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Evaluation of the Oral Toxicity of Uranium in a 4-Week Drinking–Water Study in Rats

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Uranium is an ubiquitous constituent of man's natural environment. Most exposure to uranium has occurred during the mining, processing, and transformation of the metal into fuel elements for nuclear reactors (Cothern and Lappenbusch 1983; Tasat and De Rey 1987). The enormous expansion in the operations involving uranium that has occurred over the last forty years has led to the dispersion of concentrated uranium at many sites over almost the entire world. While the dangers due to nuclear reactions are given wide play in the public press, the toxicological hazards of increased levels of uranium are less widely appreciated.

The spectrum of acute toxicity has been extensively studied in animals and to some extent in man (Tannenbaum 1951; Thun et al. 1985; Harvey et al. 1986; Domingo et al. 1987). Much less is known about the chronic effects due to the ingestion of uranium. Moreover, drinking water as a route of uranium exposure has not been extensively investigated (Cothern et al. 1983).

The study reported herein was designed to assess the toxic effects of uranium in rats when the metal was given orally for four weeks. Due to the fact that in solution the uranyl ion (UO_2^{2+}) is the most stable species of uranium and the form in which this metal is present in the mammalian body (Cothern and Lappenbusch 1983; La Touche et al. 1987), uranium has been administered as uranyl acetate.

MATERIALS AND METHODS

A total of forty male Sprague-Dawley rats (70-90 g, obtained from Interfauna Ibérica, Barcelona, Spain) were exposed to uranyl acetate dihydrate (Merck, Darmstadt, FRG) in the drinking water at levels of 0, 2, 4, 8 and 16 mg/kg body weight/day continously for four weeks. Solutions were prepared weekly to achieve a constant intake. Sugar was added to reduce the aversive effect of the metal in the water. Similar amounts of sugar were also added to the drinking water of control animals to make comparable the results. Food (Panlab diet, Barcelona, Spain) and water consumption were freely available.

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The animals were placed in individual metabolism cages. Body weights of all rats were recorded prior to treatment and daily throughout the

Table 1. Nutritional parameters[a] in rats receiving uranyl acetate at different doses[b] for four weeks.

	1st week	2nd week	3rd week	4th week
Food (g)				
0	138,0±13,7	173,9±30,2	170,9±32,6	163,8±36,9
2	139,7±5,4	161,1±22,2	125,8±21,3	148,3±12,8
4	143,5±19,5	184,1±26,3	180,2±17,5	162,1±18,1
8	134,3±11,1	174,0±17,4	172,6±18,4	164,4±20,5
16	143,7±11,9	184,3±17,0	185,5±11,3	175,0±18,2
Weight gain (ΔP in g)				
0	37,2±8,9	59,8±7,3	57,0±10,2	30,9±15,2
2	26,1±7,7	55,1±15,1	47,6±13,1	47,3±5,6
4	43,9±19,7	62,4±15,7	35,1±12,6*	45,2±13,4
8	35,7±10,3	60,3±7,0	43,3±6,7*	37,7±17,1
16	39,7±7,1	62,3±8,7	40,4±15,7*	34,6±8,8
Water (ml)				
0	182,9±62,8	223,7±73,0	259,8±94,7	250,0±73,6
2	192,3±62,1	307,4±57,5	257,7±121,0	304,6±167,8
4	214,8±64,0	250,2±73,1	262,9±122,7	280,3±76,1
8	178,1±25,3	255,8±77,7	207,5±76,1	276,4±68,3
16	212,1±47,0	257,6±79,9	314,6±116,1	320,0±104,4
Urine (ml)				
0	37,9±13,1	59,0±28,4	90,3±31,8	95,5±34,6
2	48,4±14,1	68,8±26,3	91,1±58,7	108,6±93,0
4	39,3±17,0	67,7±40,9	98,2±49,2	101,3±28,7
8	33,6±16,3	58,3±30,0	61,2±26,4	85,5±40,9
16	30,3±11,6	91,3±32,9	103,7±30,6	100,2±33,2
Feces (g)				
0	26,1±4,1	35,2±4,7	37,4±8,4	25,9±3,0
2	24,1±6,2	33,0±9,2	37,9±12,1	30,2±9,8
4	26,5±4,8	35,6±13,7	39,1±8,9	29,7±5,1
8	22,9±3,0	31,4±5,9	37,6±6,5	25,4±5,3
16	24,8±5,7	34,8±10,8	37,8±11,8	28,8±4,8

[a]Results are expressed as arithmetic means ± S.D. [b]Doses are expressed in mg/kg/day. *P<0,05 by the Mann-Whitney U test.

study. Food and water consumption, and the volume of urine and the weight of excreted feces were also measured daily. At the end of the experiment, blood samples were collected from the tail vein. Hematological parameters and concentrations of glucose, urea, creatinine, total protein, albumin, GOT and GPT in plasma, as well as the concentrations of total protein, urea and creatinine in urine were

determined according to clinical chemistry methods described earlier (Domingo et al. 1987).

