxygen as the uranyl ion, UO_2^{2+} . Naturally occurring uranium (^{nat}U) is a mixture of three radionuclides (^{234}U , ^{235}U and ^{238}U), all of which decay by both alpha and gamma emissions (Cothorn & Lappenbusch,

* This review addresses only the chemical aspects of uranium toxicity. Information pertinent to the derivation of a guideline based on radiological effects is discussed separately in a Criteria Summary on radiological characteristics.

** See Appendix I for provincial/territorial data.

*** The World Health Organization has recently established a provisional guideline value of 2 µg/L, assuming a 60-kg adult consuming 2 L of drinking water per day and a 10% allocation of the tolerable daily intake to drinking water. It is anticipated that the U.S. Environmental Protection Agency will establish a guideline of 20 µg/L (rounded from 16.8 µg/L), based on drinking water consumption of 2 L/day and an 80% allocation factor.

1983; Lide, 1992-93). The alpha energies are all clustered around 4.5 MeV. Natural uranium consists almost entirely of the ^{238}U isotope, with the ^{235}U and ^{234}U isotopes constituting about 0.71% and 0.0057%, respectively (Berlin & Rudell, 1986); 1 μg of natural uranium has an activity of 0.025 Bq (Cothorn & Lappenbusch, 1983). Uranium is widespread in nature, occurring in granites and various other mineral deposits (Roessler *et al.*, 1979; Lide, 1992-93).

The United States, Canada and South Africa produce approximately 80% of the uranium in the western world (Berlin & Rudell, 1986). It is estimated that more than 13 million kilograms were produced in Canada in 1987 (Statistics Canada, 1989). Uranium is used mainly as fuel in nuclear energy plants.

Uranium is present in the environment as a result of leaching from natural deposits, its release in mill tailings, emissions from the nuclear industry and the combustion of coal and other fuels (Dreesen *et al.*, 1982; Cothorn & Lappenbusch, 1983; Essien *et al.*, 1985; Tadmor, 1986). Phosphate fertilizers, which may contain uranium at concentrations as high as 150 mg/kg, may also contribute to the uranium content of groundwater (Spalding & Sackett, 1972).

Analytical Methods and Treatment Technology

Uranium in water is most commonly measured by solid fluorimetry with either laser excitation or ultraviolet light following fusion of the sample with a pellet of carbonate and sodium fluoride (detection limit 0.1 $\mu\text{g/L}$; Kreiger & Whittaker, 1980). Sample preparation for this method is tedious, however, and there is interference from other metals. Uranium can also be determined by inductively coupled plasma mass spectrometry, which has the same detection limit (0.1 $\mu\text{g/L}$) and a between-run precision of less than 6% (Boomer & Powell, 1987). Alpha spectrometry has been used for the determination of uranium in bottled waters (Gans, 1985) and environmental media (Singh & Wrenn, 1988), although the recovery is often highly variable owing to the low specific activity of natural uranium (Singh & Wrenn, 1988).

The effectiveness of water treatment processes for uranium removal has not been well documented in full-scale treatment plants. Laboratory studies and pilot plant tests have shown that conventional anion exchange resins are capable of removing uranium from drinking water supplies to concentrations as low as 1 $\mu\text{g/L}$ (>99% removal). Gamma radiation buildup in the uranium removal system does not appear to be a health concern (Jelinek and Sorg, 1988). However, because uranium is highly preferred by anion resins, regeneration may be difficult (Sorg, 1988). A number of other treatment methods, including conventional coagulation, lime softening, activated alumina and reverse osmosis, have also been found to reduce uranium concentrations to 1-5 $\mu\text{g/L}$ (>90% removal) in laboratory and pilot plant studies. In areas with high natural uranium levels, it may be difficult to achieve such low final uranium concentrations with the treatment technology available (Water Research Centre, 1997). Although cation exchange and granular activated carbon can remove uranium effectively, they are not considered practical methods for drinking water treatment (Sorg, 1988).

Exposure

Uranium concentrations of up to 700 $\mu\text{g/L}$ have been found in private groundwater supplies in Canada (Moss *et al.*, 1983; Moss, 1985). In a 1980-81 survey of 13 selected sites in south-central British Columbia, the mean uranium concentration ($n = 519$) in surface water and groundwater (some treated) supplies was 4.06 $\mu\text{g/L}$ (Province of British Columbia, 1981). The mean and median levels of naturally derived uranium in groundwaters of 287 wells sampled in southeastern Manitoba (1982-84) were 58.3 $\mu\text{g/L}$ and 10 $\mu\text{g/L}$, respectively; the maximum value

was 2020 µg/L (Betcher *et al.*, 1988). Uranium levels were highest in Precambrian rock aquifers (average 115.6 µg/L) and lowest in Paleozoic sedimentary rock aquifers (average 3.5 µg/L). In a radionuclide survey (n = 154) carried out by the Radiation Protection Bureau, Health Canada, in Manitoba in 1984 and 1987, levels of uranium ranged from less than the detection limit (5 µg/L) to 96 µg/L, and the mean concentration for samples with levels greater than the detection limit (45% of samples) was 16.1 µg/L (Meyerhof, 1989). In another survey conducted by Health Canada between 1975 and 1986, uranium was not found at concentrations above the detection limit (5 µg/L) in raw and treated water samples from Alberta, British Columbia, New Brunswick, Newfoundland, Nova Scotia, Quebec and the Yukon. Uranium levels above 5 µg/L were detected in water samples from Saskatchewan (80/243 samples; range 5–51 µg/L), Manitoba (7/88 samples; range 6.1–26 µg/L), Ontario (7/629 samples; range 5.2–39 µg/L) and the Northwest Territories (9/12 samples; range 19–2500 µg/L) (Hunt, 1996). In a 1990–95 survey of 130 sites (approximately 3700 samples) in Ontario, the mean of the average uranium concentrations (range <0.05–4.21 µg/L; detection limit 0.05 µg/L) in treated drinking water was 0.40 µg/L (OMEE, 1996). In Quebec, uranium was detected (detection limit <5 µg/L) in only 1.7% of samples (n = 2809) taken between 1993 and 1996 (range <5–20 µg/L) (Riopel, 1996); the survey encompassed more than 5.9 million individuals. In a 1994–95 survey of 322 wells in the Kitigan Zibi First Nation community in Quebec, uranium was detected (n = 525) at levels ranging from 0.01 to 1481 µg/L, with mean and median levels of 5.1 µg/L and 5.2 µg/L, respectively; levels in surface water (n = 11) were less than 1.1 µg/L (Moore *et al.*, 1996). Multiple sampling in some of the wells revealed wide fluctuations in uranium levels within individual wells. For example, the concentration in one well was approximately 400 µg/L on July 14, 1994, >1400 µg/L on September 1, 1994, and just over 200 µg/L on February 6, 1995. No correlations were found between the uranium concentrations and pH, redox, total dissolved solids, hardness, anions, well depth or temperature (Moore *et al.*, 1996). In New Brunswick, the mean uranium concentration from 1382 samples taken between 1994 and 1997 was 1.83 µg/L (Thomas, 1997). In a 1981 survey of 72 municipally treated systems in Nova Scotia, 98.6% of the samples tested were found to have uranium levels below 10 µg/L (Grantham, 1986).

A mean uranium concentration of 2.55 µg/L was reported in drinking water from 978 sites in the United States in the 1980s (U.S. EPA, 1990, 1991). The mean concentration of uranium in drinking water in New York City ranged from 0.03 to 0.08 µg/L (Fisenne & Welford, 1986). In five Japanese cities, the mean uranium level in potable water supplies was 0.9 ng/L (Nozaki *et al.*, 1970). Uranium may leach into water from uranium-bearing glass items (maximum of 30 µg/L) or from ceramic-glazed items in which uranium is used as a colouring agent (approximately 300 mg/L) (Landa & Councell, 1992).

On the basis of the results of the survey from Ontario (OMEE, 1996), the daily intake of uranium from drinking water for an adult consuming 1.5 L/d is estimated to be 0.6 µg.

Uranium has been detected in a variety of foodstuffs, with the highest concentrations being found in shellfish. The concentrations of uranium in fish muscle (dry weight) from a Canadian lake receiving uranium mill effluents were 7–11 times higher than those in fish caught in uncontaminated lakes (Swanson, 1985). Other dietary components that contribute to the daily intake of uranium are fresh vegetables and cereals. The predominant uranium isotope in food is ²³⁸U (Fisenne *et al.*, 1987). Levels of ²³⁴U and ²³⁸U ranging from <1 to 220 mBq/L and from <2 to 740 mBq/L (0.2–60 µg/L), respectively, were reported in bottled waters in the Federal Republic

of Germany (Gans, 1985). In a study by Cheng *et al.* (1993), the mean uranium concentration in nine different beverages was 0.98 µg/L (range 0.26–1.65 µg/L), and the mean concentration of uranium in mineral water was 9.2 µg/L.

The average daily per capita intake of uranium in food has been reported to be 1.3 µg (Fisenne *et al.*, 1987) and 2–3 µg (Singh *et al.*, 1990) in the United States and 1.5 µg in Japan (Nozaki *et al.*, 1970). In a review of naturally occurring sources of radioactive contamination in food, dietary intakes of ²³⁸U were found to range from 1.0 to 3.6 µg/d (12–45 mBq/d) in several European countries, from 0.9 to 4.8 µg/d (11–60 mBq/d) in Japan (the higher values were found in uranium mining areas) and from 1.2 to 1.4 µg/d (15–17 mBq/d) in the United States. The average daily dietary intake was of the order of 20 mBq, or about 1.6 µg. It was often difficult to determine whether these dietary intakes included that from drinking water, and it was emphasized that the latter has sometimes been found to be equal to that from the diet (Harley, 1988).

Mean levels of uranium in ambient air have been reported to be 0.02 ng/m³ in Tokyo (based on a 1979–81 survey) (Hirose & Sugimura, 1981) and 0.076 ng/m³ in New York (based on two samples, each a composite of two weekly air filter collections, from 1985 and 1986) (Fisenne *et al.*, 1987). Tracy and Prantl (1985) found the average concentration of uranium in air in a southern Ontario rural environment to be 0.1 ng/m³, based on measurements of ²²⁶Ra in dust and an assumption of equilibrium between ²³⁸U and ²²⁶Ra. Assuming a daily respiratory volume of 20 m³ and a mean urban airborne level of 0.05 ng/m³, the daily intake of uranium from air would be about 1.0 ng. Tobacco smoke (from two packages of cigarettes per day) contributes less than 0.05 µg of inhaled uranium per day (Lucas & Markun, 1970).

The daily intake of uranium from each source for adults is thus estimated to be: air, 0.001 µg; food, 2.0 µg; water, 0.6 µg. Thus, the total daily intake is approximately 2.6 µg, or 0.037 µg/kg bw for a 70-kg adult; the majority (77%) originates from food, whereas drinking water contributes most of the remainder. This is in general agreement with a review of available data for the U.S. Environmental Protection Agency, which suggested that the mean contribution of uranium from drinking water to total intake is 31.1% (U.S. EPA, 1990, 1991). The potential for uranium exposure will be greater for individuals who consume foods grown in areas with elevated concentrations of uranium in the soil and for individuals who consume drinking water containing elevated concentrations of uranium (ATSDR, 1997). In a Canadian study, Limson Zamora *et al.* (1998) found that water contributed between 31 and 98% of total daily uranium intake from food and water for individuals whose drinking water contained uranium at concentrations ranging from 2 to 780 µg/L; in contrast, intake from water was only 1–9% of total uranium intake for individuals whose municipal drinking water supply contained uranium at a concentration of 0.02 ± 0.004 µg/L.

