TOXICOLOGICAL SCIENCES 41, 117–128 (1998)
ARTICLE NO TX972367

Uranyl Nitrate: 28-Day and 91-Day Toxicity Studies in the Sprague–Dawley Rat[1]

A. P. Gilman,*[,2] D. C. Villeneuve,* V. E. Secours,* A. P. Yagminas,* B. L. Tracy,* J. M. Quinn,* V. E. Valli,† R. J. Willes,‡ and M. A. Moss§

*Environmental Health Directorate, Department of Health, Ottawa, Ontario, Canada K1A 0L2; †Department of Veterinary Pathobiology, University of Illinois at Urbana–Champaign, Urbana, Illinois 61802; ‡F. D. C. Consultants, Inc., Orono, Ontario, Canada L0B 1M0; and §Department of Pathology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

Received September 4, 1996; accepted September 2, 1997

Uranyl Nitrate: 28-Day and 91-Day Toxicity Studies in the Sprague–Dawley Rat. Gilman, A. P., Villeneuve, D. C., Secours, V. E., Yagminas, A. P., Tracy, B. L., Quinn, J. M., Valli, V. E., Willes, R. J., and Moss, M. A. (1998). *Toxicol. Sci.* 41, 117–128.

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).

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

Naturally occurring uranium (U) salts are common to many drinking water supplies, with levels ranging from 0.015 to 980 μg U/L of water and an estimated average of 3 μg/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 (Cothern *et al.*, 1983; Cothern 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; Cothern *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 (μg/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 × 0.474 = uranium equivalent.

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

[d] ± standard error of mean.

[1] This work was presented in part at the following meetings: Canadian Fed. Biol. Soc. (Edmonton), June 1982. American Public Health Association Meeting (Montreal), November 1982. Nuclear Regulatory Commission Public Meetings on Uranium (Washington), December 1985.

[2] To whom correspondence should be addressed.

1096-6080/98 $25.00

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	311 9	315.1	320.1[a]	336.9[a]	309.5
	(7.7)[b]	(7.9)	(8.5)	(6.7)	(8 9)	(6.8)
Males	487.0	521.8	513.7	494.9	496.2	503.2
	(13.94)	(11.94)	(14.69)	(15.23)	(9.30)	(12.29)
Mean body weight gain (g)						
Females	206.7	210.6	215.8	216.7	234.7[a]	210.1
	(5.7)	(8.7)	(8.3)	(6.5)	(6.6)	(7.7)
Males	389.07	417.33	408.93	391.80	393.67	398.53
	(11.81)	(10.94)	(14.79)	(15.01)	(7.39)	(13.83)
Mean feed intake (g/animal/day)						
Females	19.79	20.07	20.07	20.32	20.44	19.56
	(0 50)	(0.49)	(0.33)	(0.53)	(0.65)	(0.54)
Males	21.23	23.21	24.14	22.76	23.03	22.53
	(2.52)	(2.26)	(2.97)	(1.94)	(2.20)	(2.31)
Mean fluid consumption (ml/animal/day)						
Females	34.75	35.71	35.69	34.65	35.91	34.43
	(1.56)	(1.53)	(1.77)	(1.21)	(1 35)	(1.77)
Males	36.76	35.37	38.75	35.92	34.05	36.84
	(1.44)	(1.55)	(1.55)	(2.06)	(1.12)	(1.37)
Mean kidney weight (% body weight)						
Females	0 34	0.33	0.32	0.32	0.31	0.33
	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Males	0.28	0.29	0.29	0.30	0.30	0.30
	(0 01)	(0.01)	(0.01)	(0.005)	(0.01)	(0.01)