After four weeks, the animals were sacrificed and the weight of brain, heart, lungs, spleen, kidneys, liver, thyroid, thymus and testes measured. Histological examinations (paraffin slices, hematoxylin-eosin) of brain, kidneys, liver, thymus, stomach, thyroid and bone tissue (femur) were performed in six rats of each group. Conventional transmission electron microscopy of the kidneys was also performed in three animals of the highest dose (16 mg/kg/day).

Uranium concentrations in brain, heart, lungs, spleen, kidneys, liver, testes, thyroid, thymus, bone (femur) and abdominal muscle were measured in a computer-controlled sequential inductively coupled plasma spectrometer (Jobin Ybon JY38-VHR) after digestion as previously described (Ortega et al. 1988).

The significance of the differences in the results was determined by the Mann-Whitney U test. A difference was considered to be significant when $P<0.05$.

RESULTS AND DISCUSSION

During the treatment no deaths were observed at any of the dose levels tested and the rats appeared to be healthy throughout the study. Food and water intake, as well as the volume of urine and the amount of feces excreted showed no significant differences from the control group (Table 1, $P>0.05$). There was no significant weight-gain depression in any of the uranyl acetate-treated groups, except for the rats receiving 4, 8 or 16 mg/kg/day during the third week (Table 1).

The results of the hematological examinations are presented in Table 2. No consistent compound-related effects were observed in any of the parameters measured in rats dosed with 2, 4, 8 or 16 mg/kg/day. However, a significant increase in the values of hematocrit, hemoglobin, mean corpuscular hemoglobin concentration (MCHC) and in the number of erythrocytes was seen at 16 mg/kg/day of uranyl acetate. Plasma analyses carried out in the treated and control animals after four weeks of treatment are also summarized in Table 2. No significant changes were detected in the levels of urea, creatinine or albumin. Nevertheless, a number of statistically significant differences from control values were observed in the concentrations of glucose (increases at 4,8 and 16 mg/kg/day), total protein (increases at 2, 4, 8 and 16 mg/kg/day) and GOT or GPT (decreases at 8 and 16 mg/kg/day).

The urinary measurements (total protein, urea and creatinine) in the treated animals gave no results that consistently differed significantly from the controls (data not shown).

The concentrations of uranium in tissues of rats given uranyl acetate for four weeks are shown in Table 3. Uranium was not detected in the tissues of control animals or in those which received the lower dose of the metal (except for kidneys, liver and muscle). At both 8 and 16 mg/kg/day groups uranium could be detected in all the organs and

tissues analysed. Uranium accumulated dose-dependently and the highest concentrations of the metal were found in kidneys, thyroid, bone and muscle.

Table 2. Hematological and clinical chemical parameters[a] in rats dosed with uranyl acetate for four weeks.

	Dose (mg/kg/day)				
	0	2	4	8	16
Hematocrit (%)	43,2±1,2	45,2±2,7	42,7±3,3	42,4±1,2	45,5±1,3**
Erythrocytes (10^6/mm^3)	7,2±0,2	7,1±0,3	7,0±0,2	7,2±0,2	7,7±0,3**
Leucocytes (10^3/mm^3)	12,3±4,5	11,0±3,5	15,9±2,2	13,5±2,7	14,3±4,5
Hemoglobin (g/100 ml)	14,7±0,2	14,3±1,4	14,7±0,9	14,9±0,4	16,0±0,4***
MCV (µm^3)	60,3±2,0	62,9±4,8	60,4±4,5	59,0±1,4	59,1±2,2
MCH (pg)	20,7±0,6	21,6±1,1	20,9±1,1	20,8±0,5	20,8±0,7
MCHC (%)	34,1±0,6	30,7±2,1	34,7±1,1	35,1±0,1**	35,1±0,2**
Glucose (mg/100 ml)	140,1±12,9	151,8±7,9	175,2±5,0***	185,3±12,1***	180,1±9,4***
Total protein (g/100 ml)	6,2±1,2	6,5±2,8*	6,4±2,6*	6,4±2,4*	6,6±1,7***
Albumin (g/100 ml)	4,0±0,9	4,1±1,5	4,1±1,5	4,0±2,4	4,1±1,6
Urea (mg/100 ml)	49,5±6,4	43,8±4,7	47,2±7,3	49,8±6,1	43,9±6,4
Creatinine (mg/100 ml)	0,6±0,07	0,7±0,08	0,6±0,04	0,7±0,04	0,7±0,03
GOT (U/l)	155,2±35,1	136,9±23,7	124,9±10,1*	97,2±9,9***	101,1±9,8***
GPT (U/l)	73,1±13,9	59,8±12,8	56,6±5,7*	59,6±7,8*	49,9±11,0**

[a]Results are expressed as arithmetic means ± S.D.
*P<0,05, **P<0,01, ***P<0,001 by the Mann-Whitney U test.