Health Effects

Absorption, Distribution and Excretion

Although ubiquitous in the environment, uranium has no known metabolic function in animals and is currently regarded as non-essential (Berlin & Rudell, 1986).

Absorption of uranium from the gastrointestinal tract depends upon the solubility of the uranium compound (Berlin & Rudell, 1986), previous food consumption (Sullivan *et al.*, 1986; La Touche *et al.*, 1987), dose (La Touche *et al.*, 1987) and the concomitant administration of oxidizing agents, such as the iron(III) ion and quinhydrone (Sullivan *et al.*, 1986). The average gastrointestinal absorption of uranium in an adult human is 1–2% (Wrenn *et al.*, 1985, 1990;

Harduin *et al.*, 1994), but uptake may be less than 0.1% or as high as 5–6% under some conditions (Leggett & Harrison, 1995). Tracy and Limson Zamora (1994), in a study of 60 volunteers, reported a geometric mean gastrointestinal uptake of 1%, with a variation from 0.1 to 4%. Only 0.06% of ingested uranium was absorbed in Sprague-Dawley rats and New Zealand white rabbits fed *ad libitum* and provided free access to drinking water containing uranyl nitrate hexahydrate at concentrations up to 600 mg/L for periods up to 91 days (Tracy *et al.*, 1992).

Uranium rapidly appears in the bloodstream following ingestion (La Touche *et al.*, 1987), where it is associated primarily with the red cells (Fisenne & Perry, 1985); in the plasma, a non-diffusible uranyl–albumin complex is formed in equilibrium with a diffusible ionic uranyl hydrogen carbonate complex ($\text{UO}_2\text{HCO}_3^-$) (Moss, 1985). Because of their high affinity for phosphate, carboxyl and hydroxyl groups, uranyl compounds readily combine with proteins and nucleotides to form stable complexes (Moss, 1985). Clearance from the bloodstream is also rapid, and the uranium subsequently accumulates in the kidneys and the skeleton; little is found in the liver (La Touche *et al.*, 1987). In the skeleton, which is the major site of uranium accumulation (Wrenn *et al.*, 1985), the uranyl ion replaces calcium in the hydroxyapatite complex of bone crystals (Moss, 1985).

Based on the results of studies in experimental animals, it appears that the amount of soluble uranium accumulated internally is proportional to the intake from ingestion or inhalation (Wrenn *et al.*, 1985). It has been estimated that the total body burden of uranium in humans is 40 μg (Wrenn *et al.*, 1985; Igarashi *et al.*, 1987).

Once equilibrium is attained in the skeleton, uranium is excreted in the urine and faeces. Urinary excretion in humans has been found to account for approximately 1% of total excretion, averaging 4.4 $\mu\text{g}/\text{d}$ (Singh *et al.*, 1990), the rate depending in part on the pH of tubular urine (Berlin & Rudell, 1986). Under alkaline conditions, most of the uranyl hydrogen carbonate complex is stable and is excreted in the urine. If the pH is low, the complex dissociates to a variable degree, and the uranyl ion may then bind to cellular proteins in the tubular wall. This results in reduced uranyl ion excretion, and the protein binding can impair tubular function.

The half-life of uranium in the rat kidney has been estimated to be approximately 15 days. Clearance from the skeleton is considerably slower; half-lives of 300 and 5000 days have been estimated, based on a two-compartment model (Wrenn *et al.*, 1985). Overall half-lives for the clearance of uranium from the rat kidney and skeleton of 5–11 and 93–165 days, respectively, were determined in another study, based on a 10-compartment model (Sontag, 1986). The overall elimination half-life of uranium under conditions of normal daily intake has been estimated to be between 180 and 360 days (Berlin & Rudell, 1986). For rabbits, Tracy *et al.* (1992) found uranium half-lives of 14 days in kidney and greater than 200 days in bone. Fourteen percent of absorbed uranium was deposited in bone, and 3% in kidney.

Effects in Humans

Nephritis is the primary chemically induced effect of uranium in animals and humans (Hursh & Spoor, 1973).

Little information is available on the chronic health effects of exposure to environmental uranium in humans. In Nova Scotia, clinical studies were performed upon 324 persons exposed to variable amounts of naturally occurring uranium in their drinking water (uranium concentrations up to 0.7 mg/L), which was supplied from private wells. No relationship was found between overt renal disease or any other symptomatic complaint and exposure to uranium. However, a trend towards increasing excretion of urinary B_2 -microglobulin with increasing concentration of uranium

in well water was observed; this raises the possibility that an early tubular defect was present and suggests that this parameter might be useful as an index of subclinical toxicity. The group with the highest uranium concentration in well water failed to follow this trend, but this was attributed to the fact that most of the individuals in this group had significantly reduced their consumption of well water by the time the measurements were made, leading to the conclusion that the suspected tubular defect might well be rapidly reversible (Moss *et al.*, 1983; Moss, 1985).

In a study to determine renal effects induced by the chronic ingestion of uranium in drinking water, Limson Zamora *et al.* (1998) divided residents from two communities (Nova Scotia and Ontario) into two groups: the high-exposure group (n = 30) consumed drinking water that came from private wells and contained uranium concentrations of 2–781 µg/L, whereas the low-exposure group (n = 20) drank water that was supplied through the municipal distribution system and contained uranium at levels of ≤1 µg/L. Total uranium intake from both water and food over a three-day period was used as the indicator for establishing a correlation between uranium exposure and different biomarkers. Two types of biomarkers were used: indicators of kidney function (i.e., creatinine, glucose, total protein and β₂-microglobulin) and markers for cell toxicity (e.g., alkaline phosphatase, γ-glutamyl transferase and lactate dehydrogenase). Glucose excretion increased with increasing daily uranium intake, but creatinine and protein excretion did not; as well, alanine phosphatase and β₂-microglobulin were positively correlated with uranium intake for pooled male and female data. Taken together, these results suggest that, at the levels of uranium intake observed in this study, the segment of the nephron most at risk to injury is the proximal tubule, rather than the glomerulus.

In a pilot study conducted in 1993 in three communities in Saskatchewan, there was a statistically significant association (p = 0.03) between increasing but normal levels of urine albumin (measured as mg/mmol creatinine) and the uranium cumulative index. The cumulative index was calculated for each study participant as the product of the uranium concentration in drinking water, the number of cups of water consumed per day and the number of years lived at the current residence (Mao *et al.*, 1995). The study was conducted with 100 participants in three different areas with mean uranium levels ranging from 0.71 (control) to 19.6 µg/L. Urine albumin levels ranged from 0.165 to 16.1 mg/mmol creatinine, with eight participants having “elevated” urine albumin concentrations (>3.0 mg/mmol creatinine). Three participants had serum creatinine concentrations of >120 µmol/L (range 50–170 µmol/L), which is reportedly indicative of prevalent renal damage. It should be noted, however, that diabetics were not excluded from the study, although diabetic status and age, known risk factors for renal dysfunction, were factored into the statistical analysis of the results. According to the authors, microalbuminuria has been shown to be a sensitive indicator of early renal disease. A follow-up study is currently in progress.

Toxicological Studies

Reported oral LD₅₀s of uranyl acetate for rats and mice are 204 mg/kg bw and 242 mg/kg bw, respectively (Domingo *et al.*, 1987). Among the most common signs of acute toxicity are piloerection, significant weight loss and haemorrhages in the eyes, legs and nose.

The most common renal injury caused by uranium in experimental animals is damage to the proximal convoluted tubules, predominantly in the distal two-thirds (Berlin & Rudell, 1986; Anthony *et al.*, 1994; Domingo, 1995); the rate at which the effects occur varies with dosage level (Leggett, 1989). It has recently been shown that uranyl inhibits both Na⁺ transport-dependent and Na⁺ transport-independent ATP utilization as well as mitochondrial oxidative phosphorylation in the renal proximal tubule (Leggett, 1989; Domingo, 1995). At doses not high

enough to destroy a critical mass of kidney cells, the effect appears to be reversible, as some of the cells are replaced; however, the new epithelial lining differs morphologically, and possibly functionally, from normal epithelium (Wrenn *et al.*, 1985; Berlin & Rudell, 1986).

Histopathologically, the regenerated cells are simple flattened cells with no microvilli on luminal surfaces and with reduced numbers of mitochondria (Leggett, 1989).

There is some evidence that tolerance may develop following repeated exposure to uranium (Yuile, 1973; Durbin & Wrenn, 1976; Campbell, 1985). This tolerance does not, however, prevent chronic damage to the kidney, as the regenerated cells are quite different; although histopathologically it may appear that the repair process is well advanced, the urinary biochemical changes return to normal only slowly (Leggett, 1989). Alterations causing thickening of the glomerular basement membrane of the kidney, which results from the storage of uranium in the kidney, can be prolonged and severe enough to cause permanent damage (McDonald-Taylor *et al.*, 1992). Persistent ultrastructural changes in the proximal tubules of rabbits have also been reported to be associated with the kidney's ability to store uranium (McDonald-Taylor *et al.*, 1997). Cell damage in the proximal tubules was significantly more severe in animals allowed up to a 91-day recovery period than in animals killed at the end of the exposure period. Acquired tolerance should therefore not be considered as a practical method of protection against uranium intoxication.

Forty male Sprague-Dawley rats given uranyl ethanoate dihydrate at 0, 2, 4, 8 or 16 mg/kg bw per day (equivalent to uranium doses of 0, 1.1, 2.2, 4.5 or 9.0 mg/kg bw per day) in drinking water for 4 weeks exhibited a variety of biochemical effects, including increases in blood glucose levels at ≥ 4 mg uranyl ethanoate dihydrate/kg bw per day, decreases in aspartate aminotransferase and alanine aminotransferase levels at ≥ 8 mg uranyl ethanoate dihydrate/kg bw per day, increases in several other haematological parameters at 16 mg uranyl ethanoate dihydrate/kg bw per day and increases in total protein levels in all treated groups (Ortega *et al.*, 1989). The authors considered the no-observed-adverse-effect level (NOAEL) to be 2 mg uranyl ethanoate dihydrate/kg bw per day (1.1 mg U/kg bw per day).