[a] Significantly different from group 1: $p < 0.05$; Duncan's multiple range test.
[b] ± 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 (Cothern *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 (Cothern *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; Cothern *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 μg/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	0.27	1.32	6.85	32.09	167.21
		(0.00001)	(0.01)	(0.06)	(0.23)	(0.42)	(7.57)[b]
	Males	0.0002	0.20	1.05	5.29	27.63	124.62
		(0.00001)	(0.006)	(0.03)	(0.04)	(2.00)	(2.88)
6	Females	0.0001	0.15	0.75	3.56	18.01	100.19
		(0.00001)	(0.01)	(0.07)	(0.12)	(0.90)	(7.05)
	Males	0.0001	0.10	0.56	2.66	13.08	67.76
		(0.0001)	(0.005)	(0.03)	(0.17)	(0.66)	(3.58)
12	Females	0.0001	0.14	0.68	3.16	16.13	84.43
		(0.00002)	(0.01)	(0.04)	(0.13)	(1.28)	(4.77)
	Males	0.0001	0.07	0.43	2.18	9 83	49.77
		(0.0002)	(0.005)	(0.04)	(0.18)	(0.58)	(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	147	149	150[b]	147	148
	(1)[a]	(1)	(2)	(2)	(1)	(1)
Males	145	146	145	148	145	143
	(3)	(1)	(1)	(1)	(1)	(2)
Mean erythrocytes ($\times 10^{12}$/L)						
Females	6.69	7.16	7.18	7.36[b]	7.22	7.26
	(0.08)	(0.07)	(0.10)	(0.11)	(0.10)	(0.10)
Males	7.59	7.84	7.74	7.84	7.64	7.62
	(0.18)	(0.10)	(0.08)	(0.08)	(0.10)	(0.12)
Mean corpuscular hemoglobin (pg)						
Females	20.9	20.5	20.7	20.3[b]	20.3[b]	20.3[b]
	(0.2)	(0.2)	(0.2)	(0.1)	(0.2)	(0.1)
Males	19.2	18.7	18.8	18.8	19.0	18.8
	(0.2)	(0.2)	(0.1)	(0.1)	(0.2)	(0.2)
Mean glucose (mmol/L)						
Females	8.9	8.2	8.4	8.5	8.5	8.8
	(0.2)	(0.2)	(0.3)	(0.2)	(0.3)	(0.3)
Males	8.7	8.8	9.5[b]	9.0	9.4[b]	9.1
	(0.2)	(0.2)	(0.3)	(0.2)	(0.2)	(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 (μg/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
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 × 0.474.

[c] ± 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 μg/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 vacuolaiton	***	**	***	***	**
		×			
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					××
Tubular					
Cytoplasmic shedding	*				
Cytoplasmic inclusions			*	**	
Cytoplasmic vacuolation			*	*	**
Cytoplasmic degranulation				*	*
Apical displacement of nuclei	*				
Anisokaryosis			××		××
Nuclear vesiculation					××
Tubular dilation			**		
Tubular atrophy				**	
Protein casts		×			
Interstitial					
Collagen sclerosis					**
Lymphoid cuffing				**	**
Liver					
Accentuation of zonation				*	**
Nuclei					
Anisokaryosis		*	**	**	**
	×	××	××	××	××
Vesiculation		**			
	××	×	×		×
Hyperchromicity			**	**	**
			××	××	××
Cytoplasm					
Increased portal density				**	**
	×	××	××	××	××
Perivenous vacuolation	**				
Increased perivenous homogeneity	**	**	**	**	**
		××	××	××	××
Increased perivenous and midzone homogeneity			××		

[a] Dunnett's t test.