At necropsy, no statistically significant differences were found in the organ weights or in the relative organ weights of brain, heart, lungs, spleen, kidneys, liver, thyroid, thymus and testes (data not shown). No histopathological lesions were observed in the tissues of animals receiving 2, 4 or 8 mg/kg/day of uranyl acetate. However, a small congestion in the liver, spleen and kidneys of rats given 16 mg/kg/day could be noted in 30% of the animals. In addition, a moderate increase in the lysosomal content of the epithelial cells of the proximal convoluted tubule was seen at the ultrastructural level in animals receiving 16 mg/kg/day (Fig. 1) whereas the remaining part of the nephron was normal (Fig. 2).

Many investigations of uranium biokinetics and chemical toxicity in mammals have been undertaken (Voegtlin and Hodge 1949; Tannenbaum 1951; La Touche et al. 1987). Due to the fact that inhalation has normally been considered the main route of entry into the body, most reports about the chemical toxicity of uranium are inhalation studies

(Yuile et al. 1973). Notwithstanding, there are very few data on oral ingestion of uranium (Cothern et al. 1983). The above is a report on the results of a short-term toxicity study of uranium in rats which received the metal in the drinking water at doses which correspond approximately with 1/100, 1/50, 1/25 and 1/12.5 of the acute oral LD_{50} of uranyl acetate dihydrate previously determined (Domingo et al. 1987). Ingestion of 2, 4 or 8 mg/kg/day of uranyl acetate dihydrate by rats over a period of four weeks had little effect on the parameters investigated in this study.

Table 3. Uranium concentrations[a] in organs and tissues of rats receiving uranyl acetate for four weeks.

	Dose (mg/kg/day)				
	0	2	4	8	16
Brain	ND	ND	ND	478±110	588±100
Heart	ND	ND	216±12	425±145	477±169
Lungs	ND	ND	239±97	456±137	513±99
Spleen	ND	ND	ND	436±112	654±68
Kidneys	ND	412±116	590±147	1288±339	1429±181
Liver	ND	478±160	296±48	616±171	494±127
Testes	ND	ND	ND	280±81	340±92
Thyroid	ND	ND	260±65	610±136	1102±321
Thymus	ND	ND	ND	467±178	755±159
Bone	ND	ND	443±138	410±158	849±300
Muscle	ND	308±79	270±117	479±132	1092±325

[a]Values are expressed as arithmetic means ± S.D. Concentrations of uranium are presented for tissues as ng/g fresh weight.
ND= Not detected. Detection limit: 200 ng/g

There was no hypertrophy or hypotrophy in the organs removed after the treatment, nor were histopathological lesions evident at 2, 4 or 8 mg/kg/day. However, changes in the nephron of the animals receiving 16 mg/kg/day of uranyl acetate dihydrate could be detected. Nephritis is the primary chemically-induced toxic effect of uranium in animals and humans (Blantz 1975; Thun et al. 1985; Bentley et al. 1985).

The highest tissue concentrations of uranium were found in kidneys, thyroid, bone and muscle. Bone and kidneys have been reported as target tissues of uranium accumulation after administration of the metal (Kisieleski et al. 1952; La Touche et al. 1987).

Using a safety factor of 50-150, the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) recommends a limit of uranium in water of 100 µg/l in order to limit toxic effects in the kidney (Wrenn et al. 1985). Therefore, an average adult of 70 kg body weight, consuming 2 l water per day would not ingest more than 200 µg U/day, which is equal to 0.36 mg/day of uranyl acetate dihydrate (0.005 mg/kg/day). On the basis of the above results and discussion, it is concluded that the no-observed-adverse-effect level of uranyl acetate dihydrate was 2 mg/kg/day. Thus, a safety factor of