Groups of 15 male and 15 female weanling Sprague-Dawley rats consumed water containing uranyl nitrate hexahydrate at <0.001 (control), 0.96, 4.8, 24, 120 or 600 mg/L (equivalent to uranium doses of <0.0001 , 0.06, 0.31, 1.52, 7.54 and 36.73 mg/kg bw per day in males and <0.0001 , 0.09, 0.42, 2.01, 9.98 and 53.56 mg/kg bw per day in females) for 91 days (Gilman *et al.*, 1998a). Histopathological changes were observed mainly in the liver, thyroid and kidney. In the liver, treatment-related lesions were seen in both sexes at all doses and were generally non-specific nuclear and cytoplasmic changes. The thyroid lesions were not considered specific to the uranium treatment. The kidney was the most affected tissue. In males, statistically significant treatment-related kidney lesions (reported at all doses) included nuclear vesiculation, cytoplasmic vacuolation and tubular dilation. Other statistically significant lesions in males (≥ 4.8 mg uranyl nitrate hexahydrate/L) included glomerular adhesions, apical displacement of the proximal tubular epithelial nuclei and cytoplasmic degranulation. In females, statistically significant changes in the kidney included nuclear vesiculation of the tubular epithelial nuclei (all doses) and anisokaryosis (all doses except 4.8 mg uranyl nitrate hexahydrate/L). However, the most important changes in the female were the capsular sclerosis of glomeruli and reticulin sclerosis of the interstitial membranes; these changes occurred in all dose groups and are considered to be "nonreparable lesions." The lowest-observed-adverse-effect level (LOAEL) for adverse effects on the kidney of male and female rats, based on the frequency and degree of degenerative lesions in the renal proximal convoluted tubule, was considered to be 0.96 mg uranyl

nitrate hexahydrate/L (equivalent to 0.09 mg U/kg bw per day in females and 0.06 mg U/kg bw per day in males). The reason for the difference in sensitivity between males and females is not clear, but it did not appear to be due to differences in pharmacokinetics, as accumulation of uranium in renal tissue did not differ significantly between the two sexes at all doses and females received a larger time-weighted average dose than males.

In a similar study, groups of 10 male New Zealand white rabbits were given uranyl nitrate hexahydrate in drinking water at concentrations of <0.001 (controls), 0.96, 4.8, 24, 120 or 600 mg/L (determined to be equivalent to doses of 0, 0.05, 0.2, 0.88, 4.82 and 28.7 mg U/kg bw per day) for 91 days (Gilman *et al.*, 1998b). These rabbits were not *Pasteurella*-free, and four of them contracted a *Pasteurella* infection during the course of the study. In the same study, 10 *Pasteurella*-free female rabbits were exposed to drinking water containing <0.001 (controls), 4.8, 24 or 600 mg uranyl nitrate hexahydrate/L (equivalent to doses of 0, 0.49, 1.32 and 43.02 mg U/kg bw per day) for 91 days. In both sexes, histopathological changes were observed in the kidney tubule, liver, thyroid and aorta. In male rabbits, histopathological findings were observed in the kidney tubules at doses above 0.96 mg uranyl nitrate hexahydrate/L. When compared with controls, significant treatment-related changes included cytoplasmic vacuolation, anisokaryosis, nuclear pyknosis and nuclear vesiculation; the incidence of nuclear vesiculation and anisokaryosis appeared to be dose-related, with nuclear vesiculation having the higher frequency and severity. Other treatment-related changes included tubular dilation, hyperchromicity, tubular atrophy, changes in the interstitium collagen and reticulin sclerosis. In total, 10 different morphological indicators of tubular injury were observed in the highest exposure group. The LOAEL, based on the nuclear changes in the kidney, was considered to be 0.96 mg uranyl nitrate hexahydrate/L (equivalent to 0.05 mg U/kg bw per day). In *Pasteurella*-free female rabbits, dose-related and treatment-related nuclear changes in the kidney tubule included anisokaryosis and vesiculation, which were significantly different from effects observed in controls at all doses. Other treatment-related changes in the kidney included cytoplasmic vacuolation, tubular atrophy and nuclear pyknosis. In general, histopathological changes in the kidney in females were generally less marked than in males. The LOAEL was considered to be 4.8 mg uranyl nitrate hexahydrate/L (equivalent to 0.49 mg U/kg bw per day). In both sexes, histopathological changes in the liver, thyroid and aorta were similar. In the liver, changes may have been treatment-related, although very mildly affected animals were seen in all groups, and changes in the thyroid were mild. Changes in the aorta were not dose-dependent. It should be noted that no similar aortic changes were observed in the 91-day uranyl nitrate hexahydrate studies in rats (Gilman *et al.*, 1998a). It is interesting to note, however, that even though the female rabbits consumed on average 65% more water than the males and their average uranium intake was approximately 50% greater on a mg/kg bw per day basis, their average tissue levels were not similarly raised. The average kidney uranium level in females was 20% of that in males, whereas the average bone uranium level in females was 76% of that in males. The differences between the males and females, both qualitative and quantitative, suggest pharmacokinetic parameter differences, which contrasts with the findings in the rat study by the same authors (Gilman *et al.*, 1998a).

In an additional study to observe the reversibility of renal injury in *Pasteurella*-free male New Zealand white rabbits, groups of 5–8 animals were given <0.001 (control), 24 or 600 mg uranyl nitrate hexahydrate/L (equivalent to 0, 1.36 and 40.98 mg U/kg bw per day) in drinking water for 91 days, with a recovery period of up to 91 days (Gilman *et al.*, 1998c). Minor histopathological lesions were seen in the liver, thyroid and aorta. In the kidney, tubular injury with degenerative nuclear changes, cytoplasmic vacuolation and tubular dilation was observed in

the high-dose group, which did not exhibit consistent resolution even after a 91-day recovery period. Although the severity of the histopathological changes at 600 mg uranyl nitrate hexahydrate/L did not increase between the 45-day and 91-day recovery groups, the continued prevalence of histopathological changes during this time suggests a self-sustaining injury. Also, the presence of sclerotic changes in the tubular basement membranes and renal interstitium persisted during the recovery period and most likely represents a permanent injury (McDonald-Taylor *et al.*, 1992, 1997; Gilman *et al.*, 1998c). In general, the male rabbits did not respond as dramatically as those in the earlier study (Gilman *et al.*, 1998b), although the histopathological changes observed in this study were similar to those noted in the female rabbits of the previous study. Animals in this study consumed approximately 33% more uranium per day than the males in the previous study (Gilman *et al.*, 1998b), yet uranium residues in kidney tissue were 30% less, which would appear to indicate that *Pasteurella*-free rabbits are less sensitive than the non-*Pasteurella*-free strain to the effects of the uranyl ion in drinking water. Based on the histopathological data in the kidney, a LOAEL for the male New Zealand rabbits in this study is estimated to lie at or below 24 mg uranyl nitrate hexahydrate/L.

In an early series of experiments, very high doses (up to 20% in the diet) of a variety of uranium compounds were fed to rats, dogs and rabbits for periods ranging from 30 days to 2 years (Maynard and Hodge, 1949). On the basis of very limited histopathological investigations, renal damage was reported in each species.

Adverse reproductive effects, in terms of total number of litters and average number of young per litter, were reported in rats given 2% uranyl nitrate hexahydrate for 7 months (Maynard and Hodge, 1949). More recent studies have examined the teratogenic/embryotoxic effects and reproductive outcomes of uranyl acetate dihydrate administration to mice. Domingo *et al.* (1989a) evaluated the developmental toxicity of uranium by treating groups of 20 pregnant Swiss mice by gavage to doses of 0, 5, 10, 25 or 50 mg uranyl acetate dihydrate/kg bw per day (equivalent to 0, 2.8, 5.6, 14 and 28 mg U/kg bw per day) on days 6–15 of gestation; the animals were sacrificed on day 18. Although all dams survived until sacrifice, there was a dose-related reduction in maternal weight gain, a significant decrease in daily feed intake and a significant increase in liver weights. Exposure-related foetotoxicity, including reduced foetal body weights and length, increased incidence of stunted foetuses per litter, increased incidence of both external and internal malformations and increased incidence of developmental variations, was observed in the foetuses of mice at all doses. At doses of ≥ 14 mg U/kg bw per day, specific malformations included cleft palate and bipartite sternbrae, and developmental variations included reduced ossification and unossified skeletal variations. There was no evidence of embryoletality at any dose. Based on both the maternal and foetotoxic effects, a LOAEL of 2.8 mg U/kg bw per day could be considered.

A second study by Domingo *et al.* (1989b) evaluated the effect of uranium on late foetal development, parturition, lactation and postnatal viability. Groups of 20 female mice were treated by gavage from day 13 of pregnancy until day 21 of lactation to doses of 0, 0.05, 0.5, 5 or 50 mg uranyl acetate dihydrate/kg bw per day (equivalent to 0, 0.028, 0.28, 2.8 and 28 mg U/kg bw per day). Maternal deaths (2/20 and 3/20 at the two highest doses, respectively) were attributed to the treatment; however, maternal toxicity was not evident from changes in body weight or food consumption, although relative liver weight was significantly reduced in all treatment groups. Decreases in pup viability, as indicated by significant decreases in litter size on day 21 of lactation,

and significant decreases in the viability and lactation indexes were observed in the highest dose group. Based on developmental effects in pups, the authors established a no-observed-effect level (NOEL) of 2.8 mg U/kg bw per day.

Paternain *et al.* (1989) studied the effects of uranium on reproduction, gestation and postnatal survival in mice. Groups of 25 mature male Swiss mice were administered intragastric doses of 0, 5, 10 or 25 mg uranyl acetate dihydrate/kg bw per day (equivalent to 0, 2.8, 5.6 and 14 mg U/kg bw per day) for 60 days prior to mating with mature females (25 per group). Females were exposed for 14 days prior to mating (males and females were mated according to their respective dose levels), and exposure continued through mating, gestation, parturition and nursing of litters; half the treated dams were sacrificed on day 13 of gestation. No treatment-related effects on mating or fertility were observed. Embryo lethality (number of late resorptions and dead foetuses) was significantly increased and the number of live foetuses was decreased in the highest dose group. Lethality in pups (at birth and at day 4 of lactation) was significantly increased at ≥ 5.6 mg U/kg bw per day, and pup growth (decreases in weight and length) and development of offspring, from birth and during the entire lactation period, were significantly affected in the high-dose group. The NOEL was 5 mg uranyl acetate dihydrate/kg bw per day, equivalent to 2.8 mg U/kg bw per day.

Unspecified degenerative changes in the testes of rats have also been reported following chronic administration of uranyl nitrate hexahydrate and uranyl fluoride in the diet (Maynard and Hodge, 1949; Maynard *et al.*, 1953; Malenchenko *et al.*, 1978). In a more recent study, male Swiss mice were exposed for 64 days to uranyl acetate dihydrate in drinking water at doses of 0, 10, 20, 40 or 80 mg/kg bw per day (equivalent to 0, 5.6, 11.2, 22.4 and 44.8 mg U/kg bw per day) prior to mating with untreated females for 4 days (Llobet *et al.*, 1991). With the exception of interstitial alterations and vacuolization of Leydig cells at the highest dose, no effects were observed in testicular function/spermatogenesis. There was, however, a significant, non-dose-related decrease in the pregnancy rate of these animals.

Although bone cancer has been induced in experimental animals by injection or inhalation of soluble compounds of high-specific-activity uranium isotopes or mixtures of uranium isotopes, no carcinogenic effects have been reported in animals ingesting soluble or insoluble uranium compounds (Wrenn *et al.*, 1985).