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

specific to uranium exposure. As reported by others (Haley, 1982; Haley *et al.*, 1982; Morrow *et al.*, 1982; Cothern *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

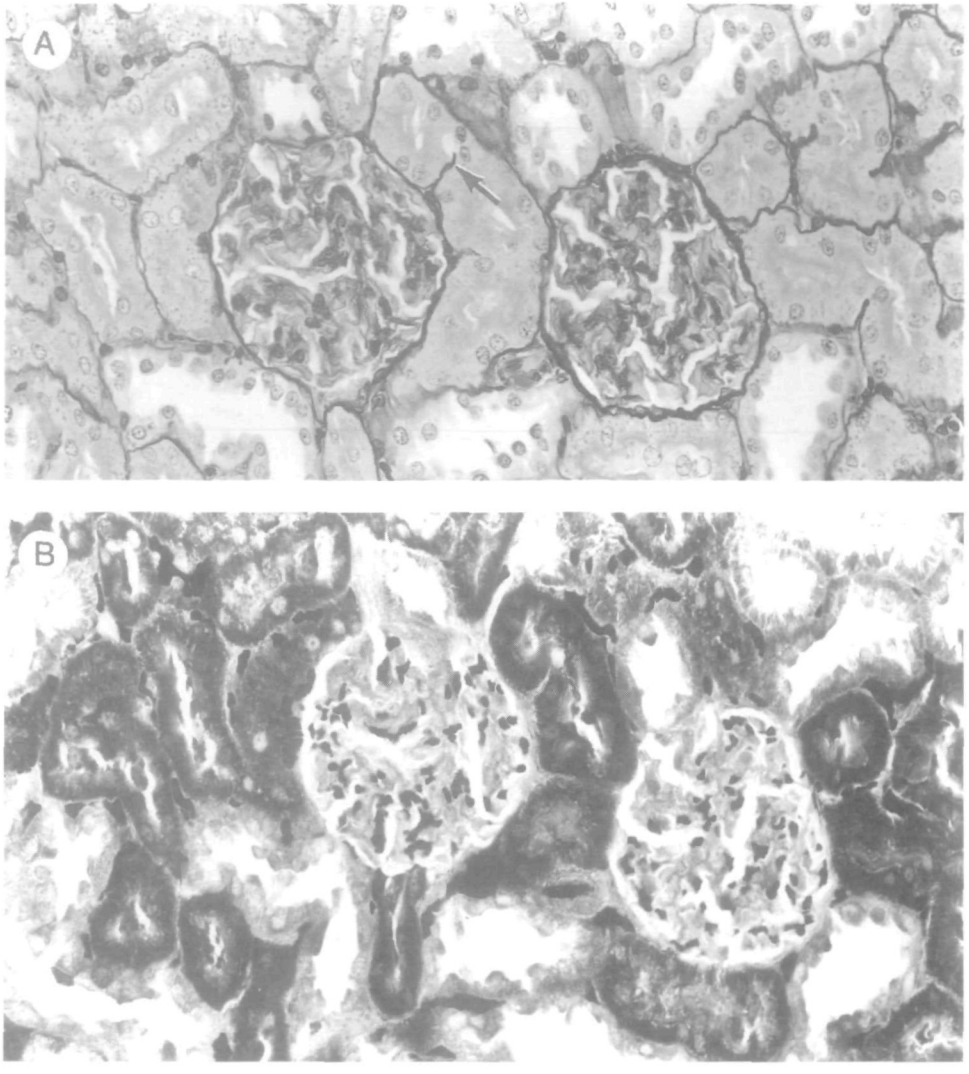

FIG. 1. Renal cortex from a female rat in the control group of the 91-day study. (A) Outer stripe cortex. Glomerular and tubular basement membranes are of normal and uniform thickness (arrow) and proximal and distal tubules have uniform diameter, cytoplasmic volume, and nuclei. PAMS ×320 (B) Section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