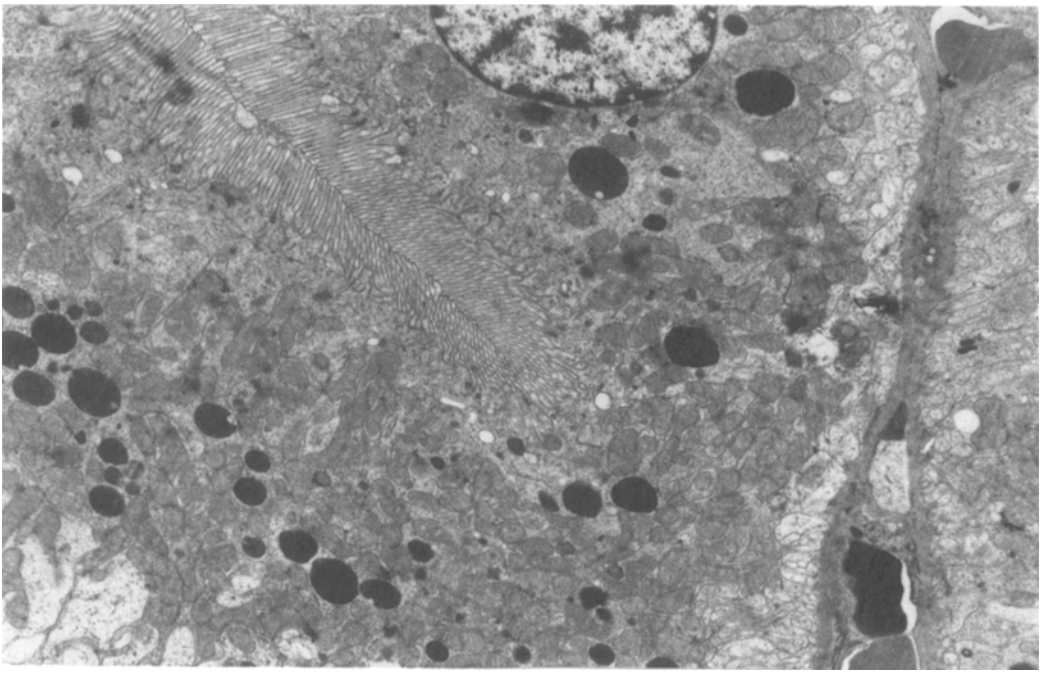

Figure 1. Epithelial cells from the proximal tubule of the nephron with abundant secondary lysosomal profiles. Rats received 16 mg/kg/day of uranyl acetate dihydrate in the drinking water for 4 weeks.

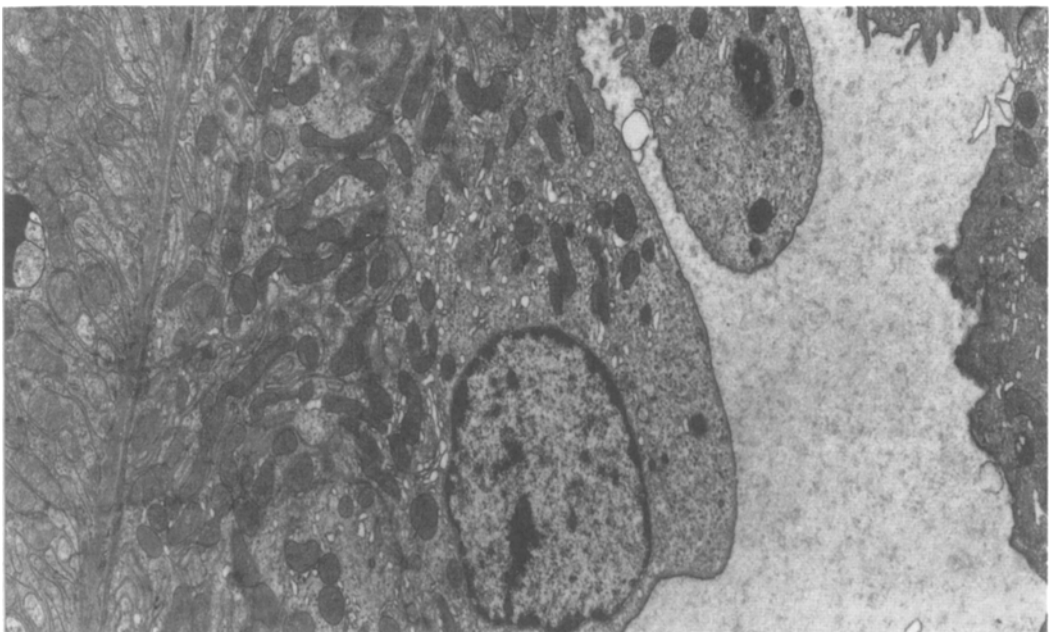

Figure 2. Normally appearing cells from a distal tubule. Rats received 16 mg/kg/day of uranyl acetate dihydrate in the drinking water for 4 weeks.

approximately 400 can be calculated. Consequently, the present study suggests that uranium does not cause adverse effects at the concentrations usually ingested by men in the drinking water.

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