Mutagenicity

Uranyl nitrate was cytotoxic and genotoxic in Chinese hamster ovary cells at concentrations ranging from 0.01 to 0.3 mmol/L. There was a dose-related decrease in the viability of the cells, a decrease in cell cycle kinetics and increased frequencies of micronuclei, sister chromatid exchanges and chromosomal aberrations (Lin *et al.*, 1993). The authors suggest that the data provide a possible mechanism for the teratogenic effects observed in the studies by Domingo *et al.* (1989a). The genotoxic effects in this study were thought to occur through the binding of the uranyl nitrate to the phosphate groups of DNA. Chromosomal aberrations have also been induced in male mouse germ cells exposed to enriched uranyl fluoride; however, these aberrations may have been produced by the radioactivity of the test compound (Hu & Zhu, 1990).

Other Special Studies

A number of studies have reported that the toxic effects of uranium can be prevented, or possibly alleviated, by the administration of chelating agents (Domingo, 1995). Three chelating agents have been found to be effective at enhancing faecal excretion of uranium and reducing

levels of uranium in bone and kidney in mice — sodium 4,5-dihydroxybenzene-1,3-disulfonate (Tiron), desferrioxamine (DFOA) and 1,2-dimethyl-3-hydroxypyrid-4-one (L1); Tiron was the most effective agent of the compounds studied. However, administration of these agents 24 hours or more after uranium exposure was not effective.

Classification and Assessment

Although the potential exists for radiological toxicity of orally administered ^{235}U , this has not been observed in humans or animals, presumably because of the relatively low specific activity of this mixture of uranium radionuclides. Experimental evidence of the carcinogenicity of uranium is restricted to highly insoluble or enriched uranium compounds delivered by inhalation or injection. Although these observations do not seem relevant to the ingestion of ^{235}U in drinking water (Wrenn *et al.*, 1985), the associated risk for induction of bone cancer has been inferred from the known risk due to ^{226}Ra exposure. The estimated excess risk of induction of bone sarcoma is considered to be insignificant compared with the normal background lifetime risk (Wrenn *et al.*, 1985). The chemical toxicity of ^{235}U has been observed in both humans and animals. Because the chemical data reviewed to date suggest a more stringent recommendation than those based upon available radiological criteria, it is recommended that the assessment of uranium toxicity in drinking water be based upon chemical criteria. Uranium has, therefore, been included in Group V (inadequate data for evaluation of carcinogenicity).

For compounds classified in Group V, the maximum acceptable concentration (MAC) is derived on the basis of the division of the NOAEL or LOAEL for the critical response (i.e., nephrotoxicity for uranium) in humans or an animal species by an appropriate uncertainty factor. As no adequate chronic study was identified, the tolerable daily intake (TDI) has been derived based on the results of the most extensive subchronic studies, in the most sensitive sex and species, conducted to date in which uranium was administered in drinking water (Gilman *et al.*, 1998a). In the 91-day study in rats, the LOAEL for degenerative lesions in the proximal convoluted tubule of the kidney in males was considered to be 0.96 mg uranyl nitrate hexahydrate/L, which is equivalent to 0.06 mg U/kg bw per day (or 60 $\mu\text{g}/\text{kg}$ bw per day). The TDI is derived as follows:

$$\text{TDI} = \frac{60 \mu\text{g}/\text{kg bw per day}}{100} = 0.6 \mu\text{g}/\text{kg bw per day}$$

where:

- 60 $\mu\text{g}/\text{kg}$ bw per day is the LOAEL for adverse effects on the kidney in male rats (the most sensitive sex and species) in a subchronic study (Gilman *et al.*, 1998a) (male rabbits were also more sensitive to the renal effects of uranium than females), and
- 100 is the uncertainty factor ($\times 10$ for intraspecies variation and $\times 10$ for interspecies variation).

There is no need to apply an additional uncertainty factor to account for the use of a LOAEL instead of a NOAEL because of the minimal degree of severity of the lesions being reported. Also, an additional uncertainty factor for the length of the study (91 days) is not required because the estimated half-life of uranium in the kidney is 15 days.

Rationale

Based on the above TDI, the proposed guideline value (recommended maximum acceptable concentration, or RMAC) may be determined as follows:

$$\text{RMAC} = \frac{0.6 \mu\text{g/kg bw per day} \times 70 \text{ kg bw} \times 0.35}{1.5 \text{ L/d}} \approx 10 \mu\text{g/L (rounded from 9.8 } \mu\text{g/L)}$$

where:

- 0.6 $\mu\text{g/kg bw per day}$ is the TDI, as derived above,
- 70 kg bw is the average weight of an adult,
- 0.35 is the proportion of daily intake of uranium allocated to drinking water; this allocation factor was determined, from data in the "Exposure" section, to best describe intake for the general population, which is generally exposed to drinking water containing uranium at concentrations below 10 $\mu\text{g/L}$,
- 1.5 L/d is the average daily consumption of drinking water by an adult.

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At an allocation factor of 70%, which would be appropriate for the population of most concern — those individuals exposed to elevated levels of uranium from naturally occurring deposits in drinking water — the resulting RMAC would be 20 $\mu\text{g/L}$, a less conservative value.

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Appendix I: Occurrence of Uranium in Provincial/Territorial Water Supplies and Costs of Treatment for Supplies Containing Uranium at Concentrations in Excess of the Recommended MAC

Prince Edward Island

Based on 58 analyses for uranium in groundwater on Prince Edward Island, mean uranium concentrations were 2.9 µg/L, with a maximum concentration of 15 µg/L; 6.9% of samples exceeded the proposed guideline value of 10 µg/L. Slightly more than half of the samples were from municipal wells, collected after pump testing. The remaining samples were from domestic wells scattered around the province.

For domestic wells, serving about half of the population, the costs of treatment for supplies with uranium concentrations in excess of the proposed guideline value are expected to be significant but modest, probably in the range of \$500 to \$1000 for point of use treatment, depending on the most appropriate technology. This would add some 25 to 35% to the cost of securing a potable water supply on Prince Edward Island. Given the limited data available, it might be expected that some 900 to 1000 households could be affected (if, in fact, all households had their water analysed for uranium).

For municipal wells, the issue is more problematic, as the most effective treatment technology to be applied in these circumstances is not known, and as there are no comparable treatment plants on the Island on which to base cost estimates. Given the absence of any treatment plants currently, costs are presumed to be high, and the response to elevated uranium might be relocation of the source of supply (also a high cost, but possibly more manageable).

Newfoundland

The sampling and analysis of water for uranium are rarely carried out in Newfoundland. Testing is carried out only if there are special reasons to suspect the presence of uranium. One spring source of water was found to contain uranium at 105 µg/L.

The cost of testing all water supplies for uranium would be quite high, given that there are few data to start with. In other words, there are no data that would allow regions less likely to have uranium to be screened out (although the possibility of correlating geochemical data on the distribution of uranium in lake sediment with uranium concentrations in water should be investigated).

It is premature to comment on the cost of removing uranium from water if any unacceptable concentrations were to be found.

Nova Scotia

Uranium occurrence

Data source	Year	n	Range	>100 µg/L	>10 µg/L
1. Municipal water supply survey	1994	125	<5-7 µg/L	0%	0%
2. Uranium Task Force					

Data source	Year	n	Range	>100 µg/L	>10 µg/L
- municipal water supplies	1981	267	<5-10 µg/L	0%	0%
- private water supplies*	1980-1982	784	<5-700 µg/L	3.2%	44.4%
3. Laboratory data					
- private water supplies	1991-1997	6148	<5-830 µg/L	1.3%	10.5%

*Note: Wells tested were in areas where bedrock was suspected or known to contain uranium.

Uranium treatment costs

1. Municipal water supplies: no extra cost

2. Private water supplies:

2500-3000 new wells constructed each year × 10% × \$1000 per treatment unit
= \$250-300 K per year for new wells (does not include treatment of existing wells)

New Brunswick

From January 1, 1994, until March 1, 1998, 1575 analyses of uranium concentrations in public water (i.e., municipal and Crown-operated systems) in New Brunswick have been recorded. The minimum value recorded was 0.01 µg/L, the maximum value was 200 µg/L, and the average value was 1.8 µg/L (standard deviation 8.1 µg/L). Fifty-one samples contained concentrations above 10 µg/L; these were clustered at approximately five locations. Three samples contained levels above 100 µg/L.

Quebec

Précisons d'abord que Le Règlement sur l'eau potable impose le contrôle obligatoire de l'uranium par les exploitants. Plus de 2300 réseaux doivent ainsi fournir des résultats d'analyse pour ce paramètre. La moitié de ces derniers sont alimentés par des eaux souterraines.

Les résultats d'uranium sont répertoriés dans la banque informatisée Eau potable. Nous avons procédé à l'analyse des résultats fournis entre 1990 et 1997 inclusivement.

Les données sont disponibles pour 1690 réseaux soit pour 75% des réseaux visés par Le Règlement. Il est cependant difficile de préciser dans quelle mesure les réseaux qui ont fourni des résultats sont alimentés par des eaux souterraines. On peut toutefois considérer que les données fournissent un portrait de la moitié de ces réseaux au Québec. On ignore également leur distribution sur le territoire québécois et par conséquent dans quelle mesure les résultats dont nous disposons sont représentatifs de la situation qui prévaut à l'échelle provinciale.

Sur plus de 9661 échantillons, seulement 167 ont présenté des concentrations égales ou supérieures au seuil de détection.

Les données recueillies sont à l'effet que 136 réseaux ont présenté au moins à une occasion une concentration supérieure au seuil de détection (0.005 µg/L). Les concentrations minimale et maximale supérieures au seuil de détection qui ont été mesurées sont respectivement de 0.005 et 0.31 µg/L.

La majorité de ces résultats correspondent toutefois au seuil de détection de 0.005 µg/L ou sont légèrement supérieurs à cette valeur. De plus, ces valeurs positives ont été le plus souvent mesurées occasionnellement dans la plupart des réseaux.

Treize (13) résultats sont toutefois supérieurs à 10 µg/L et ont été détectés dans trois (3) réseaux.

Éventuellement, le Ministère pourra mieux préciser la situation qui prévaut en regard de la présence de l'uranium dans les eaux souterraines de l'ensemble du territoire québécois à l'aide d'une méthode d'analyse dont le seuil de détection est de l'ordre de 0.2 µg/L.

Ontario

As of December 1997, Ontario's Drinking Water Surveillance Program (DWSP) had monitored 148 of Ontario's approximately 627 water supply systems for uranium. None of the water supply systems monitored had uranium levels in excess of 10 µg/L.

Uranium levels in the water supply systems not covered by DWSP are not currently available.

Many of the water supply systems that are not monitored through the DWSP are located in Central and Northern Ontario, where bedrock, and drinking water supplies, may contain uranium.

The impact of a lower uranium guideline on these systems and on private wells in the Province needs to be ascertained.

Saskatchewan

The following estimates are based on total uranium data collected from the distribution system from 1988 to 1998.

Proposed uranium MAC (µg/L)	Number of communal systems	Estimated population served	Estimated capital costs (\$)
10	67	15 515	9 000 000*
20	27	7 849	3 200 000*

* For ultra-filtration system. The estimated costs exclude operating and maintenance costs.