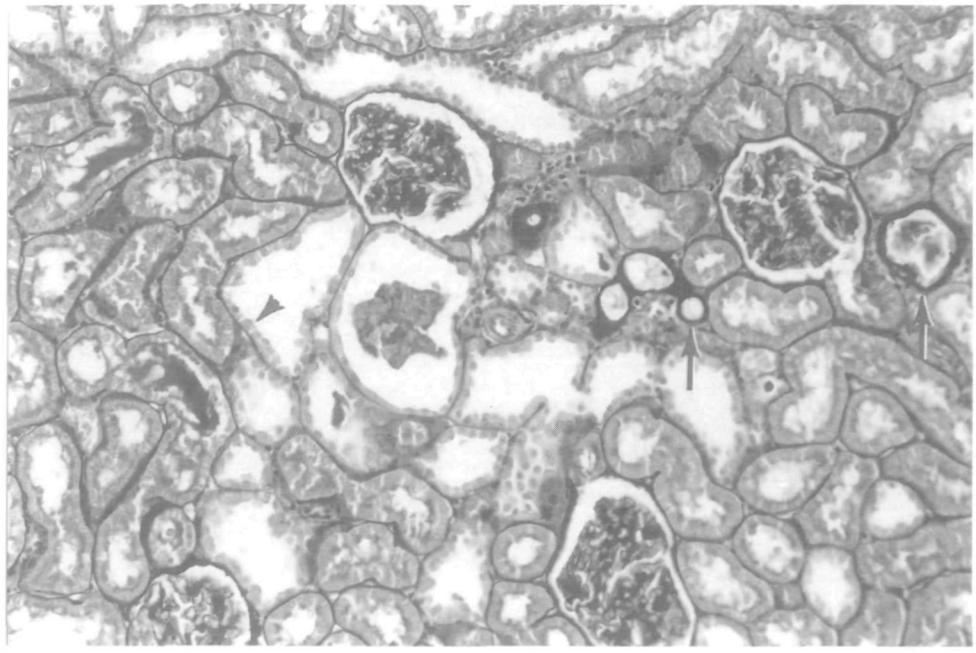

FIG. 2. Outer stripe renal cortex from a male rat which received 0.96 mg UN/L for 91 days. Multiple profiles of markedly dilated distal convoluted tubules. Epithelium is irregularly flattened and nuclei are irregularly spaced (arrowhead) and focally protruding into the lumen. Basement membranes are normal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

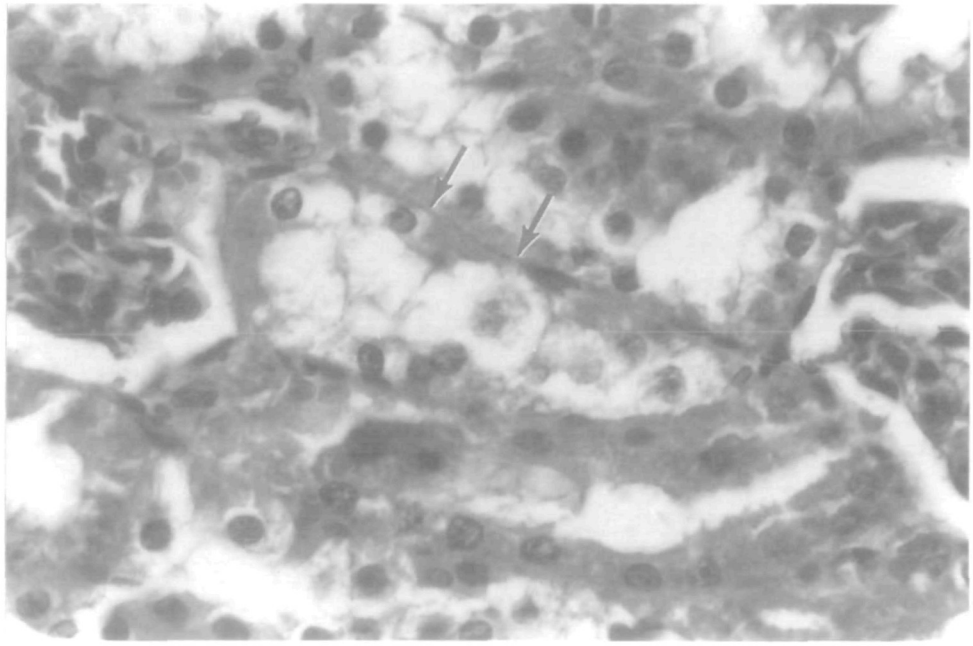

FIG. 3. Inner stripe renal cortex from a male rat which received 0.96 mg UN/L in drinking water for 91 days. Multifocal vacuolation of cytoplasm in a segment of proximal convoluted tubule with normal basement membranes. The vacuoles variably surround the nuclei and extend to the basement membrane (arrows).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

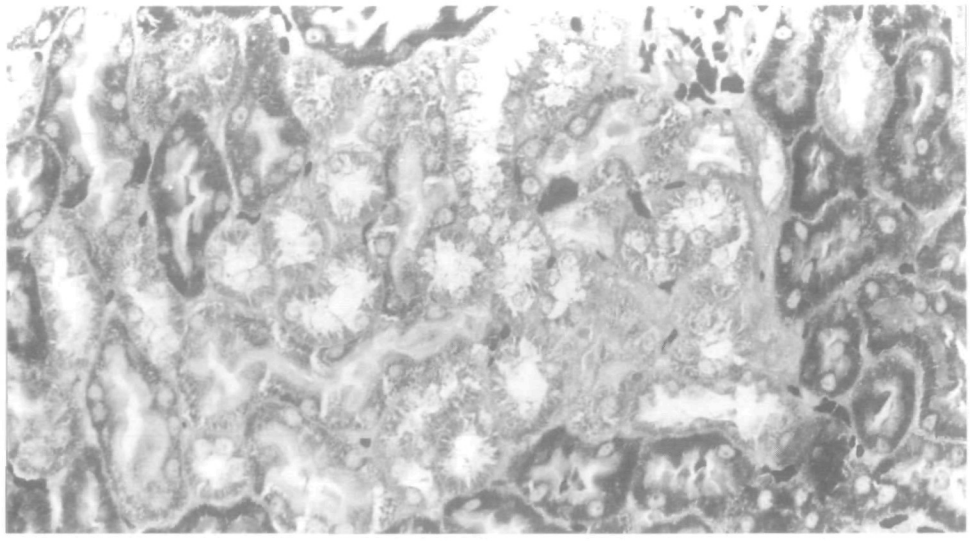

FIG. 4. Renal cortex from a male rat which received 120 mg UN/L in drinking water for 91 days. Multiple profiles of tubular injury with typical loss of density of cytoplasmic staining due to degranulation. HN ×320.

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 μg/g) at this exposure concentration.