Alberta

Below is a table listing all uranium analyses in treated drinking water in Alberta from 1997 up to March 31, 1998. The method detection limit is 0.05 mg/L (ICP-MS).

Station	Date	Uranium concentration (mg/L)	Type	Population
Alliance	18-Mar-98	0.000 8	S	230
Ardmore	27-Mar-98	0.000 36	G	224
Ardmore/Fort Kent	05-Feb-98	0.000 36	G	224/111

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Station	Date	Uranium concentration (mg/L)	Type	Population
Ashmont	25-Mar-97	0.000 31	S	153
Athabasca	25-Feb-97	0.000 34	S	2 279
	25-Apr-97	0.000 1		
	11-Jun-97	0.000 16		
	21-Jan-98	0.000 47		
Barrhead	14-Aug-97	0.000 28	S	4 160
	15-Jan-98	0.000 55		
Bear Canyon	26-Feb-97	0.000 22	G	9
Beaverlodge	27-Jan-98	0.000 22	S	1 779
Black Diamond	23-Jun-97	0.000 94	G	1 743
Bon Accord	26-Aug-97	0.005 91	G	1 460
	26-Mar-98	0.005 69		
Bow Island School	31-Mar-98	0.000 5	S	1 509
Boyle	23-Jul-97	7E-05	S	862
	21-Jan-98	6E-05		
Brooks	04-Jun-97	0.000 73	S	9 433
Bruce	14-Jan-98	0.000 81	S	88
Brule	17-Nov-97	0.000 49	S	82
Cadotte Lake	27-Feb-97	0.000 12	S	241
	07-Oct-97	0.000 11		
Calalta Waterworks Springbank Middle School	14-Jan-98	0.000 4		
Calgary Bearspaw	12-Jun-97	0.000 15	S	767 059 (total for Calgary)
Calgary Glenmore	12-Jun-97	0.000 3	S	
Camrose	15-May-97	0.000 14	S	14 121
Carson-Pegasus	10-Jul-97	0.000 17		
Carstairs	05-Feb-98	0.000 66	S	1 796
Claresholm	26-Mar-97	0.001 37	S	3 297
Cleardale	26-Feb-97	0.000 09	S	25
Clyde	18-Dec-97	0.000 35	G	441
Cochrane	02-Apr-97	0.000 57	S	6 612
	25-Mar-98	0.000 49		

Station	Date	Uranium concentration (mg/L)	Type	Population
Cold Lake	24-Feb-97	7E-05	S	4 250
	30-May-97	8E-05		
	29-Nov-97	9E-05		
	05-Feb-98	9E-05		
Craigmyle	24-Nov-97	0.000 37	G	79
Dixonville	04-Feb-98	0.005 54	G	90
Donnelly	06-Oct-97	0.000 47	S	421
Drayton Valley	26-Mar-97	5E-05	S	5 486
	10-Sep-97	7E-05		
Drumheller	09-Apr-97	0.000 98	S	6 277
Eaglesham	03-Dec-97	0.000 83	S	184
Edmonton E.L. Smith	12-Mar-97	8E-05	S	626 999 (total for Edmonton)
	18-Jul-97	5E-05		
	24-Nov-97	0.000 1		
	28-Jan-98	5E-05		
	26-Mar-98	0.000 25		
Edmonton Rossdale	23-Apr-97	0.000 1		
	19-Jun-97	0.000 12		
	22-Aug-97	0.000 11		
	23-Oct-97	0.000 4		
	18-Dec-97	0.000 33		
	09-Feb-98	0.000 36		
Edson	13-May-97	0.000 28	G	7 323
Elizabeth Metis Settlement	15-Jan-98	0.000 42		
Elk Point	26-Feb-97	0.000 26	S	1 341
	09-Oct-97	0.000 23		
Evansburg	18-Nov-97	0.000 32	G	723
Fishing Lake Metis Settlement	06-Feb-98	0.006 61		
Fort McMurray	25-Mar-97	0.000 38	S	33 698
	19-Mar-98	0.000 46		
Fox Creek	19-Mar-98	0.000 95	G	2 257
Garner Lake PP	14-Aug-97	0.001 4		
Girouxville	31-Mar-98	0.000 75	S	349
Gleichen	28-May-97	0.000 43	S	331
Grande Cache	30-Apr-97	0.000 11	S	3 842
	08-Dec-97	0.000 12		

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Station	Date	Uranium concentration (mg/L)	Type	Population
Grande Prairie	18-Dec-97	0.000 31	S	29 242
Grassland	23-Jul-97	0.000 13	S&G	66
	21-Jan-98	0.000 2		
Grassy Lake	12-Sep-97	0.001 15	S	244
Guy	22-Sep-97	0.000 22	S	54
	13-Jan-98	0.000 16		
Hay Lakes	23-Apr-97	0.000 51	S	327
	14-Jan-98	0.000 58		
High Prairie	09-Jun-97	0.000 08	S	2 932
High River	24-Apr-97	0.000 7	G	6 893
Hinton	25-Sep-97	0.000 15	S	9 341
Holden	14-Jan-98	0.000 38	S	411
Innisfree	18-Mar-98	0.000 33	G	254
Jasper	25-Sep-97	0.000 65		
Jean Cote	22-Sep-97	0	S	75
	13-Jan-98			
Keg River	05-Mar-97	0.001 73		
Kikino	11-Jun-97	0.000 16		
Lac La Biche	25-Apr-97	0.000 1	S	2 737
	03-Sep-97	7E-05		
	04-Dec-97	7E-05		
	26-Mar-98	8E-05		
Lethbridge	08-May-97	0.000 26	S	64 938
Lomond	27-Mar-97	0.002 89	S	167
Longview	23-Jun-97	0.000 38	G	304
Manning	05-Mar-97	0.001 62	S	1 139
Marlboro	18-Nov-97	0.000 08		
Mayerthorpe	28-Jul-97	0.003 6	G	1 692
McLennan	04-Mar-98	0.000 95	S	1 026
Medicine Hat	26-Mar-97	0.000 18	S	45 892
	02-Jul-97	0.000 15		
	11-Sep-97	0.000 25		
	31-Mar-98	0.000 39		

Station	Date	Uranium concentration (mg/L)	Type	Population
Millet	14-Mar-97	6E-05	G	2 005
	23-Mar-98	6E-05		
Milo	27-Mar-98	0.000 81	S	120
Morrin	24-Nov-97	0.001 16	G	251
Nampa	25-Feb-97	0.000 6	S	496
	06-Oct-97	0.000 29		
Neerlandia	11-Sep-97	0.000 34	S	71
	26-Mar-98	0.000 38		
Okotoks	24-Apr-97	0.000 72	G	7 789
Onoway	10-Sep-97	0.001 97	G	681
Paddle Prairie	28-Jul-97	0.000 1		
	19-Feb-98	0.000 29		
Parkland Village	16-Sep-97	0.000 18		
Peace River	28-Nov-97	0.000 07	S	6 696
Peavine	16-Dec-97	0.000 32		
Plamondon	23-Jul-97	9E-05	S	253
	05-Dec-97	6E-05		
	26-Mar-98	6E-05		
Ponoka	18-Mar-98	0.000 13	G	5 861
Priddis Greens	10-Sep-97	0.000 23		
Red Earth	19-Aug-97	0.000 52	S	453
Rockyford	17-Dec-97	0.001 04	S	331
Rolling Hills	04-Sep-97	0.000 89	S	175
Rycroft	19-Nov-97	0.000 94	S	634
Sangudo	28-Jul-97	0.000 14	G	405
Seven Persons	26-Mar-97	0.002 03	S	200
Silver Willows	19-Feb-98	0.004 57		
Slave Lake	10-Jun-97	0.000 09	S	6 203
Smith	24-Apr-97	0.000 4	S	323
	20-Nov-97	0.000 33		
Smoky Lake	14-Aug-97	0.002 72	G	1 057
Springbank	03-Jul-97	0.000 17		

Health Canada
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Station	Date	Uranium concentration (mg/L)	Type	Population
St. Paul	25-Apr-97	0.000 26	S	5 021
	09-Oct-97	0.000 23		
	06-Feb-98	0.000 27		
Standard	17-Dec-97	0.001	S	359
Strathmore	28-May-97	0.000 31	S	5 273
	07-Jan-98	0.000 92		
	26-Mar-98	0.000 97		
Tangent	03-Dec-97	0.000 84	S	60
Thorhild	27-Nov-97	0.000 39	S	502
Thorsby	26-Mar-97	0.000 42	S	708
	25-Feb-98	0.000 25		
Three Hills	07-Apr-97	0.000 28	S	3 298
Trochu	23-Oct-97	0.000 14	G	907
Vauxhall	04-Sep-97	0.000 94	S	1 024
Vegreville	18-Mar-98	0.000 59	S	5 138
Vilna	24-Mar-97	0.000 14	S	314
	30-May-97	0.000 11		
	27-Nov-97	0.000 21		
Wabamun	15-Jan-98	0.000 25	S	600
Wandering River	28-Aug-97	0.000 07	S	43
Wanham	03-Dec-97	0.000 66	S	216
Westlock	20-Mar-97	0.000 38	S	4 719
	15-Aug-97	0.000 4		
	27-Mar-98	0.000 81		
Whitecourt	10-Jul-97	0.000 35	S	7 056
	26-Mar-98	0.000 91		
Wildwood	17-Nov-97	0.000 17	G	353
Woking	29-Jul-97	0.000 06	S	77

G = groundwater; S = surface water.

British Columbia

Data presented below are for 358 of the approximately 2500 waterworks in British Columbia, which range from municipal systems to small systems serving two homes or a public

facility. Data are expressed as number of waterworks with an average uranium concentration in the specified range. All systems reported here with uranium levels above 20 ppb serve 300 or fewer connections with the one exception noted.

Average concentration (ppb)	Number of waterworks
<0.2	44
0.2-10	286
11-20	13
21-30	7
31-40	1
41-50	0
51-60	0
61-70	1
71-80	1
81-90	1
91-100	1
160	1 system, transient population
227	1 community system, population 2500
720	1 system, transient population

Yukon

All community supplies currently meet the existing standard. Should the limit be changed to 0.01 mg/L, one community serving some 500 persons (Champagne, First Nation land; see table below) would not meet the standard (current level at 0.023 mg/L). Should the World Health Organization level of 0.002 mg/L be adopted, water supplies to less than 2% of the population would be affected.

Data on uranium levels in community well water supplies (last measured in March 1993) follow:

Community*	Population (Yukon Statistics 1997)	Uranium concentration (mg/L)
Whitehorse	24 031	0.002
Mayo	507	0.000 3
Dawson City	2 151	0.000 66
Pelly Crossing	299	0.000 68

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Community*	Population (Yukon Statistics 1997)	Uranium concentration (mg/L)
Champagne	539	0.023
Canyon Creek		0.000 79
Takhini River		
Haines Junction	862	<0.000 05
Destruction Bay	41	0.000 86
Beaver Creek	116	0.000 22
Burwash Landing	88	0.000 25
Carmacks	478	0.001 7
Faro	1 266	0.005 4
Ross River	437	0.003
Teslin	478	0.000 08
Watson Lake	1 791	0.000 44
Carcross	431	0.007
Tagish	142	0.000 09

* Community wells missing include Mt. Lorne, Elsa and Keno.