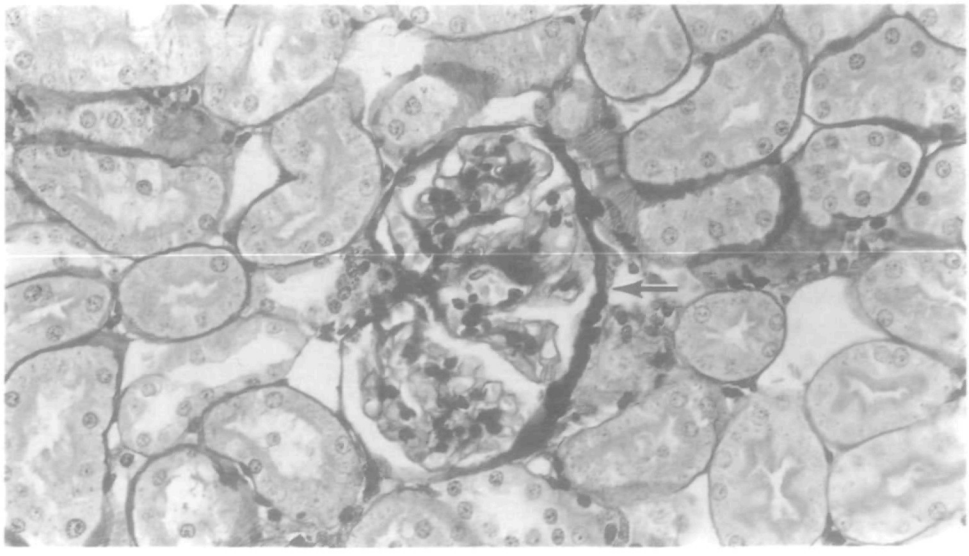

FIG. 5. Renal cortex from a female rat which received 600 mg UN/L of drinking water for 91 days. There is marked and irregular thickening of the parietal layer of Bowman's capsule typically opposite the vascular pole of the glomerulus (arrow). PAMS ×320.

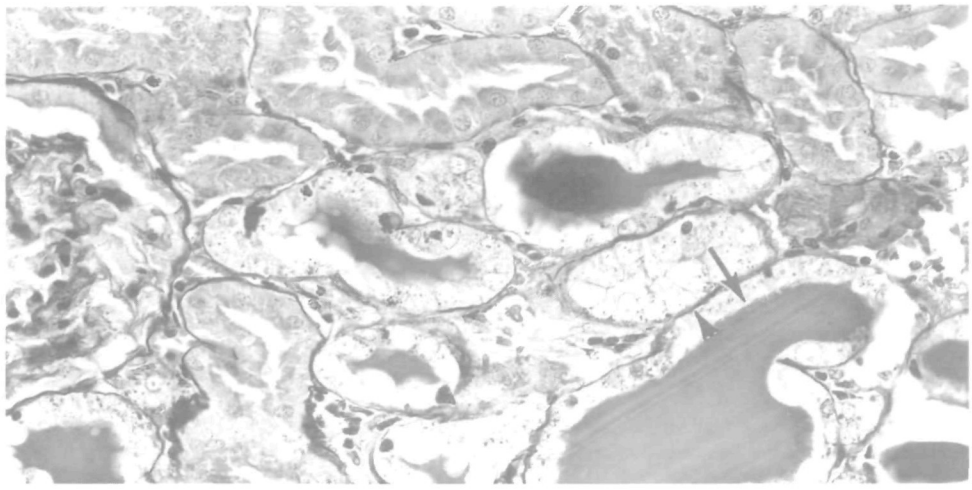

FIG. 6. Renal cortex from a male rat which received 600 mg UN/L of drinking water for 91 days Focus of glomerular injury with accumulation of protein in the urinary space of the glomerulus and protein casts irregularly dilating adjacent proximal tubules (arrow). There is irregular vesiculation and pyknosis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

REFERENCES

Ackerman, D. M., and Hook, J. B. (1984). Biochemical interactions and nephrotoxicity. *Fundam. Appl. Toxicol* **4,** 309–314.

Avasthi, P. S., Evan, A. P., and Hay, D. (1980). Glomerular endothelial cells in uranyl nitrate-induced acute renal failure in rats. *J. Clin Invest.* **65,** 121–127.

Bentley, K. W., Stockwell, D. R., Britt, K. A., and Kerr, C. B. (1985). Transient proteinuria and aminoaciduria in rodents following uranium intoxication. *Bull Environ. Contam. Toxicol.* **34,** 407–416.

Chu, I , Villeneuve, D C., and Secours, V. E. (1983). Comparative toxicity of 1,2,3,4-, 1,2,4,5-, and 1,2,3,5-tetrachlorobenzene in the rat. Results of acute and subacute studies. *J Toxicol. Environ. Health* **11,** 663–677.

Chu, I., Villeneuve, D. C., Secours, V. E , Yagminas, A., Reed, B., and Valli, V. E. (1984). Octachlorostyrene: A 90-day toxicity study in the rat. *Fundam. Appl. Toxicol.* **4,** 547–557.

Cooper, J. R., Stradling, G. N., Smith, H., and Ham, S. E. (1982). The behaviour of uranium-233 oxide and uranyl-233 nitrate in rats. *Int. J. Radiat. Biol.* **41,** 421–433.

Cothern, C. R., and Lappenbusch, W. L (1983). Occurrence of uranium in drinking water in the US. *Health Phys.* **45,** 89–99.

Cothern, C. R., Lappenbusch, W. L., and Cotruvo, J. A. (1983). Health effects guidance for uranium in drinking water. *Health Phys.* **44**(Suppl. 1), 377–384.

Drury, J. S., Reynolds, S., Owen, P T., Ross, R. H., and Ensminger, J. T. (1981). *Uranium in the US Surface, Ground and Domestic Waters* EPA-570/9-81-001.

Haley, D. P. (1982). Morphological changes in uranyl nitrate-induced acute renal failure in saline- and water-drinking rats. *Lab. Invest.* **46,** 196–208.

Haley, D. P., Bulger, R. E., and Dobyan, D. C. (1982). The long-term effects of uranyl nitrate on the structure and function of the rat kidney. *Virchows Arch. (Cell Pathol.)* **41,** 181–192.

Kobayashi, S., Nagase, M., Honda, N., and Hishida, A. (1984). Glomerular alterations in uranyl acetate-induced acute renal failure in rabbits *Kidney Int.* **26,** 808–815

Leggett, R W. (1989). The behaviour and chemical toxicity of U in the kidney: A reassessment. *Health Phys.* **57,** 365–383.

Lim, I. K., Lee, K. H., Han, B. D , Jang, J. J , and Yun, T. K. (1987) Uranyl nitrate induced polyuric acute tubular necrosis in rats. *Yonsei Med. J.* **28,** 38–48

Morrow, P., Gelein, R., Beiter, H., Scott, J., Picano, J., and Yuile, C. (1982). Inhalation and intravenous studies of UF_6/UO_2F_2 in dogs. *Health Phys.* **43,** 859–873.

Moss, M. A. (1989). *Study of the Effects of Uranium on Kidney Function.* Atomic Energy Control Board Report INFO-0306 Ottawa, Canada.

Moss, M. A., McCurdy, R. F., Dooley, K. C , Givner, M. L. G., Dymond, L. C., Slayter, J. M., and Courneya, M. M (1983). Uranium in drinking water—Report on clinical studies in Nova Scotia. In *Chemical Toxicology and Clinical Chemistry of Metals* (S Brown and J. Savory, Eds.), pp. 149–152. Academic Press, London.

Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrenner, K , and Bent, D H. (1977). *Statistical Programs for the Social Sciences.* SPSS Inc., Chicago

Novikov, Yu, V. (1972). Hygienic standardization of the permissible content of uranium in drinking water. *Gig. Sanit* **37,** 13–17.

Priest, N. D., Howells, G. R., Green, D., and Haines, J. W. (1982) Uranium in bone: Metabolic and autoradiographic studies in the rat *Hum. Toxicol* **1,** 97–114.

Tracy, B. L., Quinn, J. M., Lahey, J., Gilman, A. P , Mancuso, K., Yagminas, A. P., and Villeneuve, D. C. (1992). Absorption and retention of uranium from drinking water by rats and rabbits. *Health Phys.* **62,** 65–73.

U.S. EPA (1991) National primary drinking water regulations; radionuclides; proposed rules. Environmental Protection Agency. *Fed. Regist.* **56**(138), 33050–33127.

Villeneuve, D. C., Valli, V. E., Chu, I., Secours, V., Ritter, L., and Becking, G. C. (1979). Ninety-day toxicity of photomirex in the male rat. *Toxicology* **12,** 235–250.

Yagminas, A. P., and Villeneuve, D. C. (1977). An automated continuous-flow assay for serum sorbitol dehydrogenase activity and its use in experimental liver damage. *Biochem. Med.* **18,** 117–125