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SECOND EDITION

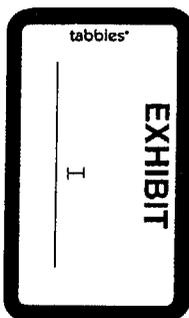
Addendum to Volume 2

*Health criteria and
other supporting information*

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URANIUM¹

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At the time of publication of the 1993 *Guidelines for drinking-water quality*, adequate short- and long-term studies on the chemical toxicity of uranium were unavailable, and therefore a guideline value for uranium was not derived. Instead, it was recommended that the limits for radiological characteristics of uranium be adopted. The equivalent for natural uranium, based on these limits, is approximately 140 µg/litre.

As new data on the chemical toxicity of uranium are now available for use in the derivation of a guideline value, the 1995 Coordinating Committee for the Updating of WHO *Guidelines for drinking-water quality* recommended that uranium be included in the 1998 Addendum.

1. GENERAL DESCRIPTION

1.1 Identity

Uranium occurs naturally in the +2, +3, +4, +5, and +6 valence states, but it is most commonly found in the hexavalent form. In nature, hexavalent uranium is commonly associated with oxygen as the uranyl ion, UO₂²⁺. Naturally occurring uranium (^{nat}U) is a mixture of three radionuclides (²³⁴U, ²³⁵U, and ²³⁸U), all of which decay by both alpha and gamma emissions (Cothem & Lappenbusch, 1983; Lide, 1992-93). Natural uranium consists almost entirely of the ²³⁸U isotope, with the ²³⁵U and ²³⁴U isotopes respectively comprising about 0.72% and 0.0054% of natural uranium (Greenwood & Earnshaw, 1984). Uranium is widespread in nature, occurring in granites and various other mineral deposits (Roessler et al., 1979; Lide, 1992-93).

Compound	CAS no.	Molecular formula
Uranium	7440-61-1	U
Uranyl ethanoate	541-09-3	C ₄ H ₆ O ₆ U
Uranyl chloride	7791-26-6	Cl ₂ O ₂ U
Uranyl nitrate	36478-76-9	N ₂ O ₈ U
Uranium dioxide	1344-57-6	UO ₂

¹ This review addresses only the chemical aspects of uranium toxicity. Information pertinent to the derivation of a guideline based on radiological effects is presented in the second edition of the *Guidelines for drinking-water quality*.

1.2 Physicochemical properties (Lide, 1992-93)

Compound	Melting point (°C)	Boiling point (°C)	Density at 20°C (g/cm ³)	Water solubility (g/litre)
U	1132	3818	19.0	insoluble
C ₄ H ₆ O ₆ U	110	275 (decomposes)	2.9	76.94
Cl ₂ O ₂ U	578	(decomposes)	-	3200
N ₂ O ₄ U	60.2	118	2.8	soluble
UO ₂	2878	-	10.96	insoluble

1.3 Major uses

Uranium is used mainly as fuel in nuclear power stations, although some uranium compounds are also used as catalysts and staining pigments (Berlin & Rudell, 1986).

1.4 Environmental fate

Uranium is present in the environment as a result of leaching from natural deposits, release in mill tailings, emissions from the nuclear industry, the combustion of coal and other fuels, and the use of phosphate fertilizers that contain uranium.

2. ANALYTICAL METHODS

Uranium in water is most commonly measured by solid fluorimetry with either laser excitation or ultraviolet light following fusion of the sample with a pellet of carbonate and sodium fluoride (detection limit 0.1 µg/litre) (Kreiger & Whittaker, 1980). Sample preparation for this method is tedious, however, and there is interference from other metals. Uranium can also be determined by inductively coupled plasma mass spectrometry, which has the same detection limit (0.1 µg/litre) and a between-run precision of less than 6% (Boomer & Powell, 1987). Alpha-spectrometry has been used for the determination of uranium in bottled waters (Gans, 1985) and environmental media (Singh & Wrenn, 1988), although the recovery is often highly variable owing to the low specific activity of natural uranium (Singh & Wrenn, 1988).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

Mean levels of uranium in ambient air have been reported to be 0.02 ng/m³ in Tokyo (based on a 1979-1981 survey) (Hirose & Sugimura, 1981) and 0.076 ng/m³

in New York (based on two samples, each a composite of two weekly air filter collections, from 1985 and 1986) (Fisenne et al., 1987). On the assumption of a daily respiratory volume of 20 m³ and a mean urban airborne concentration of 0.05 ng/m³, the daily intake of uranium from air would be about 1 ng. Tobacco smoke (from two packages of cigarettes per day) contributes less than 50 ng of inhaled uranium per day (Lucas & Markun, 1970).

3.2 Water

In a survey of 130 sites (approximately 3700 samples) in Ontario, Canada, conducted between 1990 and 1995, the mean of the average uranium concentrations (range 0.05-4.21 µg/litre; detection limit 0.05 µg/litre) in treated drinking-water was 0.40 µg/litre (OMEE, 1996). Uranium concentrations of up to 700 µg/litre have been found in private supplies in Canada (Moss et al., 1983; Moss, 1985). The mean concentration of uranium in drinking-water in New York City, USA, ranged from 0.03 to 0.08 µg/litre (Fisenne & Welford, 1986). A mean uranium concentration of 2.55 µg/litre was reported in drinking-water from 978 sites in the USA in the 1980s (US EPA, 1990, 1991). In five Japanese cities, the mean level in potable water supplies was 0.9 ng/litre (Nozaki et al., 1970).

The daily uranium intake from water in Finland has been estimated to be 2.1 µg (Kahlos & Asikainen, 1980). The daily intake from drinking-water in Salt Lake City, USA, is estimated to be 1.5 µg (Singh et al., 1990). On the basis of the results of the survey from Ontario (OMEE, 1996), the daily intake of uranium from drinking-water in Canada is estimated to be 0.8 µg.

3.3 Food

Uranium has been detected in a variety of foodstuffs. The highest concentrations are found in shellfish, and lower levels have been measured in fresh vegetables, cereals, and fish. The average per capita intake of uranium in food has been reported to be 1.3 µg/day (Fisenne et al., 1987) and 2-3 µg/day (Singh et al., 1990) in the USA and 1.5 µg/day in Japan (Nozaki et al., 1970).

In a review of naturally occurring sources of radioactive contamination in food, dietary intakes of ²³⁸U were found to range from 12 to 45 mBq/day in several European countries, from 11 to 60 mBq/day in Japan (the higher values were found in uranium mining areas), and from 15 to 17 mBq/day in the USA. The average daily dietary intake was in the order of 20 mBq, or about 4 µg. It was often difficult to determine whether these dietary intakes included intake from drinking-water, and it was emphasized that intake from drinking-water has sometimes been found to be equal to intake from the diet (Harley, 1988).

In a study by Cheng et al. (1993), the mean uranium concentration in nine different beverages was 0.98 µg/litre (range 0.26-1.65 µg/litre), and the mean concentration of uranium in mineral water was 9.20 µg/litre.

Landa & Councell (1992) performed leaching studies to determine the quantity of uranium leaching from 33 glass items and two ceramic items in which uranium was used as a colouring agent. Uranium-bearing glasses leached a

maximum of 30 µg of uranium per litre, whereas the ceramic-glazed items released approximately 300 000 µg of uranium per litre.

3.4 Estimated total exposure and relative contribution of drinking-water

The daily intake of uranium from each source for adults is estimated to be: air, 0.001 µg; drinking-water, 0.8 µg; food, 1.4 µg. Thus, the total daily intake is approximately 2.2 µg, or 0.037 µg/kg of body weight for a 60-kg adult, the majority of which originates from food.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Although ubiquitous in the environment, uranium has no known metabolic function in animals and is currently regarded as non-essential (Berlin & Rudell, 1986). Absorption of uranium from the gastrointestinal tract depends upon the solubility of the uranium compound (Berlin & Rudell, 1986), previous food consumption (Sullivan et al., 1986; La Touche et al., 1987), and the concomitant administration of oxidizing agents, such as the iron(III) ion and quinydrone (Sullivan et al., 1986). The average human gastrointestinal absorption of uranium is 1–2% (Wrenn et al., 1985).

The absorption of a uranium dose of approximately 800 mg/kg of body weight in starved female Sprague-Dawley rats increased from 0.17 to 3.3% when iron(III) (190 mg/kg of body weight) was administered simultaneously (Sullivan et al., 1986). Absorption of uranium in starved rats administered doses of uranium by gavage was reported to increase with dose; the degree of absorption ranged from 0.06 to 2.8% for doses between 0.03 and 45 mg of uranium per kg of body weight (La Touche et al., 1987). Only 0.06% of ingested uranium was absorbed in Sprague-Dawley rats and New Zealand white rabbits fed *ad libitum* and having free access to drinking-water containing up to 600 mg of uranyl nitrate hexahydrate per litre for up to 91 days (Tracy et al., 1992).

Following ingestion, uranium rapidly appears in the bloodstream (La Touche et al., 1987), where it is associated primarily with the red cells (Fisenne & Perry, 1985); a non-diffusible uranyl-albumin complex also forms in equilibrium with a diffusible ionic uranyl hydrogen carbonate complex ($UO_2HCO_3^{+}$) in the plasma (Moss, 1985). Because of their high affinity for phosphate, carboxyl, and hydroxyl groups, uranyl compounds readily combine with proteins and nucleotides to form stable complexes (Moss, 1985). Clearance from the bloodstream is also rapid, and the uranium subsequently accumulates in the kidneys and the skeleton, whereas little is found in the liver (La Touche et al., 1987). The skeleton is the major site of uranium accumulation (Wrenn et al., 1985); the uranyl ion replaces calcium in the hydroxyapatite complex of bone crystals (Moss, 1985).

Based on the results of studies in experimental animals, it appears that the amount of soluble uranium accumulated internally is proportional to the intake from ingestion or inhalation. It has been estimated that the total body burden of uranium in humans is 40 µg, with approximately 40% of this being present in the muscles,

20% in the skeleton, and 10%, 4%, 1%, and 0.3% in the blood, lungs, liver, and kidneys, respectively (Igarashi et al., 1987).

Once equilibrium is attained in the skeleton, uranium is excreted in the urine and faeces. Urinary excretion in humans has been found to account for approximately 1% of total excretion, averaging 4.4 µg/day (Singh et al., 1990), the rate depending in part on the pH of tubular urine (Berlin & Rudell, 1986). Under alkaline conditions, most of the uranyl hydrogen carbonate complex is stable and is excreted in the urine. If the pH is low, the complex dissociates to a variable degree, and the uranyl ion may then bind to cellular proteins in the tubular wall, which may then impair tubular function.

The half-life of uranium in the rat kidney has been estimated to be approximately 15 days. Clearance from the skeleton is considerably slower; half-lives of 300 and 5000 days have been estimated, based on a two-compartment model (Wrenn et al., 1985). In another study using a 10-compartment model, overall half-lives for the clearance of uranium from the rat kidney and skeleton were determined to be 5–11 and 93–165 days, respectively (Sontag, 1986). The overall elimination half-life of uranium under conditions of normal daily intake has been estimated to be between 180 and 360 days (Berlin & Rudell, 1986).

5. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

5.1 Acute exposure

Reported oral LD₅₀s of uranyl ethanoate dihydrate for rats and mice are 204 and 242 mg/kg of body weight, respectively (Domingo et al., 1987). Among the most common signs of acute toxicity are piloerection, significant weight loss, and haemorrhages in the eyes, legs, and nose.

The most common renal injury caused by uranium in experimental animals is damage to the proximal convoluted tubules, predominantly in the distal two-thirds (Berlin & Rudell, 1986; Anthony et al., 1994; Domingo, 1995); the rate of effects varies with dosage level (Leggett, 1989). It has recently been shown that uranyl inhibits both Na⁺ transport-dependent and Na⁺ transport-independent ATP utilization as well as mitochondrial oxidative phosphorylation in the renal proximal tubule (Leggett, 1989; Domingo, 1995). At doses not high enough to destroy a critical mass of kidney cells, the effect appears to be reversible, as some of the cells are replaced; however, the new epithelial lining differs morphologically, and possibly functionally, from normal epithelium (Wrenn et al., 1985; Berlin & Rudell, 1986). Histopathologically, the regenerated cells are simple flattened cells with no microvilli on luminal surfaces and with reduced numbers of mitochondria (Leggett, 1989).

There is some evidence that tolerance may develop following repeated exposure to uranium (Yuile, 1973; Durbin & Wrenn, 1976; Campbell, 1985). This tolerance does not, however, prevent chronic damage to the kidney, as the regenerated cells are quite different; although histopathologically it may appear that the repair process is well advanced, the urinary biochemical changes return to normal only slowly (Leggett, 1989). Alterations causing thickening of the

glomerular basement membrane of the kidney, which results from the storage of uranium in the kidney, can be prolonged and severe enough to cause permanent damage (McDonald-Taylor et al., 1992). Persistent ultrastructural changes in the proximal tubules of rabbits have also been reported to be associated with the kidney's ability to store uranium (McDonald-Taylor et al., 1997). Cell damage in the proximal tubules was significantly more severe in animals allowed up to a 91-day recovery period than in animals in the no-recovery group.

5.2 Short-term exposure

Forty male Sprague-Dawley rats given 0, 2, 4, 8, or 16 mg of uranyl ethanoate dihydrate per kg of body weight per day (equivalent to doses of 0, 1.1, 2.2, 4.5, or 9.0 mg of uranium per kg of body weight per day) in drinking-water for 2 weeks exhibited a variety of biochemical effects, including increases in blood glucose levels at ≥ 4 mg of uranyl ethanoate dihydrate per kg of body weight per day, decreases in aspartate aminotransferase and alanine aminotransferase values at ≥ 8 mg of uranyl ethanoate dihydrate per kg of body weight per day, increases in several other haematological parameters at 16 mg of uranyl ethanoate dihydrate per kg of body weight per day, and increases in total protein levels in all treated groups (Ortega et al., 1989). The authors considered the NOAEL to be 2 mg of uranyl ethanoate dihydrate per kg of body weight per day (1.1 mg of uranium per kg of body weight per day).

Groups of 15 male and 15 female weanling Sprague-Dawley rats consumed water containing <0.001 (control), 0.96, 4.8, 24, 120, or 600 mg of uranyl nitrate hexahydrate per litre (equivalent to doses of <0.0001 , 0.06, 0.31, 1.52, 7.54, and 36.73 mg of uranium per kg of body weight per day in males and <0.0001 , 0.09, 0.42, 2.01, 9.98, and 53.56 mg of uranium per kg of body weight per day in females) for 91 days (Gilman et al., 1997a). Histopathological changes were observed mainly in the liver, thyroid, and kidney. In the liver, treatment-related lesions were seen in both sexes at all doses and were generally non-specific nuclear and cytoplasmic changes. The thyroid lesions were not considered specific to the uranium treatment. The kidney was the most affected tissue. In males, statistically significant treatment-related kidney lesions (reported at all doses) included nuclear vesiculation, cytoplasmic vacuolation, and tubular dilation. Other statistically significant lesions in males (≥ 4.8 mg of uranyl nitrate hexahydrate per litre) included glomerular adhesions, apical displacement of the proximal tubular epithelial nuclei, and cytoplasmic degranulation. In females, statistically significant changes in the kidney included nuclear vesiculation of the tubular epithelial nuclei (all doses) and anisokaryosis (all doses except 4.8 mg of uranyl nitrate hexahydrate per litre). However, the most important changes in the female were the capsular sclerosis of glomeruli and reticulin sclerosis of the interstitial membranes; these changes occurred in all dose groups and are considered to be "nonreparable lesions." Significant treatment-related liver changes were also reported in hepatic nuclei and cytoplasm in both sexes at the lowest exposure level. The LOAEL for adverse effects on the kidney and liver of male and female rats, based on the frequency of degree of degenerative lesions in the renal proximal convoluted tubule, was

considered to be 0.96 mg of uranyl nitrate hexahydrate per litre (equivalent to 0.09 mg of uranium per kg of body weight per day in females and 0.06 mg of uranium per kg of body weight per day in males). The reason for the difference in sensitivity between males and females is not clear, but it did not appear to be due to differences in pharmacokinetics, as accumulation of uranium in renal tissue did not differ significantly between the two sexes at all doses.

In a similar study, groups of 10 male New Zealand white rabbits were given uranyl nitrate hexahydrate in drinking-water at concentrations of <0.001 (controls), 0.96, 4.8, 24, 120, or 600 mg/litre (determined to be equivalent to doses of 0, 0.05, 0.2, 0.88, 4.82, and 28.7 mg of uranium per kg of body weight per day) for 91 days (Gilman et al., 1997b). Histopathological changes were observed in the kidney tubule, liver, thyroid, and aorta. Histopathological findings were observed in the kidney tubules at doses above 0.96 mg of uranyl nitrate hexahydrate per litre. When compared with controls, significant treatment-related changes included cytoplasmic vacuolation, anisokaryosis, nuclear pyknosis, and nuclear vesiculation; the incidence of nuclear vesiculation and anisokaryosis appeared to be dose-related, with nuclear vesiculation having the higher frequency and severity. Other treatment-related changes included tubular dilation, hyperchromicity, tubular atrophy, changes in the interstitium collagen, and reticulin sclerosis. In total, 11 different morphological indicators of tubular injury were observed in the highest exposure group. The LOAEL, based on the nuclear changes in the kidney, was considered to be 0.96 mg of uranyl nitrate hexahydrate per litre (equivalent to 0.05 mg of uranium per kg of body weight per day). It should be noted, however, that these rabbits were not *Pasteurella*-free, and four of them contracted a *Pasteurella* infection during the course of the study. In the same study, 10 *Pasteurella*-free female rabbits were exposed to drinking-water containing <0.001 (controls), 4.8, 24, or 600 mg of uranyl nitrate hexahydrate per litre (equivalent to doses of 0, 0.49, 1.32, and 43.02 mg of uranium per kg of body weight per day) for 91 days. Dose-related and treatment-related nuclear changes in the kidney tubule included anisokaryosis and vesiculation, which were significantly different from effects observed in controls at all doses. Other treatment-related changes in the kidney included cytoplasmic vacuolation, tubular atrophy, and nuclear pyknosis. In general, histopathological changes in the kidney in females were generally less marked than in males. The LOAEL was considered to be 4.8 mg of uranyl nitrate hexahydrate per litre (equivalent to 0.49 mg of uranium per kg of body weight per day). In both sexes, histopathological changes in the liver, thyroid, and aorta were similar. In the liver, changes may have been treatment-related, although very mildly affected animals were seen in all groups, and changes in the thyroid were mild. Changes in the aorta were not dose-dependent. It should be noted that no similar aortic changes were observed in the 91-day uranyl nitrate hexahydrate studies in rats (Gilman et al., 1997a). It is interesting to note, however, that even though the female rabbits consumed on average 65% more water than the males and their average uranium intake was approximately 50% greater on a mg/kg of body weight per day basis, their average tissue levels were not similarly raised. The differences between the males and females, both qualitative and quantitative, suggest pharmacokinetic parameter differences, which contrasts with the findings in the rat study by the same authors (Gilman et al., 1997a).

In an additional study to observe the reversibility of renal injury in *Pasteurella*-free male New Zealand white rabbits, groups of 5-8 animals were given <0.001 (control), 24, or 600 mg of uranyl nitrate hexahydrate per litre (equivalent to 0, 1.36, and 40.98 mg of uranium per kg of body weight per day) in drinking-water for 91 days, with a recovery period of up to 91 days (Gilman et al., 1997c). Minor histopathological lesions were seen in the liver, thyroid, and aorta. In the kidney, tubular injury with degenerative nuclear changes, cytoplasmic vacuolation, and tubular dilation was observed in the high-dose group, which did not exhibit consistent resolution even after a 91-day recovery period. In general, the male rabbits did not respond as dramatically as those in the earlier study (Gilman et al., 1997b), although the histopathological changes observed in this study were similar to those noted in the female rabbits of the previous study. Animals in this study consumed approximately 33% more uranium per day than the males in the previous study (Gilman et al., 1997b), yet uranium residues in kidney tissue were 30% less, which would appear to indicate that *Pasteurella*-free rabbits are less sensitive than the non-*Pasteurella*-free strain to the effects of the uranyl ion in drinking-water. Based on the histopathological data in the kidney, a LOAEL for the male New Zealand rabbits in this study is estimated to lie between 24 and 600 mg of uranyl nitrate hexahydrate per litre.

5.3 Long-term exposure

In an early series of experiments, very high doses (up to 20% in the diet) of a variety of uranium compounds were fed to rats, dogs, and rabbits for periods ranging from 30 days to 2 years (Maynard and Hodge, 1949). On the basis of very limited histopathological investigations, renal damage was reported in each species.

5.4 Reproductive and developmental toxicity

Adverse reproductive effects, in terms of total number of litters and average number of young per litter, were reported in rats given 2% uranyl nitrate hexahydrate for 7 months (Maynard & Hodge, 1949). More recent studies have examined the teratogenic/embryotoxic effects and reproductive outcomes of uranyl acetate dihydrate in Swiss albino mice. Domingo et al. (1989a) evaluated the developmental toxicity of uranium by treating groups of 20 pregnant Swiss mice by gavage to doses of 0, 5, 10, 25, or 50 mg of uranyl acetate dihydrate per kg of body weight per day (equivalent to 0, 2.8, 5.6, 14, and 28 mg of uranium per kg of body weight per day) on days 6-15 of gestation; the animals were sacrificed on day 18. Although all dams survived, there was a dose-related reduction in maternal weight gain, a significant decrease in daily feed intake, and a significant increase in liver weights. Exposure-related fetotoxicity, including reduced fetal body weights and length, increased incidence of stunted fetuses per litter, increased incidence of both external and internal malformations, and increased incidence of developmental variations, was observed in the fetuses of mice at ≥ 2.8 mg of uranium per kg of body weight per day. At doses ≥ 14 mg of uranium per kg of body weight per day, specific malformations included cleft palate and bipartite sternbrae, and developmental

variations included reduced ossification and unossified skeletal variations. There was no evidence of embryoletality at any dose. Based on both the maternal and fetotoxic effects, a LOAEL of 2.8 mg of uranium per kg of body weight per day could be considered.

A second study by Domingo et al. (1989b) evaluated the effect of uranium on late fetal development, parturition, lactation, and postnatal viability. Groups of 20 female mice were treated by gavage from day 13 of pregnancy until day 21 of lactation to doses of 0, 0.05, 0.5, 5, or 50 mg of uranyl acetate dihydrate per kg of body weight per day (equivalent to 0, 0.028, 0.28, 2.8, and 28 mg of uranium per kg of body weight per day). Maternal deaths (2/20 at 2.8 mg of uranium per kg of body weight per day, and 3/20 at 28 mg of uranium per kg of body weight per day) were attributed to the treatment; however, maternal toxicity was not evident from changes in body weight or food consumption, although relative liver weight was significantly reduced in all treatment groups. Decreases in pup viability, as indicated by significant decreases in litter size on day 21 of lactation, and significant decreases in the viability and lactation indexes were observed in the high-dose group. Based on developmental effects in pups, a NOEL of 2.8 mg of uranium per kg of body weight per day was established.

Paternain et al. (1989) studied the effects of uranium on reproduction, gestation, and postnatal survival in mice. Groups of 25 mature male Swiss mice were exposed to oral doses of 0, 5, 10, or 25 mg of uranyl acetate dihydrate per kg of body weight per day (equivalent to 0, 2.8, 5.6, and 14 mg of uranium per kg of body weight per day) for 60 days prior to mating with mature females (25 per group). Females were exposed for 14 days prior to mating, and exposure continued through mating, gestation, parturition, and nursing of litters; half the treated dams were sacrificed on day 13 of gestation. No treatment-related effects on mating or fertility were observed. Embryoletality (number of late resorptions and dead fetuses) was significantly increased and the number of live fetuses was decreased in the high-dose group. Lethality in pups (at birth and at day 4 of lactation) was significantly increased at ≥ 5.6 mg of uranium per kg of body weight per day, and pup growth (decreases in weight and length) and development of offspring, from birth and during the entire lactation period, were significantly affected in the high-dose group.

Unspecified degenerative changes in the testes of rats have also been reported following chronic administration of uranyl nitrate hexahydrate and uranyl fluoride in the diet (Maynard and Hodge, 1949; Maynard et al., 1953; Malenchenko et al., 1978). In a more recent study, male Swiss mice were exposed for 64 days to uranyl acetate dihydrate in drinking-water at doses of 0, 10, 20, 40, or 80 mg/kg of body weight per day (equivalent to 0, 5.6, 11.2, 22.4, and 44.8 mg of uranium per kg of body weight per day) prior to mating with untreated females for 4 days (Llobet et al., 1991). With the exception of interstitial alterations and vacuolization of Leydig cells at the highest dose, no effects were observed in testicular function/spermatogenesis. There was, however, a significant, non-dose-related decrease in the pregnancy rate of these animals.

5.5 Mutagenicity and related end-points

Uranyl nitrate was cytotoxic and genotoxic in Chinese hamster ovary cells at concentrations ranging from 0.01 to 0.3 mmol/litre. There was a dose-related decrease in the viability of the cells, a decrease in cell cycle kinetics, and increased frequencies of micronuclei, sister chromatid exchanges, and chromosomal aberrations (Lin et al., 1993). The authors suggest that the data provide a possible mechanism for the teratogenic effects observed in the studies by Domingo et al. (1989a). The genotoxic effects in this study were thought to occur through the binding of the uranyl nitrate to the phosphate groups of DNA. Chromosomal aberrations have also been induced in male mouse germ cells exposed to enriched uranyl fluoride; however, these aberrations may have been produced by the radioactivity of the test compound (Hu & Zhu, 1990).

5.6 Carcinogenicity

Although bone cancer has been induced in experimental animals by injection or inhalation of soluble compounds of high-specific-activity uranium isotopes or mixtures of uranium isotopes, no carcinogenic effects have been reported in animals ingesting soluble or insoluble uranium compounds (Wrenn et al., 1985).

6. EFFECTS ON HUMANS

Nephritis is the primary chemically induced effect of uranium in humans (Hursh & Spoor, 1973).

Little information is available on the chronic health effects of exposure to environmental uranium in humans. In Nova Scotia, Canada, clinical studies were performed on 324 persons exposed to variable amounts of naturally occurring uranium in drinking-water (up to 0.7 mg/litre) supplied from private wells. No relationship was found between overt renal disease or any other symptomatic complaint and exposure to uranium. However, a trend towards increasing excretion of urinary β_2 -microglobulin and increasing concentration of uranium in well-water was observed; this raises the possibility that an early tubular defect was present and suggests that this parameter might be useful as an index of subclinical toxicity. The group with the highest uranium concentrations in well-water failed to follow this trend, but this was attributed to the fact that most of the individuals in this group had significantly reduced their consumption of well-water by the time the measurements were made, leading to the conclusion that the suspected tubular defect might well be rapidly reversible (Moss et al., 1983; Moss, 1985).

In a pilot study conducted in 1993 in three communities in Saskatchewan, Canada, there was a statistically significant association ($p = 0.03$) between increasing but normal levels of urine albumin (measured as mg/mmol creatinine) and the uranium cumulative index. The cumulative index was calculated for each study participant as the product of the uranium concentration in drinking-water, the number of cups of water consumed per day, and the number of years lived at the current residence (Mao et al., 1995). The study was conducted with 100 participants

in three different areas with mean uranium levels ranging from 0.71 (control) to 19.6 $\mu\text{g/litre}$. Urine albumin levels ranged from 0.165 to 16.1 mg/mmol creatinine, with eight participants having "elevated" urine albumin concentrations (>3.0 mg/mmol creatinine). Three participants had serum creatinine concentrations of >120 $\mu\text{mol/litre}$ (range 50–170 $\mu\text{mol/litre}$), which is reportedly indicative of prevalent renal damage. It should be noted, however, that diabetics were not excluded from the study, although diabetic status and age, known risk factors for renal dysfunction, were factored into the statistical analysis of the results. According to the authors, microalbuminuria has been shown to be a sensitive indicator of early renal disease.

7. PROVISIONAL GUIDELINE VALUE

There are insufficient data regarding the carcinogenicity of uranium in humans and experimental animals. The guideline value for the chemical toxicity of uranium was therefore derived using a TDI approach. As no adequate chronic study was identified, the TDI was derived using the results of the most extensive subchronic study conducted to date in which uranium was administered in drinking-water to the most sensitive sex and species (Gilman et al., 1997a). In the 91-day study in rats, the LOAEL for degenerative lesions in the proximal convoluted tubule of the kidney in males was considered to be 0.96 mg of uranyl nitrate hexahydrate per litre, which is equivalent to 0.06 mg of uranium per kg of body weight per day.

A TDI of 0.6 $\mu\text{g/kg}$ of body weight per day was derived using the LOAEL of 60 $\mu\text{g/kg}$ of body weight per day and an uncertainty factor of 100 (for intra- and interspecies variation). There is no need to apply an additional uncertainty factor to account for the use of a LOAEL instead of a NOAEL because of the minimal degree of severity of the lesions being reported. Also, an additional uncertainty factor for the length of the study (91-day) is not required because the estimated half-life of uranium in the kidney is 15 days, and there is no indication that the severity of the renal lesions will be exacerbated following continued exposure.

This TDI yields a guideline value of 2 $\mu\text{g/litre}$ (rounded figure), assuming a 60-kg adult consuming 2 litres of drinking-water per day and a 10% allocation of the TDI to drinking-water. This value would be protective, based on associations for subclinical renal effects reported in preliminary epidemiological studies.

Several methods are available for the removal of uranium from drinking-water, although some of these methods have been tested at laboratory or pilot scale only. Coagulation using ferric sulfate or aluminium sulfate at optimal pH and coagulant dosages can achieve 80–95% removal of uranium, whereas at least 99% removal can be achieved using lime softening, anion exchange resin, or reverse osmosis processes. In areas with high natural uranium levels, a value of 2 $\mu\text{g/litre}$ may be difficult to achieve with the treatment technology available (WRc, 1997).

The guideline value for uranium is provisional because it may be difficult to achieve with the treatment technology available, because of limitations in the key study, namely the lack of a dose-response relationship (no NOEL) despite the wide range of administered doses, and because of insufficient information on the degree and severity of the pathological examinations. It should be noted that there are several human studies under way that may provide helpful additional data.

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CYANOBACTERIAL TOXINS: MICROCYSTIN-LR

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No guideline values were proposed for cyanobacterial toxins in the second edition of the WHO *Guidelines for drinking-water quality*. As microcystins (produced by cyanobacteria, or blue-green algae) are extremely toxic and are often associated with poisonings in humans and animals, the Coordinating Committee for the Updating of WHO *Guidelines for drinking-water quality* decided that guideline values for cyanobacterial toxins were needed.

1. GENERAL DESCRIPTION

1.1 Identity

The cyanobacteria, also known as blue-green algae, owe their name to the presence of photosynthetic pigments. Cyanobacteria are a major group of bacteria that occur throughout the world. Freshwater cyanobacteria may accumulate in surface water supplies as "blooms" and may concentrate on the surface as blue-green "scums."

Some species of cyanobacteria produce toxins, which are classified according to their mode of action into hepatotoxins (e.g. microcystins), neurotoxins (e.g. anatoxins), skin irritants, and other toxins. Both hepatotoxins and neurotoxins are produced by cyanobacteria commonly found in surface water and therefore are of relevance to water supplies (Carmichael, 1992; Fawell et al., 1993).

The hepatotoxins are produced by various species within the genera *Microcystis*, *Anabaena*, *Oscillatoria*, *Nodularia*, *Nostoc*, *Cylindrospermopsis*, and *Umezakia*, although not all strains do so (Fawell et al., 1993; AWWA, 1995). Most hepatotoxins (all cyclic heptapeptides) are microcystins. At least 50 congeners of microcystins are known (Carmichael, 1994), and several of these may be produced during a bloom. The chemical structure of microcystins includes two variable amino acids and an unusual aromatic amino acid, ADDA (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid), containing a substituted phenyldecadienoic acid (Botes et al., 1985). Different microcystins have different lipophilicities and polarities, which could affect their toxicity. Microcystin-LR was the first microcystin chemically identified; to date, most work has been conducted using this microcystin. It has been associated with most of the incidents of toxicity involving microcystins in most countries (Fawell et al., 1993). Microcystin-LR is a cyclic heptapeptide with a molecular weight of about 1000 daltons.

Neurotoxins are not considered as widespread in water supplies, and they do not appear to pose the same degree of risk from chronic exposure as microcystins (Fawell et al., 1993; AWWA, 1995). The neurotoxins, such as anatoxin-a and -a(s),